

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

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ARTICLE INFO

Received 07 April 2024
Accepted 09 June 2024
Published 30 June 2024

Keywords :

spectroanalytical, Biological effectiveness, Azo compound, Ethyl p-aminobenzoate.

Citation: J. H. Al-Waeli et al., J. Basrah Res. (Sci.) 50 (1), 179 (2024).
[DOI:https://doi.org/10.56714/bjrs.50.1.15](https://doi.org/10.56714/bjrs.50.1.15)

ABSTRACT

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 (staphylococcus 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

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

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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.

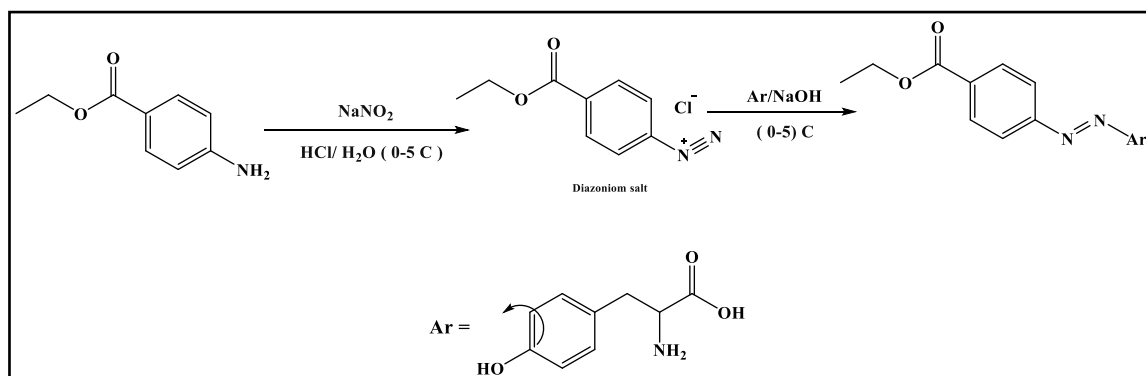
2. Experimental

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 scheme1.



Scheme1. 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 8×10^{-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 (8×10^{-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

Table1 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

Table1. chemical and physical properties of Azo(J25)

compound	M.formula	M.W (g.mol ⁻¹)	M.P °C	Yield %	Colour
J25	C ₁₈ H ₁₉ O ₅ N ₃	357.38	195-197	80	dark red

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 (145.41) 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.

Table2 . Selected infrared data of compound (J25)

Compound	$\nu(\text{O-H}) \text{ cm}^{-1}$	$\nu(\text{C=O}) \text{ cm}^{-1}$	$\nu(\text{C=C}) \text{ cm}^{-1}$	$\nu(\text{N=N}) \text{ cm}^{-1}$	$\nu(\text{C-N}) \text{ cm}^{-1}$	$\nu(\text{C-O}) \text{ cm}^{-1}$
J25	3381.21	1604.77	1514.12	1450.47	1394.53	1276.88

