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Preparation of Nano Nalidixic Acid and Study of its Biological Properties

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Abstract

Nanoparticles are of great scientific interest as they are effectively a bridge between bulk materials and atomic or molecular structures. Nalidixic acid is the first synthetic quinolone antibiotics. Nalidixic acid, in the form of nanosized particles, is prepared by ultrasonic method in tetrachloride carbon solvent. The produced nalidixic acid nanoparticles were characterized by X-ray Diffraction (XRD), Infrared Spectroscopy (IR), Scanning Electron Microscope (SEM), and other techniques. The antibacterial activities of nanoparticles tested against microorganism and compared with bulk forms (non-nano) conditions. The results show that the incorporation of the synthesized nanoparticles has a long-lasting antibacterial effect against two Gram-positive species, *Staphylococcus aureus* and *Bacillus subtilis*. Nano nalidixic acid can be injected to the human body as decontaminant agent, to prevent the growth of harmful microorganisms more effectively than the micro scale drug.

Keywords: Preparation; Nalidixic acid; Nanoparticles; Biological properties; Tempeh; Lactic acid bacteria; Antimicrobial activity; Antibiotic resistance; Fermented

Introduction

In the field of medicine, nanoparticles are being explored extensively because of their size dependent chemical and physical properties. This makes them an interesting candidate for application, both *in vivo* and *in vitro* biomedical research. The result of their integration in the field of medicine has led to their application mainly in targeted drug delivery, imaging, sensing, and artificial implants. Another advantage of nanoparticles in medicine is their use as antimicrobials to target highly pathogenic and drug resistant microbes [1]. Nanoparticles exhibited higher antimicrobial activity than micro scale drugs [2,3].

A bulk material should have constant physical properties regardless of its size, but at the nano scale size-dependent properties are often observed. Thus, the properties of materials change as their size approaches the nano scale and as the percentage of atoms at the surface of a material becomes significant. For bulk materials larger than one micrometer, the percentage of atoms at the surface is insignificant in relation to the number of atoms in the bulk of the material. The interesting and sometimes unexpected properties of nanoparticles are, therefore, largely due to the large surface area of the material, which dominates the contributions made by the small bulk of the material. Nalidixic acid (1-Ethyl-1,4-dihydro-7-methyl-4-oxo-1,1,8-naphthyridine-3-carboxylic acid) is a 4-Quinolone antibacterial agent. Quinolones as a class of antibacterial agents have been known for over 40 years [4]. Quinolone derivatives have been known to possess a variety of biological activities such as antimicrobial, cytotoxic, anti-inflammatory, antiviral, antibacterial and antiHIV [5]. Nalidixic acid has been used for selective decontamination of the gut in this patient population, either as a component of a four-drug regimen [6,7] or as part of a sequential alternating regimen [8-22].

The present study was undertaken to investigate the antibacterial effect of nanoparticles of nalidixic acid against two Gram-positive species, *Staphylococcus aureus* and *Bacillus subtilis*. Nalidixic acid is effective against both Gram-positive and Gram-negative bacteria. In lower concentrations, drugs that prevent bacterial growth and reproduction, but do not necessarily kill them. The main purpose of the present investigation was developing a new process to prepare nano balidixic acid by ultrasound method, and compare their antibacterial effects in comparison with their micro scale sizes.

Procedures

Materials and methods

Nalidixic acid was purchased from Alborz drug Company in Iran. A multiwave ultrasonic generator (Sonicator-3000; Misonix, Inc.,

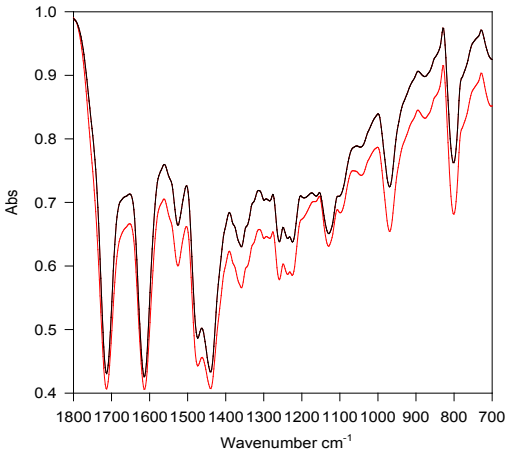

Figure 1: Comparison between the intensities of nalidixic and nanonalidixic acid (red lines) in FTIR spectra. The more intensity of nanoparticles bands are indicate that the inhibition by nano nalidixic acid is more than its micro scale size, resulting in less side effects of the nano drug. The Minimum Inhibitory Concentration of the drug prevents the bacteria resistance, which leads to better effectiveness of nano nalidixic acid.

