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Antibacterial Studies of Metal Complexes of Benzodioxane Derivative Against Gram Positive and Gram Negative Bacteria

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Abstract: 1,4-Benzodioxane is a versatile molecule widely used to design molecules capable of exhibiting diverse bioactivities. The present research work focuses on the synthesis of metal complexes involving 1,4-benzodioxane-2-carboxylic acid ligand and its subsequent evaluation for antibacterial properties. The metal complexes of cobalt, nickel and copper were synthesized and characterized by IR and mass spectral analysis. The synthesized molecules were evaluated for their antibacterial activity against Salmonella typhi, Bacillus cereus and Escherichia coli. Metal complexes exhibited promising antibacterial activity.

Keywords: Benzodioxane, metal complex, characterization, antibacterial

Introduction

1,4-Benzodioxane (BD) is a versatile starting material used to synthesize numerous molecules that can exhibit diverse bioactivities. 1,4-benzodioxane-2-carboxylic acid (Figure 1) is one of the prominent derivatives of benzodioxane. 1,4-benzodioxane-2-carboxylic acid with high enantiopurity is a very important entity in medicinal chemistry since they are the chiral building block in the design and synthesis of chiral therapeutic agents (Campbell et al., 1987; Bolognesi et al., 1999; Sun et al., 2024; Chiodini et al., 2015). Since the last few decades, its application in medicinal chemistry has led to the discovery of newer drugs (Bavo et al., 2020; Khalilullah et al., 2012; Sun et al., 2019). It is used as a chiral starting material to synthesize doxazosin mesylate, a drug used to treat benign prostatic hyperplasia (Bolchi et al., 2020; Rouf et al., 2012). Novel compounds that exhibit the potential to exhibit anti-inflammatory properties have been synthesized as antagonists of leukotriene B4 (LTB4) (Hicks et al., 2007). Among these compounds, structures featuring the dioxygenated nucleus of 1,4-benzodioxine present a promising group of LTB4 antagonists (Bouissane et al., 2023). Benzoxazole/benzothiazole-2,3-dihydrobenzo[b][1,4]dioxine derivatives

exhibited potential antidepressant activities. They also possessed binding affinities at the 5-HT1A and 5-HT2A receptors (Wang et al., 2014).

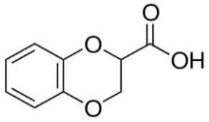


Figure 1: 1,4-benzodioxane-2-carboxylic acid

Enantioselectivity has a substantial effect on the pharmacokinetics of chiral medicines, particularly in the metabolic profile, to determine the enantiomeric toxicity (Kumari Rayala & Kandula 2022; Coelho et al., 2021). Hence it is very important to resolve the R and S- isomers of 1,4-benzodioxane-2-carboxylic acid (Figure 2).

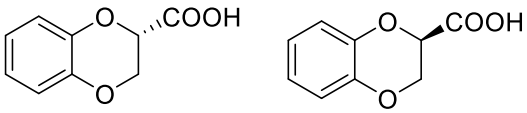


Figure 2: R- and S- isomers of 1,4-benzodioxane-2-carboxylicacid

Several works are reported to resolve (R)- and (S)- separately, kinetically resolved Ethyl 1,4-benzodioxan-2-carboxylate to get S-enantiomer of ethyl 1,4-benzodioxan 2-carboxylate in a simple lipase catalysed trans-esterification reaction (Bolchi et al., 2005). Authors synthesised (S)-doxazosin utilizing (S)-1,4-Benzodioxan-2-carboxypiperazine as a key chiral intermediate. They synthesized the target molecule using two approaches: (i) enzymatic resolution of ethyl 1,4-benzodioxan-2-carboxylate with an esterase (Serratia) followed by amide formation; (ii) direct resolution of 1,4-benzodioxan-2-carboxypiperazine with d-tartaric acid (Fang et al., 2001). Authors prepared several enantiopure 2-substituted 1,4-benzodioxanes from readily accessible (±)-1,4-benzodioxane-2-carboxilic acid advantageously using the methyl ester of 1,4-benzodioxane-2-carboxylic acid and the mesylate of 2-hydroxymethyl-1,4-benzodioxane as the synthetic intermediates (Bolchi et al., 2007).

