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Antibacterial Activity Of Bacterial Mediated Synthesized Iron Oxide Nanoparticles Using Bacillus Coagulans

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Abstract

Cells free supernatant of Bacillus coagulans gram positive, spore forming, bacteria grown in brain heart infusion broth medium (BHIB), was employed as a stabilizing and bio reducing agents to synthesize Iron Oxide Nanoparticles (IONPs) .This method was appeared as an alternative to conventional chemical and physical methods. The synthesized (IONPs) were characterized with Ultraviolet-visible spectroscopy (Uv-Vis), X-ray diffraction (XRD).and scanning electron microscopy (SEM) . The rapid produced nanoparticles resulting in small average particles size 15.18 nm. The IONPs was experimented as antibacterial agent against Uropathogenic E. coli at concentration of 100, 200, 300, 400 µg/ml, the result showed IONPs was effective at concentrated depending manner and it become more effective when the concentrated was increased .

Keywords: Iron Oxide , Magnetic nanoparticles, B. coagulans, Antibacterial, E. coli .

Introduction

Nanotechnology, is the understanding and control the matter in nanoscale at dimension between 1-100 nanometers, were unique physical ,chemical and biological properties of matter in nanoscale different from bulk form enabling novel nanotechnology application [1] nanomaterial has wide ranges use in different fields based on portability to provide specific pioneering alternative tools and resources that save money, effort, time and give hope to open up new scientific and industrial horizons, and created new opportunities, this is due to their novel specific properties[2], there is different methods of nano-synthesis and different types of nanoparticles, the biological method more adopted over physical and chemical method as it is low cost, eco-friendly and no need to use high temperature and energy[3,4]biological method via green synthesis preferred plant, alga, fungi bacteria and viruses to produce nanoparticles[5,6,7] nanoparticles can be classified according many different parameters such as their origin, chemical composition, shape and size [8].

one of most interest nanoparticles iron oxide because of the specific characteristics such as the size, shape, catalytic and magnetic behavior [9] and due to unique properties such as biocompatibility, low toxicity , biodegradable and stability, the IONPs have found more reliable in

a wide variety of biomedical and diagnostic application [10]. The superparamagntic property of IONPs was employed in the drug delivery , thermoablation and bio-separation[11,12],as well as in magnetic hyperthermal therapy(MHT) [13],and magnetic resonance imaging (MRI)[14],also the IONPs was experimented as a cancer therapy[15] and antioxidant[16],nowadays, infectious disease with bacteria resistance to antibiotic has become major challenge to global public health ,it can be considered leading cause of morbidity and mortality in the world and serious concern about increased development resistance in nosocomial pathogens of hospitalized and immunocomprimised patients after transplantation and major challenge in particular heart surgery[17] iron oxide and several metals and there oxides nanoparticles was candidate as alternative antibacterial[18],and antibiofilm formation agents[19], against pathogenic bacteria were exhibited several advantage feature over the traditional antibiotic in distractive and prevent the microbes to developed resistances[20].

Materials and Methods

Chemicals

Laboratory reagent was used without further purification iron (III) chloride hexahydrate ($FeCl_3$ .6H2O,98%) and iron (II)chloride tetrahydrate ($FeCl_2$.4H2O,99%) were purchased from THOMAS BARKER(India), and sodium hydroxide NaOH was supplied from HIMEDIA(India). All glassware used in study cleaned and sterilized following standard laboratory protocol.

Bacterial identification

The bacteria culture of Bacillus coagulans was obtained from university of Babylon, college of sciences. and the bacteria colonies grown in brain heart infusion agar was frosted glass, cream light yellow appearance but may become opaque or smooth raised wrinkly colonies. While microscopically examination to the bacteria smear was showed , gram positive rods, appear in chains or pairs, spore forming, and the single spore were ellipsoidal in shape, subterminally to Paracentrally located, take light green color when stain with malachite green . in addition, bacteria B. coagulans are motile and capable of producing lactic acid[21]

Supernatant solution collection of B. coagulans

Brain heart infusion broth medium was prepared by dissolving 37 gm of broth medium in 1000 ml of double deionized distal water, the broth medium was sterilized in autoclave at 121 ^{o}C for 15 minutes, the bacteria culture of B. coagulans was grown in BHI broth in conceal flask and incubated on shaker to mixed homogenously for overnight at 37 ^{o}C ,later the freshly bacterial culture was centrifuged at 10,000 rpm for 10 minutes, finally the cell free supernatant solution was collected to use in iron oxide nanoparticles synthesis .

