Preparation and Spectroanalytical Studies of Two New Azo Dyes Based on Luminol

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Abstract. This study involves the preparation of two new azo dyes from luminol with 4-hydroxycoumarin [3-(luminolazo)-4-hydroxycoumarin] (A1) and with 2-vanillin [5-(luminolazo)-2-vanillin] (A2). The characterization of dyes have been described by C.H.N., H¹-NMR, TGA, DTG, I.R. and visible spectroscopic techniques. The electronic spectra of these azo dyes were studied in term of acid-base properties at different pH values (2 - 12), which includes establishing isobestic points and determination of protonation and ionization constants. The other study was the effect of solvents of different polarities on the electronic spectra.

Keywords: Azo Dye, Isobestic Points, Acid-Base Properties, Solvent Effects

Introduction

The importance of azo-dyes is due to its economy, versatility, relative ease of production and good dying performance [1,2]. As a significance of n-delocalization, aryl azo compounds have powerful colors, especially reds, oranges, and yellow. Therefore, they are used as dyes, and are commonly known as azo dyes [3].

The structural features of azo-dyes, that confer color are the azo (–N=N–) group [4]. Attachment of dye to the fiber is due to salt formation between the ionic groups of both the dye and the fiber [5]. Furthermore they have been studied widely for their excellent thermal and optical properties in applications. Several studies have been published on the synthesis and spectral properties of azo dyes [6-9]. This reflects their widely important applications in different field. Most of azo dyes have acid – base properties with presence of a fixed isobestic points (which represent the number of equilibriums in such azodye), for this reason they are used as acid – base indicators [10]. Some of them can be used as metallochromic indicators, antipyretic reagents and inhibitors corrosion. The solvents of different polarities may be affected on the absorption spectra of π – conjugated compounds, exhibit n → π* transition as well as π→π* transition causing blue or red shifts. They were used as good reagents for the extraction and spectrophotometric determination of metal ions [11].

The luminol is a good reagent for forming azo dye like with 2-thiobarituric acid [12], with study of acid-base properties at different pH values. The 4-hydroxycoumarin is also a good reagent for forming azo dyes like with 2-aminothiazole, 2-aminobenzothiazole, 4-aminooantipyryne, 4-aminoacetophenone, adenine sulphate, naphthylamine and sulphadimidin [13] and also with aniline and aniline derivatives [14-16].

Two new o-vanillin-based Azo dye ligands were prepared by reaction of sulfadiazine and sulphaguanidin and with o-vanillin (2- Hydroxy-3-methoxybenzaldehyde). The characterization of
dyes have been described by C.H.N., I.R. N.M.R, mass spectra and visible. spectroscopic techniques, the electronic spectra of these azo dyes were studied in term of acid-base properties at different pH values (2-12), which includes establishing isobestic point, mechanism and determination of protonation and ionization constants of dyes. The effect of solvents of different polarities on the electronic spectra were also investigated [17]. The o-vanillin is also form azo dye 4-hydroxy-3-methoxy-5-(phenylazo)benzaldehyde-2 [18].

The present work involves preparation of two new azo dyes from luminol with 4-hydroxy-coumarin [3-(luminolazo)-4-hydroxycoumarin] and with 2-vanillin [5-(luminolazo)-2-vanillin]. The work involves two spectral studies, the first acid-base properties of deferent pH values and the second solvent effect of different polarities.

**Experimental**

All the reagents and solvents were of reagent-grad quality. The progress of reaction was monitored by TLC using silica gel coated plate sand spots were visualized under UV radiation. Infrared spectra (in KBr pellets) were recorded on FT.IR-8400S shimadzu, Melting points were determined on melting point apparatus, Element analysis (C.H.N.) were carried out by Perkin element 2400 element analysis and UV/V absorption spectra were of the dyes were recorded using Perkin Elmer Lampda EZ 210 UV/V Spectrophotometer. The pH measurements were made with pH-Meter (H. Jurgons Co. Bremen,L.Puls Munchen15).

