Influence of Cd on Anti-Bacterial Activities of $MgFe_2O_4$ synthesized by Citrate-gel method

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Abstract- **This study investigates the antibacterial activity of magnesium-cadmium (Mg-Cd) ferrite nanoparticles (NPs) with the chemical formula $Mg_{1-x}CdxFe_2O_4$ where x=0.00, 0.2, 0.4, 0.6, 0.8 and 1.0 synthesized by Citrate gel auto combustion method, and tested against Gram-positive (Staphylococcus aureus, Bacillus subtilis) and Gram-negative (Escherichia coli, Klebsiella pneumoniae) bacteria. Utilizing the disc diffusion method, inhibition zones were measured and compared with the standard antibiotic Ampicillin. Results indicate varying antibacterial efficiency among the different compositions. Sample III-5 (x = 0.8) exhibited the highest activity against *Escherichia coli* (12 mm), outperforming Ampicillin (10 mm). Sample III-4 (x = 0.6) showed the highest inhibition (20 mm) against *Klebsiella pneumoniae*. All samples were ineffective against all strains at x = 1.0. The antimicrobial activity is attributed to the nanoscale size and enhanced surface area of the ferrite particles, improving bacterial interaction. The findings highlight the potential of specific Mg-Cd ferrite compositions as effective antibacterial agents and suggest a promising alternative to conventional antibiotics, especially against resistant strains.**

Key words: ***$Mg_{1-x}CdxFe_2O_4$, anti-bacterial activity, Escherichia coli, Klebsiella pneumonia, Bacillus subtilis, Staphylococcus aureus.***

I. INTRODUCTION

Ferrites, a class of ceramic materials composed primarily of iron oxide (Fe_2O_3) combined with one or more metallic elements, have drawn significant attention in recent years due to their versatile applications across multiple fields, especially in electronics, magnetism, and biomedical sciences[1]. In the realm of biological applications, ferrites offer numerous advantages owing to their unique magnetic, electrical, and chemical properties. Among the various types of ferrites, spinel ferrites with the general formula MFe_2O_4 (where M = divalent metal ion such as Mg^{2+}, Zn^{2+}, Cd^{2+}, etc.) are of particular interest due to their tunable physical properties and nanostructured morphology[2]. The integration of magnesium (Mg) and cadmium (Cd) ions into the spinel ferrite structure, forming Mg-Cd nanoferrites, further enhances their utility in biological domains, particularly in exhibiting antibacterial activity[3].

The importance of ferrites in biological activity arises from their nano-dimensional scale, which significantly increases surface area, reactivity, and interaction with biological cells. The ability to engineer their composition, morphology, and surface characteristics has enabled researchers to tailor ferrites for specific biological interactions such as antimicrobial activity, targeted drug delivery, magnetic resonance imaging (MRI) contrast enhancement, hyperthermia treatment for cancer, and biosensing[4]. Among these, the antibacterial activity of ferrites is highly notable as it provides a promising alternative to conventional antibiotics in combating microbial infections, especially in the context of growing antibiotic resistance.

Antibacterial activity of ferrite nanoparticles is influenced by several factors, including particle size, surface area, surface charge, and chemical composition. These properties govern how nanoparticles interact with bacterial membranes, penetrate cell walls, and disrupt cellular functions. Ferrites, particularly in nano form, exhibit antibacterial effects by generating reactive oxygen species (ROS), releasing metal ions that interfere with bacterial metabolism, and disrupting membrane integrity[5]. Additionally, due to their magnetic nature, ferrites can be targeted to infection sites

using external magnetic fields, thereby enhancing localized antibacterial action with minimal systemic effects.

