Electrochemical and spectrastudies of some sulfa drug azodies and their metal complexes in aqueous solution

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Abstract

The electrochemical behavior of some azo compounds derived from sulfa drugs derivatives in B.R. buffer solutions of different pH containing 20 (v/v) ethanol was investigated at the mercury electrode using different techniques (DC, DPP, CV and Coulometry) to investigate the effect of medium on the electro reduction process and suggest the electrode reaction mechanism. The obtained results denoted that these compounds were reduced undergo a single irreversible 4-electron polarographic wave in acid and neutral solutions which represent the cleavage of the N=N center to the amine stage, whereas in alkaline solution, two wave are obtained the second is 2-electron irreversible wave corresponding to the reduction of CHO group to CH₂OH. The DPP and CV data showed a single peak in solutions of pH < 8, whereas three peaks are in alkaline solution. The dissociation constants of the investigated compounds were determined by using spectrophotometric and potentiometric methods. Also the metal — ligand formation constants were determined potentiometrically and found in the order Cu > Co > Ni > Zn.

Keywords: Sulfa drug azo; Polarography; Cyclic voltammetry; Potentiometry; Spectrophotometry

1.1. Introduction

Sulfa drugs and their derivatives as well as their metal complexes are considered to be very important compounds due to their uses and applications in several and biological purposes. They are extensively used for the treatment of diseases caused by several microorganisms [1]. Also, sulfa drugs containing azo group are of great importance due to their useful as models for biological systems, i.e., antifungal, antibacterial, anticancer and antimalarial [2, 3]. On the other hand, azo compounds occupy a good position as analytical reagents [4, 5], their applications asachromophoric and metallochromophoric reagents [6-8], photo-sensitizers [9, 10] and sensors [11-13]. Therefore, the electrochemistry of these compounds was the subject of many electro-chemists.

Although polarographic and voltammetric studies of azo compounds are frequently carried out [15-27], little attention was paid to investigate the electrochemical behavior those derived from sulfa drugs. Recently, several studies were carried on the electrochemistry of azo compounds using different techniques [28-30].

The present study aims to investigate the electro-reduction of some sulfa drugs azo compounds at the mercury electrode to elucidate their electrode reaction pathway using several techniques as well as determination of their dissociation constants using spectral and potentiometric methods. The study was extended to calculate the stability constants of complexes of these compounds with some transition metal ions in solution.

2. Experimental

2.1. Preparation of the solid azo compounds

All chemicals used in the present investigation were of analytical grade and used without further purification. The solidazo compounds (I-IV) were prepared [31] by gradual addition of an aqueous cold solution of sodium nitrite (0.01 mole) to a concentrated hydrochloride acid solution of sulfamethazine, sulfamerazine and sulfadiazine (0.01) mole with continuous stirring and kept for about 20 min in ice bath. The formed diazonium salt solutions were added gradually with sodium nitrite dissolved in 0.01M NaOH. After dilution, the obtained solid compounds were filtered off and washed with water. The crude materials were recrystallized from ethanol. These azo compounds were characterized by IR and 1H-NMR spectra.
2.2. Instrumentation
2.2.1. Voltammetric measurements
DC-polarograms were recorded on an ink recording SARGENT WELCH Polarograph model 4001. The capillary used as a dropping mercury electrode has the following characteristics in 0.1 M KCl (open circuit): \( m = 1.7 \text{ mg/s}, t = 3 \text{ sec.}, \) at mercury height \( h = 60 \text{ cm}. \) The mercury used was purified according to the recommended method\[32\]. DP polarograms and cyclic voltammograms of the studied compounds were recorded with a Polarographic Analyzer Model 264A – PARC (from EG&G) and the electrode assembly Model 303, with a hanging mercury drop electrode (HMDE) as a working electrode, Ag/AgCl as a reference electrode and Pt wire as a counter electrode. The constituents of the electrolysis cell were the same as in the DC-polarography. X-Y recorder Model RE 0091, Houston Instrument Division (from EG&G) was used for recording the DPpolarograms and voltammograms. A digital coulometer (Model 179) from EG&G was used for the coulometry measurements.

2.2.2. UV-visible spectra
Electronic absorption spectra were recorded at room temperature within the wavelength range 200- 600 nm using a Shimadzu UV- visible recording spectrophotometer Model1600 A.