The diastereomeric N-1-phenylethylamides of (S)- and (R)-1,4-benzodioxane-2-carboxylic acid displayed notable distinctions in fusibility and solubility compared to their diastereomeric 1-phenylethylammonium salts. They were effectively separated by precipitating the less soluble diastereomer (>98% de), while the more soluble one was purified via chromatography (>99% de). Authors synthesised enantiomerically pure 1,4-benzodioxane-2-carboxylic acid. They converted racemic nitriles into (R)-1,4-benzodioxane-2-carboxylic acid (Figure 3) by selective enzymatic hydrolysis by nitrilase (Scheme 1) (Fumagalli et al., 2016).

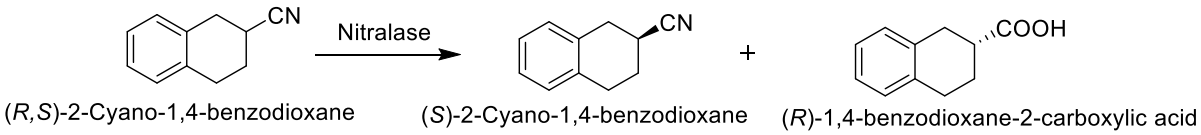
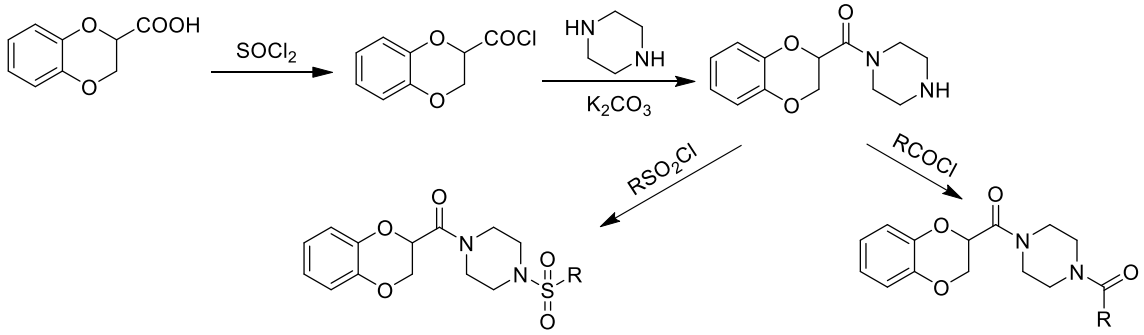


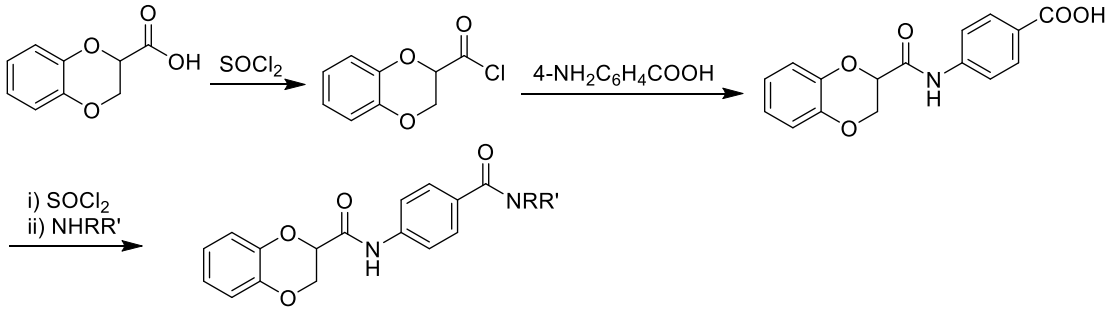
Figure 3: Selective enzymatic hydrolysis

Authors synthesised 1-(1,4-benzodioxane-2-carbonyl) piperazine by nucleophilic substitution reaction with various sulfonyl and acid chlorides using a series of novel 1-(1,4-benzodioxane-2-carbonyl) piperazine derivatives (Scheme 1). Compound 4-(2-trifluoromethyl)-benzenesulfonyl-1-(1,4-benzodioxane-2-arbonyl)piperazine showed significant antimicrobial activity against pathogenic bacterial and fungal strains (Mallesha & Mohana, 2011).



