



# Integrated Thermogravimetric and Spectroscopic Evaluation of Azo Dye and Its Polymers: Focused Analytical Characterization of the Dye and Broad Assessment of Antibacterial Activity

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## ABSTRACT

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### Keywords:

azo compounds, antimicrobial polymers, polymerization, thermogravimetric analysis

A study investigates the creation of azo-derived polymers alongside their characterization and biological evaluation with emphasis on antibacterial properties and thermal properties of these materials. This research aims to synthesize ZA1 azo substance as well as ZP3 and ZP4 polymeric derivatives and evaluate their biomedical potential. The research utilized both diazotization and coupling processes for azo compounds synthesis and incorporated phthalic acid as the crosslinking agent in the polymerization reaction. The examination of produced substances used two testing methods which included Fourier Transform Infrared Spectroscopy (FTIR) and Thermogravimetric Analysis (TGA). Antibacterial assessment of *Escherichia coli* and *Staphylococcus aureus* occurred by disk diffusion technique against standard medications ampicillin and ciprofloxacin. Laboratory research confirmed that polymerization technologies strengthened the antibacterial strength of azo compounds where ZP4 demonstrated superior outcomes compared to conventional antibiotic treatments. Thermal stability examination by TGA analysis confirmed the appropriate use of produced polymers in biomedical applications. This research is proven that polymers derived from azo compounds show strong potential as antibacterial agents to support medical functions including wound dressings, antimicrobial coatings and medication delivery methods. The discoveries enable to make alternative methods which resist antibiotic-resistant bacterial strains. The research reveals possibility of azo-based polymers adapting into healthcare materials through advanced production understanding which leads to enhanced antimicrobial performance. The research discloses which modifications improve antibacterial agent stability and effectiveness.

## 1. INTRODUCTION

Healthcare technology improvement alongside antimicrobial resistance reduction occurs through biomedical science research with azo-derived polymers starting from synthesis to characterization to implementation. The research examines biological effects of these polymers through systematic studies on their functionality alongside critical applications factors in biomedical applications [1, 2]. Urgent development of new antibacterial drugs that overcome drug-resistant bacterial strains becomes necessary because antimicrobial resistance poses a severe global health threat through its fast evolution [3, 4]. Traditional antibiotics show decreasing effectiveness because they present two main issues: cytotoxicity and bacterial resistance in combination with their unstable characteristics in physiological conditions. Scientists are studying polymeric materials as potential substitutes for these problems because of their bacterial membrane binding properties and regulated degradation rates and adjustability of physical chemistry properties. The  $-N=N-$  (azo) functional group distinguishes azo compounds thus scientists study these compounds actively because of their antibacterial and anti-

inflammatory and anticancer properties. Clinical applications of these substances are restricted by their irregular properties when present in biological conditions. Scientists report that mediating antibacterial effectiveness, stability, and clinical application occurs when researchers form well-structured macromolecules out of azo drugs [5]. Polymerizing azo compounds results in better biological system compatibility and controlled breakdown properties together with amplified antibacterial effects. The evolving attraction for azo-derived polymers exists because they show promising applications in drug delivery and biomedical coatings and wound management. The creation of azo-derived polymers happens through several polymerization methods which include condensation polymerization alongside controlled living polymerization and free-radical polymerization of azo-functionalized monomers. Researchers can now precisely control the molecular weight as well as content and architecture of polymers through these established techniques [6]. Scientists add bioactive components which enhance antibacterial outcomes while improving biological system compatibility to these polymers for functionalization. The polymers function well for biomedical use because adding

groups that are hydrophilic and hydrophobic provides adjustable breakdown rates and solubility options [7]. The truthful evaluation of polymers derived from azo compounds needs thorough procedures for both structural assessment and thermal and antibacterial investigations. The successful incorporation of azo groups into polymer matrices needs verification through analytical methods including X-ray diffraction (XRD) along with nuclear magnetic resonance (NMR) spectroscopy and Fourier transform infrared spectroscopy (FTIR) [8]. Researchers rely on TGA and DSC tests to examine the thermal stability aspects of their produced polymers for understanding degradation behavior and operating temperature range. The interaction of these polymers with bacterial cells can be microscopically analysed through transmission electron microscopy (TEM) and scanning electron microscopy (SEM) while their surface topography is evaluated [9, 10]. Azo-derived polymers show their antibacterial effect through membrane interaction as they rupture cell membranes while suppressing essential cellular functions [11]. The polymers show an additional effect of inducing oxidative stress that results in bacterial cell demise. Medical applications of azo-derived polymers consist of precision drug methods along with wound-healing materials and anti-microbial surface solutions for medical technologies [12]. The clinical performance of these products improves because their degradation time and medication release amount are both adjustable. Tissue engineering research utilizing azo-derived polymers needs development of their bioactive properties for wound healing applications and tissue regeneration [13, 14]. Concern for biomedical science has grown significantly toward azo-derived polymers because they exhibit distinctive features alongside multiple applications [15]. The antibacterial properties of polymer constructs experience a performance boost from incorporating azo groups because these groups enhance both biodegradation rates and drug encapsulation and strengthen bacterial membrane interactions [16]. Bioactive polymers present opportunities to apply them in wound-healing applications while demonstrating potential for antibacterial coatings on medical devices and drug delivery systems requiring precise breakdown kinetic control. The current scientific research offers a minimal evaluation of how azo compounds function as antibacterials compared to their equivalent polymer structures. Transformation to polymeric structures decreases both therapeutic effectiveness and thermal characteristics while affecting compatibility of azo compounds [17]. The development of biodegradable polymers represents a 50-year medical revolution that has resulted in significant biotechnological advances in drug delivery, biomaterials, tissue engineering, and medical device development, all while bringing together chemists, engineers, biologists, and physicians in a unique and collaborative manner. The timeline of this revolution corresponds to the discovery of increasingly potent treatments such as peptides, proteins, nucleic acids, and other bioactive compounds [18]. The short half-lives of many current medicines, along with the nonspecific distribution and toxicity of previously found small molecule medications, have fueled the development of polymeric drug delivery systems. The successful clinical translation of prior macro- and micro-drug-delivery systems has resulted in the development of controlled-release nanodrug delivery platforms capable of overcoming pharmacological restrictions while providing significant advantages over conventional dose forms [19]. Investigations and discoveries in synthetic methodologies,

fabrication methods, and mathematical models for studying the mechanisms of controlled drug release have resulted in the ability to create tunable polymeric nanoparticle (NP) drug delivery systems capable of localized and sustained delivery, allowing drugs to improve their therapeutic index. The capacity to manage the release of medicines, as well as the exceedingly adaptable nature of polymeric drug delivery platforms, provide multiple significant advantages [20].

