Studies of spectroanalytical and the biological effectiveness of the Azo compound (J25) prepared from Ethyl p-aminobenzoate

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A B S T R A C T

Azo compound was prepared with ethyl-4-aminobenzoate reactor with (2-amino-3(4-hydroxyphenyl) propanoic acid) and this symbol (J25) was suggested. The compound was purified and Recrystallization with absolute ethanol and then performed diagnostic and analytical techniques where the compound was diagnosed with infrared spectrometry and mass spectrometry. Optical spectrometry to pH values within the range (2-12), which included determining the highest absorption value of the compound and identifying Isopstic points. Different polarized solvents also had an impact on electronic spectrometry. The biological effectiveness of the compound (J25) was studied against two types of bacteria (E. coli) and (staphylocococcus Aureuse) and the results showed the positive effect of the compound in inhibiting the growth of bacteria. This study has been legalized and presented for medical, chemical, physical, biological and other applications

K e y w o r d s : spectroanalytical, Biological effectiveness, Azo compound, Ethyl p-aminobenzoate.

1. Introduction

Azo pigments are chemical compounds containing the group Azo (-N=N-) in their composition[1] that are bound at both ends by saturated compositions and thus are unstable Azo that quickly disintegrate in normal conditions and this type is undesirable. The second type is the part associated with the aromatic azo group and is more stable due to succession[2].Azo compounds represent the most important pigment category in textile pigments. Furthermore, Azo pigments are desirable in terms of cost and ease of use[3]. Azo pigments make up about 65% of the commercial pigment market[4]. The scientific revolution in the dye industry began in 1859 when researcher William Henry Birkin prepared the first aromatic pigments when he tried to prepare a drug for malaria and observed that the sediment became colored[5]. Previous literature confirms that about 4% of the waste production of Azo pigments in industrial wastewater. This will lead to water contamination due to its resistance to disintegration or breakdown in normal conditions[6]. Environmental pollution caused by Azo pigments does not negate that it is the most important class of industrial pigments. They have been widely used in dyeing wool textiles, industrial fiber and food color industries[7]. The diazonium formation method and the duplication reaction were used in the preparation of the...
compound (J25) for its recurrent credibility by previous literature due to the simplicity and low risk of this method. Because the compounds used to prepare Azo are pharmaceutical compounds, the biological effectiveness of two types of bacteria was studied and showed positive results.[8]. It can also be used as a evidence (acid-base) in chemical swabs and visual effectiveness due to its chemical and physical properties.

2. Experimental

2.1. Materials and Method

The reactive starter materials, solvents, and reagents were bought from trustworthy companies such as Co Aldrich and Co Merck. The SHIMADZU (FT-IR8400S) technology was utilized to record IR spectroscopy results using potassium bromide salt tablets. Using (thermo-scientific 9100), we were able to identify the melting points of the prepared compounds. A GENWAY-6305 spectrometer is used to measure visual spectrums. Using Electronic Impact technologies, mass spectrums were recorded. A scale (H Jurgons Co Bremen, L Puls Munchen) was employed to take pH measurements.

2.2. Preparation of Azo (J25)

Prepared the compound as per recommended procedures for the preparation of Azo compounds[9]. Taking (0.006) mole equivalent (0.991) g of ethyl p-aminobenzoate and (1.086) g of 2-amino-3-(4-hydroxyphenyl) propanoic acid and (1.8%) w/v. from sodium hydroxide. With the help of diagnostic techniques, proposed mechanical posture for the preparation of Azo compound (J25) as in the scheme 1.

![Scheme 1](image)

**Scheme 1.** preparation of Azo compound (J25)

J25 = (E)-2-amino-3-(3-((4-(ethoxycarbonyl)phenyl)diazenyl)-4-hydroxyphenyl)propanoic acid

2.3 Solutions

- (0.001)M of (J25).
- Solutions (pH 2-12) [10]

3. **acid-base characteristics at various pH**

Prepared standard solutions for Azo (J25) at a concentration of 8x10-5 M for different pH values (2–12). The solutions' absorbance measured at a range of (320–470) nm[11].

4. **Effect of different polar solvents**
Prepare standard solutions from the compound Azo at a concentration of \((8 \times 10^{-5})\) M with different polar solvents (Ethanol, Methanol, Water, Acetone, DMSO, Chloroform, 1,4-Dioxane, and n-Hexane). Absorption values were calculated in the range of (330-460) nm.

