Synthesis of IONPS by mixing leek extract with iron chloride salt for antibacterial application.

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Abstract: Synthesis of IONPS by mixing leek extract with iron chloride salt. By simple chemical process, with Change in PH which has a variety of effects on the optical and structural properties of IONPs. The iron oxide NPs were characterized by XRD, SEM and UV–vis techniques. XRD results showed average Crystallite size changed from (23.23) nm to (20.70) nm as pH was increased from 1.6 to 12. The particle size of (α-Fe₂O₃) NPs was about (101.60) nm in SEM pictures, but as the PH increased, the particle size decreased to(34.30) nm. UV–vis measurements showed energy band increased from (3.33- 5.62) eV. Antimicrobial activity of iron oxide NPs was determined by growth inhibition zones of the gram negative bacteria E.coli, Klebseilla spp and gram-positive bacteria S.aureus, S.epidermidis and fungal Candida albicans. It found the zones for (α-Fe₂O₃) NPs when PH 1.6 was between (12-14) mm. The zones for (α-Fe₂O₃) NPs when PH 12 was between (12-13) mm.

1. Introduction
Nanotechnology has become a common and basic technology in the recent past [1]. Nanotechnology is a field of science that concerned with the development, manipulation, and application of materials with nanometer-scale dimensions [2].

There is an increasing need to establish environmentally friendly nanoparticle synthesis processes that do not involve the use of toxic chemicals [3]. Compared to chemical and physical synthesis approaches, green synthesis has many advantages, including cost effectiveness, environment-friendly, and the absence of high pressure, energy, temperature, or toxic chemicals [4]. Plant extract is used as both a reducing and capping agent in green nanoparticle synthesis, removing the need for harmful reducing agents [5]. Organic substances from plant extracts are associated with green-synthesized nanoparticles, which increase particle stability. Plant-mediated nanoparticles are less expensive than microbes [6].

Physical, chemical, and biological methods are used to create iron oxide nanoparticles in the magnetite, maghemite, and hematite types [7]. It is still known that amorphous Fe₂O₃ and four polymorphs (alpha, beta, gamma, and epsilon) exist. Hematite and maghemite minerals include the most common polymorphs, the hexagonal corundum structure "alpha" and the cubic spinel structure "gamma." [8].

In this study, Iron oxide nanoparticles were produced using leek extract using a chemical method with a PH modification. X-ray diffraction (XRD) analysis was used to evaluate the iron oxide forms.
Scanning electron microscopy (SEM) was used to investigate the size, morphology, and distribution of iron oxide nanoparticles. UV-visible spectroscopy was used to determine the absorption spectrum of iron oxide nanoparticles. Finally, using the well diffusion process, the antibacterial activity was examined.

2. Experimental part

2.1. Material and methods

The leek leaves were collected Baghdad, Iraq (local market). Iron chloride (99.99%) was obtained from chemical supplier (Sigma-Aldrich). Gram negative bacteria (Escherichia coli, Klebseilla spp.), Gram positive bacteria (Staphylococcus, S.epidermidis), and candida albicans were collected from Mustansiriayah University, Department of Biology, College of Science.

2.2. Preparation of leek extract

To remove dust and dirt, the leek leaves were washed in distilled water. Around (10) g were chopped and boiled in a flask with (200) ml distilled water while being continuously stirred for (120) minutes to make the extract. The extract was then filtered in a burette and used as a reducing and capping agent.

2.3. Preparation of IONPs from leek extract

Drop wise slowly add 125 ml of leek extract to (0.25 M, 4.05 gm, 100 ml) of FeCl$_3$ to make iron oxide NPs. The mixture was then heated at 70°C for 40 minutes while being constantly stirred with a magnetic stirrer until the light yellow colour turned brownish black. To obtain the nano-iron oxide powder, the solution is put in the ceramic eyelid in the oven and left for 2 hours at 200°C. The chemical reaction that produces ($\alpha$-Fe$_2$O$_3$) NPs is given in equation (1) [5].

FeCl$_3$ + H$_2$O $\rightarrow$ Fe$^{3+}$O $\rightarrow$ Fe$\longrightarrow$ O (1)

The pH was changed from 1.6 to 12 by adding NaOH (14M) and the effect of the pH on the iron oxide nanoparticles was tested using a pH scale on a magnetic stirrer.
Fig. 1 Synthesis of α-Fe₂O₃ nanoparticles.

1- Leek extract 2- IONPs when PH (1.6) 3- powder When PH (1.6) 4- IONPs when PH (12) 5-powder when PH (12).

2.4. Characterization of IONPs

to determine basic properties such as optical, structural, morphological, elemental structure, and particle size using advanced techniques such as phase scan mode (XRD-6000, Shimadzu) in 2θ angles ranging from 30° to 80°, scanning electron microscope (Tescan Mira3 SEM, Czech Republic) in Iran, and spectrophotometer (UV-1800, Shimadzu).

