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Synthesis of ternary nickel-cobalt-palladium nanoparticle by microemulsion method and investigation of its antibacterial activity on Escherichia coli

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Abstract

In this research, nickel-cobalt-palladium nanoparticles were synthesized using the microemulsion method. The formation of nanoparticles, morphology, size and size distribution of nanoparticles was investigated using SEM, UV-Vis, and DLS methods. The UV-visible spectrophotometric results showed a peak in the region of 380-480 nm, confirming the presence of nanoparticles. The SEM results indicated spherical particle sizes in the range of 45-50 nm for the ratio of Ni:Co:Pd (1:1:1). Additionally, the DLS results showed a size range of produced nanoparticles between 50 and 100 nm, which is consistent with the SEM and UV-Visible results. Nickel-cobalt-palladium nanoparticles exhibited antibacterial properties against E. coli bacteria. The diameter of the zone of inhibition formed in samples containing nanoparticles was significantly larger than the diameter of the positive control sample and the negative control sample (without a zone of inhibition), thus confirming their antibacterial properties.

Keywords: nanoparticles, antibacterial properties, Escherichia coli, microemulsion method.

1. Introduction

The belief that nanotechnology represents a new era in science, combining engineering with biology, chemistry, medicine, and physics, is widely accepted by scientists [1]. Metal nanoparticles are particles of metal (either a single metal or an alloy of multiple metals) with dimensions ranging from 2 to 200 nm, and they hold particular significance due to their unique electrical, optical, medicinal, magnetic, and catalytic properties [2-5]. Nanoparticles exhibit properties distinct from those of individual molecules or bulk materials [6]. They are being considered as a new class of alternative antibiotics to combat antibiotic resistance in bacteria [7]. Metal nanoparticles, in comparison to metal ions, are less toxic, can be administered within living organisms, and have prolonged effects [8]. Nanomaterials have shown minimal toxicity throughout their life cycle and in ecosystems, making them a promising option for combating pathogenic microbes [9]. By reducing particle size, the structural and physicochemical characteristics of nanoparticles are altered. This reduction in size enhances their accessibility to living organisms, potentially increasing their toxicity [10]. Metal nanoparticles can be utilized to coat various surfaces, imparting antimicrobial properties to medical equipment and water treatment facilities [11]. Gram-positive bacteria exhibit greater resistance to metal nanoparticles compared to gram-negative bacteria, likely due to differences in cell wall structure. Research has explored the interactions between nanoparticles and macromolecules within living organisms. The electrostatic attraction between the negative charge of microorganisms and the positive charge of nanoparticles leads to the attachment of nanoparticles to cell surfaces, ultimately causing cell death through oxidation of surface molecules [12-15]. Disruption of membrane permeability ultimately results in cell death. Nanomaterials also delay bacterial adhesion and biofilm formation, inhibiting the stabilization and reproduction of bacterial colonies. Recent studies have demonstrated the antibacterial activity of nickel, cobalt, and palladium nanoparticles against pathogenic microbes [16]. This research aims to investigate the antibacterial effects of nickel-cobalt-palladium nanoparticles on Escherichia coli bacteria.

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2. Experimental

2. 1. Materials

All chemicals used were of analytical grade, including maleic anhydride (Merck, 99%), cobalt nitrate (Merck, 99%), nickel acetate (Merck, 99%), sodium hydroxide (Merck, 99%), and sodium borohydrate (Merck, 99%), palladium acetate (Merk, 99%). The equipment used included a KYKY-EM3200 scanning electron microscope, a Perkin Elmer Lambda 15 ultraviolet-visible spectrometer, a Malvern Zetasizer-Nano ZS DLS model, a Memmert-INB400 incubator model, a JENCONS ultrasonic model, and a BINDER-ED53 Four model.

2. 2. Synthesis of nickel-cobalt-palladium nanoparticles

First, solutions with the same concentration of nickel, cobalt, and palladium ions were prepared. Then, two microemulsions with a water-to-surfactant ratio (w value) of 5 were created: one containing hydrazine dissolved in water, and the other containing nickel, cobalt, and palladium ions (each microemulsion had a total volume of 5 ml). The resulting systems were vigorously stirred for half an hour to achieve stable microemulsions in the lab setting. Next, while the microemulsion of nickel, cobalt, and palladium ions was being stirred vigorously, the microemulsion containing hydrazine was slowly added drop by drop using a syringe. To fully recover nickel, palladium, and cobalt ions, a molar ratio of hydrazine to them equal to 4 was considered.