5. Interpretation of results

Table 1 summarizes the most important chemical and physical properties of Azo (J25) recorded during the preparation process, including its stability in standard conditions. Dissolved with Acetone, Dimethyl sulfoxide, and Dimethyl formamide.

<table>
<thead>
<tr>
<th>compound</th>
<th>M.formula</th>
<th>M.W (g.mol(^{-1}))</th>
<th>M.P °C</th>
<th>Yield %</th>
<th>Colour</th>
</tr>
</thead>
<tbody>
<tr>
<td>J25</td>
<td>C(<em>{18})H(</em>{19})O(_3)N(_3)</td>
<td>357.38</td>
<td>195-197</td>
<td>80</td>
<td>dark red</td>
</tr>
</tbody>
</table>

6. IR Spectra

The infrared spectrums of the Azo showed important absorption packages[12]. They were identical to the active aggregates in the new compound, and the table shows the infrared spectrums of the compound (J25) within the spectrum range (4000-400)cm\(^{-1}\). A package for the group (O-H) appears in the composite spectrum (J25) at (3431.36)cm\(^{-1}\), and we note a package for the carbonyl group (C = O) clearly within the spectral range (1620.21)cm\(^{-1}\). A package for the aromatic combination (C = C) also appears within the spectral range (1514.12)cm\(^{-1}\). The group of Azo (-N = N-) appears characteristically in the spectral range (1450.47) cm\(^{-1}\). We also note the package (C-N) clearly within the spectral range (1394.53)cm\(^{-1}\), note the package (C-O) clearly within the spectral range (1276.88)cm\(^{-1}\).

Table 2. Selected infrared data of compound (J25)

<table>
<thead>
<tr>
<th>Compound</th>
<th>(\tilde{\nu}(O-H)) cm(^{-1})</th>
<th>(\tilde{\nu}(C=O)) cm(^{-1})</th>
<th>(\tilde{\nu}(C=C)) cm(^{-1})</th>
<th>(\tilde{\nu}(N=N)) cm(^{-1})</th>
<th>(\tilde{\nu}(C-N)) cm(^{-1})</th>
<th>(\tilde{\nu}(C-O)) cm(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>J25</td>
<td>3381.21</td>
<td>1604.77</td>
<td>1514.12</td>
<td>1450.47</td>
<td>1394.53</td>
<td>1276.88</td>
</tr>
</tbody>
</table>

Fig. 1. IR spectrum of (J25).
7. Mass Spectrum

The description of the organic molecular structure depends on the measurement of the mass spectrum of the electronic fasting technique using the energy (70eV)[13]. In (figure 3) the mass spectrums are identical to the M.W (357.3) of (J25), which corresponds to the molecular ion [C18H19O5N3]+. The base ion peaked in appearance m/z = 120.2

![Mass Spectrum](image)

**Fig. 2.** Mass spectrum of (J25).

8. acid-base characteristics at various pH

used a series of pH value solutions (2–12) to study the acid and base effects of the solutions on the for (J25) [14] and Measure the ionization and protonization constants in the absorption range of 330–470 nm. The pH (2–12) values graphically represent the absorption spectrum of the (8x10-5) M solution of the prepared compounds (Figure 4). The compound (J25) has a proton shape and an isopstic point at 385 nm. Its peak absorption is 360 nm in the pH range (9–11), and 410 nm in the pH range (12).

![Absorption Spectrum](image)

**Fig.3.** At pH levels of (2-12) absorption spectrum
As shown in Figure 4, have drawn the pH absorption curves of the compound (J25) to document its ionisation and protonization constant (J25) at wavelength (360) nm. We calculated the ionisation and protonization constants (Table 3). We used the half-height method to derive the absorbance pH curve. obtained the pK values using this relation: \( pK = pH \text{ (at } A_{\frac{h}{2}} \text{) } [15], \) where \( A_{\frac{h}{2}} = \frac{(A_L + A_{\text{min}})}{2} \) and \( A_L \) and \( A_{\text{min}} \) represent the limiting and minimum absorbance, respectively.

Table 3. Ionization and protonization constant of compound (J25).

