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Chemical Synthesis of Cobalt Nanoparticles and Determination of its Minimal Inhibitory Concentrations and Minimal Bactericidal Concentrations against S. aureus and E. coli

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***Abstract* - The indiscriminate usage of antibiotics in the past few years is one of the main reasons behind increased incidences of antimicrobial resistance. The development of potent antibacterial agents to combat this problem is the need of present era. In view of this, in present study, cobalt (Co) nanoparticles were chemically synthesized by standard method. The synthesized Co nanoparticles were tested against the *S. aureus* and *E. coli* bacterial strains, and the minimal inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) values of Co nanoparticles were determined against these bacterial strains on the day of Co nanoparticles synthesis and after their storage for 30 and 60 days. The Co nanoparticles showed antibacterial actions against *S. aureus* and *E. coli* bacterial strains. The MIC values of fresh chemically synthesized Co nanoparticles in this study for *S. aureus* and *E. coli* were 140.0 µg/ml and 100.0 µg/ml, respectively. The MBC values of these nanoparticles for *S. aureus* and *E. coli* were 260.0 µg/ml and 220.0 µg/ml, respectively. The MIC and MBC values of Co nanoparticles increased on storage of its suspensions for 30 as well as 60 days. It might be considered as potent antibacterial candidate in future after some additional investigations.**
Keywords*: Cobalt nanoparticles, MIC, MBC, *S. aureus, E. coli

I. INTRODUCTION

Emergence of antimicrobial resistance has been observed as serious concerns for the living organisms throughout the world. In past few years, indiscriminate usage of antibiotics has been considered as the main reason behind the increased incidences of antimicrobial resistance. The *Staphylococcus* aureus (S. aureus) and Escherichia coli (E. coli) are common bacterial strains to which almost every human being and animal are exposed at least once in their life span. Some earlier treatable microorganisms are currently becoming untreatable such as methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant enterococcus (VRE) [1] . Bacteria can develop the resistance by different pathways like decreased influx and increased efflux mechanisms, alteration in binding target sites, production of some enzymes etc. Further, the microbial infections also increase the stress in the living organisms. Scientists of all over the world are trying their best to develop some antimicrobial compounds against many resistant microorganisms so that morbidity as well as mortality rate can be reduced along with improvement in the quality of life in patients. Thus, combating the problems of bacterial mutation, antibiotic resistance, outbreaks of pathogenic strains, etc. has been considered as urgent demand of present era. Additionally, it has been predicted that antibacterial resistance will achieve worldwide epidemic proportions by 2050 and accounting for about 10 million casualties [2,3]. So, this problem has added financial burden to the healthcare systems. Thus, there is urgent need governmental sectors, industry, healthcare professions, farmers, society etc. for the appropriate strategies and actions. Applications of nanotechnology in medical field has emerged to plays an important role in recent years.

Nanotechnology has been considered as a great favor for humanity.There are many nano metals such as silver, gold, copper, zinc etc which show structural properties as well as important biological activities [4,5]. Several health problems of humans and animals have treatment by using different metals since ancient times. Various transition metals and their complexes are of current interest from numerous points of view, like their use as antioxidant, antibacterial, antifungal, anticancer, anti-inflammatory agent etc [6,7].

Advancements in the field of nanobiotechnology and its combination with biology in the last few years have also lead to development of various metal and metal oxide nanomaterials, which can be useful for various applications in biological science and clinical medicine [8,10]. Some of these nanomaterials are also emerging as novel antibacterial agents. In the biomedical and healthcare sectors, studies on the synthesis, characterization, and applications of nanoparticles as an antimicrobial system have increased and this field has become one of the latest areas of interest.

Moreover, nanoparticles are considered better antimicrobial agents than their bulk form against drug-resistant pathogens due to size effect, doping effect, cost effective and stability, prolonged shelf-life etc. Among the various metals, Cobalt

(Co) is one, which has not been explored widely for different biological applications. It is considered as cheaper transition metals with wide applications in different industries [11]. The significant impacts of Co nanoparticles have been observed due to their exceptional magnetic and catalytic properties.

Now days, focus on Co nanoparticles in healthcare system is increasing due to its antiseptic action. Our studies have also shown the antibacterial properties of Co nanoparticles at different concentrations. However, to the best of our knowledge, the studies on the determination of minimal inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) values of Co nanoparticles are scanty and no data is available regarding these values after storage of nanoparticles for different days.

So, in view of this, the present study was aimed to chemically synthesize the Co nanoparticles to determine the MIC and MBC values on the same day and after 30 and 60 days of storage.

II. MATERIALS AND METHODS

A. Chemicals Used

The different analytical grade chemicals like cobalt chloride hexahydrate ($CoCl_2$. $6H_2O$), ethylene glycol, hydrazine hydrate, sodium hydroxide were purchased from Sigma Aldrich, USA and used for the synthesis of nanoparticles of Co. The analytical grade nutrient broth (NB), nutrient agar (NA) etc were purchased from SRL for the microbiological assay. The *S. aureus* (MTCC 1430) and *E. coli* (MTCC 2127) were the two bacterial strains against which MIC and MBC values of Co nanoparticles were determined.