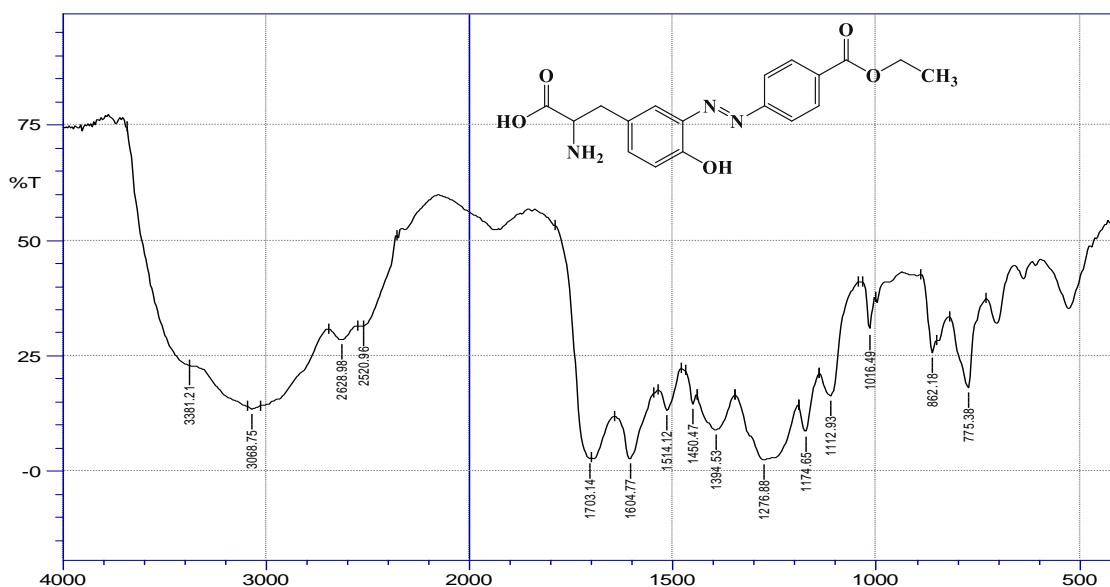


Fig. 1. IR spectrum of (J25).

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 $[C_{18}H_{19}O_5N_3]^+$. The base ion peaked in appearance $m/z = 120.2$

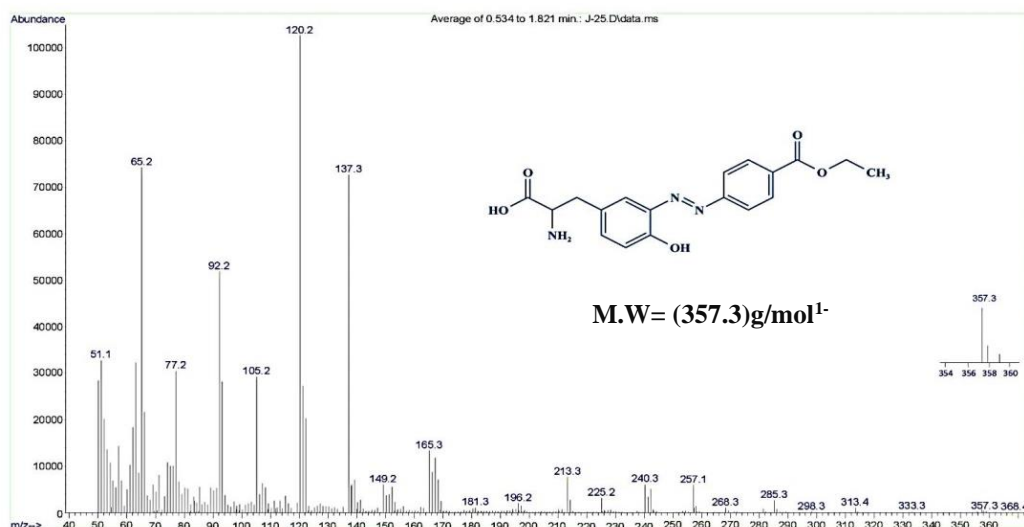


Fig. 2 . Mass spectrum of (J25).

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 (8×10^{-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 (J25) = 0.8×10^{-4} M) nm in the pH range (9–11), and 410 nm in the pH range (12).