Scheme 1: Synthesis of 1-(1,4-benzodioxane-2-carbonyl) piperazine derivatives

Thionyl chloride was employed to treat 1,4-benzodioxane-2-carboxylic acid and isochroman-1-carboxylic acid, after which the resulting acid chlorides underwent reaction with p-aminobenzoic acid in dioxane with pyridine as a catalyst, yielding the respective amido acids. These compounds were subsequently transformed into acid chlorides, which were then reacted with a variety of amines to synthesize several novel diamides (Scheme 2) (Vartanyan et al., 2012).



Scheme 2: Synthesis of 1,4-Benzodioxane-2- amides

Silver nanoparticle decorated with benzodioxane derivative was effective against anti-biofilm agent against MRSA (Karthik et al., 2021). Benzodioxane silver metal complex was synthesized and urease inhibitory activity were studied (Li et al., 2015). There is a pressing need to devise novel approaches for drug

development that can effectively combat various microorganisms, by simultaneously targeting multiple cellular processes. Recent investigations suggest that certain metal ions induce diverse forms of harm to microbial cells through mechanisms involving membrane degradation, protein malfunction, and oxidative stress. These distinct modes of action, coupled with the diverse three-dimensional configurations that metal complexes can adopt, render them promising candidates for the synthesis of novel antimicrobial therapies (Claudel et al., 2020; Zafar et al., 2023; Frei et al., 2020). In the present work we have synthesised metal complex by using the COOH group as the ligand.

Carboxylic acid can coordinate to the metal via monodenatate, bidentate or bridging mode (Figure 4) (Shahroosvand et al., 2013; Kaluderovic et al., 2010). The structures and vibrational frequencies of the acetate ion interacting with the metal ion (Na^+, Mg^{2+}, and Ca^{2+}) in the unidentate, bidentate, bridging, and pseudo-bridging forms were studied by ab initio molecular orbital calculations. Effects of water molecule coordinating to acetate ion or the metal ion were observed. The frequency of the COO^- antisymmetric stretch in the unidentate species surpasses that of the ionic species, which in turn exceeds that of the bidentate species. Conversely, for the COO^- symmetric stretch, the trend is reversed. These findings suggest a potential correlation for the analysis of Ca^{2+} binding proteins using IR spectroscopy (Nara et al., 1996).

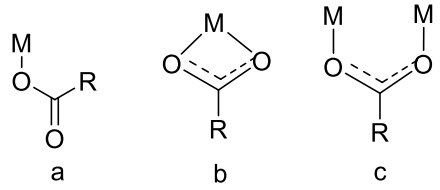


Figure 4: The possible mode of coordination of carboxylic acid. (a) Monodentate mode, (b) bidentate mode, (c) bridging mode

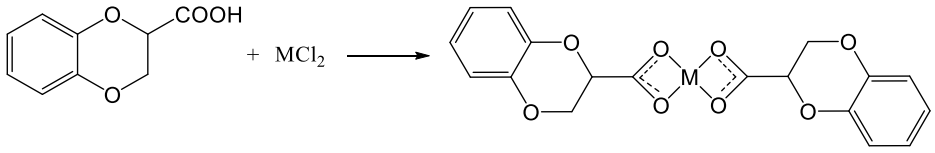
In the present work we have synthesized the metal complexes of 1,4 benzodioxane 2-carboxylic acid. The carboxylic acid group of BD was found to be coordinated to the metal, which was confirmed by mass and IR spectral techniques. Also, the synthesized molecules were evaluated for their antibacterial activity against Salmonella typhi, Bacillus cereus and Escherichia coli.

Materials and methods

All the solvents and reagents used for the synthesis were of analyticalgrade. Benzodioxane-2-carboxylic acid and metal chlorides were procured from Sigma Chemical. Mass spectral analyses were carried out in the ESI mode using HRMS mass spectrometer (Waters Q-Tof Ultima).

