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Co-precipitation method of superparamagnetic nanoparticles against some bacteria applications

Wafa Aydin Abdul AL-Latif , Hussain Salih Akbar, Husnyia Hadi Thannon

Dep. of Physics , College of Education for Pure Science , Kirkuk University , Kirkuk- IRAQ

Wafaaydin944@gmail.com , drhussainsalihakbar@yahoo.com , husnyia56@yahoo.com

ABSTRACT

Due to the exponential diversity of effective applications of superparamagnetic iron oxide nanoparticles (SPIONs) in various biological applications, the synthesis process of magnetic nanoparticles is crucial for the fabrication of small and stable particles and their successful application. In this work, we report the synthesis of a co-precipitation method based on sodium hydroxide (NaOH)-mediated precipitation of Fe^{3+} and Fe^{2+} salts in aqueous solution using trisodium citrate as surfactant in a closed system. It was carried out under an inert atmosphere of nitrogen. The resulting synthesized SPIONs were characterized by transmission electron microscopy (TEM), scanning electron microscopy (SEM), X-ray powder diffraction (XRD), and vibrating sample magnetometer (VSM) and zeta potential (Zp) analysis. TEM and SEM images showed that the particles were spherical and the particle size was ≥ 20 nm. XRD pattern during analysis showing the presence of peaks corresponding to the magnetite Fe_3O_4 phase of a sample technique Magnetic Vibration VSM. It was found that the unwrapped magnetic nanoparticles coated with SPIONs-PEI polymer possess magnetic properties but it decreases with increasing PEI concentration by 20% which is sufficient to respond to the magnetic. For the application of nanoparticles uncoated SPIONs and encapsulated SPIONs-PEI were applied to the biological activities to inhibit the growth of bacteria (*E. coli*, *P. mirabilis*, *P. aeruginosa*, *S. aureus*), which showed a significant effect on inhibiting the growth of bacteria with gram-positive and gram-negative bacteria (*P. aeruginosa* and *S. aureus*) compared to an average inhibitory effect on (*E. coli*, *P. mirabilis*).

Keywords: *E. coli*, *P. mirabilis*, *P. aeruginosa*, *S. aureus*, superparamagnetic iron oxide NPs, co-precipitation, PEI

Introduction

The manufacture of Fe_3O_4 MNPs whose dimensions and magnetism are controllable has gained much attention since time due to its distinctive properties, making it a candidate for the use of a variety of diagnostics and treatments based on nanoparticles [1-2]. At present, Fe_3O_4 SPONs is widely accepted due to its chemical and physical properties such as surface chemistry, size distribution, magnetism, modulation, etc. Multifunctional iron oxide nanoparticles have developed as an encouraging material for a wide range of for such biological, therapeutic drug, biotechnology, immunology, xemedicine, gene delivery and engineering [3-4]. Increasing bacterial resistance to antibiotics has led to an increase in the search for alternative therapies and medicines for these antibiotics, and one of the alternative treatments available in medicine is the use of metal oxide nanoparticles, including encapsulated iron oxide SPIONs and coated SPIONs, which in turn have shown supportive effects on antibiotics against bacteria when used together, as we found that SPIONs and coated and unencapsulated supermagnetic nanoparticles loaded with antibiotics

inhibited the growth rates of bacteria when coated and unencapsulated SPIONs were used at a higher rate than when using both antibiotics separately [5-8]. In this study the synthesized iron oxide magnetic nanoparticles SPIONs and effects anti-bacterial on the growth of four types of bacteria, namely (*E. coli*, *P. mirabilis*, *P. aeruginosa*, *S. aureus*), which were cultivated and bacteria were grown on them, and their inhibitory activity was studied on four types of bacteria [9-12].