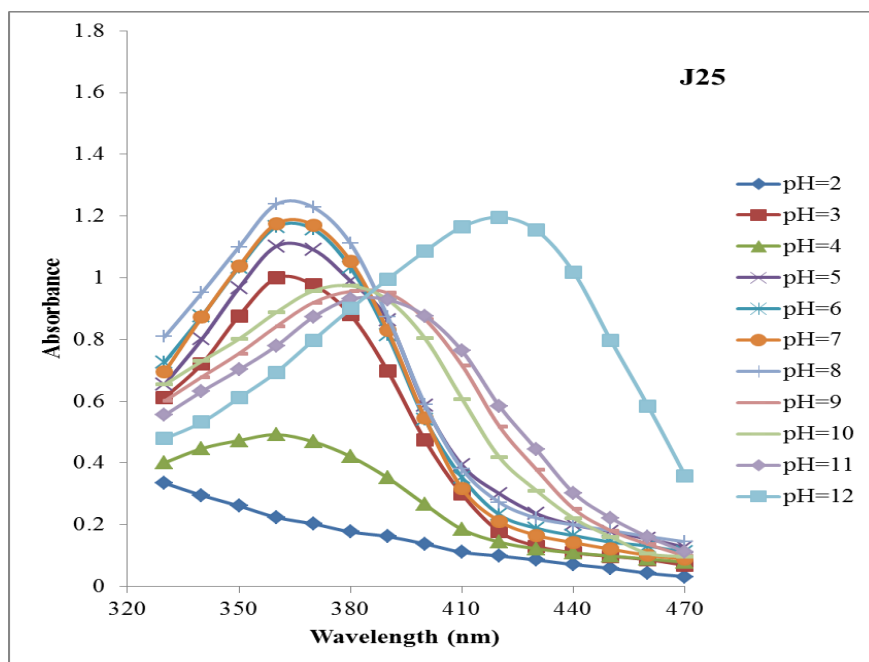


Fig.3. At pH levels of (2-12) absorption spectrum

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: $pK = pH \text{ (at } A_{1/2})$ [15], where $A_{1/2} = (A_L + A_{min})/2$ and A_L and A_{min} represent the limiting and minimum absorbance, respectively.

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

(J25) at $\lambda = (360)\text{nm}$			
A_{min}	A_L	$A_{1/2}$	pK
0.224	0.999	0.611	2.4 _{p1}
0.492	1.238	0.865	11.5 _{a1}
0.842	0.888	0.865	9.7 _{a2}

p = H's protonization constant for the phenol molecule's nitrogen atom

a = H's Ionization constant for Hydroxyl group in Phenol molecule

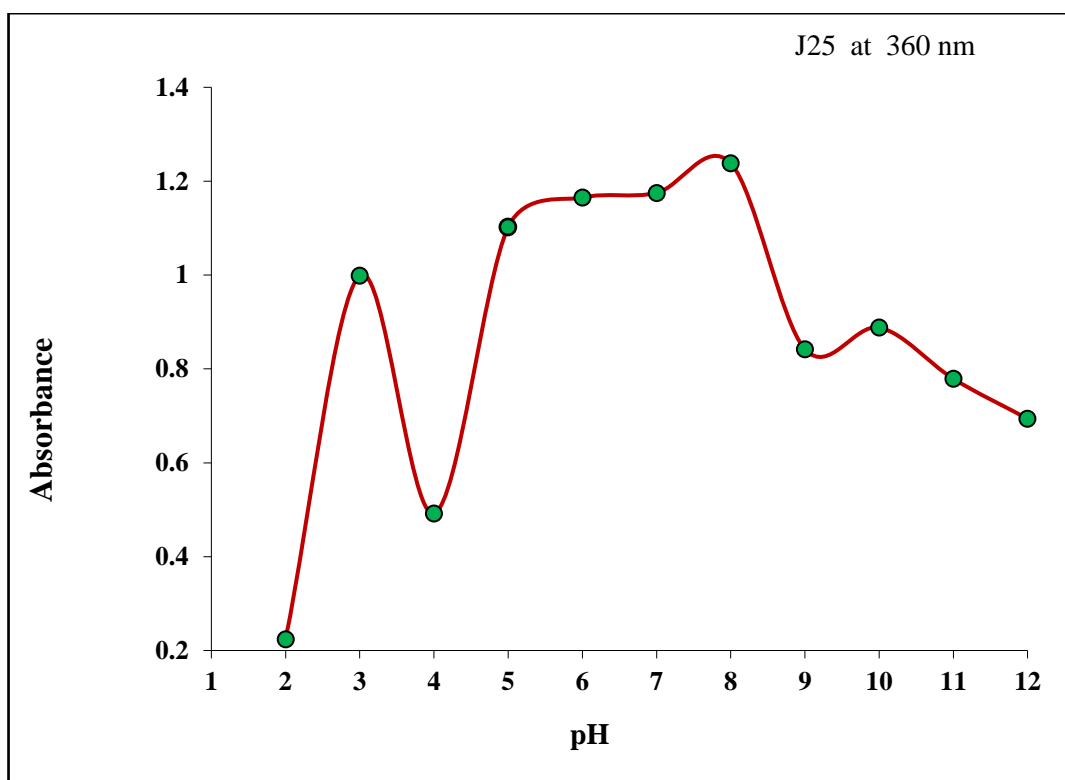
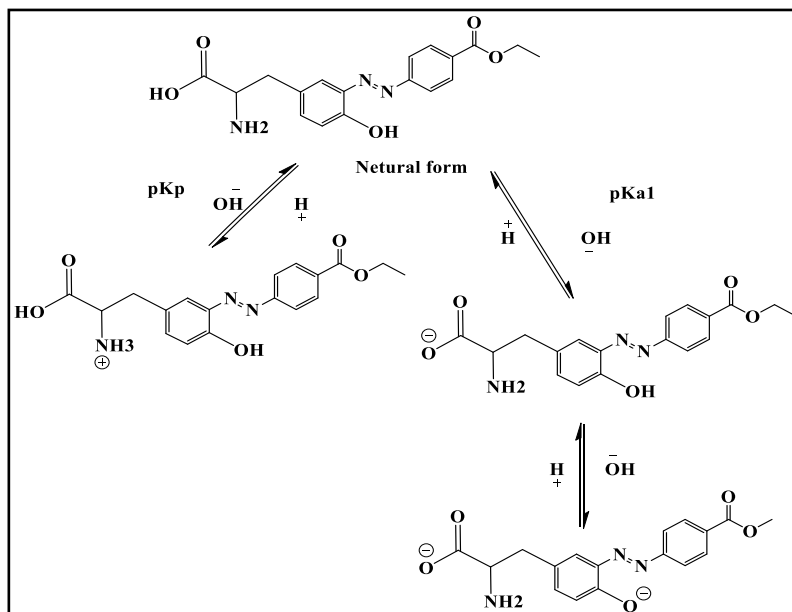