- **Preparation of azo dyes [17]**

Azo dyes were prepared by dissolving 0.004 mole of (luminol) with 1.4 ml concentrated HCl and 20 ml distilled water then adding drop by drop with stirring solution of (0.304 g. sodium nitrite in 20 ml distilled water) to form diazonium salt at -5°C temperature. The alkaline solution of 0.004 mole of (4-hydroxycoumarin or o-vanillin) in (1.2% w/v. NaOH) was added to the above diazonium salt. Then convert the prepared dye from sodium form into hydrogen form by adding of dilute HCl (keep pH value between 6.5 – 7.5). Filter the precipitate of dyes formed, and purify the dye by recrystallization from methanol and dried at 50.0°C in an oven to give azo dyes. By the aid of CHN and IR, the suggested molecular structures of azo compounds were shown in Scheme -1.

![Scheme -1](image)

Scheme -1. $A_1 = C_{17}H_{10}N_4O_5$ (M.wt.350.3) [3-(luminolazo)-4-hydroxycoumarin]

$A_2 = C_{16}H_{12}N_4O_5$ (M.wt.340.3) [5-(luminolazo)-o-vanillin]

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-Solutions
* $1 \times 10^{-3}$M of each of azo dyes A$_1$ and A$_2$
* Universal buffer solutions (pH 2-13) [19]
* Buffer solutions of pH 7 ($\text{Na}_2\text{HPO}_4\cdot2\text{H}_2\text{O} + \text{KH}_2\text{PO}_4$, hexamine, $\text{KH}_2\text{PO}_4 + \text{NaOH}$, $\text{KH}_2\text{PO}_4 + \text{borax}$, $\text{Na}_2\text{HPO}_4\cdot2\text{H}_2\text{O} + \text{citric acid}$.) [19]

-Procedure

- Acid – base properties at different pH values

A series of buffer solutions were prepared with different pH values (2 – 12) for total dye concentration for total dye concentration $8 \times 10^{-5}$M (for A$_1$) and $12 \times 10^{-5}$M (for A$_2$) via using universal buffer. The absorbance of these solutions (by using the pH value as blank solution) were recorded at range of (350 – 660 nm).

-Solvent effect of different polarities

A series of solution of dye at total concentration $8 \times 10^{-5}$M (for A$_1$) and $12 \times 10^{-5}$M (for A$_2$) were completed to the mark with, Acetone, Methanol, DMF, DMSO, Ethanol, 1,4- Dioxan, Cyclohexanol, dichloromethane and H$_2$O, the absorbance of these solutions were recorded at range of (350 – 600 nm) using the solvent as blank solution.

Results and Discussion

The prepared azo dyes are stable in air at room temperature and soluble in DMF, DMSO, methanol, ethanol and acetone.

-Elemental analysis

Table-1 shows the collects the physical properties and analytical data for the prepared azo dyes (A$_1$ and A$_2$) with good results was obtained.

**Table (1): Physical properties and elemental analysis for novel azo dyes.**

| dye | Molecular formula | m.p ºC | Yield (%) | Color | C % Cal. (found) | H % Cal. (found) | N % Cal. (found) |
|-----|-------------------|--------|-----------|-------|-----------------|-----------------|-----------------|
| A$_1$ | $\text{C}_{17}\text{H}_{10}\text{N}_4\text{O}_5$ | 200 C | 85 | yellow | 58.28 (59.10) | 2.85 (3.39) | 16.00 (15.01) |
| A$_2$ | $\text{C}_{16}\text{H}_{12}\text{N}_4\text{O}_3$ | 290-292 | 82 | red | 56.47 (58.13) | 3.66 (3.52) | 16.47 (15.67) |

-IR- Analysis

The most important IR absorption bands corresponding to the azo dyes are presented in Table 2. The observed band in the range 1450 cm$^{-1}$ is due to $\nu$(N=N) group of the ligand. The sharp bands in the range 1521-1587 cm$^{-1}$ are due to $\nu$(C=C).
Table (2): Selected infrared data of A1 and A2

| Dye | $\nu$(OH) cm$^{-1}$ | $\nu$(N=N) cm$^{-1}$ | $\nu$(C=C) cm$^{-1}$ | $\nu$(N-H) cm$^{-1}$ | $\nu$(C=O) cm$^{-1}$ |
|-----|-----------------|------------------|-------------------|------------------|------------------|
| A1  | 3450 w          | 1462.04 s        | 1558s             | 3425w            | 1651s            |
| A2  | 3425 w          | 1489.05 m        | 1543m             | 3400w            | 1651s            |