Azo dyes find widespread usage in the textile, fiber, cosmetic, leather, paint, and printing industries. Aside from their characteristic coloring effect, azo chemicals have been shown to be antibacterial, antiviral, antifungal, and cytotoxic. They can be utilized as drug carriers, either as a 'cargo' to entrap therapeutic molecules or in a prodrug strategy. As with colon-targeted drug delivery, the drug is released in response to internal or external stimuli in the region of interest. Aside from drug-like and drug carrier qualities, a variety of azo dyes are utilized in cellular staining to reveal cellular components and metabolic pathways. However, azo compounds' biological significance, particularly in cancer treatment, is still in its early stages. This could be related to early research that identified azo chemicals as one of the potential causes of cancer and mutagenesis. Researchers are now testing aromatic azo molecules for potential medicinal applications, including cancer diagnosis and treatment [21]. Many researchers have recently concentrated on the development of dispersing azo dyes, investigated their antibacterial activity, and reported polymer applications for a variety of purposes. For example, Abd El Salam et al. [22] produced and tested the antibacterial activity of various new azo-Sulfa-Based Disperse Dyes and Their Application in Polyester Printing. Mohamed et al. [21]. produced novel acid dyes based on 3-Amino-2-thioxo-4-thiazolidinone derivatives and utilized them to dye wool and silk fibers. Both synthetic dyes demonstrated good lightfastness and fastness qualities. Furthermore, all dyes have demonstrated a positive antibacterial impact. Azo dyes have gained a significant attention in an analytical chemistry [23, 24], the strongly coloured dyes, are characterized by the presence of the azo group ( $-N=N-$ ) in their structure, which stabilizes its conformation and forms a conjugated system [25]. Consequently, they are categorised as special dyes, that getting a significant interest in analytical studies. Further, the azo dyes have been identified for their varied applications, including indicators,<sup>2</sup> antidiabetic, antineoplastic, antimicrobial, DNA and RNA inhibition in microorganisms and synthesis of protein inhibitors besides [26].

This research aims to produce both ZA1 as an azo chemical and its polymeric derivatives ZP3 and ZP4. Analytical techniques approved for research will evaluate the generated substances regarding their chemical composition and structural quality. The antibacterial performance of synthetic materials along with their efficacy against traditional antibiotics will be determined through resistance zone size assessments. TGA analysis will determine the thermal resilience of these substances to shed light on their applications in heat-sensitive environments. The well-designed investigation aims to build comprehensive understanding about azo-derived polymers and multiple features of these materials.

## 2. EXPERIMENTS

The work synthesized ZA1/A1 via diazotization and

coupling processes and polymerized ZP3, ZP4, and ZP2 using phthalic acid. The disk diffusion method assessed the produced polymers' antibacterial activity. FTIR spectroscopy confirmed the chemical structure, verifying compound synthesis. TGA study revealed polymers' thermal resilience under different temperatures. The synthesis, polymerization, and characterisation of azo compounds and their polymeric derivatives were carried out using the following substances and reagents:

## 2.1 Azo compound synthesis

Azo compounds and their polymeric derivatives were synthesized using these substances and reagents: Sodium nitrite ( $\text{NaNO}_2$ ), Hydrochloric acid ( $\text{HCl}$ , 37%), Sodium hydroxide ( $\text{NaOH}$ ), and Ethanol ( $\text{C}_2\text{H}_6\text{O}$ , analytical grade). These chemicals synthesized and polymerized azo compounds.

## 2.2 Polymer synthesis

The following compounds were utilized for polymer synthesis: Phthalic acid ( $\text{C}_8\text{H}_6\text{O}_4$ ) (stoichiometric ratios 1:2) was used as a crosslinking agent, Zinc chloride ( $\text{ZnCl}_2$ ) as a polymerization catalyst, THF as a solvent, DMF as a polymer processing solvent, and Ethanol as a purification solvent.

## 2.3 Antimicrobial testing

These components were used for antimicrobial testing: *E. coli* (ATCC 25922, Gram-negative), *S. aureus* (ATCC 6538, Gram-positive), Mueller-Hinton Agar (MHA) medium, Ampicillin (10  $\mu\text{g/mL}$ ), and Ciprofloxacin (5  $\mu\text{g/mL}$ ) were used as reference antibiotics for comparison.

## 2.4 Thermal stability and structural characterization

TGAs were used to investigate thermal stability and structural characterisation. Structure was confirmed using an FTIR Spectrometer. TGA analysis also used nitrogen gas for inertness.

All chemicals were from certified suppliers and used unpurified unless otherwise stated.

## 2.5 Methods

The azo molecule (ZA1/A1) is synthesised via diazotization and coupling processes, then polymerized with phthalic acid

to create ZP3, ZP4, and ZP2, which are thermally stable and antibacterial. The disk diffusion method examined antibacterial activity, FTIR confirmed functional groups, and TGA assessed thermal stability of the produced compounds.

### 2.5.1 Synthesis of azo compound (ZA1/A1)

The azo chemical was produced by diazotization and coupling [19]. Figures below show reaction pathways.

1. Diazotization reaction: p-Hydroxybenzaldehyde was dissolved in distilled water and ice bath-cooled to 0–5°C. To the aldehyde solution, a separate sodium nitrite ( $\text{NaNO}_2$ ) solution was gently added while stirring. To create diazonium salt, concentrated hydrochloric acid ( $\text{HCl}$ ) was added dropwise. The reaction mixture was kept cool to prevent intermediate product breakdown.

2. Coupling reaction: While stirring, diazonium salt was slowly added to an alkaline p-methoxybenzaldehyde solution. The coupling process was optimized by adjusting pH (8–9) with  $\text{NaOH}$ . A colorful precipitate confirmed the azo compound (ZA1/A1) production.

3. Purification: The crude product was filtered and rinsed with cold distilled water to eliminate unreacted reagents. A pure azo compound was obtained by vacuum drying and recrystallizing with ethanol, Figure 1.

### 2.5.2 Polymerization of azo compounds (synthesis of ZP3, ZP4, and ZP2)

Polymerization with phthalic acid crosslinking improved thermal stability and antibacterial characteristics, Figure 2 shows the reaction mechanism.

### 2.5.3 Polymerization of ZP3 and ZP4

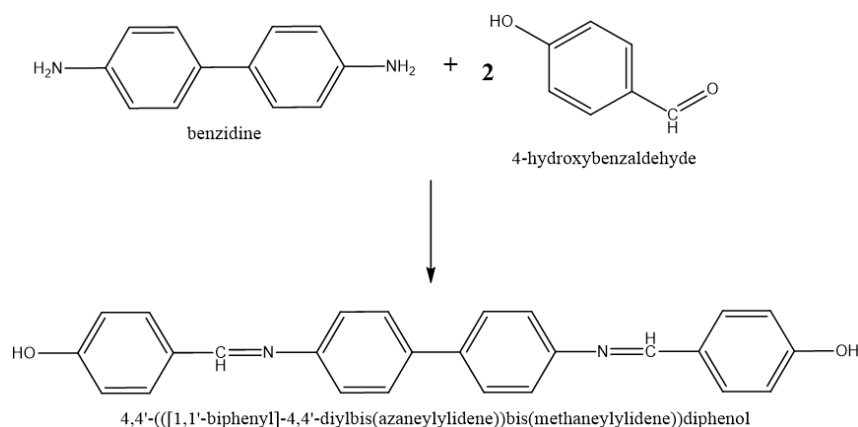
Preparation of Polymer Solution: ZA1/A1 was dissolved in THF. Phthalic acid was added in various stoichiometric ratios to produce ZP3 and ZP4.

Reaction Process: The catalyst utilized was zinc chloride ( $\text{ZnCl}_2$ ). Under reflux for 6 hours, the reaction mixture reached 60°C with constant stirring.