Synthesis of Fe_3O_4 nanoparticles

Supernatant of B. coagulans was used as stabilizing and capping agent were employed to synthesis of Fe_3O_4 NPs in simple co-precipitated method, the iron salt precursor Fe^{3+} and Fe^{2+} at a 2:1 M

ratio were added to dissolved in supernatant solution at 35 ^{o}C on magnetic stirrer, NaOH 1.0 M freshly prepared added in dropwise to adjust the pH ~11 of mixture solution with continues stirring for 30 min. after completion of reaction the synthesized Fe_3O_4 NPs was collection with external permanent magnet, then NPs was washed for three time with double deionized water and dried overnight in oven at 70^{o}C for further characteristics.

Characterization of IONPs

Uv-Vis spectral Analysis

The UV-Visible spectroscopic spectrum of biosynthesis nanoparticles clearly recording at range of 200-800 nm , the changing in iron oxide surface Plasmon reasons property band occur due to dispersion among UV/Vis spectra measuring .[22]

X-Ray Diffraction

X-Ray diffraction (XRD) analyses was used to examination the crystallinity and size of IONPs biosynthesis by B .coagulans bacteria broth supernatant , the crystallites size of Fe_3O_4 NPs could be estimated by Debye-scherrer equations (Eq.1). which reveals the relationship between XRD peak broadening and crystalline size

$$ds=K\lambda / \beta COS\ \theta \ldots\ldots\ldots\ldots\ldots\ldots(1)$$

Where ds is average crystalline size IONPs, K is the crystalline-shape factor with a scherrer constant value of 0.9 for an absence of information crystalline- shape, λ is a wavelength of X-rays= 1.5418 ^{o}A , β is a full width at half maximum (FWHM) of the XRD diffraction peak in radiant in 2θ scale and θ is the half diffraction angle of the peak[23].the result confirmed the crystalline nature of NPs synthesis in this study .the size of synthesized IONPs located within the range of nananoscale (1-100 nm) and appears to be small in size due to the acceleration of reaction in presence of NaOH and the capping agents.

Scanning electron microscope (SEM)

The (SEM) analysis confirm the information about the morphology and size of nanoparticles , SEM nanoscale images of biosynthesis of Iron Oxide NPs was showed most particles in irregular cubic shape[24]

Uropathogenic E. coli

The urine samples form UTIs patients were collected daily from out patients clinic laboratory in Al-Hakeem hospital , Baghdad, Iraq. the urine samples was labeled and culture on various types of differentiation agar medium were incubation for 24 hr. at 37 ^{O}C , then (5) ml from every urine samples were deposited by centrifuge in 2000 rpm for 5 minutes in order to examined by light microscopic with high power objective lens(40x) . The presence of 10 pus cells or more in one

field of microscope and from 50-200 pure colonies found in one plate culture conceder as positive result .

Antibacterial activity of IONP

The Antibacterial activity of Iron Oxides Nanoparticles biosynthesis by B .coagulans were used against growth of Uropathogenic E . coli cultured on Muller Hinton agar plates medium with standardized cell suspension with 0.5 McFarland turbidity(10^8x1.5) for 24 hr.at 37 ^{O}C, the method agar well- diffusion was used to detecting the antibacterial activity of biosynthesis Fe_3O_4 NPs at concentration 100. 200, 300, 400 µg/ml to verify the most effective concentration in Nanoparticles application .

Result and Discussion

B .coagulans one of biologically active bacteria belong to soil-based probiotic spore forming, which can secreted bacteriocin and has ability to produce many types of enzymes at an economical level, identification through biochemical tests and morphological characteristics[25,26]the supernatant of bacteria take yellowish color after addition of iron chloride salt precursor with continuously stirring and added of NaOH the mixture color gradually turn to black brown, indicating to IONPs formation (Fig .1). External magnet used to separation and collection of the Fe_3O_4 NPs from solution that confirm the magnetic property of synthesized nanoparticles, the presence of base and biomolecules available from bacterial supernatant acting as stabilizer agent in mixture reaction control the nucleation and precipitant of nanoparticles

