



Green synthesis of silver nanoparticles using grape pomace extract obtained by betaine-based deep eutectic solvent

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Introduction. Green synthesis of silver nanoparticles is an emerging technique drawing more attention recently because of several advantages over the conventional chemical approaches. Among various synthesis protocols such physical, chemical and biological, solution phase chemical synthesis methods widely used owing to their versatile nature, simple operation, high production yields of NPs with hierarchical and controlled morphologies and high stability [1-2]. This study investigated the rapid and green synthesis of silver nanoparticles (AgNPs) using the grape pomace extract obtained by betaine-based deep eutectic solvent (Betaine - D,L-lactic acid). A DES is composed of two or more components that are capable of self-association through hydrogen bonding to form a mixture with a melting point lower than that of each individual component, preferably below room temperature. Although DESs exhibit almost identical physicochemical properties to traditional ionic liquids, their lower cost, no complicated synthesis and environmentally friendlier nature are expected to project them as favorable media in several areas of material sciences. In this regard, they have been explored for a wide range of applications, including the synthesis of nanomaterials.

Methods. Briefly, betaine and hydrogen bond donor HBD (D,L-lactic acid were mixed in sealed 100 mL glass flasks in molar ratios. The mixtures were placed in a round-bottom flask and continuously stirred at 60 °C, 300 rpm in a magnetic stirrer until the mixture formed a clear solution. Water solvent (10%) was added to the mixture to get a better extraction yield. The grape pomace powder was added into DES in a solvent/solid ratio of 20:1. The mixture was ultrasonic extracted in an ultrasonic bath with an ultrasonic input power of 100 W and a frequency of 30 kHz under the desired conditions. After the extraction, the solution was filtered through paper filter and used to synthesize AgNPs. The AgNPs were characterized by UV-Vis spectroscopy (absorption peak at 432 nm). The grape pomace extract was obtained by maceration and used as a reducing and capping agent in the synthesis of nanoparticles. The samples were diluted with deionized water and UV-visible spectra were recorded using 1 cm Quartz cuvette at 25 °C. The standard broth dilution method (CLSI M07-A8) was used to study the antimicrobial activity of silver nanoparticles by detecting the visible growth of microorganisms in the agar broth.

Results.

In general, the formation and stability of metal nanoparticles in the reaction mixture can be detected by UV-Vis absorption spectroscopy methods. The absorption spectra of silver nanoparticles were observed in the visible region 300-700nm. Fig.1 shows the UV-visible spectrum of AgNPs. The appearance of characteristic peak corresponding to the maximum absorbance at around 435 nm confirms the formation of AgNPs. The silver nanoparticles have strong absorption peaks because of high surface plasmon resonance. Fig.2 reveals that the as synthesis AgNPs have globular morphology in which several NPs aggregates upon each other. These small sized globules randomly connected with each other and through physical interactions and impart meso and macro-porosity to the material. Antimicrobial properties of AgNPs were tested and showed strong antibacterial activity against *E. Coli*. and gram-positive bacteria (*Bacillus subtilis*) (Fig.3). AgNPs increase antibacterial properties due to their effective surface area of nanoparticles, atomic size, and reactivity. Due to this, nanoparticles can penetrate with bacterial cells, causing a more extensive zone of inhibition of silver nanoparticles. In Fig. 3. shows the zone of inhibition of *E-coli* bacteria treated with AgNps.

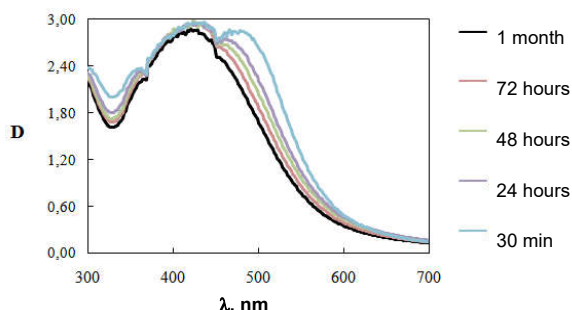


Fig.1 The UV-vis spectra of the colloidal AgNPs solution synthesized by the grape pomace extract obtained by deep eutectic solvent

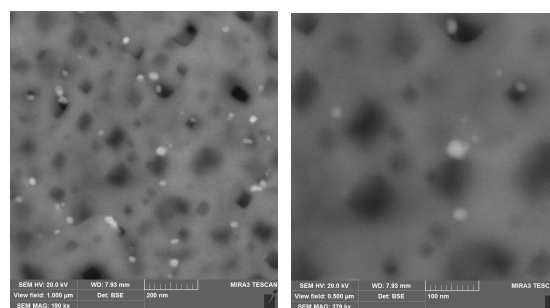


Fig. 2 SEM microphotographs of AgNPs obtained with grape pomace extract obtained by deep eutectic solvent



Fig.3 Antibacterial activity via agar disk diffusion (gram-negative bacteria (*E. Coli*) and gram-positive bacteria (*B. Subtilis*)).

Conclusions. In this work, stable and nearly spherical silver nanoparticles were synthesized by using the grape pomace extract in deep eutectic solvent as a reducing and capping agent. The synthesized AgNPs showed antimicrobial activities (gram-negative bacteria (*E. Coli*) and gram-positive bacteria (*B. Subtilis*)). The tested AgNPs showed potential anti-Gram-negative and anti-Gram-positive bacterial activity, with more effects in response to Gram-negative bacteria. It is owing to the variation in the construction of a crucial agent of the cell membrane, named peptidoglycan

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References

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