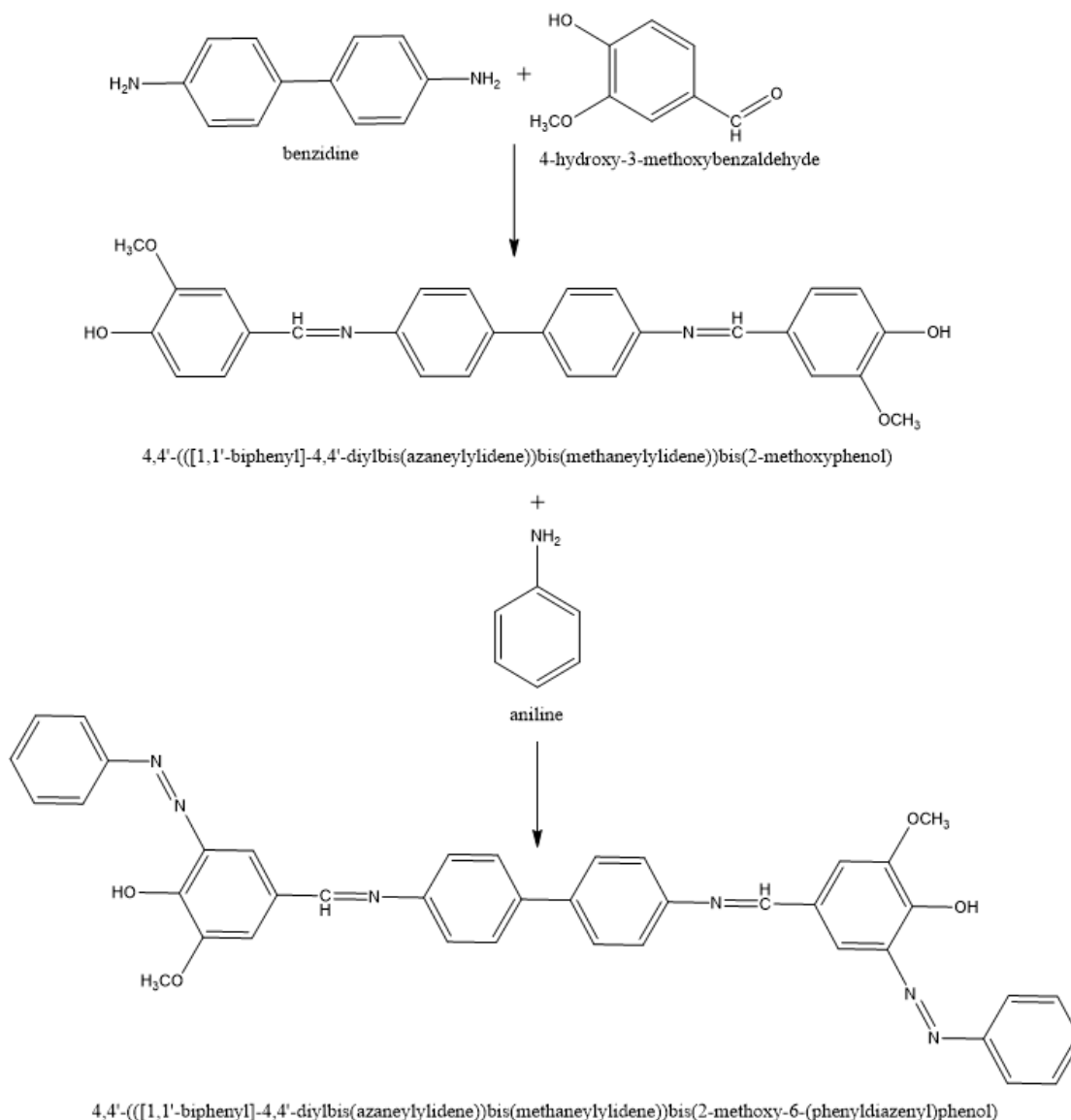
Precipitation and Purification: The polymer precipitated with ethanol after cooling the reaction mixture. Filter, ethanol wash, and vacuum oven drying of the polymer.

## 2.6 Antibacterial activity testing

ZA1, ZP3, and ZP4 were tested for antibacterial activity using the disk diffusion assay, a standard antimicrobial method [20].



**Figure 1.** Synthesis of 4,4'-((1,1'-biphenyl)-4,4'-diylbis(azanylidene))bis(methanylylidene)diphenol (4)



**Figure 2.** Mechanism of synthesis of azo compounds from schiff base

### 2.6.1 Preparation of bacterial cultures

A conventional antimicrobial disk diffusion experiment was used to assess ZA1, ZP3, and ZP4's antibacterial activities. In this step, *E. coli* and *S. aureus* were inoculated in Mueller-Hinton Broth (MHB) and incubated overnight at 37°C. Bacterial suspensions were standardized to 0.5 McFarland Standard (around  $1.5 \times 10^4$  CFU/mL). The prepared bacterial suspensions were injected onto Mueller-Hinton Agar (MHA) plates with sterile cotton swabs. The compounds (ZA1, ZP3, and ZP4) were impregnated on sterile filter paper disks at doses of 1000, 500, and 250 µg/mL. For proper evaluation, control disks were prepared as follows:

Positive Control: Ampicillin (10 µg/mL) and Ciprofloxacin (5 µg/mL)

Negative Control: Solvent-only (THF)

Inoculated plates were incubated at 37°C for 24 hours. After incubation, the inhibition zone diameter (millimetres) was measured to evaluate the compounds' antibacterial activity.

### 2.7 Fourier transform infrared spectroscopy (FTIR) analysis

FTIR research confirmed functional groups in polymer samples AZ, ZP2, ZP3, and ZP4. Each sample was prepared

as potassium bromide (KBr) pellets and examined using an FTIR spectrometer. The spectra were acquired between 4000 and 400  $\text{cm}^{-1}$ . Identified peaks for functional groups such as azo ( $-\text{N}=\text{N}-$ ), hydroxyl ( $-\text{OH}$ ), ester ( $-\text{C}=\text{O}$ ), and ether ( $-\text{OCH}_3$ ) revealed the chemical structure of produced polymers.

### 2.8 Thermogravimetric analysis (TGA)

TGA was used to evaluate ZA1, ZP3, and ZP4 thermal stability and breakdown.

**Sample Preparation:** Analysis began with meticulous drying and weighing of generated samples. The samples were then placed in the TGA chamber under nitrogen to prevent oxidation.

**Heating Procedure:** Heat was regulated at 10°C/min from 30°C to 600°C for the samples. This technique measured sample weight loss as a function of temperature.

**Data Interpretation:** Analysis included start degradation temperature and total weight loss (%). To assess synthetic polymer stability, heat degradation profiles were examined.

### 2.9 Data analysis and experimental controls

Antibacterial effectiveness was studied between inhibition

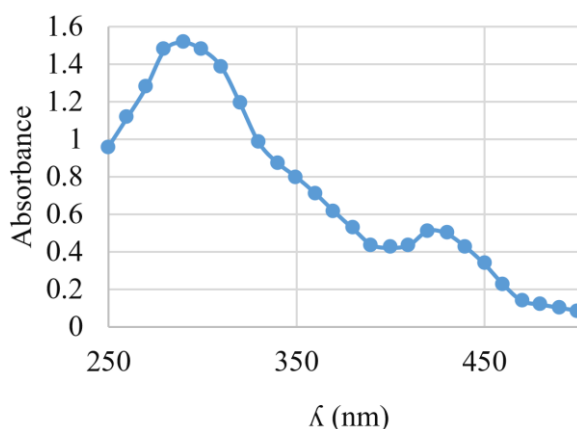
zones and conventional antibiotics. TGA study assessed degradation patterns of produced materials, while FTIR spectra confirmed polymerization-induced structural changes. Triplicate trials with solvent-only and commercial antibiotic controls ensured repeatability. Throughout the investigation, experimental conditions were standardized for consistency and reliability.