Fig.4. The Absorbance pH curves of compound (J25)

Scheme 2 illustrates the proposed chemical mechanisms of protonization and ionization.



Scheme2 . mechanism of (J25)

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

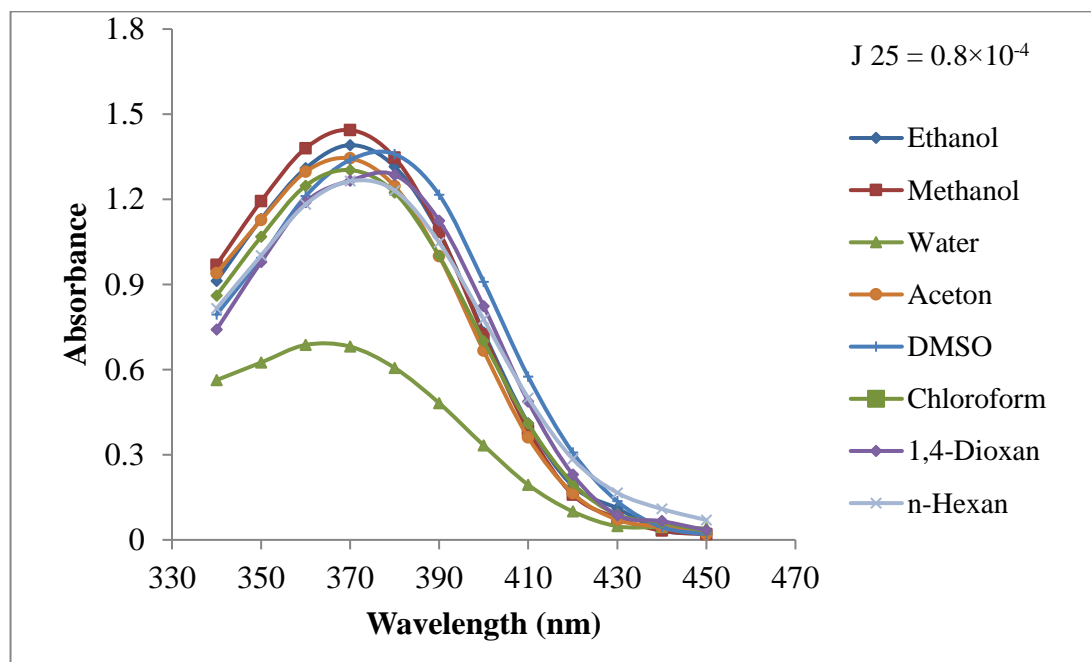


Fig.5 . The electronic spectrums of compound J25 at different polar solvents

From the shape of the spectrum of the compound(J25) in different polar solvents, the results recorded in (Tables4,5) 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 the (table.5)

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

compound (J5)	λ_{\max} to 1,4-Dioxane	λ_{\max}	Blue shift	Red shift	Notes shift
	380	Methanol	Ethanol, , Acetone, Chloroform, and n-Hexane	-----	Medium shift

Solvent	n-Hexane	1,4-Dioxane	Chloroform	Acetone	Ethanol	Methanol	DMSO	Water
λ_{\max}	370	380	370	370	370	370	380	360
$\epsilon 10^4$	1.58	1.60	1.62	1.68	1.73	1.80	1.69	0.85

