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# Synthesis, Characterization and Biological Activity of Iron(II) Complex from Recycled Expired Metformin

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**Abstract:**

In this study, an iron(II) complex was successfully synthesized utilizing recycled, expired metformin hydrochloride as a ligand source. The synthesized complex was thoroughly characterized using a range of analytical techniques, including proton nuclear magnetic resonance ($^1$H-NMR) spectroscopy, Fourier-transform infrared (FTIR) spectroscopy, ultraviolet-visible (UV-Vis) spectroscopy, and molar conductivity measurements. Spectral data and conductivity results indicate that the complex adopts a square planar geometry, with metformin functioning as an anionic bidentate ligand. The biological activity of the complex was evaluated against two bacterial strains: *Staphylococcus aureus* and *Escherichia coli*. Its antimicrobial efficacy was quantitatively assessed and compared to that of metformin at varying concentrations.

**Keywords:** Recycled drugs, metformin, iron(II) complex, biological activity.

# التحضير و التشخيص و الفعالية الحيوية لمعقد الحديد (II) من الميتفورمين منتهي الصلاحية المعاد تدويره.

زهراء فاضل هاشم ، عادل علي عبد الحسن الفريجي ، رائد كاظم زيدان
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**مستخلص:**

في هذه الدراسة، تم تحضير معقد الحديد(II) بنجاح باستخدام الميتفورمين هيدروكلوريد المنتهي الصلاحية كليجند. تم تشخيص المعقد المُحضر بشكل شامل باستخدام مجموعة من التقنيات التحليلية، بما في ذلك مطيافية الرنين المغناطيسي النووي للبروتون ($^1$H-NMR)ومطيافية الأشعة تحت الحمراء (FTIR)و مطيافية الأشعة فوق البنفسجية-المرئية (UV-Vis) وقياسات التوصيلية المولارية. تشير البيانات الطيفية ونتائج التوصيلية إلى أن المعقد يتخذ شكلًا مربعًا مستويًا، حيث يعمل الميتفورمين كليجند ثنائي السن سالب الشحنة. تم تقييم النشاط الحيوي للمعقد ضد سلالتين من البكتيريا: المكورات العنقودية الذهبية (*Staphylococcus aureus*) ، والإشريكية القولونية (*Escherichia coli*) كما تم تقييم الفعالية المضادة للميكروبات للمعقد بشكل كمي ومقارنتها بفعالية الميتفورمين بتركيزات مختلفة.

**الكلمات المفتاحية** : الأدوية المعاد تدويرها، الميتفورمين، معقد الحديد(II)، النشاط الحيوي .

## Introduction

Metformin is a widely prescribed antihyperglycemic agent for the management of type 2 diabetes mellitus. Its primary mechanisms involve decreasing hepatic gluconeogenesis, reducing intestinal glucose absorption, and enhancing peripheral glucose uptake and utilization by increasing insulin sensitivity.(World Health Organization, 2003; Kathuria et al., 2018)

Chemically, metformin belongs to the biguanide class, with the systematic name 1,1-dimethylbiguanide. Its molecular formula is $C_4H_{11}N_5$.(Mahmoud et al., 2024) The chemical structure is depicted in Figure 1.

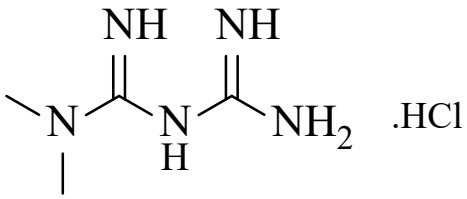



**Figure 1 : Structure of Metformin hydrochloride**

Metformin ($C_4H_{11}N_5$) exhibits a notably high nitrogen-to-carbon ratio, which contributes to its distinctive chemical characteristics.(Rebitski et al., 2018) It appears as a white crystalline solid, highly soluble in polar solvents such as water, due to its polarity arising from amino and guanidine groups. Conversely, metformin is slightly soluble in ethanol and practically insoluble in non-polar solvents like acetone, ether, and chloroform. (Janaki et al., 2016; Al-Zubaid et al., 2005).

