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Optimizing Dissolution and Dispersion Conditions for Metal and Metal oxide Nanoparticles Used as Antimicrobial Agents

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ABSTRACT

Background: The growing global danger of antimicrobial resistance (AMR) to human health highlights the urgent need to develop new treatments for infections brought on by multidrug-resistant (MDR) bacteria. The dual impact of nanoparticles (NPs) is due to their ability to act as both an antibacterial agent and an antibiotic carrier is one of its advantages. **Objectives**: The goal of this work was to enhance the nanoparticle dispersing conditions by using different solvents or applying sonication, we also tested different microbial growth conditions and to examine whether using shaking vs. static conditions is more crucial for activity of NPs. **Methods**: Water, DMSO and acetic acid were used as solvents for testing NPs with or without sonication. The antibacterial activity of tested NPs was examined by twofold serial microdilution technique under shaking and static conditions. **Conclusion**: all the investigated NPs require sonication to generate a stable suspension; however, DMSO was needed for silver hydroxyapatite NPs and magnesium oxide NPs, water was needed for Zinc oxide NPs, and an acidic aqueous solvent, like 1% acetic acid, was needed for chitosan hydroxyapatite NPs. Meanwhile there was no difference between shaking and static conditions used in investigating the minimal inhibitory concentration of NPs.

Keywords: *Magnesium oxide, zinc oxide, silver hydroxyapatite, chitosan hydroxyapatite, nanoparticles, resistance.*

INTRODUCTION

The antibiotic era began with Sir Alexander Fleming (1881–1955) discovery of penicillin, followed by a surge in antibiotic research resulting in the "golden era of novel antibiotic discovery" between the 1950s and 1970s. However, since then, no new classes of antibiotics have been discovered. After that, the method used was altering already-approved antibiotics to find new medications[1].

However, as time went on, bacteria developed resistant mechanisms as a result of overuse and misuse leading to an antibiotic resistance crisis which is considered as a global threat. According to a 2013 center of disease control and prevention (CDC) report, humanity is currently living in the "post-antibiotic era", that forced the research to develop new alternative approaches to combat resistance such as nanoparticles (NPs). [2–6]

Nanoparticles not only have intrinsic broad bactericidal activity, but they also serve as nanocarriers for antibiotics. Additionally, NPs are widely used as bacterial growth inhibitors in applications like coatings for medicinal materials, implantable devices, and dental composites. [4,5,7,8] The aim of the current work was to optimize the dispersing conditions of nanoparticles' powder to give stable suspensions to be used as antimicrobial agents and tested against multidrug resistant (MDR) bacteria.

MATERIALS AND METHODS

Nanoparticles

The nanoparticles used in the present study namely, magnesium oxide nanoparticles (MgO NPs), chitosan hydroxyapatite (ChHa NPs) and silver hydroxyapatite (AgHa NPs) were purchased from Nawah scientific research institute, Cairo, Egypt. Moreover, Zinc oxide nanoparticles (ZnO NPs) were purchased from nanotech Egypt, 6th of October, Giza, Egypt.

Solvents and sonication conditions

Water, DMSO and acetic acid were used as solvents with and without applying sonication using Ultrasonic processor (UP50H) Hielscher, Germany, intermittent pulse and amplitude 100% to form stable suspension for the tested NPs.

Incubation conditions

Shaking incubator and static incubator were used to investigate whether there will be a difference in the antibacterial activity of the tested NPs. The antibacterial activity of tested NPs was studied using twofold serial microdilution technique as described elsewhere [9] with minor modifications. The starting concentrations were 8, 10, 10 and 13.3 mg/mL for MgO NPs, AgHa NPs, ChHa NPs, and ZnO, respectively. Subsequently, the microtiter plates were incubated at 37 °C for 18-20 hrs. All experiments were performed in triplicates.

Microbial strains

Standard strains of *Klebsiella pneumoniae* (ATCC 13883) and *E. coli* (ATCC 25922) were obtained from the Egyptian microbial culture collection (EMCC), Faculty of agriculture, Ain Shams University.