## 2.10 Different solvents polarities effect in A1

A standard solution of ligand 2A1 ( $1 \times 10^{-3}$  M) was prepared in ethanol. Subsequently, a series of diluted solutions ( $1 \times 10^{-4}$  M) were prepared using various solvents with different dielectric constants, including water, ethanol, methanol, hexane, 1,4-dioxane, and DMSO.

## 3. RESULTS AND DISCUSSION

The UV–visible spectrum of the A2 was recorded in ethanol, revealing distinct absorption features within the wavelength range of 250 to 500 nm, as illustrated in Figure 3.



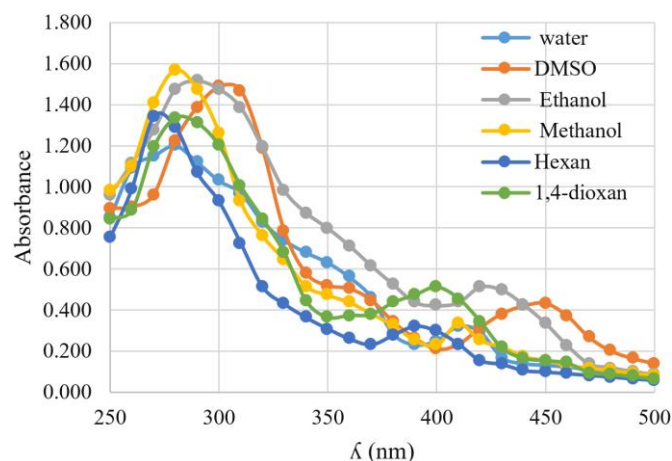
**Figure 3.** The UV-vis spectrum of A2 in ethanol

An absorption spectrum of A1 was showed a band at 290 nm and 420 nm corresponding to a  $\pi \rightarrow \pi^*$  and  $n \rightarrow \pi^*$  electronic transitions respectively. These finding were matching the structure of A1, which is contain a long  $\pi$ -conjugated system across multiple aromatic rings and imine ( $-\text{CH}=\text{N}-$ ) groups. Also, it has azo ( $-\text{N}=\text{N}-$ ) groups and electron-withdrawing groups (EWGs) groups beside the phenyl rings and this conjugated system allows  $\pi \rightarrow \pi^*$  and  $n \rightarrow \pi^*$  transitions. Therefore, the effects of different solvents in the structure of A2 were studied through the variation in their dielectric constant (D), see Figure 4.

Figure 4 shows that the polar solvents can stabilize LUMO more, reducing the HOMO–LUMO gap. Also, the stronger conjugation in the structure of A2 due to the smaller gap, resulting a red shift. The polar solvents (Ethanol, Water, DMSO and Methanol), absorbance is higher and slightly red-shifted. But, the nonpolar solvents (Hexane and 1,4-Dioxane), the absorbance is lower and blue-shifted was observed. Therefore, the designated absorption spectrum of A1 was also affected by meaning of dielectric constant (D), (Eq. (1)) [24] due to calculate related functions:  $F(D)$ ,  $\phi(D)$  and  $(D-1)/(D+1)$  that presents in Table 1.

Table 1 shows that the dielectric constants of various solvents are playing a crucial role in the modulating their

physicochemical interactions, and directly influencing the functional behaviour and spectral properties of A1. these solvents-dependent dielectric variations significantly affect its maximum absorption wavelength ( $\lambda_{\text{max}}$ ), highlighting the impact of solvent polarity on its electronic transitions. Thus, the linear relationships of these chosen solvents (1-6) were generated in depend on D and its functions.



**Figure 4.** The UV-vis spectrum of A1 in different solvents

$$\Delta\tilde{\nu}=[(a-b)(n^2-1/2n^2+1)]+b(D-1/D+1) \quad (1)$$

$$F(D) = \frac{2(D-1)}{2D+1} \quad (2)$$

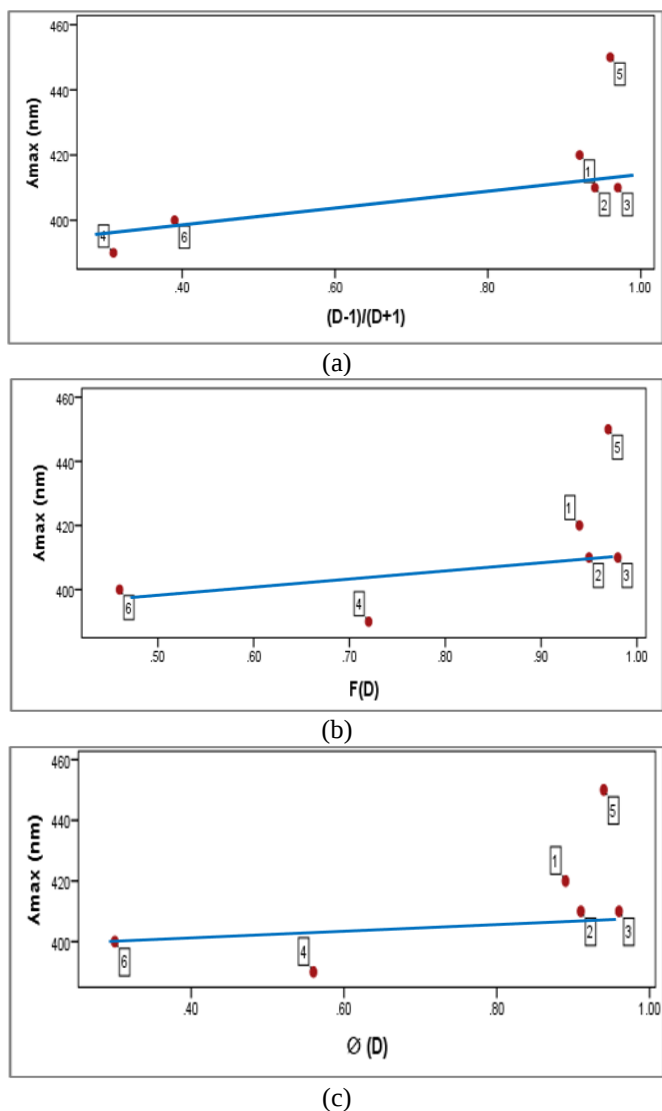
$$\phi(D) = \frac{D-1}{D+2} \quad (3)$$

**Table 1.** The effect of dielectric constants of various solvents and their functions in spectral properties of A1

| Id. No. | Solvents    | D     | $\frac{(D-1)}{(D+1)}$ | $F(D)$ | $\phi(D)$ | $\lambda_{\text{max}}$ (nm) |
|---------|-------------|-------|-----------------------|--------|-----------|-----------------------------|
| 1       | Ethanol     | 24.55 | 0.92                  | 0.94   | 0.89      | 420                         |
| 2       | Methanol    | 32.70 | 0.94                  | 0.95   | 0.91      | 410                         |
| 3       | Water       | 78.30 | 0.97                  | 0.98   | 0.96      | 410                         |
| 4       | n-Hexane    | 1.890 | 0.31                  | 0.72   | 0.56      | 390                         |
| 5       | DMSO        | 46.68 | 0.96                  | 0.97   | 0.94      | 450                         |
| 6       | 1,4-Dioxane | 2.300 | 0.39                  | 0.46   | 0.30      | 400                         |

Figure 5 (a-c) above shows, that the dielectric environment of a solvent serves as a dynamic medium that can be affected electronic landscape of A1, subtle shifts in the solvent polarity, driven by differences in the dielectric constants and induce marked changes in its absorption behavior, particularly in the position of  $\lambda_{\text{max}}$ . This solvatochromic response reveals the profound influence of the solvent's dielectric nature on A1 electronic transitions and overall photophysical profile.