The biguanide structure of metformin enables it to act as an effective N-donor bidentate ligand, coordinating readily with various transition metals to form complexes that are typically cationic and exhibit diverse colors, contingent on the metal ion and its oxidation state.(Rusanvo et al., 2022; Olar et al., 2007) The synthesis of these metal–metformin complexes is of significant interest, as the biguanide moiety can form hydrogen bonds, facilitating interactions with biomolecules. This interaction potentially enhances the biological activity of the complexes, increasing their viability as metallodrugs.(Bentefrit et al., 1997)

In this context, we utilized expired pharmaceutical tablets of metformin and ferrous sulfate—specifically, Diaphage and Ferrous Sulphate + Folic

Acid tablets as sources for the ligand and metal ion, respectively. This approach led to the synthesis of bis(1,1-dimethylbiguanide)iron(II) sulfate with a high yield of 85%.

## 2. EXPERMINTAL

### 2.1 CHEMICALS

Metformin hydrochloride was extracted from the expired drug "Diaphage 500 mg" by UNITED PHARMACEUTICALS. Ferrous sulphate was extracted from the expired drug Ferrous Sulphate + Folic Acid tablet 200 by "CYGNUS", All chemicals were purchased and used without further purification, were obtained from BDH and Fluka Companies. Other chemicals and solvents were used as received, without purification.

### 2.2 Instrumentation

• Infrared (IR) Spectroscopy: IR spectra were recorded using a Bruker ALPHA II FT-IR Spectrophotometer.

• Molar Conductivity: Measurements were conducted in dimethyl sulfoxide (DMSO) at room temperature with a concentration of $10^{-3}$ M, utilizing a Peak Instrument equipped with a standard platinum electrode (cell constant: 1.002 $cm^{-1}$) at the University of Basrah, Iraq.

• Ultraviolet-Visible (UV-Vis) Spectroscopy: Spectra were obtained on a SHIMADZU spectrophotometer (200–900 nm range) in DMSO at the University of Basrah, Iraq.

• Proton Nuclear Magnetic Resonance ($^1$H-NMR) Spectroscopy: Spectra were recorded on a Bruker Ascend 400 MHz spectrometer using DMSO-$d_6$ as the solvent at the University of Basrah, Iraq.

• Biological Activity Assessment: The antimicrobial activity of the synthesized complex was evaluated against two bacterial strains at the University of Basrah, Iraq.

### 2.3 Extraction of Active Pharmaceutical Ingredients

#### 2.3.1 Extraction of Metformin Hydrochloride

Thirty "Diaphage" tablets (each containing 500 mg of metformin hydrochloride) were dissolved in 500 mL of cold water. The solution was filtered to remove insoluble excipients, and the filtrate was evaporated under reduced pressure using a rotary evaporator. This process yielded white, needle-like crystals with a 75% recovery rate. The melting point was determined to be

226°C, consistent with the reported value for pure metformin hydrochloride.(Chandira et al., 2010)

**2.3.2 Extraction of Ferrous Sulfate**

"Ferrous Sulphate + Folic Acid 200 mg" tablets were dissolved in 500 mL of water. The solution was filtered and washed multiple times with ethanol to remove dye components. An off-white solid was obtained with a 65% yield. The compound exhibited a decomposition temperature above 300°C, aligning with literature values for ferrous sulfate.(Kobe & Dickey, 1945)

**2.4 Synthesis of Bis(1,1-dimethylbiguanide)iron(II) Sulfate**

Recycled ferrous sulfate (2 mmol, 304 mg) was dissolved in 15 mL of distilled water. Subsequently, a solution containing metformin hydrochloride (4 mmol, 660 mg) and potassium hydroxide (4 mmol, 224 mg) was added incrementally. The reaction mixture was stirred continuously for six hours at room temperature. Upon completion, the mixture was filtered, yielding a dark brown solid with an 85% yield. The product decomposed at temperatures exceeding 300°C.

Characterization Data:

• Molar Conductance ($\Lambda_m$): 12 $\Omega^{-1}$ $cm^2$ $mol^{-1}$ in DMSO.

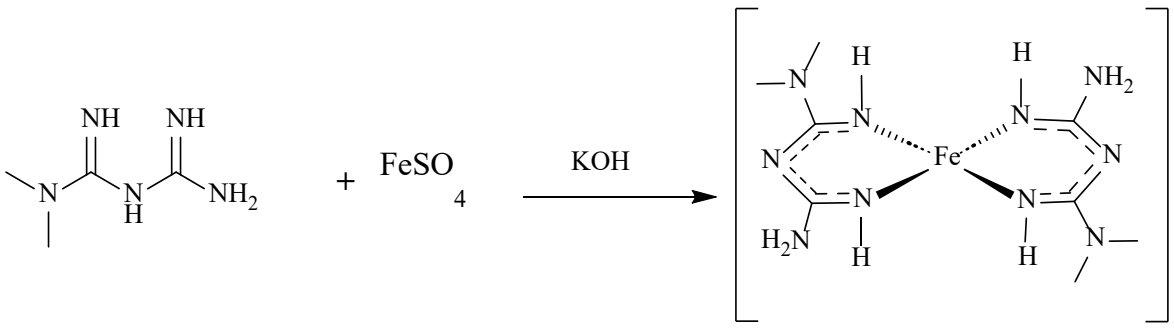
• FT-IR ($cm^{-1}$): 3368 (m), 3296 (m), 3149 (s), 1625 (s), 1209 (w), 1052 (s), 633 (s), 578 (s).

