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Research Article

In vitro evaluation of antimicrobial property of nanoparticles and chlorhexidine against *Streptococcus pneumoniae*

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ABSTRACT

This study used ionic liquids to successfully create a positively charged copper nanocomplex that served as a nanocarrier for chlorhexidine. Interestingly, this nanocomplex may deliver the antibacterial drug with a synergistic outcome. In this study, positively charged zinc nanoparticles (CZNPs) loaded with chlorhexidine were examined using UV-visible spectroscopy, TEM, X-ray diffraction, FTIR, and PSA. The effectiveness of loading, the drug release profile, and the antibacterial properties of chlorhexidine were then assessed. For CZNPs, an average size of 42.65 nm was found. As a result of their consistent release of chlorhexidine, the CZNPs are a more potent antibacterial agent while treating *Streptococcus pneumoniae*.

Keywords: *Antibacterial, Bacteriostatic, Chlorhexidine, Nanoparticle*

INTRODUCTION

The gram-positive pathogenic bacteria *Staphylococcus pneumoniae* spreads through both community and hospital acquisitions[1]. The development of multi-drug resistant strains like MRSA (Methicillin-Resistant Staphylococcus Pneumoniae) makes treatment difficult. S. aureus can penetrate internal organs or

bloodstreams and produce a number of potentially dangerous infections[2].

Chlorhexidine's di-cationic composition is thought to be the cause of its antibacterial capabilities. Chlorhexidine continues to be the benchmark by which other gingivitis and antiplaque medications are evaluated[3]. Its antibacterial and bacteriostatic properties as well as its substantial presence in the oral cavity account for its efficiency. Understanding chlorhexidine biochemical characteristics can help you appreciate its effectiveness and how to utilize it to treat skin infections[4].

In place of antibiotics, nanoparticles (NPs) are being employed more frequently to target microorganisms. The use of NPs in antibacterial vaccines to prevent bacterial infections, in antibiotic delivery systems to treat disease, in bacterial detection systems to produce microbial diagnostics, and in antibacterial coatings for implantable devices and pharmaceutical materials to prevent infection and promote wound healing[5].

RESEARCH METHODOLOGY and OUTCOMES

Materials

Chlorhexidine was procured from Hi-Media Pvt. Ltd. Mumbai (India). All chemicals used in the present research were of analytical reagent grade.

Preparation of Chlorhexidine loaded Zinc nanoparticles (CZNPs)

We sonicated 2 mg of zinc NP powder in 8 ml of ordinary saline. Similarly, we created a stock solution using 2% chlorhexidine gluconate solution that was readily accessible in the market. To reach a concentration of 100 g/ml, we added 0.2 ml of chlorhexidine to 9.8 ml of sterile distilled water. The resulting solution was then stirred magnetically at 500 rpm for 18 hours or overnight before being centrifuged at 10000 rpm at 4°C for 40 minutes[6]. The supernatant was gathered and examined for the unbound Chlorhexidine molecule using a UV spectrophotometer. The produced CZNPs were then separated, twice-rinsed with double-distilled water, and lyophilized.

Particle size and Zeta potential

CZNPs were analyzed for size and zeta (stability) potential measurements by dynamic light scattering. The optimized nanoparticles size was found to have 132.8

nm. CZNPs have a zeta potential of -29.5 mV (Fig 1), signifying nanoformulation stability[7].

In vitro release profile of CCNPs

The pace at which chlorhexidine is released from nanoparticles' polymeric matrix has been shown to protect them from rapid metabolism and degradation[8]. According to in-vitro drug release, 78.29% of the chlorhexidine was released in less than three hours. However, a continuous release was noticed in CZNPs, with Chlorhexidine released at rates of 38.22% and 74.48% after 3 h and 24 h, respectively. Because of the hydrophobic (nonpolar) nature of chlorhexidine and the drug release profile of CZNPs, chlorhexidine was released continuously throughout time.

Percentage encapsulation efficiency

The method used, the molecule's degree of polarity, the molecular composition of the encapsulating components, and the media used for nanoparticles formation are all factors that affect encapsulation efficiency[9]. For CZNPs, the respective encapsulation efficiency rate was 89%.

Morphological characterization of CZNPs by TEM

The size, shape and dimension of nanoparticles affects drug release rate, solubility rate and dissolution rate of a molecule/drug. The CZNPs were found to be segregated, spherical having particle size range of 28 - 55 nm[10].

FTIR Analysis of Drug Samples

The FTIR spectroscopy was used to confirm the loading of Chlorhexidine in CZNPs. FTIR spectrum of Chlorhexidine shows absorption bands at 3112 cm^{-1} for -OH represent intermolecular H- bonding and 2833 cm^{-1} & 2211 cm -1 showing stretching bond for the terminal – CH_3 groups[11].

Antibacterial Activity

Antimicrobial potential of CZNPs had evaluated on *Streptococcus Pneumoniae*. CZNPs have a potency to effectively kill as compare to Chlorhexidine as a control. Surface area of these nanoparticles is very high that potentially raise its antibacterial activity[12].