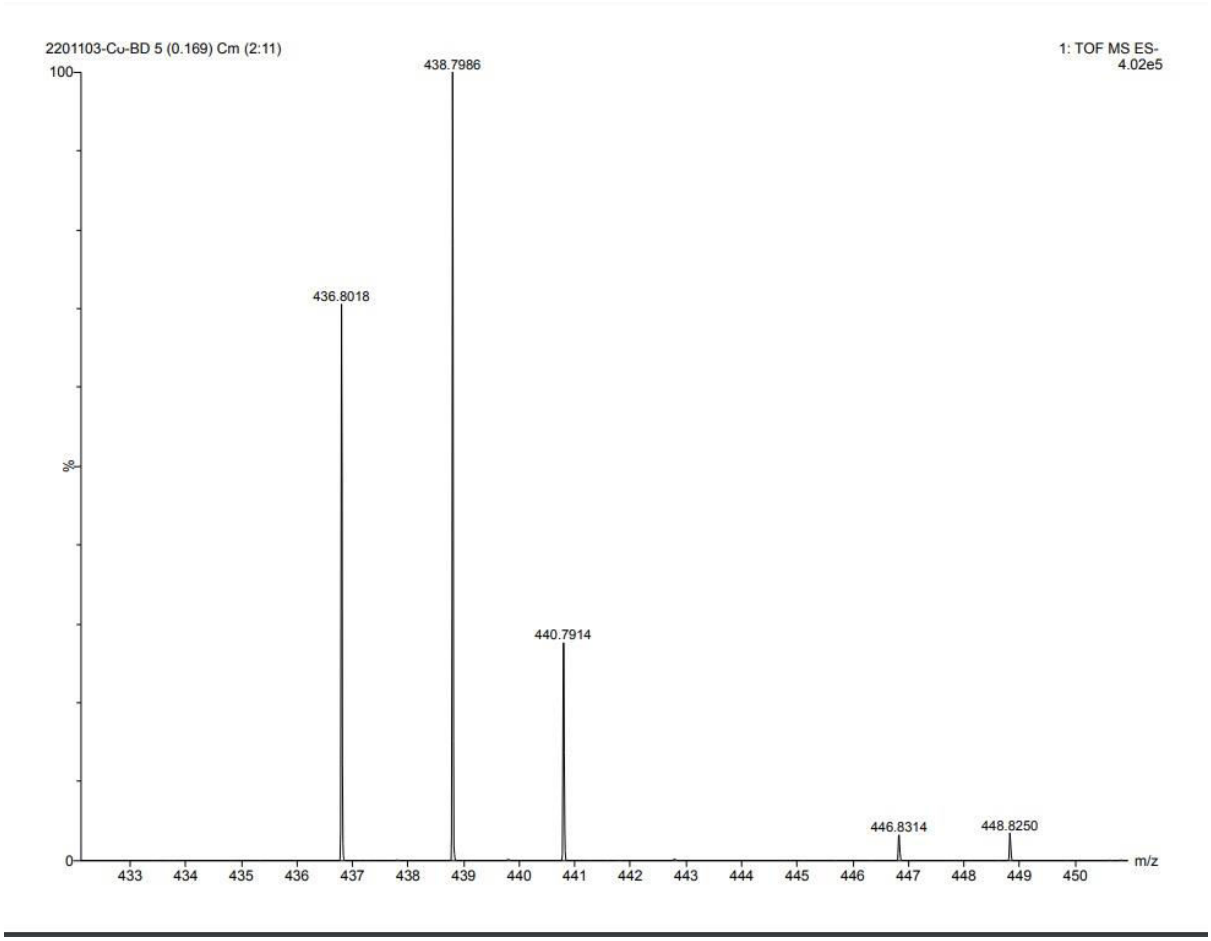
General procedure for the synthesis of metal complexes

To a solution of BD (0.5 mmol) in methanol, metal chloride (0.5 mmol) in methanol was added slowly, then 0.5ml of 1N NaOH solution was added. The reaction was refluxed for 2 hrs. The metal complex formed was filtered and dried. The product was confirmed by IR and mass spectral analysis (Figures 5-10).



(M=metal, Cu, Fe, Co, Ni)

Scheme 3: General scheme for the synthesis of metal complex



Calculated m/z= 440.0090, Observed m/z = 440.7914
Figure 5: Mass spectra of Cu-BD