• $^1$H- NMR (400 MHz, DMSO-$d_6$, δ/ppm): 2.91 (s, 12H, 4 × $CH_3$), 6.57 (s, 4H, NH), 7.16 (s, 3H, $NH_2$).

UV-Vis ($\lambda_{max}$, nm): 262, 275 (in • .(DMSO

**Results and discussion**

This study explores the utilization of expired pharmaceutical tablets as starting materials for synthesizing an iron(II) complex, specifically focusing on the coordination properties of recycled metformin. The complex, bis(1,1-dimethylbiguanide)iron(II) sulfate, was synthesized by reacting metformin hydrochloride with ferrous sulfate in distilled water at room temperature, maintaining a 2:1 molar ratio. This straightforward aqueous synthesis yielded a dark brown solid with a high yield of 85%. The successful formation of this complex underscores the potential of metformin as a bidentate ligand, effectively coordinating with iron(II) ions under mild conditions.




**Scheme 1 : Synthesis of bis(1,1-dimethylbiguanid)iron(II) sulfate**

The molar conductivity of the synthesized complex was measured in dimethyl sulfoxide (DMSO) at room temperature, with a concentration of $10^{-3}$ M. Molar conductivity measurements are essential for understanding the electrolytic behavior of coordination compounds in solution. The observed value of 12 $\Omega^{-1}\cdot cm^2\cdot mol^{-1}$ suggests that the complex behaves as a non-electrolyte, indicative of its non-ionic nature in DMSO solution.(Thawarkar et al., 2015; Feltham & Hayter, 2015)

Infrared (IR) spectral analysis further elucidated the coordination behavior of metformin in the complex. The free metformin ligand exhibited characteristic absorption bands: N–H stretching vibrations at 3373 $cm^{-1}$ and symmetric N–H stretches between 3159–3298 $cm^{-1}$. Additionally, the C=N stretching vibration appeared at 1583–1626 $cm^{-1}$. Upon complexation with iron(II), notable shifts were observed in these bands. The C=N stretching band shifted to 1612–1629 $cm^{-1}$, indicating coordination through the imine nitrogen atoms. Similarly, the N–H stretching bands shifted to the 3149–3296 $cm^{-1}$ range, suggesting involvement of the amine groups in bonding. These spectral changes confirm the formation of a stable coordination environment around the Fe(II) center.(Das et al., 2021)