The results in table6 indicate that the linear relationship between solvent electrical insulation constant and absorption peaks is equivalent to $(D-1)/(D+1)$ [17]. and this gives the impression that electrical insulation constant is the factor influencing absorption, where (D)[18] value represents solvent electrical insulation constant according to the literature

Table6 . Solvent effect on spectra of (J25)

Solvent	symbol	D	$(D-1)/(D+1)$	λ_{\max} (J25)
n-Hexane	1	1.89	0.308	370 Strong
1,4-Dioxane	2	2.30	0.394	380 Strong
Chloroform	3	4.80	0.655	370 Strong
Acetone	4	20.60	0.907	370 Strong
Ethanol	5	24.00	0.920	370 Strong
Methanol	6	33.60	0.942	370 Strong
DMSO	7	46.67	0.958	380 Strong
Water	8	78.30	0.975	360 Weak

As seen in Fig. 6, $(D-1)/(D+1)$ against the λ_{\max} of compound (J25) yields about a high linear relation with solvents of moderate polarity

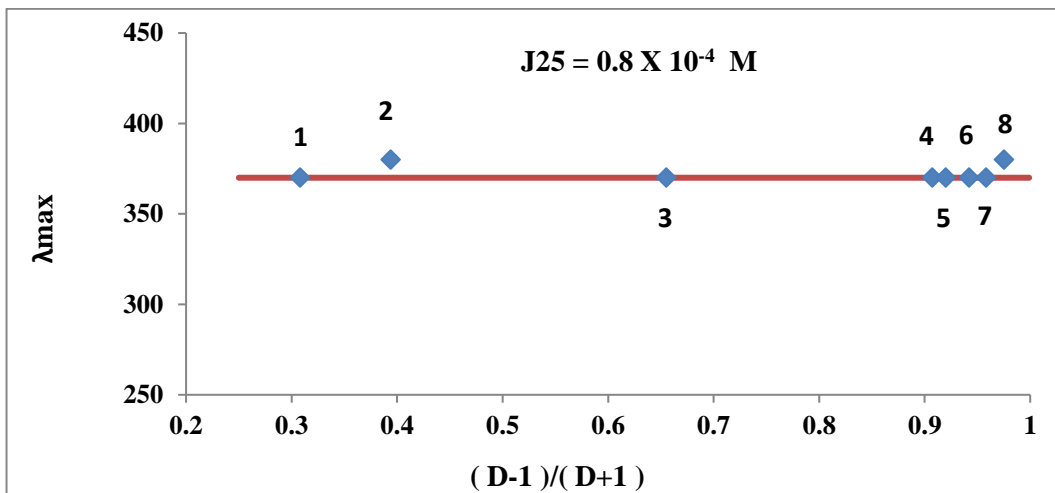


Fig. 6 . The relationship between (λ_{max}) and (D-1)/(D+1)for compound(J25)