Thus, in the antibacterial investigation, polymerization considerably increased the antibacterial activity of the azo compound (A1). ZP4 and 2P4 showed the maximum efficacy against E. coli (30 mm) and S. aureus (35 mm) at 1000  $\mu\text{g/mL}$ . Improved membrane contacts, molecular weight, and surface charge effects make polymerized azo compounds intriguing medicinal prospects.



**Figure 5.** The functions of solvent dielectric constants namely: a)  $(D-1)/(D+1)$ , b)  $F(D)$  and c)  $\text{Ø}(D)$ , in correlating to  $\lambda_{\text{max}}$  of 2A1

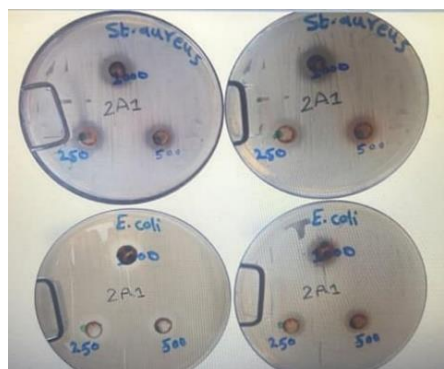
### 3.1 Antibacterial activity of azo compound (A1) and its polymers (ZP3, ZP4, 2P3, 2P4)

**Table 2.** Antibacterial activity of A1, ZP3, ZP4, 2P3, and 2P4

| Sample            | Concentration | E. Coli | S. aureus (mm) |
|-------------------|---------------|---------|----------------|
| A1 (Azo Compound) | 1000          | 15      | 17             |
|                   | 500           | 11      | 12             |
|                   | 250           | 12      | 14             |
|                   | 1000          | 25      | 20             |
| ZP3 (polymer)     | 500           | 23      | 16             |
|                   | 250           | 22      | 8              |
|                   | 1000          | 30      | 35             |
| ZP4 (polymer)     | 500           | 25      | 30             |
|                   | 250           | 10      | 15             |
|                   | 1000          | 25      | 20             |
| 2P3 (Polymer)     | 500           | 23      | 16             |
|                   | 250           | 22      | 8              |
|                   | 1000          | 30      | 30             |
| 2P4 (Polymer)     | 500           | 25      | 35             |
|                   | 250           | 10      | 15             |

The disk diffusion experiment was used to test the

antibacterial activity of the produced azo drug (A1) and its polymeric derivatives (ZP3, ZP4, 2P3, 2P4) against Gram-negative (*Escherichia coli*) and Gram-positive (*Staphylococcus aureus*). The inhibition zone diameters (mm) were evaluated at 1000, 500, and 250  $\mu\text{g/mL}$  concentrations to evaluate antimicrobial efficacy. Chemical structure, polymerization, and interactions with bacterial membranes give these substances antibacterial characteristics. Due to higher molecular weight, solubility, and surface charge interactions, polymeric derivatives have better bacterial membrane permeability and intracellular penetration, increasing their antibacterial efficacy. Table 2 shows how polymerization improves antibacterial effectiveness [21].



**Figure 6.** Disk diffusion assay for A1 against *E. coli* and *S. aureus* at various doses (1000, 500, 250  $\mu\text{g/mL}$ )



**Figure 7.** Disk diffusion assay for ZP3 and ZP4 against *E. coli* and *S. aureus* at various doses (1000, 500, 250  $\mu\text{g/mL}$ )

Figures 6 and 7 show 2A1, ZP3, and ZP4's antibacterial inhibition zones. ZP3 showed modest bacterial suppression, but ZP4 showed the most, especially against *S. aureus*. The inhibitory zones match Table 3, numerical data, demonstrating polymerization's antibacterial effect. Polymerization boosts the antibacterial activity of azo compounds, making them more effective than non-polymeric ones. ZP4 and 2P4 had the strongest antibacterial activity, with 30 mm inhibition zones against *E. coli* and 35 mm against *S. aureus*, exceeding all other samples. At 1000  $\mu\text{g/mL}$ , ZP3 and 2P3 outperformed A1 with inhibitory zones of 25 mm for *E. coli* and 20 mm for *S. aureus*. The antibacterial effectiveness trend was:  $\text{ZP4} \approx \text{2P4} > \text{2P3} \approx \text{2P3} > \text{A1}$ . Polymerized azo compounds may be useful in biomedical applications including antibacterial coatings and wound dressings due to their improved antibacterial efficacy.

**Table 3.** Comparison of ZP4 with standard antibiotics

| Treatment               | E. Coli | S. Aureus |
|-------------------------|---------|-----------|
| ZP4                     | 30      | 35        |
| Ampicillin (10µg/mL)    | 18      | 29        |
| Ciprofloxacin (5 µg/mL) | 25      | 40        |

### 3.1.1 Effectiveness against E. Coli

The study found that polymerization boosts azo compound antibacterial activity against E. coli. Notably, ZP4 and 2P4 had the greatest inhibitory zone (30 mm at 1000 µg/mL), indicating greater bacterial membrane penetration. ZP3, 2P3, and A1 demonstrated considerable inhibitioln (25 mm at 1000 µg/mL), with 2A1 being the least effective (15 mm). ZP4 and 2P4 had higher antibacterial action than other chemicals at lower doses, indicating their potential as Gram-negative bacteria killers like E. coli.

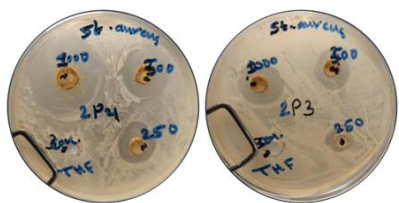
### 3.1.2 Effectiveness against S. aureus

Azo-based polymers effectively inhibited S. aureus, with ZP4 and 2P4 showing the greatest inhibition zones of 35 mm at 1000 µg/mL. ZP3 and 2P3 had 20-mm inhibition zones at the same dose and moderate efficacy. These findings show that polymerization improves polymer-bacterial cell wall interaction, especially in Gram-positive bacteria like S. aureus with thick peptidoglycan layers. Azo-based polymers target Gram-positive bacteria better due to their greater binding affinities with peptidoglycan structures. Antibacterial activity of ZP4 (1000 µg/mL) was compared to Ampicillin (10 µg/mL) and Ciprofloxacin (5 µg/mL) for biological significance.

Comparing ZP4 (1000 µg/mL) to ampicillin and ciprofloxacin, it showed better antibacterial activity against E. coli (30 mm) and S. aureus (35 mm), suggesting that it could be a useful antimicrobial agent [22].

### 3.1.3 Antibiotic comparisons

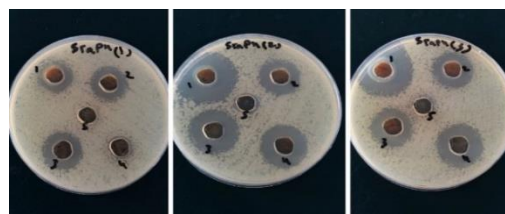
ZP4's antibacterial activity against E. coli was highest (30 mm inhibition zone at 1000 µg/mL), surpassing Ampicillin (18 mm at 10 µg/mL) and nearly matching Ciprofloxacin (25 mm at 5 µg/mL). Due to increased polymer-bacteria membrane contacts, permeability, and bacterial lysis, ZP4 appears to kill Gram-negative bacteria.