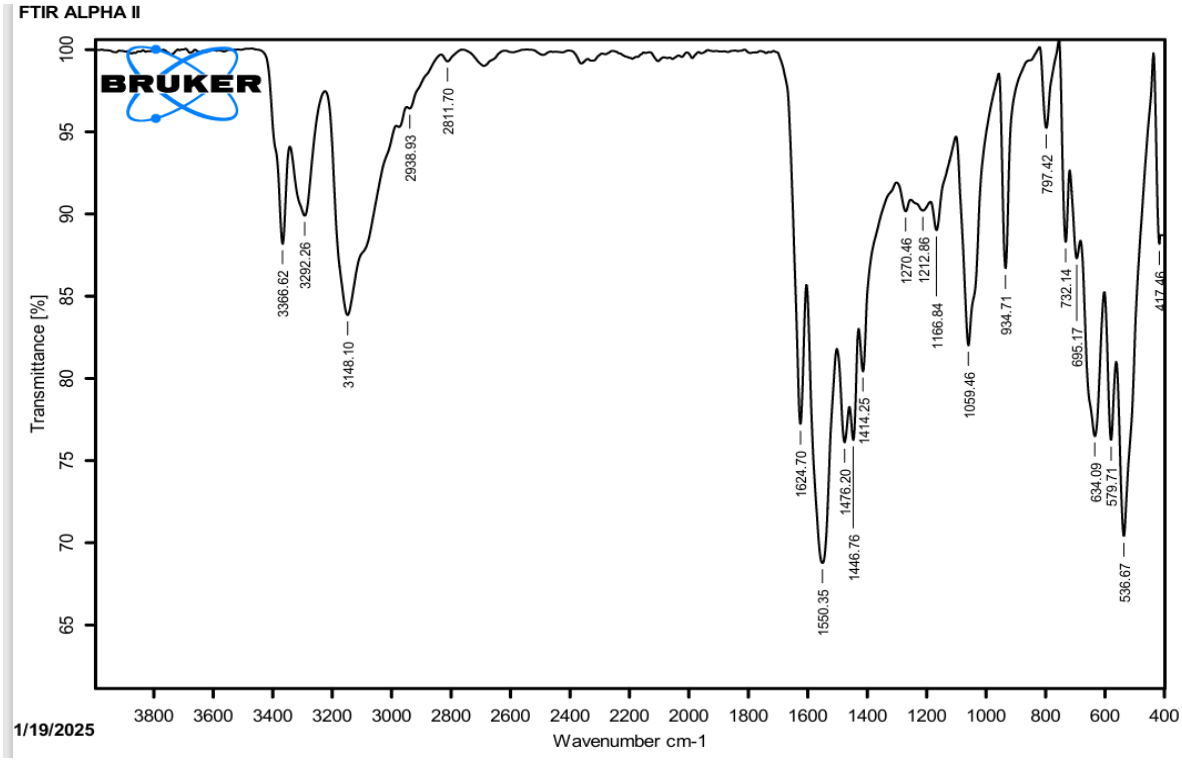



**Figure 2: FT-IR spectrum of Metformin**

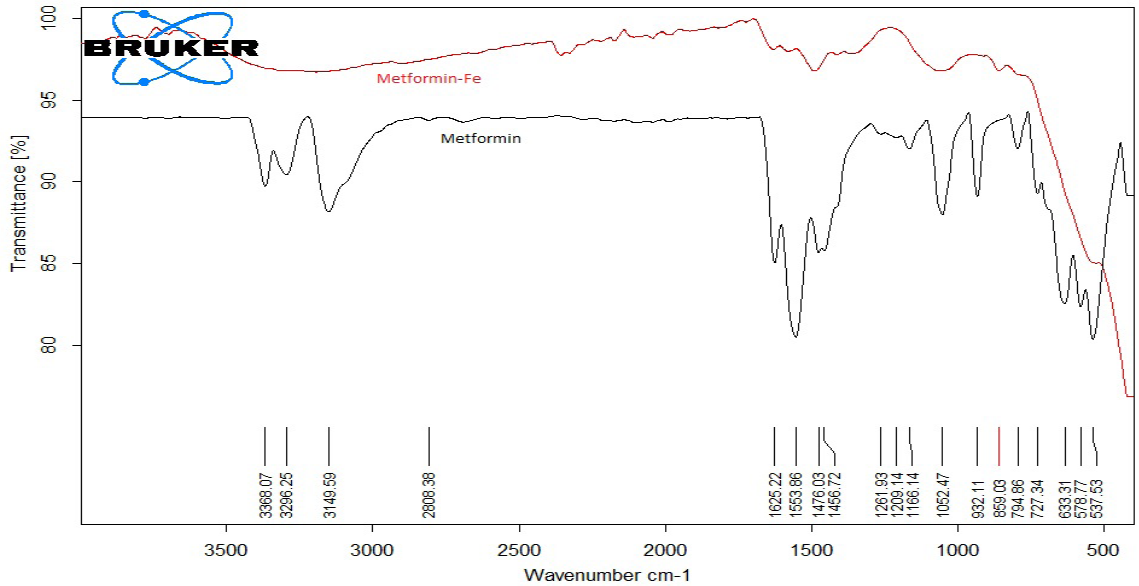



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**Figure 3: FT-IR spectrum of the complex**

The $^{1}$H-NMR spectrum of free metformin exhibits characteristic signals corresponding to its molecular structure. A broad singlet at approximately 7.24 ppm is attributed to the imine protons (–C=NH–) within the guanidine group, influenced by hydrogen bonding and exchange effects. The amine protons (–$NH_2$ and –NH–) appear as a broad signal around 6.84 ppm, reflecting their involvement in hydrogen bonding. The methyl groups (–$N(CH_3)_2$) present a singlet at 2.93 ppm, indicative of their chemical equivalence.(Rena et al., 2017)

Upon coordination with iron(II), notable shifts in these signals are observed. The imine proton signal shifts slightly downfield to 7.16 ppm, suggesting coordination through the guanidine nitrogen atoms to the iron center. The amine protons' signal shifts to 6.57 ppm, and the methyl groups' signal moves marginally to 2.91 ppm. These chemical shift changes indicate alterations in the electronic environment due to complexation, confirming the interaction between metformin and the iron(II) ion.(Gadape & Parikh, 2011) .