<table>
<thead>
<tr>
<th>( A_{\text{in}} )</th>
<th>( A_L )</th>
<th>( A_{\frac{h}{2}} )</th>
<th>( pK )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.224</td>
<td>0.999</td>
<td>0.611</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.492</td>
<td>1.238</td>
<td>0.865</td>
<td>11.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.842</td>
<td>0.888</td>
<td>0.865</td>
<td>9.7</td>
</tr>
</tbody>
</table>

\( p = H\text{'s protonization constant for the phenol molecule's nitrogen atom} \)

\( a = H\text{'s Ionization constant for Hydroxyl group in Phenol molecule} \)

**Fig.4.** The Absorbance pH curves of compound (J25)

Scheme 2 illustrates the proposed chemical mechanisms of protonization and ionization.
9. Effect of different polar solvents

Figure 5 shows the compound (J25)'s spectrums in different solvents different polarisations that have[16]. The solvents include (Ethanol, Methanol, Water, Acetone, DMSO, Chloroform, 1,4-Dioxane, and n-Hexane). The wavelength at its longest point is 370 nm, and measurements were made of the constant protonation and ionisation. From Figure 5, compound (J25) With water, there is a blue shift at 360 nm. There is a blue shift at 370 nm with ethanol, methanol, acetone, chloroform, and hexane.

![Scheme 2. mechanism of (J25)](image)

![Fig.5. The electronic spectrums of compound J25 at different polar solvents](image)
From the shape of the spectrum of the compound (J25) in different polar solvents, the results recorded in (Tables 4, 5) were found. From the results, the maximum wavelength of the compound, the (red shift), and the (blue shift) were determined. The great wavelengths and molar absorption coefficient compound (J25) were also recorded in different polar solvents as shown in (table 5).

**Table 4,5. Information obtained from the use of different polar solvents**

<table>
<thead>
<tr>
<th>Compound (J5)</th>
<th>λ&lt;sub&gt;max&lt;/sub&gt; to 1,4-Dioxane</th>
<th>λ&lt;sub&gt;max&lt;/sub&gt;</th>
<th>Blue shift</th>
<th>Red shift</th>
<th>Notes shift</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>380</td>
<td>380</td>
<td>Ethanol, Acetone, Chloroform, and n-Hexane</td>
<td>------</td>
<td>Medium shift</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Solvent</th>
<th>n-Hexane</th>
<th>1,4-Dioxane</th>
<th>Chloroform</th>
<th>Acetone</th>
<th>Ethanol</th>
<th>Methanol</th>
<th>DMSO</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>λ&lt;sub&gt;max&lt;/sub&gt;</td>
<td>370</td>
<td>380</td>
<td>370</td>
<td>370</td>
<td>370</td>
<td>370</td>
<td>380</td>
<td>360</td>
</tr>
<tr>
<td>ε&lt;sup&gt;10&lt;/sup&gt;&lt;sup&gt;-4&lt;/sup&gt;</td>
<td>1.58</td>
<td>1.60</td>
<td>1.62</td>
<td>1.68</td>
<td>1.73</td>
<td>1.80</td>
<td>1.69</td>
<td>0.85</td>
</tr>
</tbody>
</table>

The results in table 6 indicate that the linear relationship between solvent electrical insulation constant and absorption peaks is equivalent to (D - 1/D + 1)<sup>17</sup>. and this gives the impression that electrical insulation constant is the factor influencing absorption, where (D)<sup>18</sup> value represents solvent electrical insulation constant according to the literature.

**Table 6. Solvent effect on spectra of (J25)**

<table>
<thead>
<tr>
<th>Solvent</th>
<th>symbol</th>
<th>D</th>
<th>(D-1)/(D+1)</th>
<th>λ&lt;sub&gt;max&lt;/sub&gt; (J25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-Hexane</td>
<td>1</td>
<td>1.89</td>
<td>0.308</td>
<td>370 Strong</td>
</tr>
<tr>
<td>1,4-Dioxane</td>
<td>2</td>
<td>2.30</td>
<td>0.394</td>
<td>380 Strong</td>
</tr>
<tr>
<td>Chloroform</td>
<td>3</td>
<td>4.80</td>
<td>0.655</td>
<td>370 Strong</td>
</tr>
<tr>
<td>Acetone</td>
<td>4</td>
<td>20.60</td>
<td>0.907</td>
<td>370 Strong</td>
</tr>
<tr>
<td>Ethanol</td>
<td>5</td>
<td>24.00</td>
<td>0.920</td>
<td>370 Strong</td>
</tr>
<tr>
<td>Methanol</td>
<td>6</td>
<td>33.60</td>
<td>0.942</td>
<td>370 Strong</td>
</tr>
<tr>
<td>DMSO</td>
<td>7</td>
<td>46.67</td>
<td>0.958</td>
<td>380 Strong</td>
</tr>
<tr>
<td>Water</td>
<td>8</td>
<td>78.30</td>
<td>0.975</td>
<td>360 Weak</td>
</tr>
</tbody>
</table>