2.5. Antimicrobial activity of IONPs:

To investigate the herbal functionality of NPs, antimicrobial properties of synthesized (α-Fe₂O₃) NPs were studied. The agar well diffusion method was used to assess antimicrobial activity [9]. Gram negative bacteria were used in the antimicrobial tests (Escherichia coli, Klebsella spp). Candida albicans and Gram-positive bacteria (Staphylococcus, S.epidermidis). Using the equation below, the percentages of inhibition zones were determined [10].

\[
\text{Inhibition Zone(%) = } \frac{\text{Diameter of the inhibition zone in mm}}{\text{Diameter of pertiplate(90mm)}} \times 100\% \tag{2}
\]

3. Characterization:

According to the XRD patterns shown in ‘Figure 2-a’ and ‘Figure 2-b’, the crystal planes of (104), (110), (202), (116), and (122) specify the formation of Hematite (α-Fe₂O₃) nanoparticles in both cases. This result is in accordance with [11]. All of the reflection peaks agree well with the expected rhombohedral structure of (α-Fe₂O₃). When PH 1.6 was used, The average size of crystallites was discovered to be around (23.23) nm, and when PH 12 was used, The average size of crystallites was discovered to be around (20.70) nm. Impurities are removed and the substance becomes more pure when the pH is raised to 12. This may be due to the FeCl₃ is not reduced completely to nano Fe, when
the pH is raised; all FeCl₃ has reduced to nano Fe. When (pH 12), the peak intensities of Fe NPs increase and become more normal than when (pH 1.6).

Fig. (2-a) XRD data of α-Fe₂O₃ nanoparticles at PH 1.6 Fig. (2-b) XRD data of α-Fe2O3 nanoparticles at PH 12.

The SEM was used to study the morphology and particle size. ‘Figure 3-a’ shows a micrograph of the rod structures observed at PH 1.6 with a particle size of (101.60) nm. ‘Figure 3-b’ shows FE-SEM images of synthesized (α-Fe₂O₃) NPs when PH 12 is applied. The shape of the particles was influenced by the experimental conditions, as shown by a micrograph. Small NPs aggregate into nanoparticles with a particle size of (34.30) nm.

Fig. (3-a) FESEM image showing morphology; when PH 1.6 Fig. (3-b) FESEM image showing morphology; when PH 12.
‘Figure 4’ indicates the energy band gap of (α-Fe₂O₃) NPs, which was calculated by plotting the square (αhυ)² against the photon energy (hυ). Extrapolating the straight line to (αhυ)², the energy band gap is determined. The value of the optical band gap of (α-Fe₂O₃) NPs is (3.33) eV. An increase in the absorbance was observed at (223) nm with an increase in the pH value until the pH of 12 [12]. As a result, increasing the pH raises the energy gap; the band gap of (α-Fe₂O₃) NPs at pH 12 is (5.62) eV.

![Image](image-url)

**Fig. 4 Energy band gap of nano α-Fe₂O₃ NPs.**

### 4. Antimicrobial activity of α-Fe₂O₃ NPs

Gram-positive (S.aureus, S.epidermidis) and Gram-negative (Escherichia coli, Klebseilla spp) bacterial cultures, as well as fungal cultures (Candida albicans), were tested for antimicrobial activity of iron oxide NPs synthesized (Candida albicans). The potential of the antibacterial agent NPs to rupture bacterial cells was tested using the well diffusion process. Owing to the presence of phytochemical elements, plant extracts, in addition to NPs, may have antibacterial activity [13]. (25) mL of nutrient agar was poured onto 90mm sterile petri plates for the assay. The plates were spread with the bacterial sample and incubated at 37 °C for 24 hours to recover the strain. The pure colony was picked under sterile conditions, transferred to nutrient broth, and cultured for 24 hours at 37 ° C. Spread plate techniques were used to create (0.8) cm diameter wells in fresh nutrient agar plates and inoculate them with broth culture. A total of (80) µL of iron oxide nanoparticles were loaded into two separate wells on the same plate. The plates with iron oxide nanoparticles and test strains were incubated for 24 hours at 37 ° C. The IONPs had antibacterial properties, and both gram-negative and gram-positive bacteria were affected in the same way. Despite of Gram-negative bacteria have an extra layer of lipopolysaccharides and peptidoglycans on their outer surface. As shown in Table 1 , the ‘Figure 5’ and the ‘Figure 6’.

| The name of the microbe | PH 1.6 | PH 12 |
|------------------------|--------|-------|
| S. aureus              | 13 mm  | 13 mm |
| S.epidermidis          | 12 mm  | 12 mm |
| E.coli                 | 13 mm  | 13 mm |
| Klebseilla spp         | 12 mm  | 13 mm |
| Candida albicans       | 14 mm  | 13 mm |
Fig. 5 Antimicrobial activity of $\alpha$-Fe$_2$O$_3$.

Fig. 6 Antimicrobial potency of biosynthesized.

5. Conclusion:
Leek extracts with FeCl$_3$ were used to synthesize iron oxide NPs using a simple chemical method at 200 °C for 2 hours. With increasing pH to 12, XRD results showed average Crystallite size changed from (23.23) nm to (20.70) nm as pH was increased from 1.6 to 12. The particle size of (α-Fe$_2$O$_3$) NPs was about (101.60) nm in SEM pictures, but as the pH increased, the particle size decreased to (34.30) nm. UV–vis measurements showed energy band increased from (3.33-5.62) eV. Antimicrobial activity of iron oxide NPs was determined by growth inhibition zones of the gram negative bacteria E.coli, Klebsiella spp and gram-positive bacteria S.aureus, S.epidermidis and fungal Candida albicans. The zones for (α-Fe$_2$O$_3$) NPs when PH 1.6 was between (12-14) mm. The zones for (α-Fe$_2$O$_3$) NPs when PH 12 was between (12-13) mm.

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