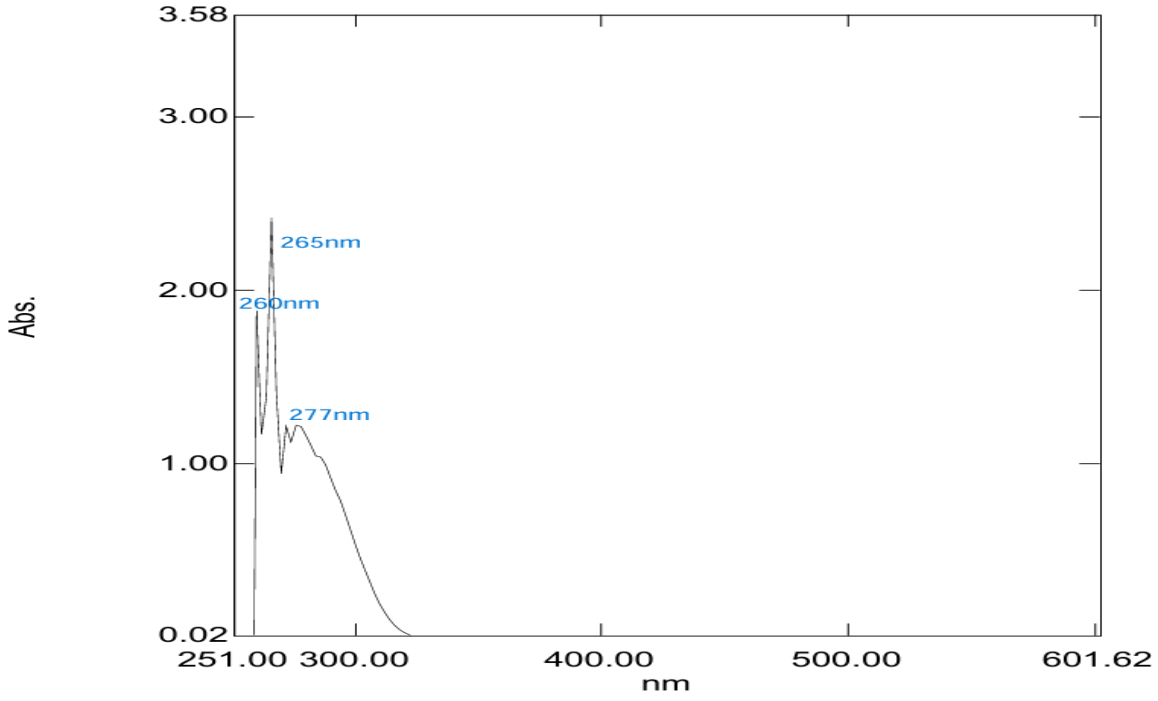


**Figure 4 : 1H-NMR spectrum of Metformin**

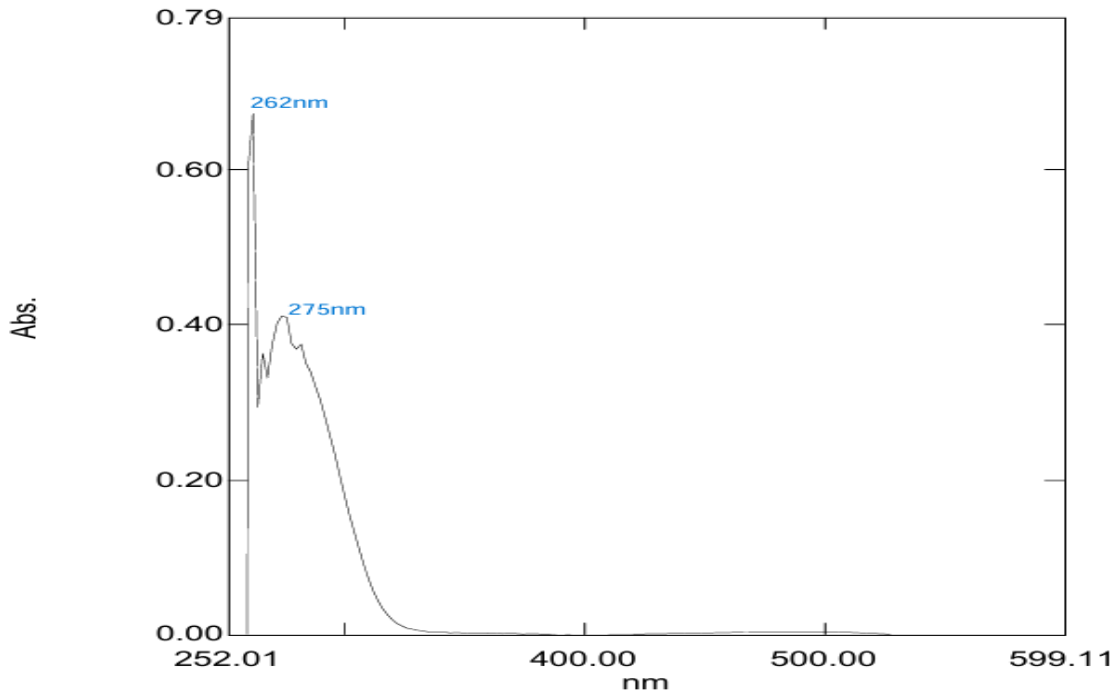


**Figure 5 : $^{1}$H-NMR spectrum of the complex**

The UV-Vis absorption spectra of metformin and its iron(II) complex were recorded in dimethyl sulfoxide (DMSO) at a concentration of $1\times10^{-4}$ M. Free metformin exhibited prominent absorption bands at 260 nm and 265 nm, corresponding to $\pi \rightarrow \pi^*$ transitions primarily associated with the C=N bonds in the biguanide moiety. An additional absorption band was observed around 277 nm, attributed to $n \rightarrow \pi^*$ transitions involving non-bonding electrons, typically from nitrogen atoms.

Upon complexation with iron(II), the UV-Vis spectrum displayed absorption bands at 262 nm and 275 nm. These slight shifts (approximately 2–3 nm) in the absorption maxima are consistent with the coordination of metformin to the iron center, indicating changes in the electronic environment due to complex formation. The square planar geometry of the Fe(II) complex facilitates ligand-to-metal charge transfer (LMCT) transitions, which are characteristic of such coordination compounds. These observations cor-

roborate the successful coordination of metformin with iron(II), leading to the formation of a stable complex. (Mahadeva & Revanaisddappa, 2024; Gispert, 2008; Karami et all., 2017).