Experimental

Materials and Chemical Synthesis Method

Nanoscale crystallites of iron oxide were synthesized by an inexpensive coprecipitation method. Mix 1.35 g ferric chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) and 0.69 g ferrous sulfate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) with 1.77 g trisodium citrate and 1.2 g NaOH. Initially, all materials were dissolved in 20 mL of deionized water. The reaction mixture and reaction temperature were slowly raised to 80°C under reflux and reacted under continuous stirring for 60 minutes, and then a solution of iron salts in a mixture of trisodium citrate and NaOH was added dropwise. Immediate formation of a black

precipitate was observed, which was then rinsed thoroughly with ethanol and then filtered with distilled water to remove impurities. During each rinse step, the sample is separated from the supernatant with a permanent magnet. (10g) PEI (Branched) polymer was used, and then dissolved in (100ml) deionized water, taking into account the pH of the substance, which should be (7.9). Where three concentrations of nanoparticles ($R = 10, 30, 50$) and polymer coating (PEI) were used.

Superparamagnetic Iron Oxid Characterization

The structural characterization of the nanoparticles was investigated using an X-ray diffractometer (XRD) system with a $\text{CuK}\alpha$ radiation source generated at 30 mA 40 kV. The average hydrodynamic diameter and zeta potential of the samples were determined using a Malvern Zetasizer ZS (Malvern Instruments Ltd., U.K.). While TEM and SEM techniques are used to identify nanoparticle size, crystal structure and morphology. Vibrating Sample Magnetometer (VSM) Magnetic Characterization was used for SPION in this work [13-15].

Preparation of the culture media

Urine samples were collected for the purpose of investigating the cause of urinary tract infection, especially *Escherichia coli*, as well as *Proteus mirabilis*. Samples were collected to investigate the bacteria from extraction samples of cases of respiratory tuberculosis. Samples were collected for *Pseudomonas aeruginosa*. From the burn parlors. Samples were collected from the boils on the outer surface of the skin to check for *Staphylococcus aureus*. Aerobic cultivation was used by transferring each specimen onto blood agar and McConkey's medium using a sterile bacteriological cabin, then the bacterial dishes were incubated in an incubator at a temperature of 37 °C for a period of (18-24) Isolates Diagnosis watch. The outward appearance of the developing colonies on the surfaces of the culture media was observed in terms of shape, color and texture. Slides were made for the purpose of manufacturing the isolated bacteria using a gram-negative method, in order to distinguish between gram-positive and gram-negative bacteria. Biochemical diagnostic tests were performed and included:

Sugar consumption on a medium (TSI). Consumption of the amino acid (Tryptophan) during the (Indol) assay. Using the enzyme (Urease) to deduce the cleavage of urea to (NH_3 , CO_2) by the bacteria (*Proteus mirabilis*). Citrate assay with a feature to differentiate between bacteria (*Klebsiella*, *Escherichia coli*). Coagulase Yest test to confirm the presence of *Staphylococcus aureus*. This was done by examining the method of diffusion on the standard Muller Hinton medium, as colonies (2-3) were transferred on the above medium, and according to the four isolated types, traditional drug allergy diseases were developed and according to the type of

pathogenic bacteria, including the use of (Amoxicilin, Ampicillin, Amikacin, Azthnmycin, Meropenim, Pipralisin, Refadin, Ceftazidim, Refadin). The purpose of using these types of drug allergy tablets was to test the best allergy tablet for the purpose of conducting a comparison with nanoparticles prepared during the current study.

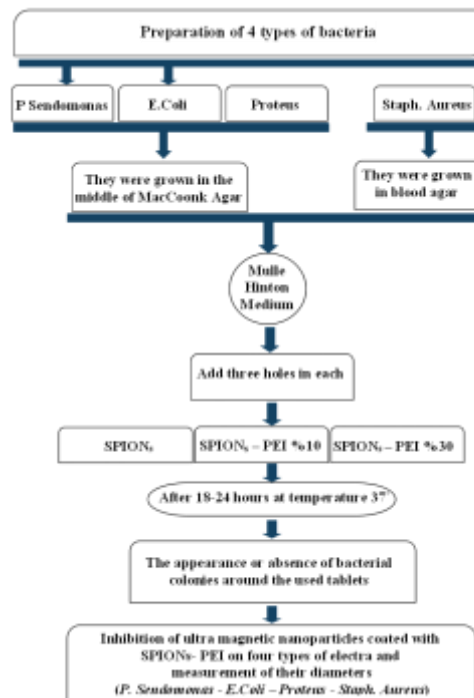


Fig. 1: A flowchart showing the biological application of supermagnetic coated nanoparticles SPIONs - PEI .