In this context, Mg-Cd nanoferrites emerge as a novel and effective antibacterial agent. The substitution of magnesium and cadmium into the ferrite lattice modifies both the structural and functional characteristics of the material. Magnesium, a biocompatible and physiologically relevant metal, contributes to structural stability and enhances biological compatibility. On the other hand, cadmium, although toxic at high concentrations, when incorporated into the ferrite matrix in controlled amounts, imparts strong antimicrobial properties due to its ability to disrupt microbial enzyme function and protein synthesis[6]. The significance of Mg-Cd nano ferrites as antibacterial agents extends beyond direct bacterial inhibition. Their incorporation into wound dressings, coatings for medical implants, and drug delivery systems presents enormous potential for practical biomedical applications[7,8]. These nanoparticles can reduce the risk of post-surgical infections, improve the longevity of implants, and support healing in chronic wounds by preventing microbial colonization. Furthermore, their magnetic properties open avenues for targeted delivery and remote activation, making them ideal candidates for smart antimicrobial systems.

In recent experimental studies, including the evaluation presented in the provided document, Mg1-xCdxFe2O4 nanoparticles with varying Cd concentrations (x = 0.00 to 1.00) were tested for their antibacterial efficacy against both Gram-positive and Gram-negative bacterial strains. The strains selected Escherichia coli, Klebsiella pneumoniae, Bacillus subtilis, and Staphylococcus aureus represent common pathogens associated with human infections.

II. EXPERIMENTAL STUDIES

The antibacterial activity of the prepared Mg-Cd nano ferrite NPs $Mg_{1-x}CdxFe_2O_4$ where x=0.00, 0.2, 0.4, 0.6, 0.8 and 1.0 (Coaded as III-1, III-2, III-3, III-4, III-5, to III-6) was evaluated in conjunction with the conventional medication Ampicillin[9]. Gram-positive strains of Staphylococcus aureus and Bacillus subtilis, as well as Gram-negative strains of Escherichia coli and Klebsiella pneumonia, were the microbes used in this investigation. By identifying the presence of inhibition zones, the disc diffusion agar method was utilized to assess the antibacterial properties of the produced Mg-Cd ferrites' nanoparticles. 0.1 ml from 108 CFU/ml of the four disease causing bacteria viz., Gram-negative strains of Escherichia coli, Klebsiella pneumonia and Gram-positive strains of Staphylococcus aureus and Bacillus subtilis suspensions were dispersed on agar plates individually that are nourished with LB (Luria Bertani) media. Over the agar plates, filter paper discs (of 5 mm in diameter) were positioned and then nano particles of various compositions of the prepared Mg-Cd ferrites (10 µl.) were incorporated onto the discs in different amounts of concentrations[10,11]. To measure the antimicrobial activity, 5 µl (10 µg/10 µl. concentration) of Ampicillin has served as the standard. Then, the agar plates were incubated for twenty-four h. at 37^{o}C. the observed inhibition zones were measured around each disc in (mm).

III. RESULTS AND DISCUSSIONS

A. Anti-Bacterial activity

The synthesized Mg-Cd ferrite NPs (Mg-Cd-1 to Mg-Cd-6 samples) were tested for them antimicrobial activity along with the standard drug Ampicillin. The bacteria used in this study are viz., Gram-negative strains of Escherichia coli, Klebsiella pneumonia and Gram-positive strains of Staphylococcus aureus and Bacillus subtilis. The disc diffusion agar method was used for the evaluation of antimicrobial activities of the nanoparticles of the prepared Mg-Cd ferrites by determining the presence of inhibition zones[12]. The disc diffusion assay showed that the prepared ferrite NPs are inhibiting the growth of the bacteria efficiently. It is due to the fact that the prepared nanoferrites have enormously large surface area that provides good interaction with micro-organisms.

The result of antibacterial growth around the ferrite NP's is an estimate of the potential of NPs to inhibit the growth. The antibacterial study results were shown in in Fig. 1. The figure presented illustrates the antibacterial activity and zones of inhibition (ZOI) of ferrite samples $Mg_{1-x}Cd_xFe_2O_4$ where x=0.00, 0.2, 0.4, 0.6, 0.8 and 1.0 (III-1 to III-6) tested against both Gram-negative (Escherichia coli and Klebsiella pneumoniae) and Gram-positive (Staphylococcus aureus and Bacillus subtilis)

bacterial strains. Each Petri plate in the figure corresponds to one bacterial strain, where distinct inhibition zones around each sample indicate the extent of antibacterial effectiveness. The clear zones surrounding the samples reflect the diameter of bacterial growth inhibition, with a larger zone indicating higher antibacterial activity[13].