JSS COLLEGE , B.N.ROAD , MYSURU -25

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Sample Scans:64
Background Scans:64
Resolution:4
System Status:Good
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User:JSSRL
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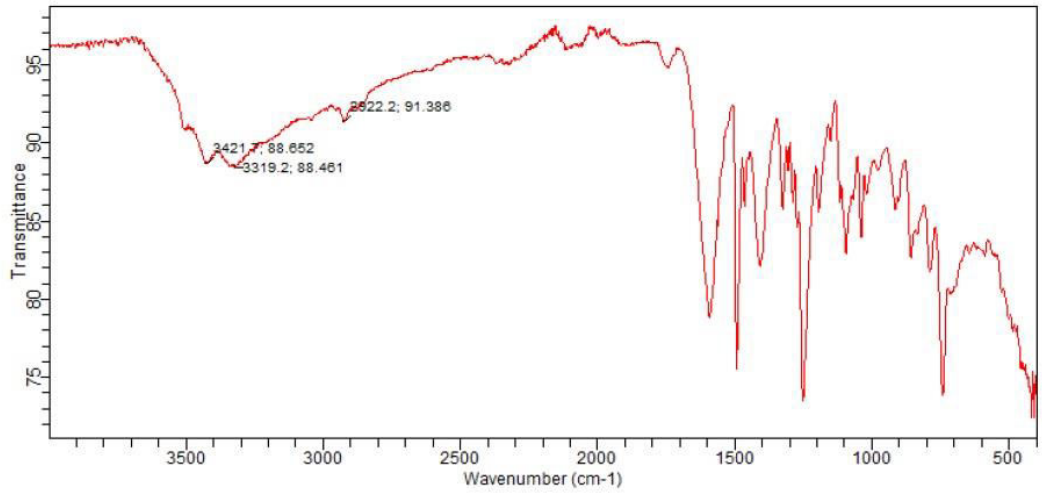
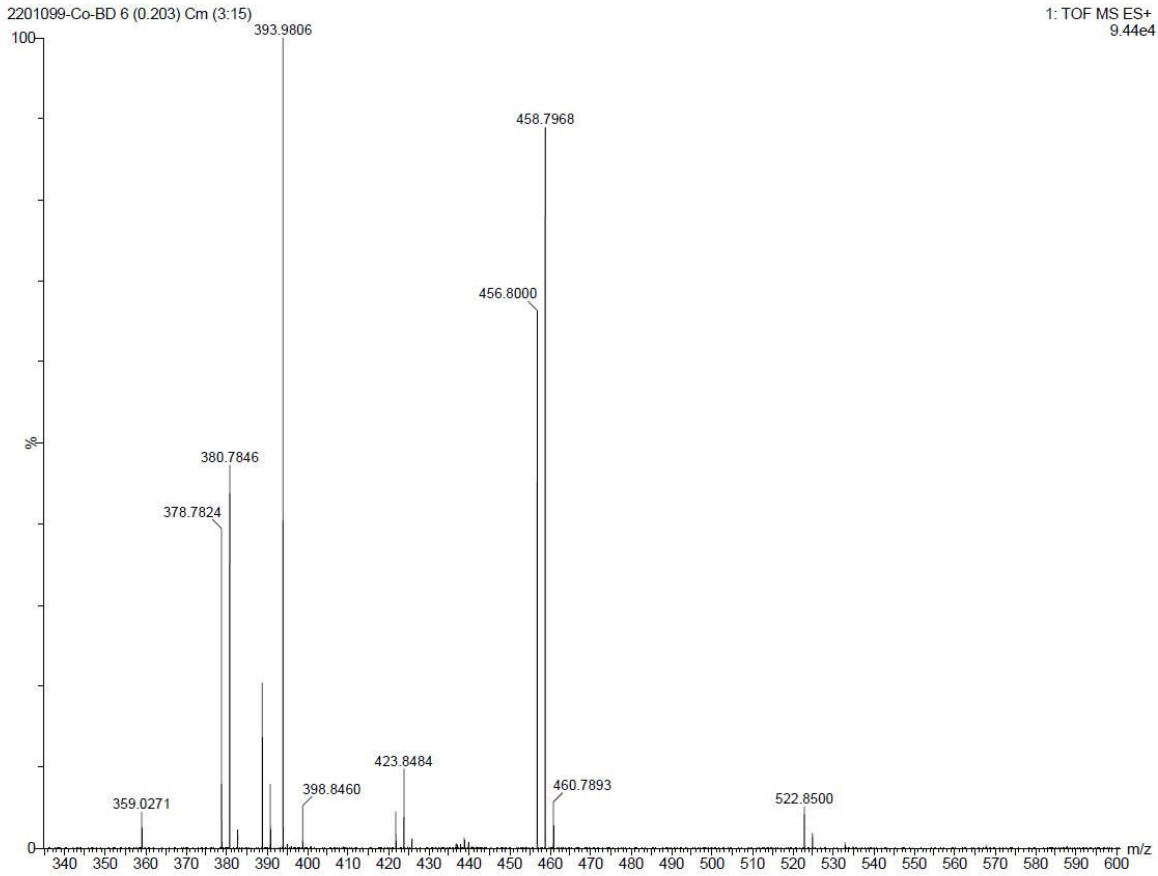