Results and discussion

X-ray diffraction Analysis of SPIONs Structure

X-ray diffraction (XRD) technique provides information about the crystal structure and size of the synthesized particles, where the degree of arrangement of the crystal structure is determined, Fig. 2 shows that the supermagnetic non-sheathed iron oxide nanoparticles (SPIONs) are shown in X-ray diffraction analysis. The presence of black iron oxide (Fe_3O_4) within the crystal structure and according to Miller's coefficients indicated at the angles of the detector (θ_2) obtained from the results of the study that the average crystal size of uncoated iron oxide calculated using the Debye-Scherrer equation is about (~ 10 nm).

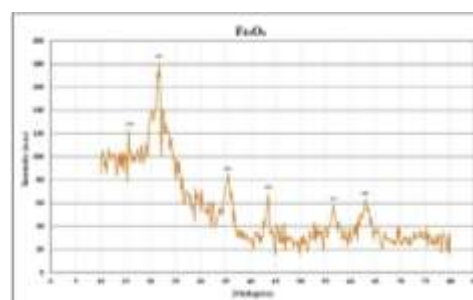


Fig.2. X-ray diffraction for Fe_3O_4 uncoated iron oxide powders prepared at 80 °C.

Magnetic Characterization (VSM) Analysis

Vibration sample magnetometer (VSM) was used to study the magnetic properties of iron in supermagnetic iron oxide nanoparticles uncoated and coated with SPIONs-PEI prepared by co-precipitation method at conditions (temperature 80°C and rotation speed (RPM) 900 cycles) per minute in the presence of nitrogen gas N₂. Fig.3 shows the magnetic properties of the supermagnetic SPION (naked) and coated SPIONs-PEI nanoparticles that showed saturation magnetization (Ms) when the magnetic field applied to the object was increased.

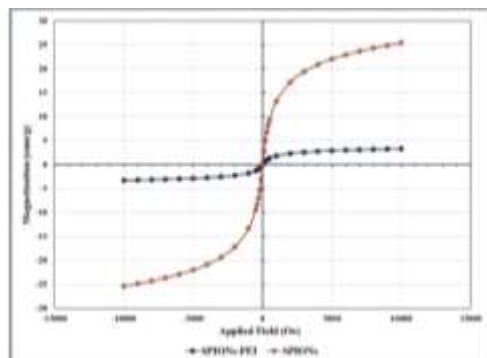


Fig. 3: Magnetic properties of supermagnetic iron oxide nanoparticles SPIONs and SPIONs-PEI-coated (hysteresis ring) nanoparticles with a concentration of 30% of PEI.

Zeta Potential Analysis

Colloidal stability plays a very important role in the zeta potential (ZP) analysis of magnetic nanoparticles of uncoated iron oxide (SPIONs) and coated with polyethylene amine (SPIONs-PEI) when they are introduced into biological applications in vivo. Fig.4 show the zeta potential of supermagnetic iron oxide nanoparticles SPIONs (Naked) and SPIONs-PEI coated nanoparticles prepared by co-precipitation method under conditions and at a concentration of (NaOH = 1.2g)and a rotational speed of 900 cycles per minute and a temperature (80°C) and the stirring continues for one hour.

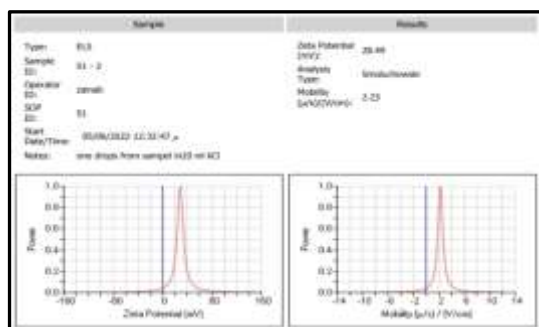


Fig. 4: The zeta potential at a ratio and concentration of polyethylene amine PEI 10%.