2.2.3. Potentiometric measurements
Potentiometric measurements were performed using a Digital ORION pH-meter Model 201 accurate to \( \pm 0.02 \text{ pH} \) units having combined glass and calomel electrode, with a magnetic stirrer and a semi micro burette with divisions of 0.01 ml. The electrode was standardized before andafter titration with buffer solutions produced by FISHER (New Jersey, USA). The following solution mixtures (A, B & C) were prepared:

(A) 5 ml HCl (0.001 M) + 5 ml KCl (1M) + 10 ml ethanol+ 30 ml bi-distilled water.
(B) 5 ml HCl (0.001 M) + 5 ml KCl (1M) + 5 ml azo compound \( \left( 5 \times 10^{-3} \text{M} \right) \) + 5 ml ethanol + 30 ml bi-distilled water.
(C) 5 ml HCl (0.001 M) + 5 ml KCl (1M) + 5 ml azo compound \( \left( 5 \times 10^{-3} \text{M} \right) \) + 5 ml of metal ions \( \left( 1 \times 10^{-3} \text{M} \right) \) + 5 ml ethanol + 25 ml bi-distilled water.

These solutions were titrated potentiometrically with 0.02 M NaOH solution. The NaOH solution was titrated against a standard solution of sodium carbonate.

3. Results and Discussion
3.1. Electrochemical studies
3.1.1. DC Polarography
The polarograms of \( 1 \times 10^{-4} \text{ M} \) of salicyldehydeazomethazine (I), salicyldehydeazo-sulfamerazine (II),salicyldehydeazo-sulfadiazine (III) and sulfamethazineazo-phenol (IV) in Britton- Robinson buffer solutions of pH 2-11 containing 20\% (v/v) ethanol were recorded. The addition of ethanol is due the low solubility of these compounds in pure aqueous media. The polarograms of the azo compounds (I-III) consist of two reduction waves of unequal heights. The second wave in acidic solutions (pH < 7) is ill-defined and coalesces with the hydrogen evolution. The first wave is an irreversible 4-electron step represents the reduction of the azo group, whereas the second one is an irreversible 2-electron wave and due to the reduction of the aldehyde group. Fig. 1Arepresents the polarograms of azo compound (I) as atypical example of this series. However, in solutions of pH > 8.0, the first wave of azo compounds (I-III) splits into two waves of unequal heights. On increasing the pH of the solution, the limiting current \( \left( i_{l} \right) \) of the second splitting wave increases at the expense of the first splitting one whereas the total limiting current is remained constant. The \( i_{l} \)-pH curves of the single wave and the two splitting waves recalled a dissociation curve \( (Z \text{-shaped}) \) and an association curve \( (S \text{-shaped}) \) (Fig. 1B), the behaviour which may be attributed to an acid-base equilibrium in that pH range\[33\].
The half-wave potential (E_{1/2}) of the polarographic wave shifted to more negative values on increasing the pH of the solution, denoting the consumption of protons in the reduction process and the proton uptake precedes the electron transfer [34]. The effect of mercury height (h) on the limiting current (i) of the polarographic waves using the equation [34]: i = k h^1, revealed that the reduction process for all azo compounds is controlled mainly by diffusion with some adsorption contribution. The value of the exponent (x) at different pH values amounts to 0.5-0.75 (Table 1). Analysis of the polarographic waves at different pH values using the basic equation for reversible polarographic wave [32] showed that the electrode reaction proceeds irreversibly. The value of the transfer coefficient (α) and the number of electrons participating in the rate-determining step (n_a) were determined from the reciprocal slopes (S_1) of the logarithmic analysis plots and given in Table 1. It was found that, the most probable (α) values were obtained at n_a equals to one or two depending on the pH of the medium, which revealing that the rate-determining step of the reduction process may involve two electrons in acidic solution and neutral solutions, and one electron in alkaline ones (Table 1).

The E_{1/2} – pH plots of all azo compounds gave straight lines of three segments and the breaks occur at pH 4-5 and 8-9. From the slope values (S) of these plots and the slopes of logarithmic analysis (S_1), the number of protons (Z_{H+}) participating in the rate-determining step was determined using the following relation [34]:

\[ Z_{H+} = \left( \frac{\delta E_{1/2}}{\delta \text{pH}} \right) \left( \frac{0.0591}{\alpha n_a} \right) = S_2 / S_1 \]

The value of Z_{H+} for all compounds were calculated at different pH values and found approximately equal to unity (Table 1).