-1H-NMR spectrum of azodyes

The 1H-NMR spectra of the azo dyes (Figs. 1 and 2) was measured using in DMSO as a solvent. The azo dyes spectral results show peaks at $\delta = 7.27$-7.28 ppm attributed to the phenyl ring. The single peak at $\delta = 10.38$ ppm due to the OH groups in azo dyes. The signal peak at 10.5 ppm due to S,1H, CHO. The result shows in Table.(3)

Table (3): 1H-NMR data of the azo dyes.

| Dye | $\delta$ (ppm) |
|-----|----------------|
| A1  | 2.5 (s, 6H, DMSO), 3.3(s, H, HOD), 5.5 (s, 2H, NH), 12.5 (s, H, OH), 6.5-8.5 (m, 7H, Ar-H) |
| A2  | 2.5 (s,6H,DMSO), 3.3 (s,H,HOD), 3.8 (s,3H, OCH$_3$), 6.5-8.5 (m,5H,Ar-H), 12.2 (s, H,OH), 4 (s,2H,NH), 10.3 (s,H,CHO) |

s = Singlet, m = Multiplet

Figure. (1): 1H-NMR spectrum of A1
-Thermal Analysis

To study the thermal stability of azo dyes A1 and A2 (Table 4) at temp. range (0 - 700°C) to fix the decomposition temperature (initial Ti, T max ant final Tf) and carbonated level at temp. 700°C. For the high stability of dyes, it was found that the weight loss not more than 14% and half loss weight is not reach 50%, the weight loss is mostly as evaporated gases.

| Dye | Charting content at 700°C | R Min % | T(i) °C | T(max) °C | T(f) °C |
|-----|--------------------------|--------|---------|-----------|---------|
| A1  | 86.00%                   | 1.03   | 20.00   | 32.91     | 110.00  |
|     |                          |        | 310.00  | 355.77    | 385.00  |
|     |                          |        | 580.00  | 667.09    | 700.00  |
| A2  | 89.30%                   | 0.79   | 27.00   | 59.81     | 140.00  |
|     |                          |        | 300.00  | 328.86    | 415.00  |
|     |                          |        | 627.00  | 672.86    | 700.00  |

Acid-Base properties

For study the effects of acidity and basicity of universal buffer solutions on the dyes and to calculate the ionization and protonation constants, a series of universal buffer solutions were prepared at different pH values (2-12) for each dye. The absorption spectra of 8 x 10⁻⁵ M solution of A1 dye of varying pH values (2–12) were represented graphically at 340-630 nm. (Fig. 3). The spectra characterized by three maximal bands the first at 420 nm. in pH range (2 - 6) which represents protonated form (cation form) and the second at wavelength range (380-410 nm.) at pH value range (7-9), which represent the neutral form and the third at 425 nm. at pH range of (10-12), which represent basic form (an ionic form). From the figure it was found that there is one isobestic point at 400 nm. In case of A2 azo dye (Fig. 4), the all spectra characterized by two maximal bands first at 350 nm. in pH range (2 - 7) which represents protonated form (cation form). The second at 360 nm.
at pH range (10-12), there were found two isobestic points at 450 and 460 nm. From Figs. 5 and 6 the best pH value that gives the highest absorbance was at 7.

![Absorption spectra of azo dye A1 at different pH values](image1.png)

**Fig. (3): Absorption spectra of azo dye A1 at different pH values**

![Absorption spectra of azo dye A2 at different pH values](image2.png)

**Fig. (4): Absorption spectra of azo dye A2 at different pH values**

To determine the ionization and protonation constants of the azo dyes A1 and A2, at certain wavelength 360 nm. and 420 nm. of azo dyes A1 and A2 respectively (Figs. 3 & 4), the absorbance – pH curves were plotted (Fig. 5). The ionization and protonation constants were
calculated (Table 5). From Absorbance–pH curve and by the aid of half-height method [17], the pK values were obtained by this relation

$$pK = pH (at \ A_{1/2})$$

where : \(A_{1/2} = \frac{(A_L + A_{min})}{2}\)

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

Fig. (5): The Absorbance – pH curves of Azo Dyes A1 and A2

Table (5): The ionization and protonation constants of azo dyes A1 & A2

| Azo dye A1 at λ = 360 nm | Azo dye A2 at λ = 420 nm |
|--------------------------|--------------------------|
| A_m | A_1 | A_{1/2} | pK  | A_m | A_1 | A_{1/2} | *pK |
| 0.44 | 0.50 | 0.470 | 4.51 (p) | 0.31 | 0.370 | 0.340 | 3.41 (a1) |
| 0.48 | 0.80 | 0.640 | 6.51 (a1) | 0.32 | 0.446 | 0.380 | 7.25 (a1) |
| 0.72 | 1.19 | 0.955 | 9.40 (a2) | 0.38 | 0.518 | 0.450 | 10.20 (a2) |