2. 3. Characterization

After adding the microemulsion containing hydrazine, we analyzed the resulting mixture at various time points using a double-beam UV-Vis spectrophotometer. The color of the microemulsion system was a pale gray and it became more stable over time. The blank sample for the microemulsion containing nickel, cobalt, and palladium nanoparticles consisted of all the components except for these specific nanoparticles. Using the DLS device, we determined the size of the mobile nanoparticles in the solvent. The diameter of the nickel-cobalt-palladium nanoparticles was measured using SEM.

2.4. Antibacterial activity

A specific number of desired bacteria (Escherichia coli) is cultured on a Mueller plate and placed in an incubator for 24 hours to activate the bacteria [10, 11]. Using a sterilized swab, a few colonies of activated bacteria are placed in physiological serum to prepare a half-McFarland solution. Simultaneously, the appropriate amount of prepared nanoparticle powder is dissolved in the solvent (DMSO or DMSO with distilled water). A disc is then coated with 40 microliters of the prepared nanoparticle solution and considered a sample containing nanoparticles. Additionally, a disc impregnated with a solution without nanoparticles is prepared as a negative control sample, and a Genta Mycen disc (with antibacterial properties) is prepared as a positive control sample. The swab stained with bacteria is removed from the half-McFarland solution and cultured on a Mueller plate. The prepared discs (all three types) are placed on the plate and incubated for 24 hours to assess their antibacterial properties. If a zone of inhibition is observed around the discs on the cultured plate, it indicates that the disc containing nanoparticles has antibacterial properties.

3. Results and discussion

3. 1. Analysis of nanoparticles

The analysis of nanoparticles using UV-Vis spectrophotometry is a widely employed technique to study their optical properties, particularly surface plasmon resonance (SPR). Surface plasmon resonance is a phenomenon that occurs where electrons in a thin metal sheet become excited by light that is directed to the sheet with a particular angle of incidence, and then travel parallel to the sheet. Using UV Vis spectroscopy is very common to find the information about the plasmonic resonance of nanoparticles. Confirmation of silver nano-particle formation and plasmonic resonance can be found by analyzing the absorbance data of UV-VIs spectroscopy. It can also roughly estimate the size of a particle. When nanoparticles, especially metallic ones like gold, silver, nickel, cobalt or palladium, are exposed to light, the collective oscillation of conduction electrons at the surface results in SPR, which is observed as a characteristic absorption peak in the UV-Vis spectrum. The position, shape, and intensity of this peak provide

valuable information about the nanoparticle size, shape, dispersion, and dielectric environment. For instance, smaller nanoparticles typically exhibit a sharp SPR peak at shorter wavelengths, while larger particles or aggregates show broader peaks that are red-shifted. Additionally, changes in the local refractive index around the nanoparticles can shift the SPR peak, making UV-Vis spectrophotometry a sensitive tool for monitoring surface modifications or interactions. Thus, the UV-Vis spectrum serves as a fingerprint for nanoparticle characterization, offering insights into their physicochemical properties and behavior in various media. The UV-Visible results confirmed the presence of nickel-cobalt-palladium nanoparticles, showing a peak in the 380-480 nm region, which was not present before nanoparticle formation (see Fig. 1).