(a_1) First splitting wave  (a_2) Second splitting wave  (b) second wave

\[ S_1 = 0.0591 / \alpha n_a \quad S_2 = \left( \frac{\delta E_{1/2}}{\delta \text{pH}} \right) \]

### 3.1.2. Cyclic voltammetry measurements

The cyclic voltammetry of azo compounds (I-IV) was measured in buffer solutions of different pH values containing 20% (v/v) ethanol. The voltammograms were recorded at different scan rates (20 – 500 mV/s). As shown in Fig. 2A (as a typical example), a single cathodic peak was observed for azo compounds (I-III) in solutions of pH < 8, whereas in alkaline solutions (pH > 8) three cathodic peaks were observed. This behavior agrees with those of DCPolarography. However, for azo compound IV, a single cathodic peak is observed within the entire pH range. The absence of any peaks in the reverse scan (anodic direction) for all the voltammograms confirmed the irreversibility of the reduction process of these compounds. The peak potential (E_p) shifts to more negative values on increasing the pH of the solution. The values of the transfer coefficient (α) were determined using Galus equation [35]:

\[ E_p = -1.14(\text{RT}/\alpha n_a F) \ln(k^{1/2}/D^{1/4}) - \text{RT}/2\alpha n_a F \ln(\alpha n_a) \]

### Table 1 DC-polarographic data of 1 x 10^{-4}M of azo compound values, containing 20% (v/v) ethanol.

| Compd | pH | \(i_i\) (μA) | \(-E_{1/2}\) (V) | \(S_i\) (mV) | \(αn_a\) | \(α_{n_a=2.0}\) | \(S_2\) (mV) | \(Z_{H+}\) | \(\delta \log i/ \log h\) |
|-------|----|-------------|----------------|--------------|---------|--------------|-------------|-----------|-----------------|
| I     | 2.1 | 1.09        | 0.05           | 85.3         | 0.70    | 0.35         | 66.3        | 0.78      | 0.55            |
|       | 7.0 | 1.02        | 0.33           | 121.5        | 0.49    | 0.25         | 66.3        | 0.55      | 0.49            |
|       | 10.1| 0.56 (a_1)  | 0.61           | 115.6        | 0.36    | 0.18         | 90.1        | 0.78      | 0.60            |
|       |     | 0.53 (a_2)  | 0.82           | 109.5        | 0.54    | 0.27         | 77.7        | 0.71      | 0.65            |
|       |     | 0.46 (b)    | 1.33           | 133.3        | 0.44    | 0.22         | 12.5        | 0.09      | 0.57            |
| II    | 2.1 | 1.15        | 0.04           | 87.1         | 1.03    | 0.52         | 89.3        | 1.03      | 0.55            |
|       | 7.1 | 1.04        | 0.46           | 100.0        | 0.59    | 0.30         | 89.3        | 0.89      | 0.49            |
|       | 10.1| 0.38 (a_1)  | 0.62           | 87.0         | 0.68    | 0.34         | 81.1        | 1.26      | 0.45            |
|       |     | 0.68 (a_2)  | 1.04           | 92.5         | 0.72    | 0.36         | 110         | 1.19      | 0.60            |
|       |     | 0.6 (b)     | 1.6            | 90.6         | 0.53    | 0.27         | 93.0        | 0.9       | 0.55            |
| III   | 2.1 | 1.10        | 0.03           | 78.0         | 0.87    | 0.44         | 90.0        | 1.15      | 0.58            |
|       | 7.1 | 1.05        | 0.28           | 90.0         | 0.66    | 0.33         | 90.0        | 1.00      | 0.55            |
|       | 10.1| 0.55 (a_1)  | 0.45           | 110.0        | 0.54    | 0.27         | 100         | 0.91      | 0.60            |
|       |     | 0.40 (a_2)  | 1.30           | 120.0        | 0.49    | 0.25         | 95.0        | 0.79      | 0.75            |
|       |     | 0.55 (b)    | 1.58           | 112.0        | 0.53    | 0.27         | 90.0        | 0.80      | 0.70            |

https://cirworld.com/
(a1) First splitting wave  (a2) Second splitting wave  (b) second wave

\[ S_1 = 0.0591/αn \quad S_2 = \delta E_{1/2} / \delta \text{pH} \]

Where, \( k_{\text{h,h}} \) is the heterogeneous formal rate constant (cm/s), \( D_0 \) is the diffusion coefficient (cm\(^2\)/s), \( R \) is the gas constant, \( T \) is the absolute temperature, \( F \) is the faraday constant, \( αn \) has its usual significant.