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

pK_p is protonation constant for oxygen atom of luminol molecule
pK_{A1} is ionization constant for hydroxyl group of 4-hydroxycumarine
pK_{A2} is the second ionization constant for hydroxyl group (in enol form) of luminol
*pK_p is protonation constant for oxygen atom of aldehyde group of o-vanillin
*pK_{A1} ionization constant for hydroxyl group of o-vanillin
*pK_{A2} is the second ionization constant for hydroxyl group (in enol form) of luminol

Kind of buffer solution of pH7

To fine the effect of kind of pH 7, several solution of buffer that gives pH 7 like (Na₂HPO₄·2H₂O + KH₂PO₄, hexamine, KH₃PO₄ + NaOH, KH₂PO₄+ borax, Na₂HPO₄·2H₂O + citric acid.) [19]. The best kind which gives highest absorbance were Universal Buffer solution with respect of azo dye A1 and Na₂HPO₄·2H₂O + citric acid or Na₂HPO₄·2H₂O + KH₂PO₄ with respect to dye A2.

Solvents effect

Figures 6 and 7 show the spectra of azo dyes (A1 & A2) at different solvents with one band at \(\lambda_{max}\) in the range of (410-430nm.) for azo dye A1 and with two bands with respect azo dye A2, the first at \(\lambda_{max}\) at 350 nm. and the second at range of \(\lambda_{max}\) (450-490 nm.) except for the solvent DMF and DMSO having three bands at the first at \(\lambda_{max}\) of 350 nm., the second at range of \(\lambda_{max}\) of (455
and 465 nm.) and the third at range of $\lambda_{\text{max}}$ of (530 and 540 nm.) for the solvents DMF and DMSO respectively. The Figures also show the solvents dichloromethane and DMSO give beigest absorbance of azo dyes $A_1$ and $A_2$ respectively. It was shown for azo dye $A_1$ (Fig.6) there were red shift to the bands w.r.t DMF and DMSO and little red shift with other solvent except for with water there is blue shift. In case of azo dye $A_2$ (Fig.7) it was found observed red shift w.r.t DMF and DMSO and little red shift with other solvents.

![Fig.(6): Electronic spectra of azo dye $A_1$ at different solvents](image)

The absorption spectra in various solvents are influenced by salvation and / or dielectric effects of solvents. To verify whether the band shift ($\Delta\nu$) is due to change in salvation energy or pure dielectric. Table (6): The Functions $(D-1)/(D+1)$ ($D$ is dielectric constant) of solvent and $\lambda_{\text{max}}$ of azo dyes $A_1$ and $A_2$. 

| Solvent       | $\lambda_{\text{max}}$ (nm) | Absorbance |
|---------------|-------------------------------|------------|
| Aceton        | 340                           | 1.2        |
| DMF           | 360                           | 1.1        |
| 1,4 Dioxane   | 380                           | 1.0        |
| Di Chloro Methan | 400                  | 0.9        |
| DMSO          | 420                           | 0.8        |
| Ethanol       | 440                           | 0.7        |
| Methanol      | 460                           | 0.6        |
| Cyclo hexanol | 480                           | 0.5        |
| Water         | 500                           | 0.4        |

Fig. (6): Electronic spectra of azo dye $A_1$ at different solvents
Fig. (7): Electronic spectra of azo dye A2 at different solvents

Table (6): The Functions (D-1)/(D+1) of solvent and $\lambda_{\text{max}}$ of azo dyes A1 and A2

| Symbol No. | Solvent     | D [19] | (D-1)/(D+1) | $\lambda_{\text{max}}$ nm A1 | A2         |
|------------|-------------|--------|-------------|------------------------------|------------|
| 1          | Acetone     | 20.60  | 0.907       | 420                          | 360m, 455m |
| 2          | DMF         | 36.71  | 0.923       | 425                          | 360m, 470m, 540s |
| 3          | 1,4-Dioxan  | 2.20   | 0.375       | 425                          | 360m, 455m |
| 4          | Dichloromethan | 9.10  | 0.802       | 430                          | 360w, 455m |
| 5          | DMSO        | 47.00  | 0.958       | 430                          | 360w, 455m, 555s |
| 6          | Ethanol     | 24.30  | 0.921       | 425                          | 360w, 470m |
| 7          | Methanol    | 32.70  | 0.941       | 420                          | 360w, 470m |
| 8          | Cyclohexanol| 15.00  | 0.851       | 435                          | 360w, 470m |
| 9          | Water       | 78.40  | 0.975       | 415                          | 360w, 470m |