RESULTS

Effect of solvents and sonication in dispersion of nanoparticles

Different solvents were used with or without sonication to form stable suspension of the tested NPs as illustrated in (**Tables 1, 2, 3** and **4**).

Table 1. Effect of solvent alone and in combination with sonication on formation of MgO NPs suspension

Solvents	Water	DMSO	DMSO and sonication
Effect on MgO NPs	White mass formed at high concentration	Insoluble	Suspension was formed

The best condition for dispersing MgO NPs was in DMSO and applying sonication to form stable suspension. Using DMSO on its own without sonication leads to no suspension formation while using water resulted in formation of white mass at high concentrations.

Table 2. Effect of solvent alone and in combination with sonication on formation of ZnO NPs suspension: ZnO NPs

Solvent	Water	water and sonication
Effect on ZnO NPs	Insoluble	Suspension was formed

The best condition for dispersing ZnO NPs was using water and applying sonication to form stable suspension. On the other hand, no suspension was formed when water was used alone without sonication.

Table 3. Effect of solvent alone and in combination with sonication on formation of Aga NPS suspension

Solvent	DMSO	DMSO and sonication
Effect on AgHa NPs	Insoluble	Suspension was formed

The best condition for dispersing AgHa NPs was obtained using DMSO with sonication to form stable suspension. On the other hand, no suspension was formed when DMSO was used on its own without sonication.

The best condition for dispersing ChHa NPs was in 1% acetic acid with applying sonication to form stable suspension. While on the other hand, when only DMSO and water were used even with applying sonication no suspension was formed. In addition, high concentration of acid dissolved the ChHa NPs, in contrast no suspension was formed when low acid concentration was used without applying sonication.

Table 4. Effect of solvent alone and in combination with sonication on formation of ChHa NPs suspension

Solvents	Water	Water and sonication	10% acetic acid	DMSO	DMSO and sonication	1% acetic acid	1% acetic acid and sonication
Effect on ChHa NPs	Insoluble	Insoluble	Soluble	Insoluble	Insoluble	Insoluble	Suspension

Table 5: Effect of incubation conditions on NPs antibacterial activity

	MIC (µg/mL)							
	Shaking incubator				Static incubator			
NPs	ZnO NPs	AgHa NPs	MgO NPs	ChHa NPs	ZnO NPs	AgHa NPs	MgO NPs	ChHa NPs
E. coli (ATCC 25922)	2500	1875	1500	469	2500	1875	1500	469
Klebsiella pneumoniae (ATCC 13883)	2500	1875	1500	469	2500	1875	1500	469

Effect of incubation conditions on NPs antibacterial activity

There was no difference between the minimum inhibitory concentration of the tested NPs between static and shaking conditions.

DISCUSSION

Antimicrobial resistance (AMR) is a growing global threat to humanity which leads to an urgent need to find another treatment approach for infections caused by MDR bacteria. [10,11] Nanoparticles have the advantage of being antimicrobial agent itself as well as to be carrier for antibiotics, in other words dual effect.[5,12]

The utmost aim of the current work was to find the best conditions to form a stable suspension of the tested NPs. According to the literation Agha NPs was solubilized in DMSO but when tested it needed also sonication to form stable suspension. [13] While ChHa NPs need aqueous high concentrated acidic medium to be soluble [14], so ChHa NPs best condition to form stable suspension was to be add to 1% acetic acid and sonicated.

MgO NPs due to its hygroscopic property another solvent than water was used which was DMSO and sonication was applied to form suspension. ZnO NPs suspension was formed through adding the powder to water and sonicated. Moreover, between a shaking incubator and a static incubator, there was no variation in the tested NPs' minimal inhibitory concentration.

CONCLUSION

Collectively, to form stable suspension for the tested NPs all need sonication but AgHa NPs and MgO NPs required DMSO as a solvent, ZnO NPs required water and ChHa NPs required aqueous acidic solvent such as 1% acetic acid. The studied NPs minimal inhibitory concentration did not differ between a shaking incubator and a static incubator. Furthermore, we prefer to buy NPs in powder form rather than suspension because powder is less expensive, and suspensions have the drawback of having a one-month stability period.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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