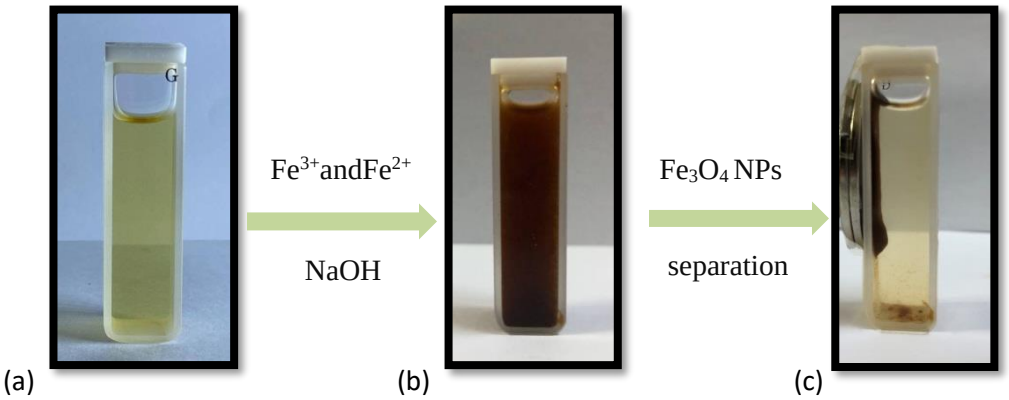

Figure1. (a)supernatant of B. coagulans (b)reactants (c) separation of Fe_3O_4 NPs from the mixture reaction solution by external magnet .

In Uv-Vis spectral analysis, the absorption spectra beak of IONPs was observed at range of 200-300 nm as a result of excitation for surface plasmon resonance which is identical to the characteristic of UV spectral analysis for metallic iron (Fig.2). Among the expected peak , the absorbance maximum at 250.5nm in current study was the characteristic peak of the IONPs and

this value is much closer to the another studies early reported the absorbance of magnetic NPs almost in this range value [27].

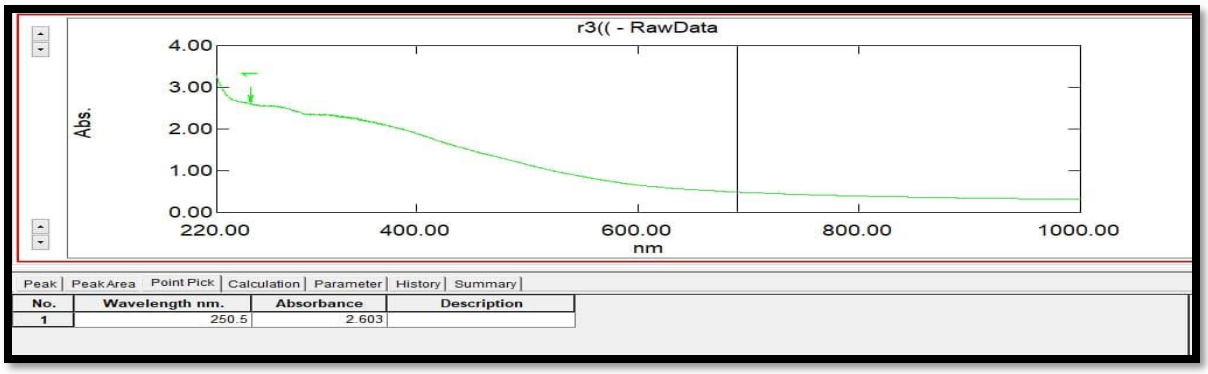

Figure 2. Uv-Vis absorption spectra of IONPs

X-Ray diffraction (XRD) analyses was used to examination the crystallinity and size of IONPs biosynthesis by B .coagulans bacteria broth supernatant from scherrer equation (Eq.1) the calculated of crystalline mean size of IONPs was (15,13 nm) , the Bragg's reflection intense peaks at 2θ value in XRD pattern recorded many relative intensity for magnetite (Fe3O4) NPs corresponding to the standard 2θ value .furthermore the result determined that the relative intensities and positions of reflection peaks 2θ=31.50,35.50,43.12 (Fig.3). for synthesis magnetic nanoparticles by