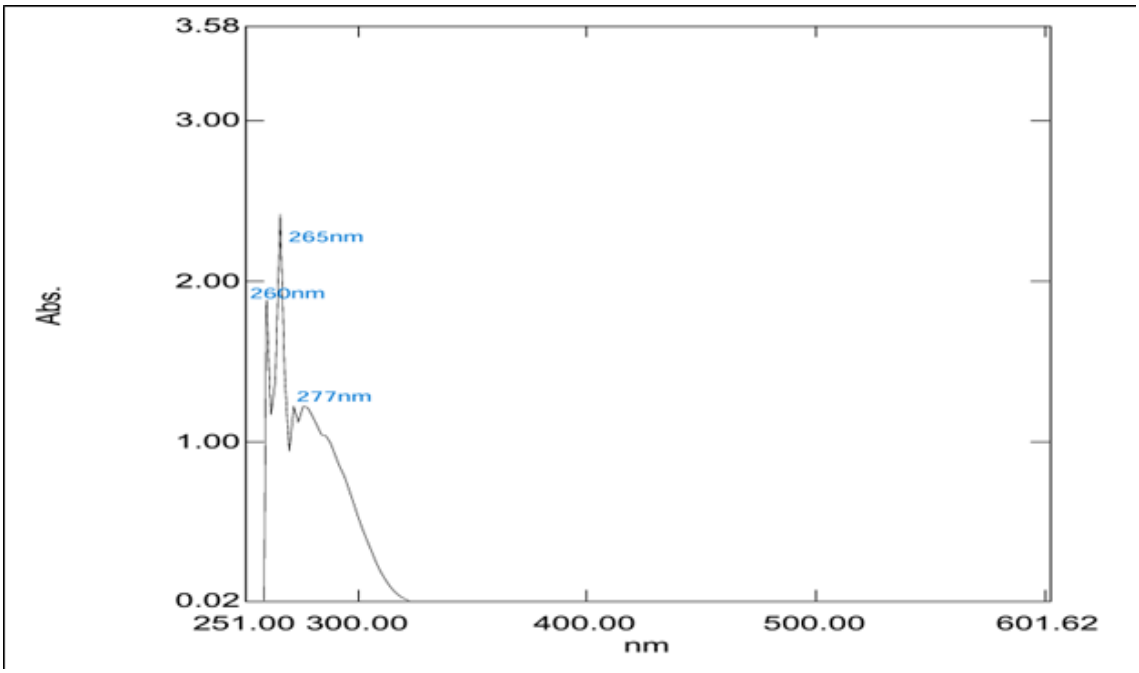


**Figure 6: Uv-vis spectrum of Metformin**

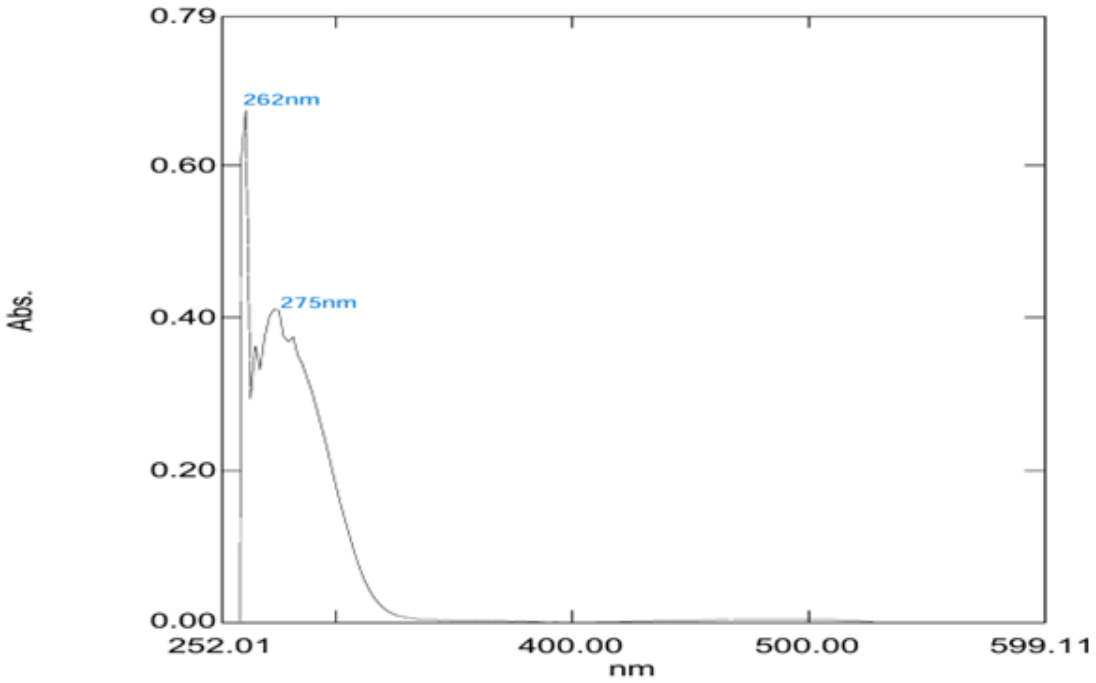


**Figure 7: Uv-vis spectrum of the complex**

The antibacterial efficacy of the synthesized iron(II) complex was assessed against *Staphylococcus aureus* and *Escherichia coli* using the disk diffusion method. The complex was tested at concentrations of 0.1 mg/mL and 0.05 mg/mL, and the resulting inhibition zones were measured in millimeters, as detailed in Table 1.

**Table 1: Inhibition Zones of Iron(II) Complex Against Bacterial Strains**

| compounds | Diameter of Inhibition Zone (mm) | | | |
|---|---|---|---|---|
| | Gram Positive Bacteria (*Staphylococcus aureus*) | | Gram Negative Bacteria (*Escherichia coli*) | |
| | **0.05** | **0.1** | **0.05** | **0.1** |
| Complex **1** | 40 | 40 | 40 | 40 |
| Metformin | 0 | 0 | 0 | 20 |

These results indicate that the iron(II) complex exhibits antibacterial activity against both Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria, with similar degrees of efficacy at different concentrations. The observed inhibition zones suggest a concentration-dependent antibacterial effect, highlighting the potential of the complex as an antimicrobial agent.

These findings align with existing literature, which highlights the enhanced antimicrobial properties of metal complexes compared to their uncoordinated counterparts. For instance, studies have shown that certain metal complexes exhibit superior antibacterial activity against various strains, including S. aureus and E. coli, due to the metal center's role in disrupting microbial cell structures.