This equation relates the values of \( E_p \) and \( αn \). The plots of \( E_p \) versus \( \ln \nu \) (logarithm of sweep rate) were straight lines, from their slopes; \( α \) values were calculated and given in Table 2. It was found that the most probable values of \( α \) were obtained at \( n_a = 1.0 \) in all pH solutions. This may be due to the high resolution of cyclic voltammetry compared to DC-polarography.

On plotting the peak current (\( i_p \)) as a function of the square root of sweep rate (\( \nu \)) according to the following equation [36]:

\[ i_p = 3.01 \times 10^5 n(αn)^{1/2} A \text{C}_o v^{1/2} D_0^{1/2} \]

In which \( n \) is the total number of electrons, \( A \) is the electrode surface area (cm\(^2\)), \( C_o \) is the concentration of the depolarizer and the remainder terms have their usual significance.

Linear correlations were obtained which deviated from the origin, confirming that the reduction process is mainly controlled by diffusion with some adsorption contribution [37].

**Table 2.** Cyclic voltammograms of azo compound (I–IV) in aqueous buffer solutions of different pH values, containing 20% (v/v) ethanol.

| Compd | pH | Scan rate (mV/s) | \( -E_p \) (V) | \( \delta \ln E_p / \delta \nu \) | \( α \) | \( n_a = 1.0 \) | \( n_a = 2.0 \) |
|-------|----|-----------------|----------------|---------------------|-----|----------------|----------------|
| I     | 2.2| 100             | 0.07           | 0.035               | 0.34| 0.17           |
|       | 6.9| 100             | 0.30           | 0.023               | 0.51| 0.26           |
|       | 10.1| 100            | 0.56 (a1)      | 0.018               | 0.66| 0.33           |
|       |     |                 | 0.82 (a2)      | 0.021               | 0.36| 0.18           |
|       |     |                 | 1.35 (b)       | 0.018               | 0.30| 0.15           |
| II    | 2.2| 100             | 0.10           | 0.022               | 0.54| 0.27           |
|       | 6.9| 100             | 0.32           | 0.018               | 0.66| 0.33           |
|       | 10.1| 100          | 0.50 (a1)      | 0.019               | 0.32| 0.16           |
|       |     |                 | 0.92 (a2)      | 0.025               | 0.47| 0.24           |
|       |     |                 | 1.33 (b)       | 0.023               | 0.39| 0.19           |
| III   | 2.2| 100             | 0.12           | 0.020               | 0.59| 0.30           |
|       | 6.9| 100             | 0.35           | 0.027               | 0.44| 0.22           |
|       | 10.1| 100         | 0.54 (a1)      | 0.022               | 0.54| 0.27           |
|       |     |                 | 0.85 (a2)      | 0.025               | 0.47| 0.24           |
|       |     |                 | 1.37 (b)       | 0.021               | 0.56| 0.28           |
| IV    | 2.2| 100             | 0.31           | 0.018               | 0.65| 0.33           |
|       | 6.9| 100             | 0.56           | 0.035               | 0.30| 0.17           |
|       | 10.1| 100        | 0.71           | 0.018               | 0.66| 0.33           |

(a1) First splitting wave  (a2) Second splitting wave  (b) second wave
3.1.3. DP-Polarography

The DP-polarograms of all azo compounds (I-IV) showed a single dp-polarographic peak in acidic solutions (pH < 8), whereas in alkaline ones three peaks were obtained. On the other hand, the DP-polarograms of azo compound (IV) showed a single peak in all pH solutions. Fig. 1C represents the DP-polarograms of azo compound (I) as a typical example of this series in solution of pH 2.2. The results of the DPP measurements are in agreement with those obtained from DC and CV measurements.