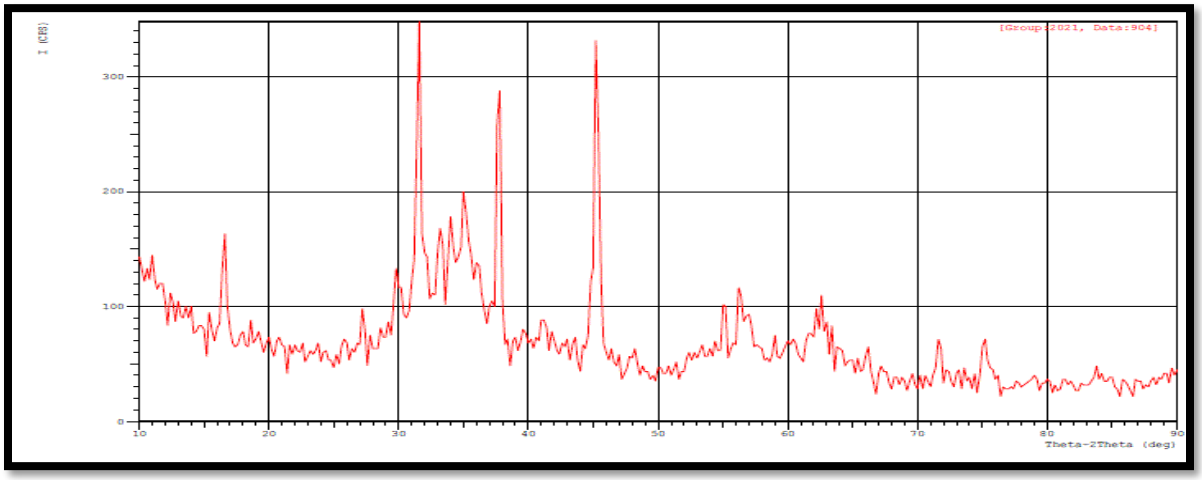

Figure 3. X-ray diffraction of IONPs

bacteria biosynthesis agree with standard diffraction Fe3O4 NPs [28] . the above result confirmed the crystalline nature of synthesized NPs form .

The (SEM) Analysis confirm the information about the morphology and size of Nanoparticles ,SEM Nanoscale images (Fig .4) of biosynthesis Iron Oxide NPs were mostly irregular cubic shape and the size of NPs between(4 to 33) nanometers which is within the range size of nanoparticles

, the small size and irregular shape of this NPS were seen probably due to low level of agglomeration as result of fast formation of precipitation and short time of reaction incubation , furthermore abundance of active biomolecules and capping agent secreted by bacteria in growth medium when supernatant collection ,that could be considered protected agent by covered surface area of NPs which increased physical stability[28].

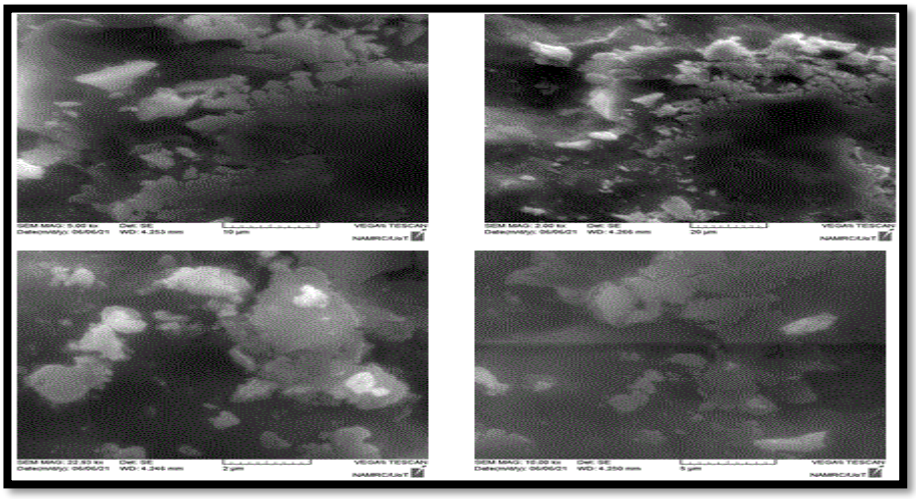

Figure 4. Scanning electron microscope (SEM) of IONPs.

The antimicrobial activity of IONPs was investigated against Uropathogenic E. coli most common urinary tract infection(UTIs) pathogen by agar well diffusion method, four concentration of iron oxide NPs was used 100, 200, 300 and 400 µg/ml on Muller-Hinton agar medium (Fig.5) ,the result showed that Fe_3O_4 NPs has antibacterial activity against E. coli bacteria in dose depended manner that mean the highest inhibition was observed at 400 µg/ml[29] ,the antibacterial activity of IONPs still unknown. However, the NPs involve in generation of reactive oxygen species (ROS) resulting in cell wall and bacterial membrane permeability disruption leading to cell death[30,31]

Figure.5: Antibacterial activity of IONPs against E.coli at concentration 100,200,300 and 400µg/ml.

Conclusion

Magnetic iron oxide nanoparticles was synthesized by supernatant B. coagulans were used as a green stabilizing agent in simple co-precipitated method. Antibacterial activity of IONPs were evaluated against E. coli showed that efficiency increases when NPs concentration increases .

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