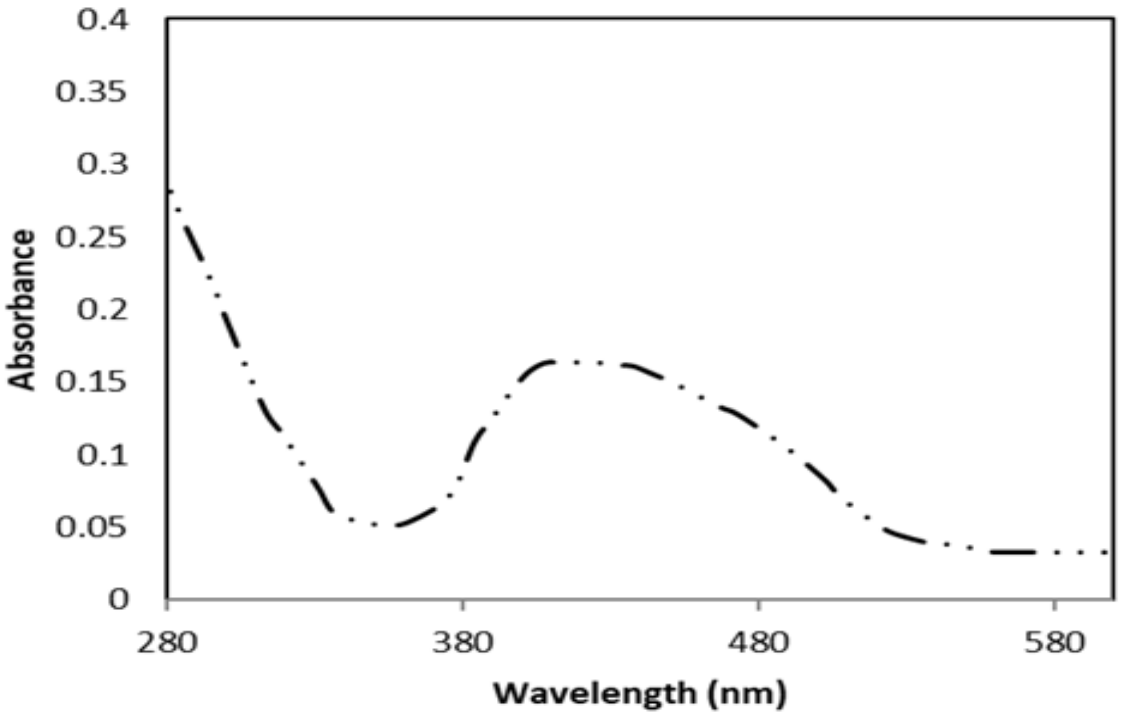

Fig. 1. UV-Visible spectrum of nickel-cobalt-palladium nanoparticles

Dynamic Light Scattering (DLS) is an established and precise measurement technique for the characterization of particle sizes in suspensions and emulsions. DLS is most commonly used to analyze nanoparticles. Examples include determining nanogold size, protein size, latex size, and colloid size. In general, the technique is best used for submicron particles and can be used to measure particle with sizes less than a nanometer. In this size regime (microns to nanometers) and for the purposes of size measurement (but not thermodynamics) the distinction between a molecule (such as a protein or macromolecule) and a particle (such as nanogold) and even a second liquid phase (such as in an emulsion) becomes blurred. Dynamic light scattering can also be used as a probe of complex fluids such as concentrated solutions. However, this application is much less common than particle sizing. Using the DLS device, we obtained the size of the mobile nanoparticle along with layers of solvent around it, resulting in a larger size measurement. DLS results confirmed a particle size range between 50-100 nm (see Fig. 2). Small particles in suspension undergo random thermal motion known as Brownian motion. This random motion is modeled by the Stokes-Einstein equation. Below the equation is given in the form most often used for particle size analysis. In conclusion, the size of the synthesized nickel-cobalt-palladium nanoparticles was confirmed using SEM, UV-visible, and DLS methods.