B. Synthesis of Co nanoparticles

The synthesis of cobalt nanoparticles was carried as per the procedures explained in our earlier report [12]. Briefly, cobalt chloride was dissolved in ethylene glycol and water mixture (2:1). The vigorous stirring of solution was done till complete dissolution.

The aqueous NaOH solution was used to adjust the pH of the solution to 12. The solution of $CoCl_2$ was then treated with the mixture of hydrazine hydrate (50%). After about 30 min, black solid particles appeared.

After the completion of reaction, the reaction mixture was centrifuged. The black particles collected were washed several times with triply distilled water and absolute ethanol to remove hydrazine, sodium, and chlorine ions.

The final product was then dried in an oven at 60^{o}C. The size of synthesized Co nanoparticles was determined by Malvern Instruments Zetasizer Nano-ZS instrument.

C. Determination of MIC and MBC of Co nanoparticles

The Co nanoparticles were suspended in different concentration (40, 60, 80, 100, 140, 180, 220, 260, 300 and 340 µg/ml) in DMSO and further used on the same day for *in vitro* determination of MIC and MBC against *S. aureus* and *E. coli* bacterial strains. The different suspensions of Co nanoparticles were kept at room temperature and further evaluated for these MIC and MBC on day 30 and 60 in order to determine the stability of nanoparticles suspension.

For the calculation of MIC and MBC, the overnight incubated suspension of *S. aureus* and *E. coli* bacterial strains were aseptically inoculated (about 10^6 CFU/ml) to 10ml tube nutrient broth medium. Ten different dilutions of Co nanoparticles were prepared in DMSO.

Each concentration was evaluated in triplicate for each bacterial strain. The inoculated sets were incubated at 37ºC overnight. The visible turbidity in each tube was evaluated after incubation period. The lowest concentration with no turbidity was considered as the MIC for the tested strain.

The tubes showing no turbidity were further cultured on nutrient agar plates and incubated at 37ºC overnight. Next day, growth of bacterial colonies was checked and the concentration at which no growth observed was considered as the MBC for the tested bacterial strain.

III. RESULTS AND DISCUSSION

In present study, the average size of Co nanoparticles was 178.2 nm with a polydispersity index (PDI) of 0.321 (Fig. 1 and Table 1). On day 30, the average size and polydispersity index of nanoparticles were 323.1 nm and 0.411, respectively (Fig. 1 and Table 1). In the suspensions of day 60, the average size and polydispersity index of Co nanoparticles were 501.6 nm and 0.347, respectively (Fig. 1 and Table 1). So, the size of the Co particles in present study was in nano range, which markedly increased with their storage for 30 and 60 days. However, the size was still in the nano range.

The MIC (Fig. 2a) and MBC (Fig. 2b) values of the synthesized Co nanoparticles against *S. aureus* and *E. coli* bacterial strains on different days are presented in Table 2. Both the bacterial strains were susceptible for the antibacterial actions of Co nanoparticles, which revealed the broad spectrum anti-bacterial activity of Co nanoparticles. The values of MIC and MBC of Co nanoparticles also showed that their action was more against the *E. coli* than *S. aureus*. It was also evident that the MIC and MBC values of Co nanoparticles for *S. aureus* and *E. coli* were increased on its storage for 30 as well as 60 days. It was considered that this was might be due to agglomerations of the nanoparticles during the storage, which lead to increased particle size and decreased antibacterial actions of the nanoparticles. Growth and survival of bacteria is also affected by the size, morphology, and concentration of nanoparticles.

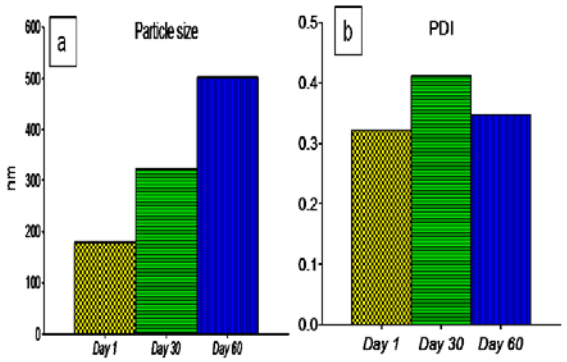

Fig.1 (a) Average Particle Size and (b) PDI Value of Co Nanoparticles on Different Days.

TABLE I THE AVERAGE PARTICLE SIZE (NM) AND POLYDISPERSITY INDEX OF CO NANOPARTICLES (NPS) ON DIFFERENT DAYS

Particle size (nm)			**Polydispersity index**		
Day 1	**Day 30**	**Day 60**	**Day 1**	**Day 30**	**Day 60**
178.2	323.1	501.6	0.321	0.411	0.347