CONCLUSION

As nanotechnology advances, new, effective methods to treat various illnesses are produced. There are several different nanoformulations on the market now to effectively battle cancer types caused by free radicals. We examine the in vitro release rates, antioxidant capacity, and anticancer effects of polymeric nanoformulations of chlorhexidine nanoparticles on both a qualitative and quantitative level. This brand-new polymeric nanocarrier of chlorhexidine nanoparticles contains a lot of tremendous

potential for preclinical and clinical trials in the near future and may offer a hopeful future in the fight against bacteria.

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Fig 1 PSA of CZNPs.

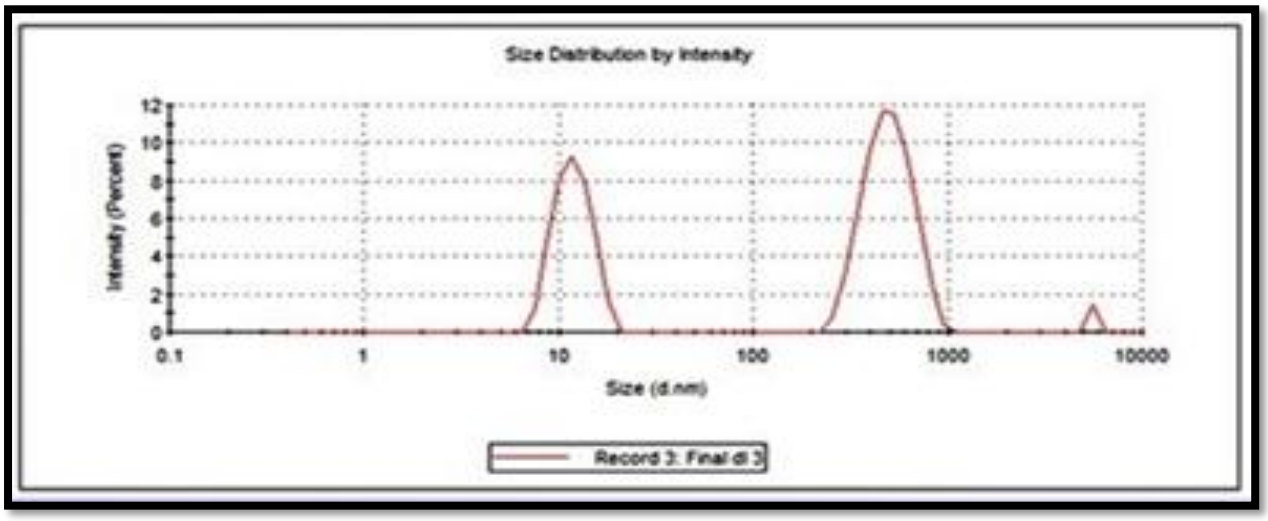

Figure 2. Zeta potential of CZNPs

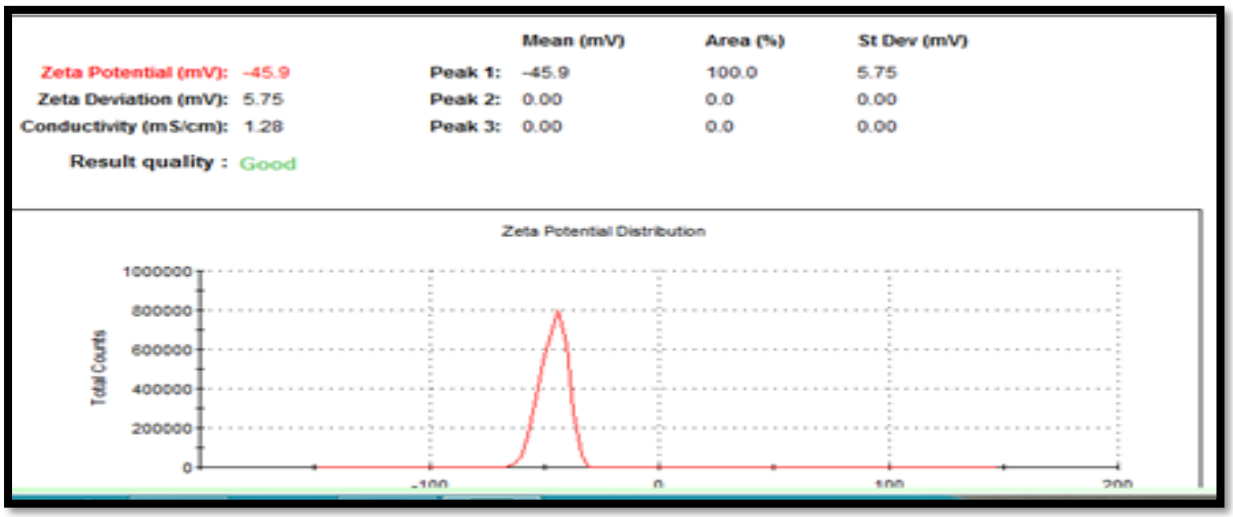

Figure 3: SEM image of CZNPs