3.1.4. Controlled potential electrolysis measurements (Coulometry)

The total number of electrons involved in the electrode reaction was determined using controlled potential electrolysis technique (coulometry) using a mercury pool cathode. The accumulated charge (Q) was taken from the digital coulometer at a potential corresponding to the limiting current of the polarographic wave. Applying the equation:

\[ Q = n \cdot F \cdot w / M \]

where \( w \) is the weight of the sample (in grams) and \( M \) is the gram molecular weight, the values of \( n \) along the first wave of azo compounds (I-III) and the single wave of azo compound (IV) was found to be 4 electrons which represent the reduction of azo group whereas for azo compounds (I-III) along the second wave \( n \) equals to two electrons representing the reduction of aldehyde group attached to the phenyl ring. The data of coulometry are given in Table (3).

Table 3. Data of controlled potential electrolysis (coulometry) of azo compounds (I-IV) buffer solutions of different pH values.

| Compd | Mol. wt | pH  | Applied potential | Weight of sample | Coulomb | Total number of electrons (n) |
|-------|---------|-----|-------------------|------------------|---------|-------------------------------|
| I     | 399     | 2.2 | - 0.40           | 3.99             | 0.381   | 3.95 ~ 4 (a)                  |
|       |         |     | - 0.85           |                  | 0.179   | 1.85 ~ 2 (b)                  |
|       |         |     | 10.1             | - 0.00           | 0.362   | 3.75 ~ 4 (a)                  |
|       |         |     |                  | - 1.40           | 0.164   | 1.70 ~ 2 (b)                  |
| II    | 385     | 2.2 | - 0.40           | 3.85             | 0.372   | 3.85 ~ 4 (a)                  |
|       |         |     | - 0.95           |                  | 0.183   | 1.90 ~ 2 (b)                  |
|       |         |     | 10.1             | - 1.30           | 0.357   | 3.70 ~ 4 (a)                  |
|       |         |     |                  | - 1.65           | 0.169   | 1.75 ~ 2 (b)                  |
| III   | 371     | 2.2 | - 0.4            | 3.71             | 0.391   | 4.05 ~ 4 (a)                  |
|       |         |     | - 0.9            |                  | 0.178   | 1.85 ~ 2 (b)                  |
|       |         |     | 10.1             | - 1.35           | 0.376   | 3.90 ~ 4 (a)                  |
|       |         |     |                  | - 1.60           | 0.174   | 1.80 ~ 2 (b)                  |
| IV    | 383     | 2.2 | - 0.40           | 3.83             | 0.400   | 4.15 ~ 4 (a)                  |
|       |         |     | 10.1             | - 1.10           | 0.381   | 3.95 ~ 4 (a)                  |

(a) First wave (b) Second wave

3.1.5. Identification of the electrolysis products

The completely electrolysed solutions of azo compounds (I) at pH 2 and 10 were chosen as an example for the compounds under consideration. These solutions were concentrated and the buffer ingredients were removed by extracting the slurry with ether. Thin layer chromatography (TLC) was applied for the ethereal extract in chloroform – ethanol mixture (90:10); two products (two spots) were observed. On comparing with authentic sulfamethazine sample, it is concluded that the later is one of the two electrolysis products which indicated the cleavage of N=N centre. On the other hand, the UV-spectra of the continuously electrolysed solution of azo compounds (I) at pH 2 and different time intervals showed that the band appeared at \( \lambda_{max} = 350 \text{ nm} \) which characteristic to \( \pi - \pi^* \) transition of N=N group decreases gradually and disappeared completely for the completely electrolysed solution which confirmed also the cleavage of the N=N bond. (Fig.2C).
3.1.6. Mechanism of the electrode reaction

The data obtained from coulometric measurements along the first wave for azo compounds (I-III) and the single wave for azo compound (IV) revealed that the electro-reduction process of these compounds involved 4 electrons and 4 protons leading to the cleavage of the N=N center within the entire pH range and the corresponding amine is obtained.

According to Zuman [33], if the limiting current (i_l) is pH-independent and the half-wave potential (E_{1/2}) is pH-dependent, the sequence of the electrode reaction may be H^+, e^-, H^+, e or H^+, e, e, H^+. The results obtained agreed with the later sequence thus; the electrode reaction mechanism of azo group can be represented as follows (Scheme 1):

Scheme (1): Reduction mechanism of N=N group of compounds I-IV.
On the other hand, coulometry measurements along the second wave of the azo compounds (I-III) revealed that 2 electrons are involved in the reduction process which represents, the reduction of the electroactive aldehyde group (CHO) to (CH₂OH) The reduction mechanism can be represented as follows (Scheme 2):

\[
\text{(vi)} + H^+ \xrightarrow{E_3} \text{(vii)}
\]

\[
\text{(vii)} + e^- \xrightarrow{E_4} \text{(viii)}
\]

\[
K_3 = K_4 = K_{1/2} \text{ (second wave)}
\]