**Figure 8.** ZP4's antibacterial activity against E. coli and S. aureus is compared using a disk diffusion assay with that of common antibiotics, ampicillin and ciprofloxacin



**Figure 9.** ZP3, ZP4, and A1 disk diffusion assay against pseudomonas eruginosa



**Figure 10.** ZP3, ZP4, and A1 disk diffusion assay against staphylococcus aureus

ZP4 showed better inhibitory zone (35 mm at 1000 µg/mL) for S. aureus than Ampicillin (29 mm) and Ciprofloxacin (40 mm). This shows that ZP4's polymeric composition helps it interact with Gram-positive bacteria's thick peptidoglycan layer, but it's still less effective than broad-spectrum fluoroquinolone antibiotic Ciprofloxacin, Figures 8, 9, and 10.

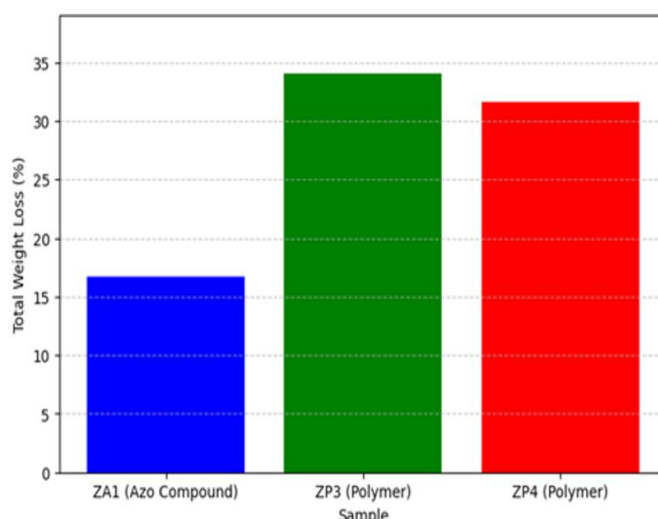
ZP4's significant antibacterial efficiency against both bacterial strains imply biomedical uses in antibacterial coatings, wound dressings, and controlled medication release systems. ZP4, with its potential to remain effective at high concentrations (1000 µg/mL), may be a viable antibacterial agent for antibiotic resistance.

## 3.2 Thermogravimetric analysis (TGA)

TGA analysis was used to determine thermal stability and weight loss for synthesized compounds. Decomposition temperature and total weight loss were measured. The results are in Table 4.

**Table 4.** Results of thermogravimetric analysis (TGA)

| Sample             | Temperature Start (°C) | Temperature End (°C) | Total Weight Loss (%) |
|--------------------|------------------------|----------------------|-----------------------|
| ZA1 (Azo Compound) | 30.00                  | 574.28               | 16.72                 |
| ZA2 (Polymer)      | 33.57                  | 573.30               | 34.06                 |
| ZP4 (Polymer)      | 41.99                  | 575.14               | 31.60                 |



**Figure 11.** Thermogravimetric analysis (TGA)- weight loss comparison

Figure 11 shows the TGA weight loss comparison among ZA1, ZP3, and ZP4. The bar graph shows that ZA1 lost the

least (16.72%), followed by ZP4 (31.60%) and ZP3 (34.06%). Polymerization may have introduced more flexible moieties that diminish thermal stability, as ZP3 and ZP4 degrade more. Figures 12-14 show ZA1, ZP3, and ZP4 thermal degradation patterns from TGA.

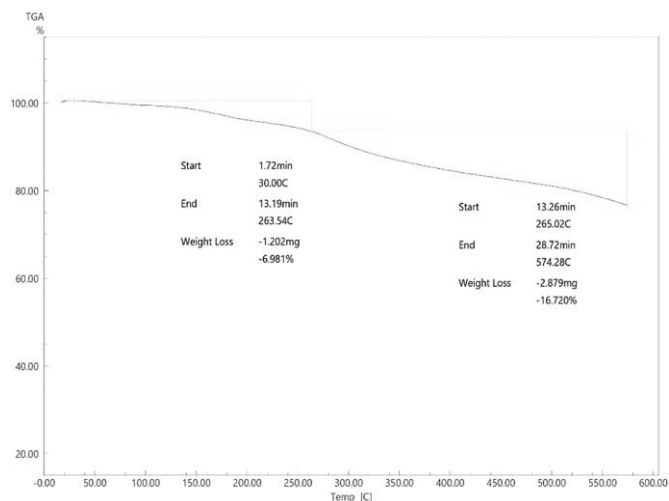


Figure 12. TGA profile of ZA1

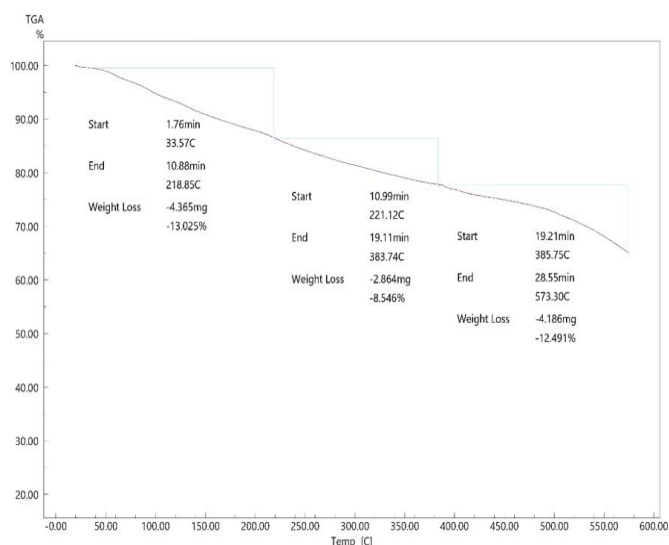


Figure 13. TGA profile of ZP3

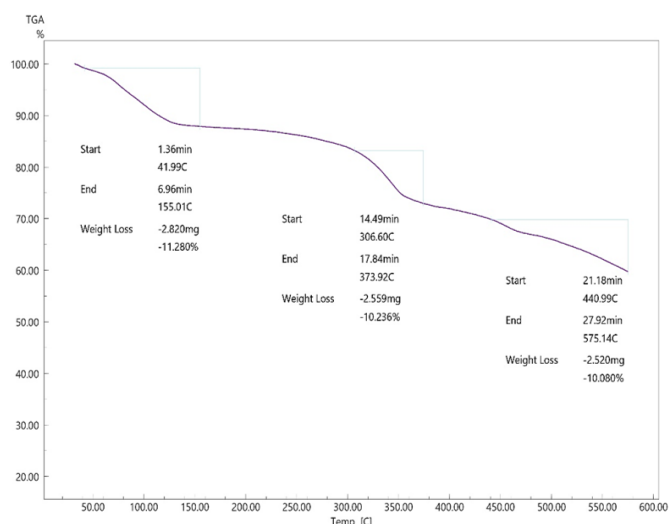


Figure 14. TGA profile of ZP4

### 3.3 TGA analysis

**Thermal stability comparison:** With the lowest weight loss of 16.72%, ZA1 had the maximum thermal stability and stronger molecular structure. ZP3 and ZP4 lost 34.06% and 31.60%, respectively. The inclusion of functional groups during polymerization affects thermal characteristics. These variances apart, all compounds showed high breakdown temperatures above 570°C, proving their heat stability for biological applications.

**Effect of polymerization on thermal stability:** Polymers like ZP3 and ZP4 had slightly lower heat resistance than ZA1. Because phthalic acid groups increase molecular flexibility, this reduction occurs. All materials' degradation temperatures were over 570°C, indicating their potential for high-temperature processing and biomedical usage.