Figure 6: IR spectra of Cu-BD



Calculated m/z = 456.0232, Observed m/z = 456.8000

Figure 7: Mass spectra of Co-BD

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Resolution:4
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User:JSSRL
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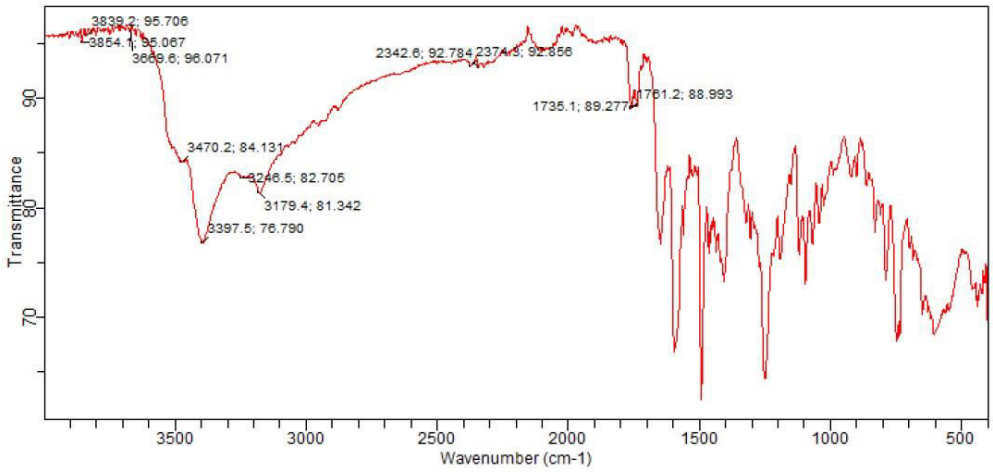
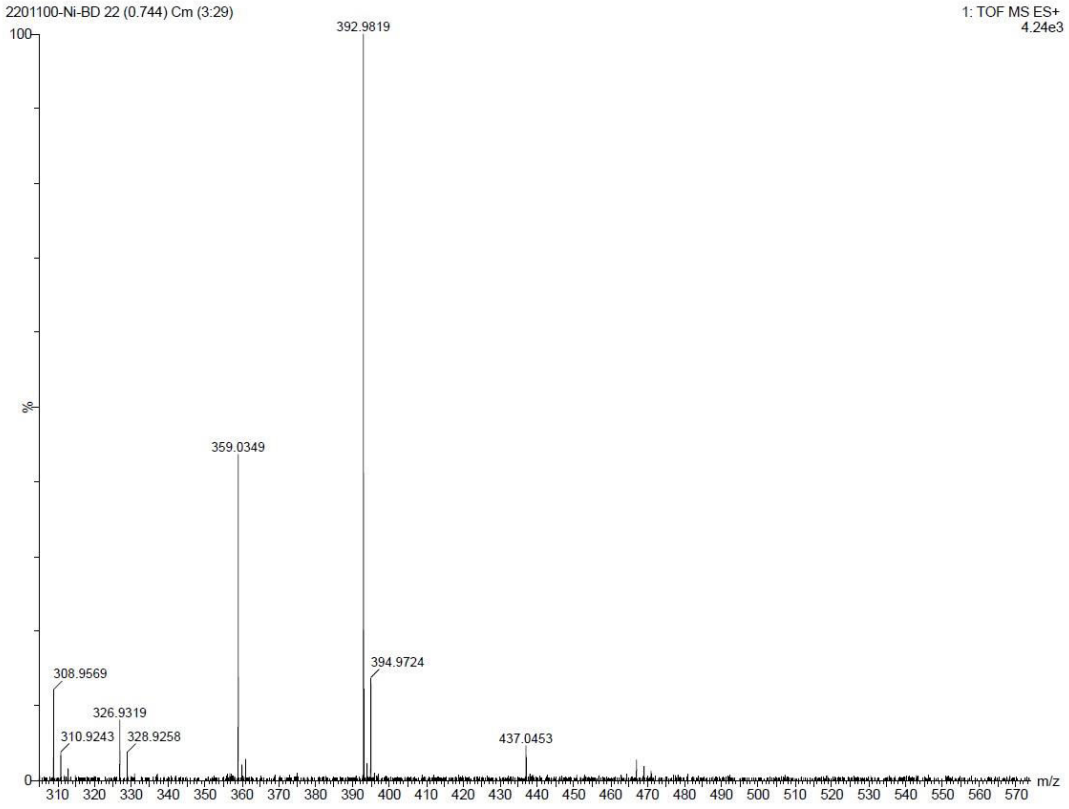