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

J25	staphylococcus Aureuse	Escherichia Coli
Effect compound	+++	-

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

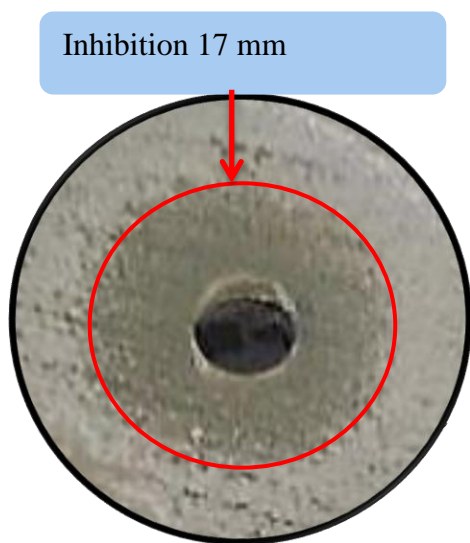


Fig7a . Staphylococcus Aureuse

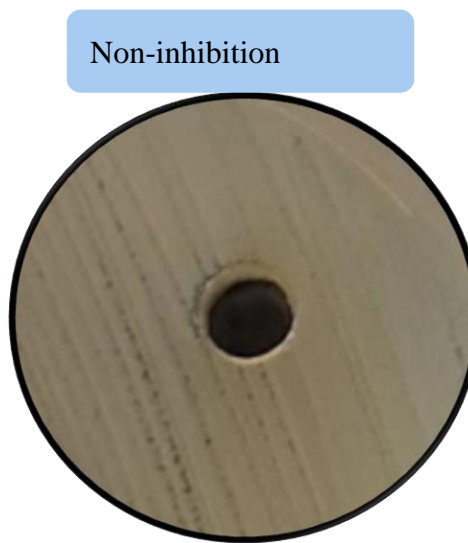


Fig7b . Escherichia Coli

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 viruse

13. References

- [1] R. Khanum, R. A. Shoukat Ali, H. R. Rangaswamy, S. R. Santhosh Kumar, A. G. Prashantha, and A. S. Jagadisha, "Recent review on Synthesis, spectral Studies, versatile applications of azo dyes and its metal complexes," *Results Chem.*, vol. 5, Jan. 2023.
- [2] M. M. Aftan, A. A. Talloh, A. H. Dalaf, and H. K. Salih, "Impact para position on rho value and rate constant and study of liquid crystalline behavior of azo compounds," *Mater. Today Proc.*, vol. 45, no. xxxx, pp. 5529–5534, 2021. Doi: <https://doi.org/10.1016/j.matpr.2021.02.298>.
- [3] M. N. Khan, D. K. Parmar, and D. Das, "Recent Applications of Azo Dyes: A Paradigm Shift from Medicinal Chemistry to Biomedical Sciences," *Mini-Reviews Med. Chem.*, vol. 21, no. 9, pp. 1071–1084, Nov. 2020. Doi: <https://doi.org/10.2174/138955752099201123210025>.
- [4] L. H. Ahlström, C. Sparr Eskilsson, and E. Björklund, "Determination of banned azo dyes in consumer goods," *TrAC - Trends Anal. Chem.*, vol. 24, no. 1, pp. 49–56, 2005. Doi: <https://doi.org/10.1016/j.trac.2004.09.004>.
- [5] R. M. Christie, "Colour: A brief historical perspective," in *Colour Chemistry*, The Royal Society of Chemistry, 2007, pp. 1–11. Doi: <https://doi.org/10.1039/9781847550590-00001>.
- [6] S. Sandhya, S. Padmavathy, K. Swaminathan, Y. V. Subrahmanyam, and S. N. Kaul, "Microaerophilic-aerobic sequential batch reactor for treatment of azo dyes containing simulated wastewater," *Process Biochem.*, vol. 40, no. 2, pp. 885–890, 2005. Doi: <https://doi.org/10.1016/j.procbio.2004.02.015>.
- [7] H. A. S. A. Majeed, "Synthesis , Characterization , and study of the Spectral and Electronic Properties of a New Azo Dyes Compounds," *Univ. Thi-Qar J. Sci.*, vol. 4, no. 1, pp. 91–101, 2019. Doi: <https://doi.org/10.32792/utq/utjsci/vol4/1/8>.
- [8] C. P. Locher et al., "Anti-microbial activity and anti-complement activity of extracts obtained from selected Hawaiian medicinal plants," *J. Ethnopharmacol.*, vol. 49, no. 1, pp. 23–32, 1995. Doi: [https://doi.org/10.1016/0378-8741\(95\)01299-0](https://doi.org/10.1016/0378-8741(95)01299-0).
- [9] L. A. Mohammed, N. I. Mahdi, and R. A. B. Aldujaili, "Preparation, characterization and the biological activity study of a new heterocyclic (Azo-Schiff base) ligand and their complexation with {Co,Ni,Cu,Zn(II)}Ions," *Egypt. J. Chem.*, vol. 63, no. 1, pp. 289–300, 2020. Doi: <https://doi.org/10.21608/ejchem.2019.19821.2195>.
- [10] T. A. Fahad, A. A. Ali, And A. H. Baty, "Synthesis, Characterization And Analytical Studies Of Some New Azodyes Driven From O-Vanillin," *World J. Pharm. Res.*, Vol. 8, 2019, [Online Available: <https://www.researchgate.net/publication/331486640>].
- [11] Y. H. Ebead, M. A. Selim, and S. A. Ibrahim, "Solvatochromic, acid-base features and time effect of some azo dyes derived from 1,3-benzothiazol-2-ylacetone nitrile: Experimental and