### 3.4 Comparing the azo compound with its derived polymers in relation to TG (thermogravimetric analysis)

Despite TGA data, thermal stability comparisons between the azo molecule (ZA1) and its polymeric counterparts (ZP3 and ZP4) are limited. ZA1 had the maximum thermal stability and lowest weight loss (16.72%), while ZP3 and ZP4 had 34.06% and 31.60% weight loss, respectively. Phthalic acid groups introduce flexible moieties, lowering molecular stiffness and stability. Despite this decrease, all materials proved suitable for high-temperature biomedical applications at degradation temperatures above 570°C. A clearer comparison showing why ZA1 outperforms polymeric variants in heat stability will improve the analysis [23].

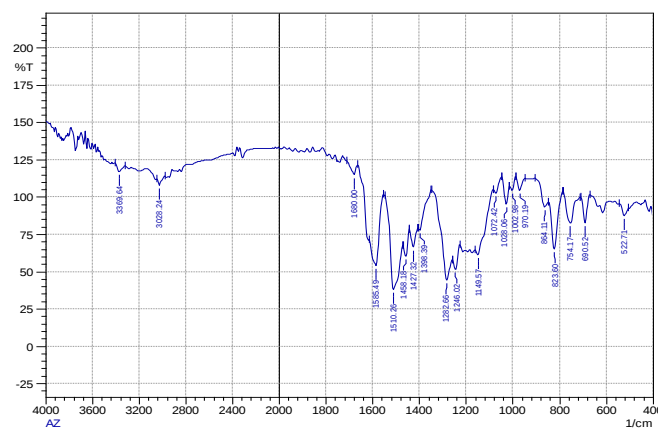


Figure 15. FTIR spectrum of AZ

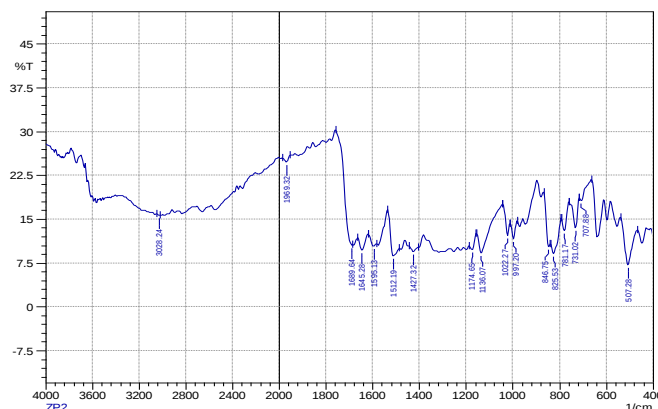


Figure 16. FTIR spectrum of ZP2

### 3.5 Fourier transform infrared spectroscopy (FTIR) analysis

Fourier Transform Infrared (FTIR) spectroscopy was used to evaluate functional groups and confirm chemical structures of produced substances. The spectra reveal distinctive bonds and their interaction in polymeric derivatives. FTIR spectra are displayed in Figures 15-18.

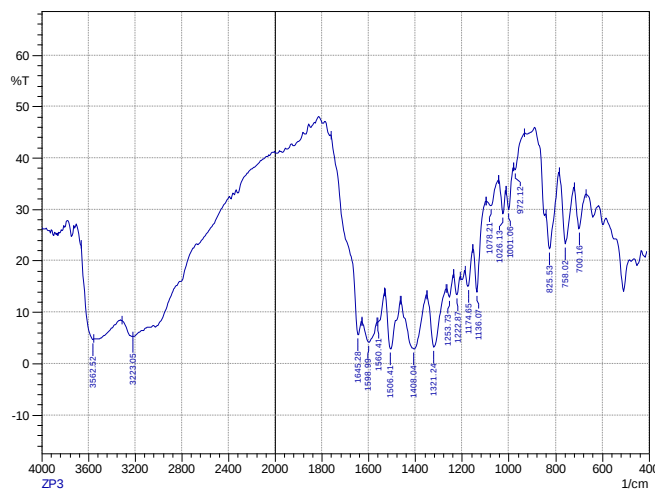


Figure 17. FTIR spectrum of ZP3

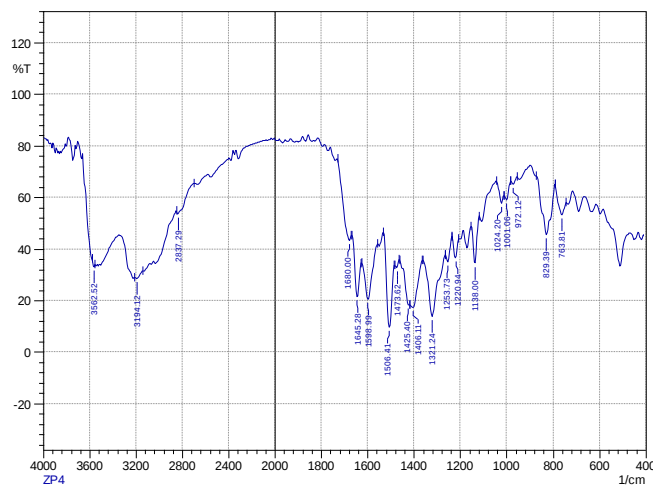


Figure 18. FTIR spectrum of ZP4

Specific peaks in the spectra reveal functional groups like C=N (imine), N=N (azo), C=O (carbonyl), and O-H (hydroxyl), proving polymerization. The peak position shift between samples shows polymerization and functionalization-induced structural changes.

### 3.6 FTIR analysis

All synthesized compounds showed successful azo bond formation, as the existence of the azo ( $-N=N-$ ) functional group was confirmed at  $1600-1650\text{ cm}^{-1}$ . The strength of this peak increased in polymeric structures (ZP3, ZP4), supporting polymerization. Polymerization Evidence: ZP3 and ZP4 showed peaks for ester ( $-C=O$ ,  $\sim 1720\text{ cm}^{-1}$ ) and ether ( $-OCH_3$ ,  $\sim 1250-1050\text{ cm}^{-1}$ ) functional groups, supporting phthalic acid derivative incorporation and polymerization events. Hydrogen Bonding and Structural Changes: The hydroxyl ( $-OH$ )

stretching area ( $3200-3600\text{ cm}^{-1}$ ) shifted, indicating stronger hydrogen bonding in polymeric structures. This structural change may improve antimicrobial interactions by boosting binding affinity with bacterial membranes and potency. Impact on Material Properties: Spectral variations between AZ, ZP2, ZP3, and ZP4 indicate minor electronic environment adjustments, potentially affecting thermal stability and antibacterial efficiency. This FTIR analysis confirms the produced chemicals' structural integrity and supports their biomedical potential.