Scheme 2: Reduction mechanism of CHO group of compounds I-III

3.1.7. Analytical micro-determination

Analytical microdetermination of azo compound (I) as an example of this series was investigated in buffer solution of pH 2. The micro-determination was done using the more sensitive dp-polarography. The total volume of the electrolysis solution in the polarographic cell was kept to be 10 ml containing the same percent of ethanol. The concentration of the depolarizers was increased from $1.43 \times 10^{-8}$ to $5.26 \times 10^{-8}$ mol/L. The dp-polarograms were recorded and represented in Fig. 3. The diffusion current ($i_d$) was recorded versus the corresponding concentrations [C] and the plot is illustrated in Fig 3.

Figure 3. DP-polarograms of sulfamethazinazo compound I at different concentrations; 1 = $5.26 \times 10^{-7}$, 2 = $1 \times 10^{-6}$, 3 = $1.43 \times 10^{-6}$, 4 = $1.82 \times 10^{-6}$, 5 = $2.7 \times 10^{-6}$, 6 = $3.22 \times 10^{-6}$, 7 = $4.26 \times 10^{-6}$, 8 = $6.25 \times 10^{-6}$, 9 = $8.16 \times 10^{-6}$, 10 = $1 \times 10^{-5}$, 11 = $1.43 \times 10^{-5}$, 12 = $1.82 \times 10^{-5}$, 13 = $2.17 \times 10^{-5}$, 14 = $5.2 \times 10^{-5}$, 15 = $7.67 \times 10^{-5}$, 16 = $1.43 \times 10^{-4}$ mole/L.
The plot gives straight line passing through the origin within the concentration up to (9x10^6), indicating the applicability of analytical micro-determination of this compound within this range of concentration.

### 3.2. Spectrophotometric Studies

#### 3.2.1. Electronic absorption spectra of the sulfas drug azo compounds

The electronic absorption spectra of 1 x 10^-4 M of azo compounds (I-IV) investigated in buffer solutions of varying pH (2-11) were recorded within the range 200-700 nm. Fig. 4A, represents the spectra of azo compound (I) as a typical example of this series.

**Figure 4.** (A) The electronic absorption spectra of salicylaldehyde sulfamethazineazo compound (I), (B) Potentiometric titration curves of salicylaldehydesulfamethazineazo compound (I)

All the investigated compounds exhibited three bands; the first band (not shown) appeared at 230-250 nm is due to the local excitation of the π-π* transition of the aromatic moiety, while the second and third bands appeared within the range 380-450 nm are attributed to the charge transfer (C.T) interaction within the whole molecule. Also, a clear one or two isobestic points were observed indicating more than one species are present in equilibrium.

#### 3.2.2. Determination of the dissociation constant values

To determine the dissociation constants of the azo compounds (I-IV); the limiting absorbance method (LAM) [37], and the modified limiting absorbance method (MLAM) [38] were used. The measurements were applied at two different wavelengths for each compound.

In the (LAM), the dissociation constant values (pk_a) were calculated using the relation [37]:

\[
pH = pk_a + \log \left( \frac{A}{A_{max} - A} \right)
\]

The plots of pH vs log (A / A_{max} - A) gave linear correlations, from which the pk_a values were obtained. The pk_a values equal to the pH at which log (A / A_{max} - A) amounts to zero.

In the (MLAM), the pk_a values in very dilute solutions were calculated using the relation [38]:

\[
pH = pk_a + \log \left( \frac{A - A_{min}}{A_{max} - A} \right)
\]

The plots of log (A / A_{min}) / (A_{max} - A) vs. the pH give linear correlations. At log (A / A_{min}) / (log A_{max} - A) equal to zero, the pk_a value equals to the pH. The pk_a values of azo compounds (I-IV) obtained from the two previous methods are given in Table (4). The results indicated that two pk_a values for each azo compound (I-IV) were obtained; the first is due to the dissociation of proton of amide group whereas the second is attributed to the dissociation of the hydroxyl group in the phenyl ring.
Table 4. Dissociation constants of azo compounds (I-IV) determined using spectrophotometric and potentiometric methods.