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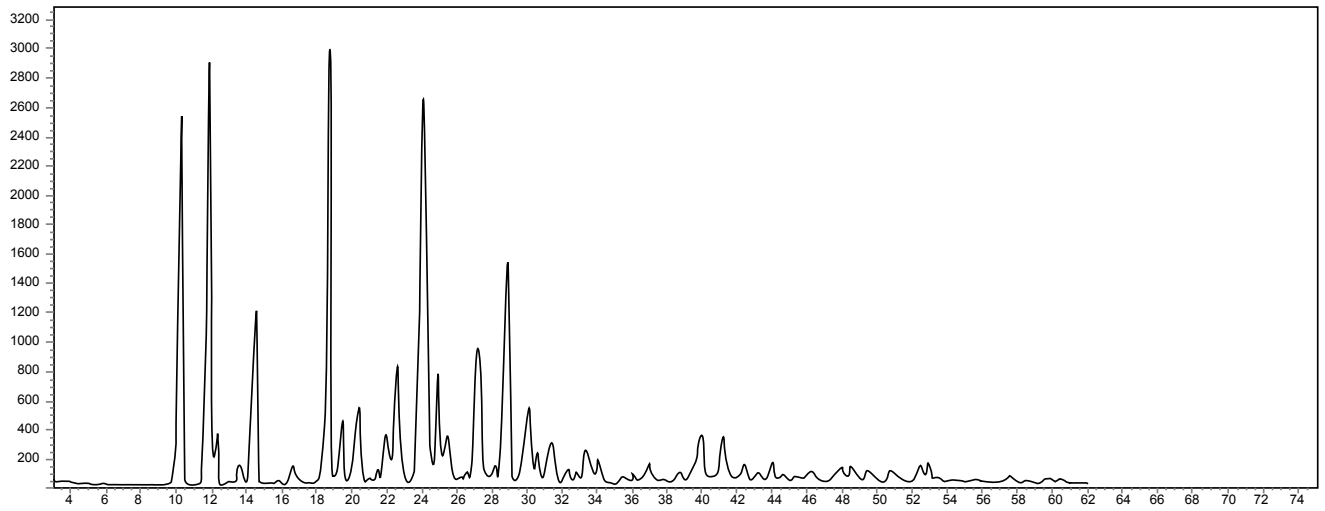

Figure 2: X-ray diffraction spectrum of nano nalidixic acid. The average size of the particles was found to be around 50-60 nm for nano nalidixic acid, respectively, which is in agreement with the value obtained from the SEM image.

Farmingdale, NY), equipped with a converter/transducer and titanium oscillator (horn), 12.5 mm in diameter, operating at 20 kHz with a maximum power output of 600 W. The morphology of synthesized sample was studied using Scanning Electron Microscopy (KYKY-EM3200), by a sputtering technique, with gold as covering contrast material. The XRD measurements of synthesized samples were carried out using a Philips X-pert PRO powder diffractometer with Cu-K_α radiation (λ=1.54 Å), in the scan range 0-100°. The IR spectra were recorded using Bruker spectrometer, with KBr pellets in the range from 400-4000 cm^{-1}. The micro dilution method was used to determine the antibacterial activity of nalidixic acid against the bacteria: *S. aureus* ATCC 6538, *B. subtilis* ATCC 6633.

Synthesis of nanoparticles

Nalidixic acid and carbon tetrachloride was mixed to prepare nanoparticles by ultrasonic method. The suspension was ultrasonically (90 kHz and 79 W/cm^2) irradiated with a high-density ultrasonic probe, immersed directly into the solution. The obtained suspension was allowed to age for 15 min. The precipitate was separated from mother liquor by using a centrifuge at 6000 rpm for 15 min, and at least 8 cycles. Finally, the solid product was extracted and then, kept in a desiccator over silica gel.

Results and Discussion

Figure 1 shows the typical FTIR spectrum pattern of the nalidixic and nano nalidixic acid in the region of 400–4000 cm^{-1}. The ring carbon-carbon stretching vibrations occur in the region 1444.06-1617.19 cm^{-1}. The N-C-H deformation bands occur in the regions, 1518.80, 1470.51, 1294.81-1050.60 and 705.87-776.90 cm^{-1}. The band observed at 1712.97 cm^{-1} is assigned to C=N stretching vibration mode, and C-N stretching usually lies in the region 1227.54 cm^{-1}. The band due to C=O stretching vibrations is observed in the region 1800-1518.80 cm^{-1}. As it is clear in figure 1, the ultrasonic wave has no destructive effects on the structure of nalidixic acid. The intensities of the bands for nanonalidixic acid should be responsible for higher inhibition of harmful microorganism.

The lowest concentration of antimicrobial agent which inhibits the growth of the microorganism, minimum inhibitory concentration of nalidixic acid for inhibiting *Staphylococcus aureus* and *Bacillus subtilis*, have been determined. The method used tubes of growth broth containing a test level of preservative, into which nalidixic acid was added. With decrease of the added concentration of nalidixic acid, the diameter zone for microbial growth would be increased. Minimum inhibitory concentration of usage for nano nalidixic acid is *Staphylococcus aureus* and *Bacillus subtilis*, prevents harmful microorganism growth, decreasing dose of nalidixic acid during healing are the advantages of this nano drug.