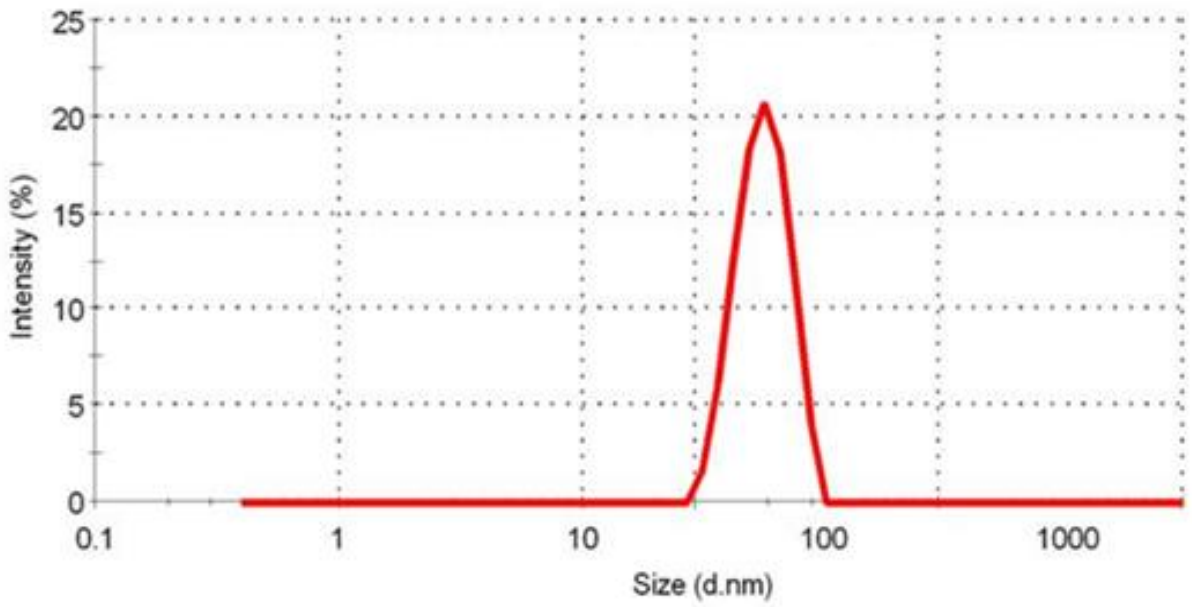

Fig. 2. DLS diagram of nickel-cobalt-palladium nanoparticles

SEM results confirmed that the nickel-cobalt-palladium nanoparticles produced had a uniform spherical morphology and varied in size between 30 and 60 nm (seen Fig.3). This spectroscopy technique is a form of high-resolution surface imaging that uses the principle of light microscopy.