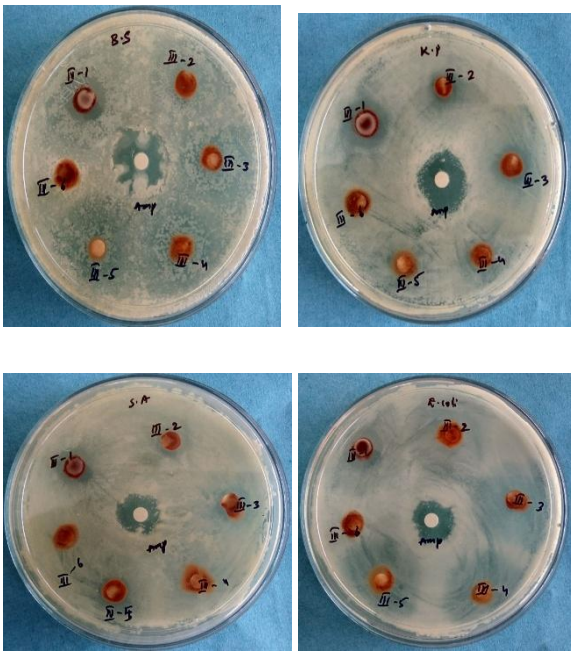

Fig 1. An antibacterial activity and Zone of Inhibition of $Mg_xCd_{1-x}Fe_2O_4$ (III-1 to III-6 samples) ferrites on Gram-negative strains of Escherichia coli, Klebsiella pneumonia and Gram-positive strains of Staphylococcus aureus and Bacillus subtilis.

Table 1 illustrates the Zone of inhibition (in mm) of $Mg_xCd_{1-x}Fe_2O_4$ where x=0.00, 0.2, 0.4, 0.6, 0.8 and 1.0 (III-1 to III-6) system against Escherichia coli, Klebsiella pneumonia, Bacillus subtilis, and Staphylococcus aureus" presents antibacterial activity results for various compositions, compared to standard antibiotic Ampicillin. Sample 5 exhibits the highest antibacterial activity against Escherichia coli among all tested samples. The zone of inhibition recorded for Sample 5 is 12 mm, which clearly indicates a strong antimicrobial effect. This value is notably greater than the zones observed for Samples 1 to 4, which range from 8 mm (Samples 1 and 2) to 11 mm (Sample 4). In contrast, Sample 6 shows no inhibition zone (0 mm), suggesting that it is ineffective against E. coli[14].

Table 1 Zone of inhibition (in mm) of $Mg_xCd_{1-x}Fe_2O_4$ system against Escherichia coli, Klebsiella pneumonia (Gram negative strains) and Staphylococcus aureus and Bacillus subtilis (Gram positive strains).

Name of Micro Organism	1 (30 µg /30 µl)	2 (30 µg /30 µl)	3 (30 µg /30 µl)	4 (30 µg /30 µl)	5 (30 µg /30 µl)	6 (30 µg /30 µl)	Ampicillin (30µg /30 µl)
Escherichia coli	8	8	9	11	12	0	10
Klebsiella pneumonia	8	5	6	20	0	0	12
Bacillus subtilis	8	8	9	10	12	0	12
Staphylococcus aureus	6	4	3	5	6	0	10