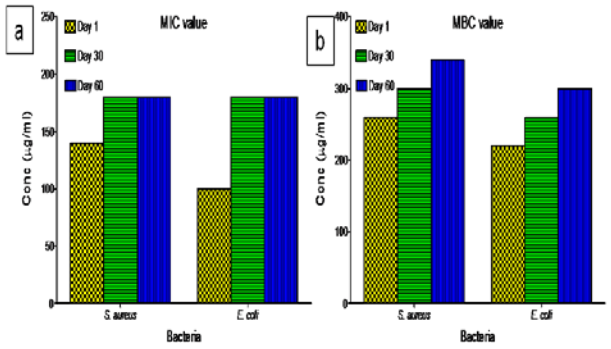

Fig.2 (a) MIC and (b) MBC values of Co nanoparticles on Different Days Against *S. aureus* and *E. coli*

TABLE II THE MIC AND MBC VALUES (MG/ML) OF CO NANOPARTICLES (NPS) AGAINST *S. AUREUS* AND *E. COLI* ON DIFFERENT DAYS

Bacteria	**MIC**			**MBC**		
	Day 1	**Day 30**	**Day 60**	**Day 1**	**Day 30**	**Day 60**
S. aureus	140.0 µg/ml	180.0 µg/ml	180.0 µg/ml	260.0 µg/ml	300.0 µg/ml	340.0 µg/ml
E. coli	100.0 µg/ml	180.0 µg/ml	180.0 µg/ml	220.0 µg/ml	260.0 µg/ml	300.0 µg/ml

Previous studies have suggested that metal and metal oxide nanoparticles having size in nano range might have promising antimicrobial actions [13]. Thus, the synthesized Co nanoparticles in our study might be considered promising antimicrobial candidate. Smaller size of the metal nanoparticles than the pores of bacterial cell membrane helps the easy transmission of nanoparticles across the cell wall to the cytoplasm, which further causes cell death [14]. Thus, the potent antibacterial potentials of Co nanoparticles might be attributed to their relatively smaller sizes and high amount of surface-area-to-volume ratio, which facilitated close interaction with the membranes of bacteria [15]. Earlier, it has been also reported that the inhibition of bacterial growth increases with the increase in concentration of nanoparticles [16].

Various metal nanoparticles like Ag, Cu, Zn etc. have shown the antimicrobial activities in many previous reports [17]. Accumulated metal ions can affect the microbes directly as well as indirectly. In direct method, metal dissolution causes the development of reactive radicals, which directly have an effect on bacteria and causes breakage of the cell wall and cell death. Indirect method includes the incorporation of dissolved ions into bacterial protein, which leads to formation of malfunctioned or non-functional protein. Nanoparticles produces, reactive oxygen species (ROS) in bacterial cells, and intracellularly accumulated ROS further causes apoptosis [18]. We also hypothesized that smaller sized synthesized Co nanoparticles get easy diffusion in the bacteria, where it leads to generation of ROS and resulting in disruption of bacterial membrane permeability and death. Additionally, positive surface charge of Co has electrostatically attraction to the negative charge of bacterial cell surface, thus direct contact or accumulation of Co ions itself might hinder the bacterial cell activity followed by cell lysis in present study. A study has also mentioned that the mechanism behind antibacterial activity of Co nanoparticles is due to increased lipophilicity, which later allows the dispersion of microbes over bilayer phospholipid of the cell membrane, through obstructive the metal-obligatory sites on the enzymes of microbes [19]. Development of bacterial resistance has not been observed against metal and they are considered safe as well as potential antimicrobial alternatives for clinical applications [20].

Different nanoparticles of metal like Ag, Cu, Zn etc are widely applied in different fields for various purposes including as bactericides in dental practices, antibacterial soaps, catheters, burn wound care etc. [21]. Some reports have revealed that pigmentation effect of Ag nanoparticles on teeth, biological safety issues etc. has declined the use Ag nanoparticles [22]. Thus, Co nanoparticles may have the hope to be a alternative for the silver nanoparticles in future as antibacterial. The Co is also considered cheaper than silver metal. Higher durability, more stability, lower mammalian cell toxicity etc. of metal nanoparticles in

comparison to organic nanoparticles make them better antimicrobial [23]. Further, applications of Co nanoparticles can be extended to reduce the morbidity as well as mortality associated with the bacterial infections in humans and animals. These nanoparticles might be very helpful to reduce the bacterial problems associated in agricultural fields. Coating of cellulose bandages, bed linen, army uniforms, medical equipments etc. by these Co nanoparticles may have significant impact on the reduction of bacterial contamination. In future, application of Co nanoparticles can be attempted in combinations with other antimicrobial agents, as combination therapy of metal nanoparticles with antibiotics has showed some synergistic effects [24,25]. However, *in vivo* studies of Co nanoparticles and studies on their ecological impact are pre-requisite for their application in the ecosystem as well as for clinical uses. Therefore, future studies of Co nanoparticles for using it as potent antimicrobial are interesting and challenging.

IV. CONCLUSION

The MIC values of fresh chemically synthesized Co nanoparticles for *S. aureus* and *E. coli* were 140.0 µg/ml and 100.0 µg/ml, respectively. The MBC values of these Co nanoparticles for *S. aureus* and *E. coli* were 260.0 µg/ml and 220.0 µg/ml, respectively. The MIC and MBC values of Co nanoparticles increased on storage of its suspensions for 30 as well as 60 days. In future, the Co nanoparticles might be considered as potent antibacterial candidate after conducting some additional investigations.

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