| Compd | Spectrophotometry | Potentiometry |
|-------|-------------------|---------------|
|       | HHM               | LAM           | Mean value |
|       | pk	sub{a}Sup{1}   | pk	sub{a}Sup{2} |                  |
| I     | 3.8               | 4.0           | 4.2           | 4.2           | 4.00 | 8.7    | 3.60 | 8.80 |
| II    | 4.0               | 7.9           | 3.8           | 7.7           | 4.2  | 8.4    | 4.00 | 8.00 |
| III   | 3.8               | 8.3           | 4.5           | 7.9           | 4.5  | 7.6    | 4.30 | 8.00 |
| IV    | 4.0               | 8.4           | 4.2           | 8.6           | 4.2  | 8.6    | 4.10 | 8.50 |

\( H HM = \) half-height method. \( L AM = \) limiting absorbance method. \( M LM A = \) modified limiting absorbance method.

3.3. Potentiometric studies

3.3.1. Proton-ligand dissociation constants

The acid dissociation constants of the azo compounds (I-IV) were calculated from the titration curves of the hydrochloric acid with sodium hydroxide solution in absence as well as in presence of azo compounds. However, potentiometric titration of the ligands with sodium hydroxide in the presence of \( (10^{-4} M) \) hydrochloric acid was carried out at ionic strength \( (\mu=0.1 M) \) adjusted by the addition of potassium chloride. Potentiometric titration curves of azo compounds (I) as a typical example of this series are shown in Fig. 4B. Proton-ligand dissociation constants of these azo compounds were determined potentiometrically using the method of Irving and Rossotti [39, 40] as adapted by Fronaeus [41]. The average number of protons associated with the reagent molecules \( (n_A) \) is determined at different pH values applying the Fronaeus equation [42]:

\[
\frac{-n_A}{Y} = Y \pm \frac{(V_1 - V_2)(N^o - M^o)}{V^o - V_1 (T^o_{CL})}
\]

where: \( Y \) is the total number of dissociable protons attached to the ligand, \( N^o \) is the molarity of the sodium hydroxide solution, \( M^o \) is the molarity of the hydrochloric acid solution, \( V_1 \) and \( V_2 \) are the volumes of sodium hydroxide required to reach a definite pH value for the free acid titration curve and for that acid in presence of a ligand, respectively, \( V^o \) is the total concentration of the ligand in molar scale and \( T^o_{CL} \) is the initial volume of the titrated solution (50 ml).

On plotting \( n_A \) vs. pH gives the proton-ligand formation curves (Figure omitted for brevity) of azo compound (I) as a typical example of this series. The values of proton-ligand dissociation constants of the investigated compounds were calculated by interpolation at half \( n_A \) values, at \( n_A = 0.5, 1.5 \) and 2.5 and given in Table (4). These values indicated that the azo compounds (I- IV) have two dissociable protons (two \( pk_a \) values). The second is due to dissociation of proton of phenolic OH group in the phenyl ring whereas the first is attributed to dissociation of amide group (NH) within the sulfa drug moiety. It is important to noted that, the data obtained from spectrophotometric measurements are in agreement with those calculated by potentiometric ones.

3.3.2. Stability constants of metal complexes of the sulfa drug azo compounds

The pH-metric titration of the investigated compounds in presence of \( 10^{-4} M \) of \( Co^{2+}, Ni^{2+}, Cu^{2+} \) and \( Zn^{2+} \) ions was carried out at ionic strength \( \mu = 0.1 M \) (KCl) and \( 10^{-3} M \) hydrochloric acid. The pH-metric curves of azo compounds (I) in presence of the metals ions are shown in Fig. 4A. It is clear that the metal – ligand titration curves separated well from those of the ligand titration curve, indicating that the complexation took place with the liberation of hydrogen ions. The metal – ligand stability constants were evaluated from the formation curves.

where \( \bar{n}_L \) is the average number of ligand molecules attached per metal ion and is calculated using the equation [42]:

\[
\bar{n}_L = \frac{-n_A}{Y} = Y \pm \frac{(V_1 - V_2)(N^o - M^o)}{V^o - V_1 (T^o_{CL})}
\]
pL is the free ligand exponent and is calculated using the equation [43]:

\[
pL = \log \left( \frac{\sum_{n=0}^{\infty} \beta_n^H}{TC^o_L - \frac{1}{\text{anti log pH}}} \right) \frac{V^o + V_3}{V^o}
\]

Where \( V_3 \) is the volume of sodium hydroxide required to reach the same pH in the titration of the complex formation, \( TC^o_M \) is the total metal ion concentration and \( \beta^H \) is the overall proton – ligand formation constant. The values of \( \beta^H \) and pL for the investigated compounds were calculated at different pH values.