Characterization of nanoparticles

X-ray Diffraction (XRD) technique was used to determine the ingredients of the sample. Figure 2 shows the XRD patterns of nano nalidixic acid prepared by the sonochemical process. The nanoparticles size was calculated from the full width at half maximum (FWHM) technique, using Scherer's formula $D= K\lambda/(\beta \cos \theta)$, where K is the constant (0.99), λ is the wavelength of Cu-K_α (1.54 Å) line, β is the FWHM, and θ is the diffraction angle. The nanoparticles size obtained in the range 50-60 nm.

The SEM image is shown in figure 3. The morphology of nanoparticles was observed using a Scanning Electron Microscopy (SEM).

Determination of the minimum inhibitory concentration (MIC)

Minimum Inhibitory Concentration (MIC) is the lowest concentration of an antimicrobial drug that will inhibit the visible growth of a microorganism, after overnight incubation. MICs can be determined on plates of solid growth medium, or broth dilution methods. Broth dilution is a technique in which containers holding in dental volumes of broth with antimicrobial solution in incrementally (usually geometrically) increasing concentration, are inoculated with a certain number of bacteria. The MIC of the synthesized nano nalidixic acid and micro scale nalidixic acid, determined by conventional agar dilution method in table 1, with respect to different microorganism test, including Gram-positive bacteria. Two milliliters cultures of two bacterial strains of *S. aureus* and *B. subtilis* were prepared and placed in a water bath overnight at 37°C. The overnight cultures were diluted with sterile Muller–Hinton broth. The compounds were resuspended

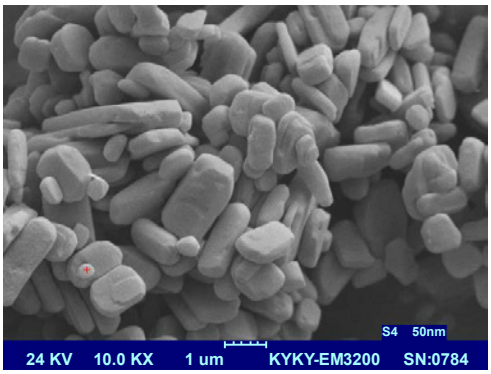

Figure 3: SEM image of nano nalidixic acid demonstrate amorphous morphology with a dimension of 50-60 nm.

Bacterium	Zone of Inhabitation (mm)	
	Nalidixic acid	Nano nalidixic acid
S. aureus	<13	21
B. subtilis	<13	19

Table 1: Antibacterial activities of nalidixic acid. The more efficacy of the drug, the more the inhabitation zone.

to a concentration of 30 μg/ml (in ethanol), with sterile distilled water in a 10-well micro-plate. A similar twofold serial dilution of gentamycin (Sigma) was used as positive control against each bacterium. One hundred microliters of each bacterial culture was added to each well. The plates were covered and incubated overnight at 35-37°C. Bacterial growth in the wells was indicated by a red color, whereas clear wells indicated inhibition.

The obtained results have been shown that nanoparticle has inhibited the microbial activity much more that the micro nalidixic acide, resulting in a smaller dose of nanoparticles to inhibit the growth of the bacteria.

Solubility of nano nalidixic acid

Importance of solubility in pharmaceutical preparations is very important. If a drug is not getting dissolved or miscible in vehicle, then it becomes very difficult to administer it and hence forth, it shows poor bioavailability [23-28]. One of the problems of nalidixic acid is its very low aqueous solubility [29]. Importance of solubility enhancement is for the absorption of drug from the site of absorption. Poor water soluble drug shows poor bioavailability and *vice-versa* [30-32]. The size of the solid particle influences the solubility, because as a particle becomes smaller, the surface area to volume ratio increases of the particle. The larger surface area allows a greater interaction with the solvent. Increased aqueous solubility with the nanoparticle size increases the efficiency, and/or reducing side effects for certain drugs.

Conclusion

In this paper, an attempt has been made to build nano drug with new method. Drugs by having the unique property of ultrasonic waves are transformed into nano, by the use of ultrasonic device. Nanoparticles would be recognized with the use of spectroscopic techniques. The size of particles was measured by using the current relations and methods like XRD and SEM. In addition, the antibacterial and antifungal properties of these substances were studied both in normal and nano conditions. The result nanoparticles have antibacterial activities more than bulk (non-nano) form. When substances are transformed into nano forms, the proportion of surface to volume will be increased. This is because the increased surface energy due to the smaller particles, fusing back to one large particle decreases the total energy, favorable in thermodynamic. The change in the distance among atoms of particles will have the same impact on the properties of substances. Therefore, the synthesized nano particles have the capability of being more antibacterial, in comparison to their normal forms.

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