Figure 8: IR spectra of Co-BD



Calculated m/z = 435.0148, Observed m/z = 436.0453

Figure 9: Mass spectra of Ni-BD

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Background Scans:64
Resolution:4
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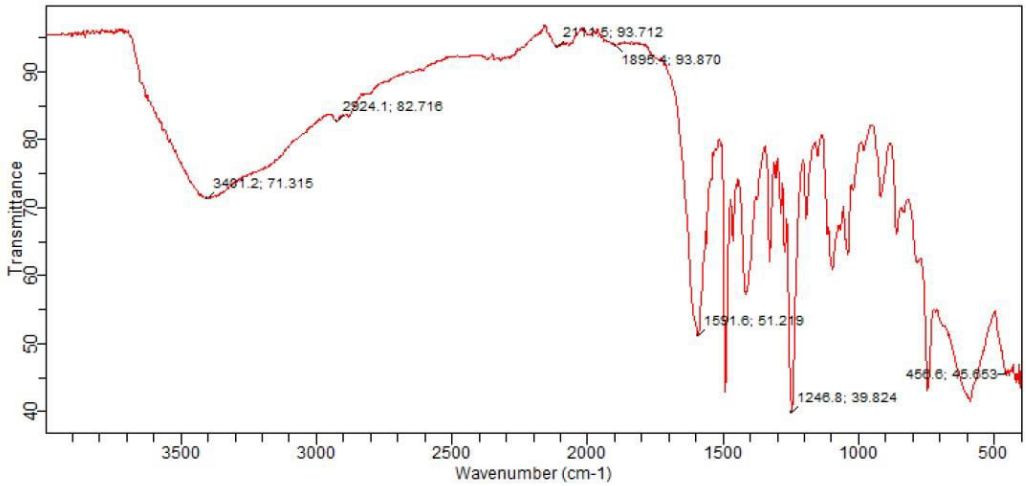

Figure 10: IR spectra of Ni-BD

Antibacterial activity

Determination of antibacterial activity by disk-diffusion method

The pure cultures obtained were revived and maintained in nutrient agar at 37 °C. The microorganisms were cultured in nutrient broth at 37 °Cover-night. The test organisms were subculture using nutrient agar medium. The tubes containing sterilized medium were inoculated with the respective bacterial strain. After incubation at 37 °C ±1 °C for 18 hours, they were stored in a refrigerator. The nutrient agar medium was sterilized by autoclaving at 121 °C for 15 min. Into each sterilized Petri plate, was poured about 25 ml of nutrient agar medium. The plates were left at room temperature aseptically to allow the solidification. After solidification, the appropriate bacterial cultures were inoculated over the surface of the agar using glass beads. The discs of each compound were placed individually on nutrient agar medium with fresh bacteria respectively. Each test compound was dissolved in dimethyl sulfoxide. Each test compound was added separately in the plates were kept undisturbed for at least 2 hours in a refrigerator to allow diffusion of the solution properly into nutrient agar incubated at 37±1 ^{0}C for 24 hours. After incubation, the diameter of zone of inhibition surrounding each of the dishes was measured with the help of an antibiotic zone reader.

Result and Discussion

In the present work we have synthesised the metal complexes of 1,4-benzodioxane-2-carboxylic acid. The antibacterial activity of metal complexes was determined against Salmonella typhi, Bacillus cereus and Escherichia coli.