The results from Sample 5 indicate this formulation provides enhanced antibacterial properties. This could be attributed to optimized magnesium and cadmium content, particle size, surface charge, or crystal structure in Sample 5, which possibly enables better interaction with the bacterial cell membrane, leading to disruption of cell wall integrity and bacterial death. Interestingly, when compared to the standard antibiotic Ampicillin, which shows a 10 mm inhibition zone, Sample 5 actually performs better in inhibiting *E. coli[15]*. This result highlights the potential of Sample 5 as a strong antibacterial agent, possibly even superior to conventional antibiotics in certain cases. Moreover, the gradual increase in inhibition zone from Samples 1 to 5 (8 mm → 12 mm) suggests that modifications in the composition of the ferrite nanoparticles have a direct influence on antimicrobial activity. It also implies that there is a specific compositional range that maximizes efficacy, which is achieved in Sample 5[16]. Klebsiella pneumonia shows a dramatic response to sample 4, with a 20 mm inhibition zone, significantly higher than any other sample, including Ampicillin (12 mm). However, its sensitivity to other samples is lower, ranging from 5 to 8 mm for samples 1 to 3, and zero for samples 5 and 6. Bacillus subtilis, a Gram-positive strain, exhibits uniform sensitivity across samples 1 to 5, with inhibition zones increasing gradually from 8 mm to 12 mm. Like other strains, it shows no response to sample 6 but responds strongly to Ampicillin (12 mm)[17]. Staphylococcus aureus displays the least sensitivity overall. Inhibition zones range from 3 to 6 mm for samples 1 to 5,

indicating mild to moderate activity. No activity is observed for sample 6. Ampicillin shows a 10 mm inhibition zone against this strain.

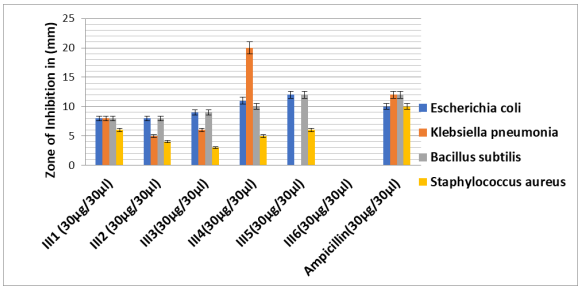

Fig. 2 Graphical representation of Zone of Inhibition exhibited by system and standard drug ampicillin against Gram-negative strains of Escherichia coli, Klebsiella pneumonia and Gram-positive strains of Staphylococcus aureus and Bacillus subtilis.

The graphical representation of Zone of Inhibition exhibited by system and standard drug ampicillin against Gram-negative strains of Escherichia coli, Klebsiella pneumonia and Gram-positive strains of Staphylococcus aureus and Bacillus subtilis illustrated in Fig. 2. Among all, Sample III4 shows the highest zone of inhibition, especially against *Klebsiella pneumonia* (~20 mm), surpassing Ampicillin. *Escherichia coli* and *Bacillus subtilis* also exhibit strong responses (~12 mm) to Sample III5. *Staphylococcus aureus* shows mild inhibition but improves with higher-performing samples. Sample III6 is ineffective against all strains[18]. The results confirm that Sample III4, with optimized composition, demonstrates broad-spectrum and superior antibacterial potential compared to conventional drugs, highlighting its promising application in combating both Gram-positive and Gram-negative bacterial infections.

IV. CONCLUSION

The experimental results demonstrate that Mg-Cd ferrite nanoparticles exhibit significant antibacterial properties, highly dependent on their composition. Among all tested variants, Sample III-5 (x = 0.8) and Sample III-4 (x = 0.6) showed superior antibacterial activity, especially against *Escherichia coli* and *Klebsiella pneumoniae*, respectively. The trend suggests that increasing cadmium substitution up to a certain threshold enhances antibacterial performance, likely due to improved nanoparticle-bacteria interaction via increased surface area, optimal particle size, and favorable physicochemical properties. Conversely, complete substitution (x = 1.0) resulted in complete loss of antibacterial activity. Compared to the standard drug Ampicillin, certain samples demonstrated even better efficacy, underlining their therapeutic potential. The absence of inhibition in fully cadmium-substituted ferrites indicates a critical compositional balance is required for antimicrobial effectiveness. These findings position Mg-Cd ferrite nanoparticles as potential alternatives in antimicrobial treatments, particularly in the context of rising antibiotic resistance. Further in vivo studies and toxicity evaluations are warranted to advance clinical applications.

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