- semiempirical investigations,” *Spectrochim. Acta - Part A Mol. Biomol. Spectrosc.*, vol. 75, no. 2, pp. 760–768, 2010. Doi: <https://doi.org/10.1016/j.saa.2009.11.051>.
- [12] S. A. Mohammed and H. Y. S. Zebary, “Spectrophotometric Determination of Sulfadiazine via Diazotization and Coupling Reaction - Application to Pharmaceutical Preparations,” *Rafidain J. Sci.*, vol. 24, no. 11, pp. 61–73, 2013. Doi: <https://doi.org/10.1155/2012/206076>.
- [13] S. M. Kadhim and S. M. Mahdi, “Preparation and Characterization of New (Halogenated Azo-Schiff) Ligands with Some of their Transition Metal Ions Complexes,” *Iraqi J. Sci.*, pp. 3283–3299, Aug. 2022. Doi: <https://doi.org/10.24996/ij.s.2022.63.8.4>.
- [14] K. Tanaka, K. Padermpole, and T. Hisanaga, “Photocatalytic degradation of commercial azo dyes,” 2000. Doi: [https://doi.org/10.1016/S0043-1354\(99\)00093-7](https://doi.org/10.1016/S0043-1354(99)00093-7).
- [15] A. A. Al-Muhsin, T. A. Fahad, and A. A. Ali, “Preparation and characterization azo dyes derived from 4- hydroxycoumarin and studying their analytical Applications,” *J. Phys. Conf. Ser.*, vol. 1999, no. 1, 2021. Doi: <https://doi.org/10.1088/1742-6596/1999/1/012010>.
- [16] T. Stalin and N. Rajendiran, “Intramolecular charge transfer effects on 3-aminobenzoic acid,” *Chem. Phys.*, vol. 322, no. 3, pp. 311–322, 2006. Doi: <https://doi.org/10.1016/j.chemphys.2005.09.002>.
- [17] Y. G. Sidir, I. Sidir, H. Berber, and E. Taşal, “UV-spectral changes for some azo compounds in the presence of different solvents,” *J. Mol. Liq.*, vol. 162, no. 3, pp. 148–154, 2011. Doi: <https://doi.org/10.1016/j.molliq.2011.07.002>.
- [18] A. Airinei, M. Homocianu, and D. O. Dorohoi, “Changes induced by solvent polarity in electronic absorption spectra of some azo disperse dyes,” *J. Mol. Liq.*, vol. 157, no. 1, pp. 13–17, 2010. Doi: <https://doi.org/10.1016/j.molliq.2010.07.011>.
- [19] M. A. Zoroddu, S. Zanetti, R. Pogni, and R. Basosi, “An electron spin resonance study and antimicrobial activity of copper(II)-phenanthroline complexes,” *J. Inorg. Biochem.*, vol. 63, no. 4, pp. 291–300, 1996. Doi: [https://doi.org/10.1016/0162-0134\(96\)00015-3](https://doi.org/10.1016/0162-0134(96)00015-3).

دراسات التحليل الطيفي والفعالية البيولوجية لمركب الأزو (J25) المحضر من بارا-امينو بنزوات الأيثيل

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الملخص

معلومات البحث

تم تحضير مركب الأزو مع مفاعل إيثيل 4-أمينوبنزوات مع 2-أمينو-3 (4-هيدروكسي فينيل) حمض البروبانويك واقتراح هذا الرمز (J25). تم تنقية المركب وإعادة بلورته باستخدام الإيثانول المطلق ثم أجرى تقنيات 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 الإيزوبستية. كان للمذيبات مختلفة القطبية أيضاً تأثير على قياس الطيف المرئي. تمت دراسة الفعالية البيولوجية للمركب (J25) ضد نوعين من البكتيريا (الاشريكية القولونية) و (المكورات العنقودية الذهبية) وأظهرت النتائج التأثير الإيجابي للمركب في تثبيط نمو البكتيريا. تم تقنين هذه الدراسة وتقديمها كدراسة مستقبلية للتطبيقات الطبية والكيميائية والفيزيائية والبيولوجية وغيرها من التطبيقات المهمة بمركبات الأزو.

الاستلام 07 نيسان 2024

القبول 9 حزيران 2024

النشر 30 حزيران 2024

الكلمات المفتاحية

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Citation: J. H. Al-Waeli et al.,
J. Basrah Res. (Sci.) 50 (1), 179
(2024).

[DOI:https://doi.org/10.56714/bjrs.50.1.15](https://doi.org/10.56714/bjrs.50.1.15)