The formation curves (Figure omitted for brevity) are constructed on plotting the average number of ligand attached per metal ion ( \( \bar{n} \) ) versus the free ligand exponent (pL).

In the present study, the maximum \( \bar{n} \) value was found to be 2.0 confirming the possibility of formation of ML and ML2 types of complexes. The values of the stepwise stability constants were determined by interpolation of \( \bar{n} \) values at 0.5 and 1.5 according to the half \( \bar{n} \) method and summarized in Table (5).

**Table 5.** Stepwise stability constants of ML and ML2 complexes of ligands (I-IV) in water-ethanol mixture containing 0.1 M KCl.

| Compd. | \( \bar{n} \) | Cu | Co | Ni | Zn |
|--------|--------------|----|----|----|----|
| I      | 0.5          | 8.2| 7.6| 7.2| 7.2|
|        | 1.5          | 4.1| 3.6| 4.1| 4.2|
| II     | 0.5          | 9.2| 8.1| 8.3| 8.4|
|        | 1.5          | 5.4| 5.0| 5.0| 5.3|
| III    | 0.5          | 8.4| 8.1| 7.6| 7.4|
|        | 1.5          | 3.5| 3.5| 3.7| 4.2|
| IV     | 0.5          | 8.0| 8.0| 7.9| 7.8|
|        | 1.5          | 5  | 4.5| 4.6| 4.5|

The stability constants of the produced metal complexes follow the order [44]:

\( \text{Cu}^{2+} > \text{Co}^{2+} > \text{Ni}^{2+} > \text{Zn}^{2+} \)

Which is in agreement with that of Irving and Williams.

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Figure captions:
Fig 1 (A) DC – Polarograms of salicylaldehyde sulfamethazineazo compound (I), (B) i-pH plots of azo compound (I), (C) E1/2-pH plots of azo compound (I)
Fig 2 (A) Cyclic voltammograms of salicylaldehyde sulfamethazineazo compound (I), (B) DP – Polarograms of salicylaldehyde sulfamethazineazo compound (I), (C) UV spectra of salicylaldehyde sulfamethazineazo compound (I) at different time intervals
Fig 3 DP- polarograms of sulfamethazino compound I at different concentrations; 1 = 5.26x10^{-7}, 2 = 1 x10^{-6}, 3 = 1.43 x 10^{-6}, 4 = 1.82 x 10^{-6}, 5 = 2.7 x 10^{-6}, 6 = 3.22 x 10^{-6}, 7 = 4.26 x 10^{-6}, 8 =6.25 x 10^{-6}, 9 = 8.16 x 10^{-6}
10 = 1 \times 10^{-6}, 11 = 1.43 \times 10^{-5}, 12 = 1.82 \times 10^{-5}, 13 = 2.17 \times 10^{-5}, 14 = 5.2 \times 10^{-5}, 15 = 7.67 \times 10^{-5}, 16 = 1.43 \times 10^{-4} \text{ mole/L.}

Fig 4  (A) The electronic absorption spectra of salicylaldehyde sulfamethazineazo compound (I)

(B) Potentiometric titration curves of salicylaldehydesulfamethazineazo compound (I)

Table captions:

Table (1): DC-polarographic data of $1 \times 10^{-4}$M of azo compound (I-IV) in aqueous buffer solutions of different pH values, containing 20% (v/v) ethanol.

Table (2): Cyclic voltammograms of azo compound (I-IV) in aqueous buffer solutions of different pH values, containing 20% (v/v) ethanol.

Table (3): Data of controlled potential electrolysis (coulometry) of azo compounds (I-IV) buffer solutions of different pH values.

Table (4): Dissociation constants of azo compounds (I-VI) determined using spectrophotometric and potentiometric methods.

Table (5): Stepwise stability constants of ML and ML2 complexes of ligands (I-IV) in water-ethanol mixture containing 0.1 M KCl.

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