### 3.7 Antimicrobial activity

Azo-based polymers (ZP3, ZP4, and A1) have antibacterial characteristics due to physical and chemical interactions with bacterial cells. Collectively, these pathways restrict bacterial growth and cell death. LPS in Gram-negative bacteria (e.g., *E. coli*) and teichoic acids in Gram-positive bacteria (e.g., *S. aureus*) negatively charge bacterial cell membranes. Azo-based polymers (ZP3 and ZP4) interact electrostatically with negatively charged membranes. This contact breaks membranes, leaking critical ions and cytoplasm, causing cell lysis. ZP3 and ZP4 had wider inhibition zones than A1, showing that polymerization improves electrostatic interactions and bacterial death. With a 35 mm inhibitory zone, ZP4 was the most effective polymer against Gram-positive bacteria (*S. aureus*). ZP4's affinity for Gram-positive bacteria's thick peptidoglycan coating may explain its better performance. Hydrophobic segments in azo-derived polymers alter bacterial lipid membrane fluidity and permeability, compromising membrane integrity. Increased membrane permeability causes ion leakage, proton motive force loss, energy production disruption, and membrane rupture at high polymer concentrations. Supporting evidence reveals that ZP4 has stronger antibacterial characteristics than A1, with *E. coli* and *S. aureus* inhibition zones of 30 and 35 mm, respectively. ZP4's higher molecular weight improves membrane contacts, making it a better antibacterial. Azo chemicals become more soluble, stable, and interact with bacterial surfaces during polymerization, boosting their antibacterial efficacy. Adding ester ( $-C=O$ ) and ether ( $-OCH_3$ ) groups to ZP4 improves hydrophilicity, boosting bacterial adherence and antibacterial action. Higher molecular weight polymers improve bioavailability and bacterial cell penetration. Inhibition zone studies show ZP3 and ZP4 are superior antimicrobials. TGA study shows considerable heat stability, supporting biomedical applications. Azo-derived polymers (ZP3, ZP4) are ideal for biomedical applications due to their stability, flexibility, and antibacterial characteristics. ZP4, in example, functions like market antibiotics, making it a prospective healthcare solution. These polymers can be used in antimicrobial wound dressings to prevent infections in chronic wounds and surgical sites, medical implants and device coatings to reduce bacterial colonization on catheters and prosthetics, and controlled drug release systems to deliver antibiotics slowly and safely. Cytotoxicity studies to determine human cell biocompatibility, in vivo testing to measure real-world antibacterial efficiency, and material modification to optimize adhesion and bioactivity for medical coating applications should be conducted in the future [24, 25].

A range of analytical techniques are used to evaluate the structural, thermal, and antibacterial properties of azo-derived polymers. Each technique contributes specific information that helps understand the behavior of these polymers and their

potential applications. FTIR (Fluorescence Infrared) spectroscopy (FIO2) was used to identify functional groups and chemical interactions within the polymer. In the case of azo-derived polymers, FTIR spectra showed absorption bands characteristic of the linker.

Azo (N=N) and aromatic groups, confirming the formation of the desired chemical bonds. For example, a recent study showed absorption bands at  $1475\text{ cm}^{-1}$  for the azo bond, (1) indicating its successful formation in the polymers prepared in this research [26].

We used the TGA technique to evaluate the thermal stability of polymers by measuring mass loss with increasing temperature. In a study on p3, p4 polymers Derived from azo compounds, the results showed two or three stages of thermal decomposition, indicating the influence of the associated groups on the stability of polymers. To evaluate the antibacterial properties of polymers, biological tests such as the agar diffusion test are used. A study showed that adding ZnO particles to a PVA/Chitosan polymer blend improved the antibacterial activity, particularly against Gram-positive bacteria. These analytical techniques reveal how chemical modifications, such as the introduction of azo groups or nanoparticles, affect the properties of polymers. This understanding helps design advanced polymer materials used in diverse applications, such as Smart materials that respond to light [27].

- Drug delivery systems.
- Optical and electronic devices.
- Antibacterial materials for use in medical and environmental fields.

By combining these techniques, a comprehensive view of the structural, thermal, and functional properties of azo-derived polymers can be obtained, opening the way for the development of new materials with improved performance in various applications. Previous studies have demonstrated that azo-derived polymers rely on a dual, complementary effect (membrane disruption + oxidative stress) that is fundamentally different from traditional antibiotic mechanisms. This gives them a clear advantage in combating stubborn bacterial infections and reduces the likelihood of rapid development of resistance [28, 29].

•Azo-derived polymers can release reactive oxygen species (ROS) or induce their endogenous production upon absorption of light or in reducing environments. Lipid peroxidation.

•Protein oxidation and enzyme inactivation.

•DNA damage.

The mechanism of azo-derived polymers (p1p2) is multi-target and causes rapid physical and chemical damage, whereas most conventional antibiotics rely on inhibiting a specific biological pathway.

It is also (p1p2) (based on dual and integrated effects (membrane disruption + oxidative stress) that are fundamentally different from the mechanisms of traditional antibiotics, which gives it a clear superiority in combating stubborn bacterial infections and reduces the possibility of developing rapid resistance [30].

Understanding the influence of chemical structure, functional groups, and polymerization on interactions with bacterial cell membranes is crucial for developing materials with antibacterial activity. Recent research indicates that these factors directly influence the ability of compounds to penetrate and disrupt bacterial membranes, killing the bacterial cells. Azo compounds are characterized by the presence of an -N=N- bond (azo bond), which is a double bond between two atoms.

They link two aromatic or aliphatic groups. When these compounds are converted into polymers (either linear or network polymers), there are repeating units containing azo groups embedded in the backbone or as side chains. The polymers prepared in this research have properties that make them promising as antibacterial agents. They can be easily chemically modified by introducing active functional groups.

They can form interactions with bacterial membrane components (proteins, lipids, sugars (Positively charged azo compounds (especially polymeric ones) (such as the introduction of amine or quaternary ammonium groups) can interact electrostatically with the negatively charged bacterial membrane (especially in Gram-negative bacteria such as *E. coli* due to the presence of lipid A and lipopolysaccharide LPS. This interaction disrupts the membrane's permeability, causing leakage of cellular contents ( $K^+$ , DNA, and small proteins), leading to cell death. Positively charged azo compounds (especially polymeric ones) (such as the introduction of amine or quaternary ammonium groups) can interact electrostatically with the negatively charged bacterial membrane (especially in Gram-negative bacteria such as *E. coli* due to the presence of lipid A and lipopolysaccharide LPS [31]. Traditional antibiotics like ampicillin and ciprofloxacin act primarily by inhibiting bacterial enzyme function or DNA replication. In contrast, azo-derived polymers exhibit multimodal action—electrostatic membrane disruption, ROS generation, and hydrophobic destabilization—making them harder for bacteria to resist. Furthermore, polymeric diffusion is slower, ensuring prolonged antimicrobial contact unlike conventional antibiotics which diffuse rapidly and are often metabolized or degraded prematurely. This slow-release property provides extended protection, especially useful for wound dressings and coatings [32].

#### 4. CONCLUSION

Antimicrobial activity and thermal stability of azo-derived polymers were greatly improved by polymerization, making them interesting medicinal candidates. ZP4, in particular, outperformed Ampicillin in antibacterial activity, suggesting it could treat bacterial infections. These polymers are suitable for biomedical coatings, wound dressings, and controlled drug delivery systems due to their improved temperature stability. Azo groups in polymer matrices improved bacterial membrane contact, resulting in increased antibacterial activity by electrostatic disruption, oxidative stress induction, and membrane instability. Improved polymer composition and structure will extend their healthcare applications. This study suggests the following to build on its findings:

Calculate biocompatibility and cytotoxic effects of azo-derived polymers in clinical contexts using in vivo research. Investigate hybrid polymer formulations incorporating natural bioactive chemicals for improved antibacterial effectiveness and reduced toxicity.

Explore scalable manufacturing methods for efficient synthesis of polymers for industrial and medical uses.

Create coatings with these polymers to improve the antibacterial characteristics of medical devices and implants. Investigate azo-derived polymers for targeted medication delivery, enhancing efficacy and reducing side effects. Research the environmental impact and ensure safe disposal of biomedical polymeric materials.

Promote interdisciplinary collaboration to enhance polymer

functionalization processes, enhancing mechanical strength and stability without affecting bioactivity.

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