Three metal carboxylates of BD were synthesised were synthesised by the reaction of metal chlorides with BD. The formed complexes were stable at room temperature and commonly soluble in DMF and DMSO. FT-IR spectra exhibited the changes in the carbonyl region. The carboxylic group in 1,4-benzodioxane-2-carboxylic acid creates electronegative cavities to host divalent cations. We synthesised the metal complex with and without sodium hydroxide. When sodium hydroxide solution was used, a better yield of the metal complex was observed. This may be due to the formation of the carboxylate ion, which binds to the metal more efficiently. In the proposed model, the M^{2+} coordinates in a cavity created by carboxylate group (COO^-) may be involved in the coordination with the metal cation (Figure 11). Based on mass spectral data and from literature, we have proposed the structure of the molecule (Martínez et al., 2010; Sutton et al., 2015).

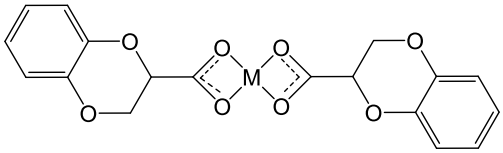

Figure 11: Proposed structure of the metal complex

The antibacterial activity of compounds was tested against the three different bacteria, Salmonella typhi, Bacillus cereus and Escherichia coli. The diameter (mm) of the inhibition zone as tabulated in Table 1. Compared to the ligand the metal complexes exhibited better antibacterial activity (Figure 12). Co-BD shows higher antibacterial activity when compared to BD, at 200 μg of samples. Copper complex exhibited similar MIC when compared to the parent compound. Ni-BD showed lesser antibacterial activity. Among the metal complexes Fe-BD showed higher zone of inhibition in S. typhi and less in E coli. This may be due to the fact that iron capably carries the bioactive ligands and increase their concentrations inside the target microbial cells. This property may help to increase the antibacterial property of the metal complex (Sharma et al., 2022).

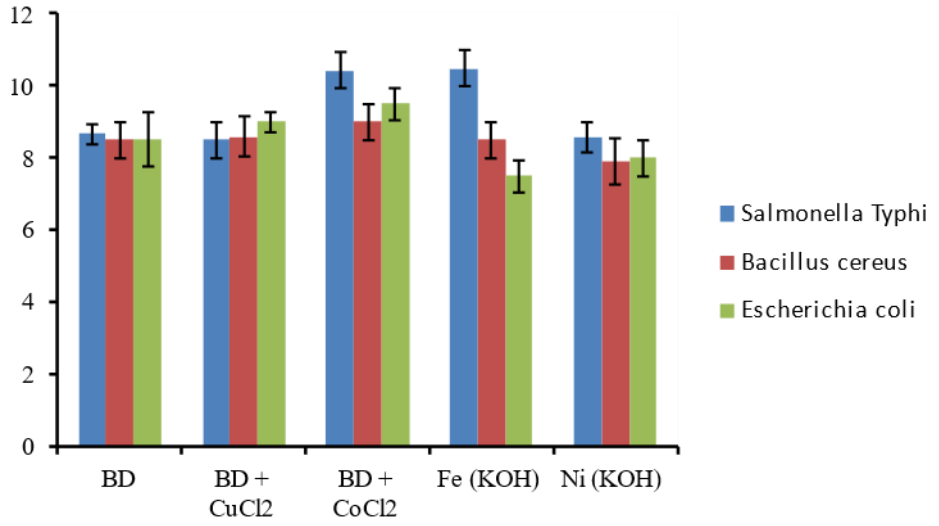

Figure 12: Antibacterial activity of compounds

Conclusion

In the present research work, we have synthesized the metal complexes of 1,4-benzenedioxane-2-carboxylic acid. The metal complexes of iron, cobalt, nickel and copper were synthesised and characterised by IR and mass spectral analysis. The BD metal complex exhibited promising antibacterial activity. Its potential as an antibacterial agent could find applications in the development of new antibacterial compounds. Further research is required to elucidate the precise mechanisms underlying its antibacterial effects.

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Table 1: Antibacterial activity of test compounds

Compound	Salmonella Typhi	Bacillus cereus	Escherichia coli
BD	8.67 ± 0.29	8.5 ± 0.5	8.5 ± 0.76
Cu-BD	8.50 ± 0.50	8.6 ± 0.53	9 ± 0.29
Co-BD	10.43 ± 0.51	9 ± 0.5	9.5 ± 0.45
Fe-BD	10.47 ± 0.50	8.5 ± 0.5	7.5 ± 0.45
Ni-BD	8.57 ± 0.40	7.90 ± 0.66	8 ± 0.50