Zeta Size (DLS) Analysis

Zeta size analysis was performed using Dynamic Light Scattering DLS to determine the hydrodynamic size of the supermagnetic nanoparticles of uncoated iron oxide (Naked) SPIONs coated with a polyethylene amine (SPIONs-PEI) polymer prepared

by co-precipitation method at a constant rotation speed (RPM) 900 cycles. per minute and at a constant temperature of 80°C and using NaOH at a concentration of (1.2g). Where the value of the effective diameter of the zeta size of the non-coated supermagnetic nanoparticles SPIONs (7.27 nm) appeared, which is identical to the results reached by the researchers in their study[15,18] .As for the results of the zeta volume analysis of ultra-magnetic iron oxide nanoparticles coated with SPIONs-PEI polymer, which plays an important role in increasing the zeta volume according to the bonding strength between PEI and the surface of the SPIONs nanoparticles, as the packaging was done by ex-Situ Method with different concentrations of polyethylene amine (PEI R = 10%,30%,50%).



Fig.5: Volume of the zeta DLS of uncoated iron oxide supermagnetic encapsulated with a concentration of PEI 10%

Scanning Electron Microscope (SEM)

Scanning electron microscopy (SEM) of non-encapsulated supermagnetic iron oxide nanoparticles (SPIONs) demonstrates the nature of the particle surface, particle size, shape and distribution method. Where samples of supermagnetic iron oxide nanoparticles Fig.6 [16] shows the results of the SEM assay for uncoated iron oxide SPIONs .We find that the uncoated SPIONs tend to aggregate in spherical shapes into groups with an average diameter of about (~25 nm) . And after super-magnetic iron oxide nanoparticles were coated with a polyethylene amine (SPIONs-PEI) polymer using ex-Situ Method, at a concentration of R =30%. indicates that the nanoparticles were spherical with an average diameter of about (~20 nm).

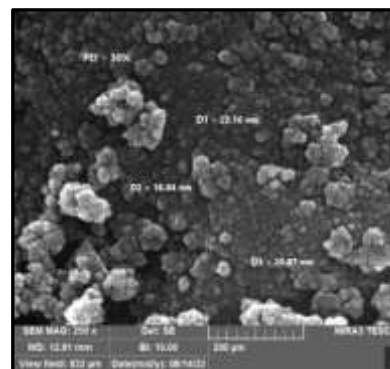


Fig.6: SEM electron microscopic photograph of SPIONs-PEI PEI=30% .

Transmission Electron Microscopic Technique (TEM)

The prepared Fe_3O_4 tested by TEM Technique to obtain actual size of SPIONs by TEM [17]. Fig.7 shows the illustrated the actual size of TEM synthesized via co-precipitation method, the actual size of Fe_3O_4 SPIONs after using the image analysis program ImageJ and the Origin Lab program, it was found that the size of nanoparticles before encapsulation is (5nm).

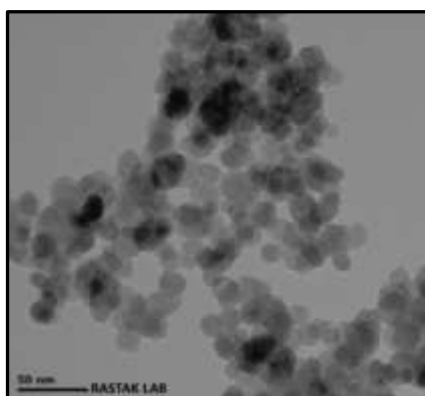


Fig .7: Transmission electron microscopic photographs of Fe_3O_4 nanoparticles coated of SPIONs-PEI at PEI (R=30%).