As seen in Fig. 6, (D-1)/(D+1) against the λ<sub>max</sub> of compound (J25) yields about a high linear relation with solvents of moderate polarity.
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Fig. 6. The relationship between $(\lambda_{\text{max}})$ and $(D-1)/(D+1)$ for compound(J25)

10. Biological effectiveness

The biological effectiveness of Azo (J25) against the negative Staphylococcus Aureuse and Escherichia Coli are studied. The effect of compound(J25) is evident in inhibiting the growth of staphylococcus Aureuse[19], and has not affected Escherichia Coli. The effect of compound(J25) was measured by measuring the diameter of the inhibition area, which was (17) mm and which was within range (10-25) mm

<table>
<thead>
<tr>
<th>J25</th>
<th>staphylococcus Aureuse</th>
<th>Escherichia Coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effect compound</td>
<td>+++</td>
<td>-</td>
</tr>
</tbody>
</table>

+ = low inhibition effectiveness  
++ = Medium inhibition effectiveness  
+++ = strong inhibition effectiveness  
= = Non-inhibition

Fig7a. Staphylococcus Aureuse  
Fig7b. Escherichia Coli
11. Conclusions
1. The synthesis of the compound (J25) has been validated by the use of the diagnostic methods mentioned in this paper.
2. The stability of compound (J25) has been confirmed through spectral and weight analysis methods.
3. Applicability of Pierre's law and linearity using compound (J25) solutions at greater wavelength
4. The study showed the effectiveness of the biological compound (J25) towards some types of bacteria

12. Recommendations
1. By studying the effect (pH) on the compound (J25) can be suggested as evidence in the correction (acid-base)
2. It is possible to propose the compound (J25) as a corrosion inhibitor
3. Usage of compound (J25) prepared for dyeing types of clothing fabrics and Usage of Azo for polymeric fiber dyeing
4. Biological study of other types of bacteria, fungi and virus

13. References


دراسات التحليل الطيفي والفعالية البيولوجية لمركب الأزو (J25) المحضر من بارامينو بنزوات الأيثيل

جاسم حميد الولائي، حنين عبد الصمد عبد المجيد، صادق محمد حسن أسماعيل
قسم الكيمياء، كلية التربية للعلوم الصرفة، جامعة البصرة، البصرة، العراق.

المستند

تم تحضير مركب الأزو مع مفاعل إيثيل 4-أمينوبنزوات مع 2-أمينو-3-(4-هيدروكس فينيل) حمض البروبانويك (J25). تم تناول المركب واعداد بلورته باستخدام الإيثانول المطلق. تم أخرى تكنيات تشخيصية وتحليلية حيث تم تشخيص المركب بقياس الطيف بالأشعة تحت الحمراء وقياس الطيف الكتلي. قياس الطيف المرئي لقيم الأس الهيدروجيني ضمن النطاق (2-12) pH، والتي تضمنت تحديد أعلى قيمة امتصاص للمركب وتحديد النقاط الزواياوية. كان للمذيبات مختلفة القطبية أيضًا تأثير على قياس الطيف المرئي. تم دراسة الفعالية البيولوجية للمركب (J25) ضد نوعين من البكتيريا (الاشريكيا القولونية) والمكورات العنقودية الذهبية) وأظهرت النتائج التأثير الإيجابي للمركب في تثبيط نمو البكتيريا. تم تقييم هذه الدراسة وتقديمها كدراسة مستقبلية للتطبيقات الطبية والكيميائية والفيزيائية والبيولوجية وغيرها من التطبيقات المهتمة بمركبات الأزو.

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- الفعالية البيولوجية
- مركب الأزو
- بارامينو بنزوات الأيثيل

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