Figures **8** and **9** illustrate the antibacterial effects of metformin and its iron(II) complex, providing visual representation of the inhibition zones against the tested bacterial strains.

In summary, the iron(II) complex exhibits potent antibacterial properties, with effectiveness maintained at lower concentrations, underscoring its potential as an antimicrobial agent.

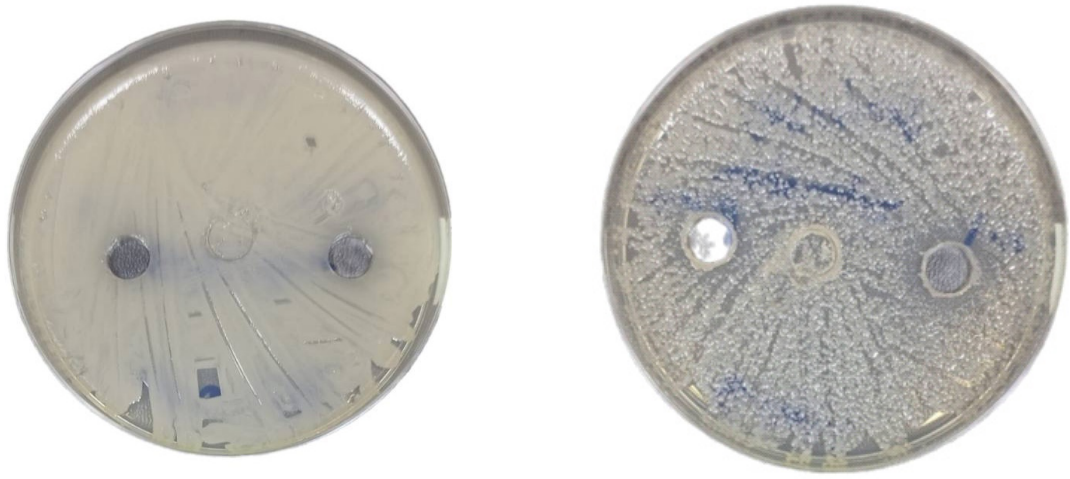

Figure **8**: antibacterial activity of metformin against both *S. aureus and E. coli*

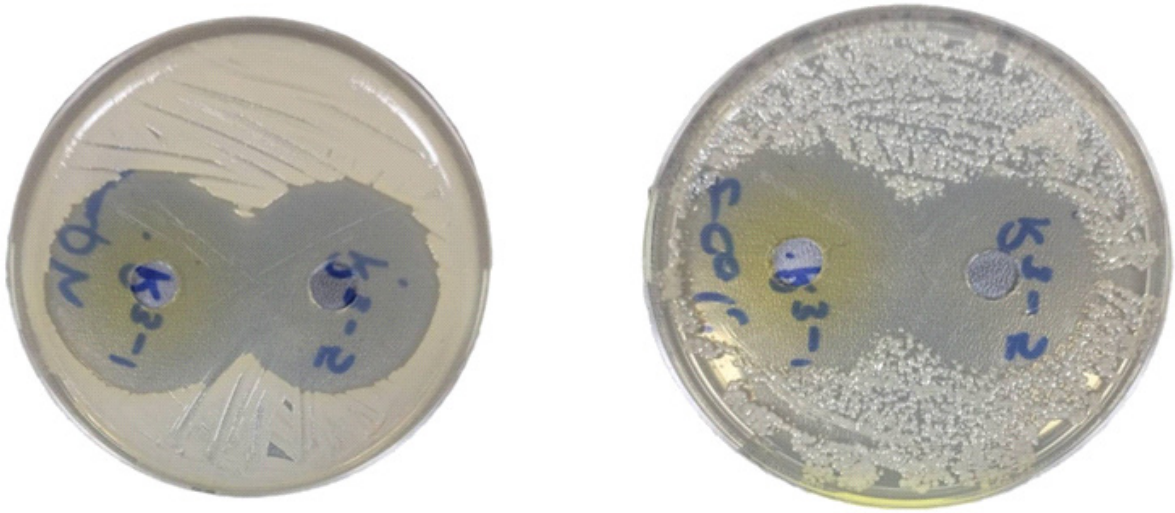

**Figure 9: antibacterial activity of complex 1 against both S. aureus and E. coli**

**Conclusion:** A novel iron(II) complex was synthesized using recycled expired metformin as the ligand. The complex was characterized through UV-Vis, FT-IR, and $^{1}$H-NMR spectroscopy, confirming its chemical structure. Molar conductivity measurements indicated that the complex behaves as a non-electrolyte in solution.

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