s = Strong, m = Medium, w = Weak

Table (7) shows the values of maximum wavelengths, the molar absorption coefficients of azo dyes A1 and A2 at different polarities solvents.
Table (7): The values of $\lambda_{\max}$ and $\varepsilon_{\max}$ azo dyes A1 and A2 at different solvents

| No. | Dye | Solvent       | $\lambda_{\max}$ | $\varepsilon_{\max} \times 10^4$ | $\lambda_{\max}$ | $\varepsilon_{\max} \times 10^4$ | $\lambda_{\max}$ | $\varepsilon_{\max} \times 10^4$ |
|-----|-----|---------------|-------------------|---------------------------------|-------------------|---------------------------------|-------------------|---------------------------------|
|     |     |               | $\pi \rightarrow \pi^*$ | 1 mol$^{-1}$ cm$^{-1}$ | $\pi \rightarrow \pi^*$ (azo) | 1 mol$^{-1}$ cm$^{-1}$ | $\pi \rightarrow \pi^*$ (hydrazo) | 1 mol$^{-1}$ cm$^{-1}$ |
| 1   | A1  | Acetone       | 360              | 0.42                           | 455               | 0.46                           | ---                | ---                |
| 2   |     | DMF           | 360              | 0.36                           | 470               | 0.40                           | 540               | 0.45               |
| 3   |     | 1,4-Dioxane   | 360              | 0.38                           | 455               | 0.38                           | ---                | ---                |
| 4   |     | Dichloromethan| 360              | 0.38                           | 455               | 0.38                           | ---                | ---                |
| 5   |     | DMSO          | 360              | 0.38                           | 455               | 0.38                           | 555               | 0.58               |
| 6   |     | Ethanol       | 360              | 0.43                           | 455               | 0.43                           | ---                | ---                |
| 7   |     | Methanol      | 360              | 0.43                           | 470               | 0.46                           | ---                | ---                |
| 8   |     | Cyclohexanol  | 360              | 0.38                           | 470               | 0.43                           | ---                | ---                |
| 9   |     | Water         | 360              | 0.38                           | 470               | 0.32                           | ---                | ---                |

The plot of (D−1)/(D+1) against the $\lambda_{\max}$ of azo dye A1 gives more or less high linear relation with solvents of moderate polarities (Fig.8). This denotes that the dielectric constant of the medium is the main factor governing the band shift in such solvents. While in azo dye A2, all solvents having the same effect of linearity except for the solvents DMF and DMSO out of linearity, this attributed may be by hydrogen bonding between the solute and solvent (Fig.9).
Beer’s law, sensitivity and precision

It was studied the sensitivity and the linearity of Beer’s law of azo dyes A₁ and A₂ at pH 7. From Calibration Curve, the molar absorptivity coefficient at maximum wavelength (\( \varepsilon_{\text{max}} \)), Sandell Sensitivity (S), Specific Absorpitivity (a) and correlation coefficient (\( R^2 \)) were calculated [20]. The Standard Deviation (S.D.) and Detection Limit (DL) were also calculated. Table (8) shows good result were obtained for sensitivity, precision and linearity (that observed from correlation coefficient which is very near from unity).

Table(8): Data obtained from Beer’s law, sensitivity and precision of A₁ & A₂
Dye | $\lambda_{\text{max}}$ nm. | $\varepsilon_{\text{max}}$ x10$^4$ a ml.g$^{-1}$.cm$^{-1}$ | S µg.cm$^{-2}$ | R$^2$ | S.D | DL x10$^{-5}$ µg.ml$^{-1}$ | Beer's level ppm
---|---|---|---|---|---|---|---
A$_1$ | 420 | 0.54 | 0.0154 | 0.065 | 0.9948 | 0.0082 | 0.26 | 126.1
A$_2$ | 470 | 0.12 | 0.0035 | 0.286 | 0.9916 | 0.0071 | 1.38 | 190.6

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