Biological Applications

Supermagnetic nanoparticles of ferric oxide coated and uncoated with polyethylene amine SPIONs-PEI were prepared as an antibacterial on the growth of types of bacteria namely (*E. coli*, *P. mirabilis*, *P.*

aeruginosa, *S. aureus*), which were cultured and the bacteria were grown and studied. Its inhibitory activity on four types of bacteria. Where the results showed the use of encapsulated super-magnetic nanoparticles SPIONs on them, where the inhibitory effect was minimal on bacterial growth when compared with antibiotics, and so these uncoated nanoparticles can be considered non-toxic, and this property can be taken advantage of by using them as a drug carrier, as for the super-magnetic nanoparticles coated with polyethylene amine SPIONs-PEI, which had a significant effect on bacterial growth, where the inhibitory effect was especially on bacteria positive for gram dye and negative for gram (*S.aureus*, *P. aeruginosa*) with a coating concentration of (10%,30%) Fig.8. compared to an average inhibitory effect on gram-negative bacteria (*E. coli*, *P.mirabilis*). Through this study and by using super-magnetic nanoparticles coated with polyethylene amine (SPIONs-PEI) polymer, it can be used to inhibit the growth of both types of pathogenic bacteria, gram-positive and gram-negative, and this unique property is due to the presence of inhibition by gram-positive bacteria in the present study is *Staphylococcus aureus* due to the presence of peptidoglycan polysaccharides that are present in value-positive bacteria. The thickest 80% of the wall chemical content of the cell may be from this substance, while the polysaccharide polysaccharide found in value-negative bacteria may be positive[18-22].

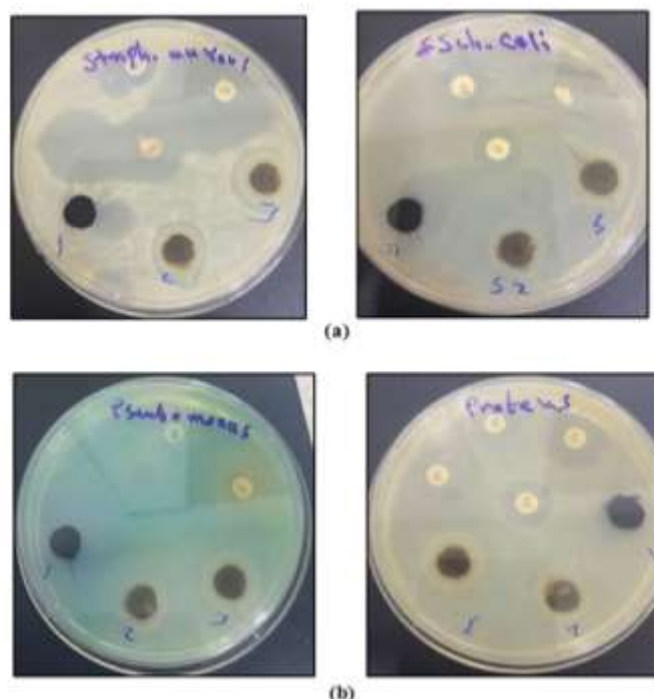


Fig. 8 (a,b): shows the effect of SPIONs-PEI-coated supermagnetic nanoparticles coated with SPIONs-PEI on four types of bacteria with different percentages and concentrations of PEI (R=0,10,30).

The nanoparticles meaning that penetrated the peptidoglycan and worked to inhibit and resist bacteria. The intermediate inhibitory effect of

SPIONs-PEI-coated supermagnetic nanoparticles on the gram-negative bacteria can be explained by the fact that the gram-negative bacteria contain in the

outer wall a thick layer of phosphorylated lipids, which can prevent the entry of nanoparticles into the bacteria cell, which led to reducing the inhibitory effect on these negatively charged particles. While the Gram-positive bacteria, the supermagnetic nanoparticles encapsulated with SPIONs-PEI showed a clear and high inhibitory effect, which is due to the ability of the nanoparticles to penetrate the cell wall of the Gram-positive bacteria due to the absence of a phosphorylated lipid layer as in the Gram-negative bacteria[23-25].

Since the supermagnetic nanoparticles coated with SPIONs-PEI polymer carry a positive charge which is highly attracted to the binding on the cell wall of the negatively charged bacteria. Thus, PEI works on changing the permeability of the cell membrane and increasing the permeability of the cell membrane, and thus the difficulty of controlling the entry and exit of toxic substances through the cytoplasmic membrane, thus leading to the death of the bacteria cell. Table 1 shows the types of bacteria used and the inhibitory size for each of the ratios and concentrations[26-27].