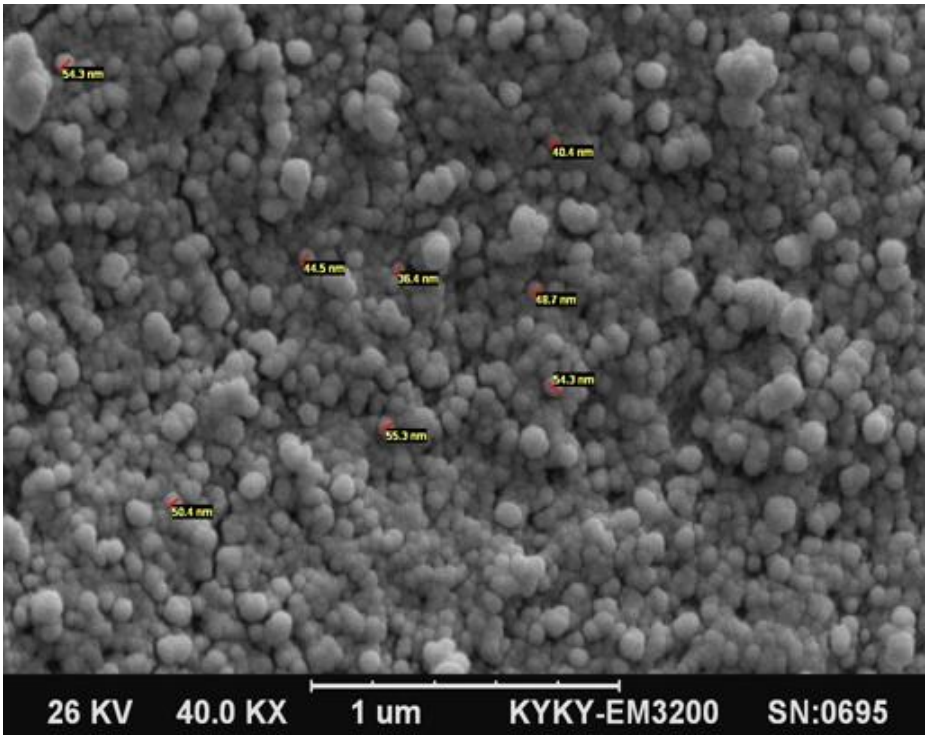

Fig. 3. SEM image of nickel-cobalt-palladium nanoparticles

3. 2. Antibacterial activity of nickel-cobalt-palladium nanoparticles

The antimicrobial activity of nanoparticles was assessed by observing and measuring the diameter of the zone of inhibition. Bacteria surrounding the discs containing nickel-cobalt-palladium nanoparticle solution were observed in the medium cultured with E. coli bacteria. The solution containing ternary nickel-cobalt-palladium nanoparticles (disc containing nanoparticle sample) exhibited antimicrobial activity against E. coli bacteria. A detectable zone of inhibition, resulting from the growth inhibition of microbes by nanoparticles, was observed around the disc containing the nanoparticle sample. In contrast, no zone of inhibition was observed around the negative control sample (Fig. 4). Nanoparticles have been imposed as an excellent antimicrobial agent being able to combat bacteria in vitro and in vivo causing infections. The antibacterial capacity of nanoparticles covers Gram-negative and Gram-positive bacteria, including multidrug resistant strains.

Furthermore, the diameter of the halo formed around the sample containing nanoparticles was 4.8 cm, which was larger than the positive control (GM) sample with a diameter of 2.4 cm, and the negative control (without a zone of inhibition) (Table 1).

Table 1. The diameter of the obtained halo of the ternary nickel-cobalt-palladium nanocatalyst sample on *E. coli* bacteria.

Nanoparticles	Positive control	Negative control
4.8	2.4	0

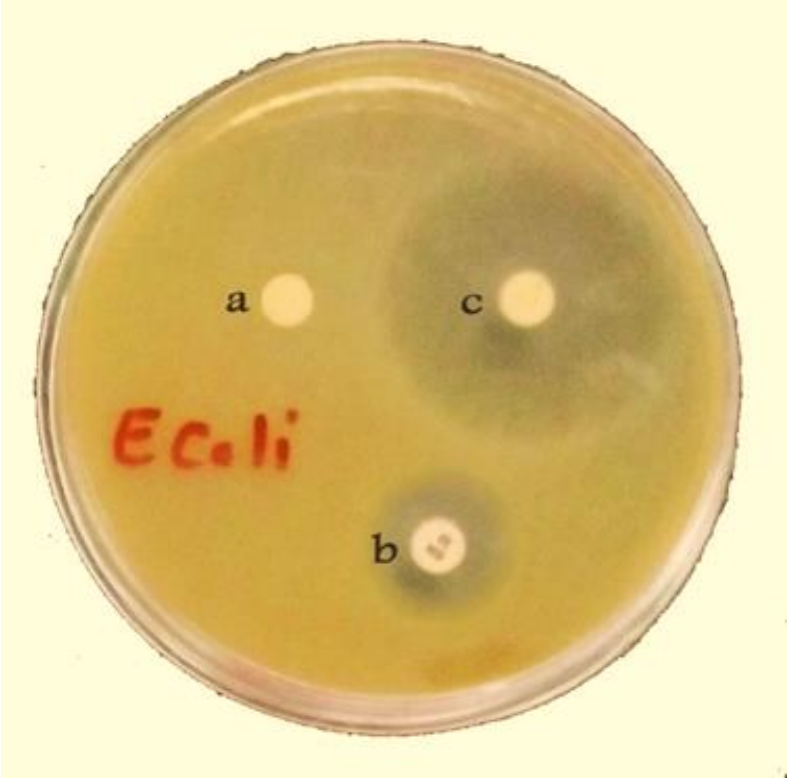

Fig. 4. Obtained the zone of inhibition of the ternary nickel-cobalt-palladium nanoparticles sample on E. coli bacteria.

4. Conclusion

The aim of the present study was to investigate the antibacterial activity of synthesized nickel-cobalt-palladium nanoparticles on E. coli. Previous research has already confirmed the antibacterial activity of individual nanoparticles of nickel, cobalt, and palladium. In this experiment, the synthesized nanoparticles were confirmed using SEM, UV-visible, and DLS. The nickel-cobalt-palladium nanoparticles demonstrated antibacterial activity against E. coli, as indicated by a larger halo formation compared to both the positive and negative control samples. This confirmed the effectiveness of these nanoparticles in combating bacterial growth.

Conflict of Interest

The authors declare no conflict of interest.

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