Tayps of bacteria	Inhibiting effect		
	SPIONs (mm)	SPIONs – PEI (mm)	
		10%	30%
E.Coli	Resist	6	4
P.aeruginosa	18	13	10
P.mirabilis	8	6	4
S.aureus	2	8	12

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Conclusions

XRD technique showed that the nanoparticles of pure black iron oxide Fe₃O₄ are within the crystalline structure of standard nanoparticles and within the nanoscale. Analysis of VSM technique showed that the nanoparticles coated with SPIONs-PEI have magnetic property, but it decreases with increasing concentration of polyethylene amine, but it is sufficient to respond to the magnetic field. The zeta potential analysis of the surface charge was negative for supermagnetic iron oxide nanoparticles before encapsulation and was positive after encapsulation with SPIONs-PEI polyethylene polymer. The prepared particles coated with PEI polymer can be prepared in large quantities and at low cost for use in biological applications. The polymer used in the packaging of supermagnetic nanoparticles SPIONs led to the stability and effectiveness of these particles in biological applications. Bacterial media (*Escherichia coli*, *Proteus*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*) used showed resistance to non-SPIONs-coated supermagnetic nanoparticles and ineffective inhibition effect. The action of patients with bacterial isolates of gram-negative (*Pseudomonas* and *Staphylococcus aureus*). The prepared particles were coated with polyethylene amine polymer. The effect of inhibition was greater if it was used with antibiotics for gram-negative and gram-positive bacteria.

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معالجة بعض البكتريا بجسيمات نانوية فائقة المغناطيسية المحضرة بطريقة الترسيب المشترك

وفاء ايدن عبد اللطيف ، حسين صالح اكبر ، حسنية هادي ذنون

قسم الفيزياء ، كلية التربية للعلوم الصرفة ، جامعة كركوك ، كركوك ، العراق

الملخص

نظراً للتغير الأسّي للتطبيقات الفعالة للجسيمات النانوية لأكسيد الحديد الفائق المغناطيسية (SPIONs) في التطبيقات البيولوجية المختلفة ، فإن عملية تحضير الجسيمات النانوية المغناطيسية ضرورية لتصنيع الجسيمات الصغيرة والمستقرة وتطبيقها الناجح. في هذه الدراسة ، استخدم طريقة الترسيب المشترك من مواد هيدروكسيد الصوديوم (NaOH) لأملاح Fe^{2+} و Fe^{3+} في محلول مائي باستخدام سترات الصوديوم كمادة خافضة للتوتر السطحي في نظام مغلق من اجواء نيتروجين. تم تشخيص SPIONs الناتجة باستخدام تقنيات الفحص المجهر الإلكتروني (TEM) ، والمسح المجهر الإلكتروني (SEM) ، وحيود مسحوق الأشعة السينية (XRD) ، ومقياس المغناطيسية لعينة الاهتزاز (VSM) وتحليل إمكانات زينتا (Zp). أظهرت صور TEM و SEM أن الجسيمات كانت كروية وأن حجم الجسيمات كان حوالي (20 نانومتر). ومن نتائج نمط XRD أثناء التحليل اظهرت وجود القمم المقابلة لجسيمات المغنتيت Fe_3O_4 ، وان نتائج تقنية العينة الاهتزاز المغناطيسي VSM وجد أن الجسيمات النانوية المغناطيسية غير المغلفة ببوليمر PEI-SPIONs تمتلك خصائص مغناطيسية ولكنها تتناقص مع زيادة تركيز PEI بنسبة 20% والتي يكفي للاستجابة للمغناطيسية و الجسيمات النانوية SPIONs غير المغلفة و-SPIONs PEI المغلفة المحضرة تم تطبيقها على التطبيقات البايولوجية لتثبيط نمو البكتيريا (E. coli ، P. mirabilis ، P. aeruginosa ، S. aureus) والتي أظهرت تأثيراً كبيراً في تثبيط نمو البكتيريا مع البكتيريا موجبة الجرام وسالبة الجرام (S. aureus ، P. aeruginosa) مقارنة بمتوسط التأثير المثبط على (E. coli ، P. mirabilis) .