Chitosan-modified superparamagnetic iron oxide nanoparticles: design, fabrication, characterization and antibacterial activity

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Introduction
Composites are multiphase systems in which the macroscopic properties are dominated by interfacial interactions. These materials have recently become particularly interesting because of dispersed phase formed by metallic or metallic oxides nanoparticles in a matrix which can be amorphous or crystalline. These systems, now usually referred to as nanocomposites (NC), have gained increasing attention because of the new physics of the two phases heterojunctions which can lead to improved physical and mechanical properties [1].

Chitosan (CTS) has become a promising new research field as a valuable natural polymeric matrix because of its excellent chemical and biological properties. It is a cationic, hydrophilic polymer that is also nontoxic, biocompatible, biodegradable, bioactive, bioabsorbable and antibacterial [2, 3] which has made it a popular material for medical applications in recent years, but only recently being used in combination with magnetic nanoparticles [4]. Chitosan is a weak base and is insoluble in water, but soluble in dilute aqueous acidic solutions below its pKa (~6.3), in which it can convert units (-NH3+) into the soluble protonated form (-NH3+2) [5]. Chitosan film is regarded as biofunctional material, well tolerated by living tissues, particularly applicable as edible coatings to prolong shelf-life and preserve quality of fresh foods [6]. In medical field, chitosan films have been tested as curative wound dressing and as scaffolds for tissue and bone engineering [7]. Chitosan has been investigated as an antimicrobial material against a wide range of target organisms like algae, bacteria, yeast and fungi in experiments involving in vivo and in vitro interactions with chitosan in different forms (solutions, films and composites). Early research describing the antimicrobial potential of chitosan and its derivatives date from the 1980-1990s [8]. The antimicrobial activity of chitosan differs with respect to fabric samples. The sorption of chitosan on cellulose/protein fibrous structure is due to ionic interaction between negative charges and protonated amino groups of chitosan (-NH3+), hydrogen bonding and van der Waals’ forces. However, its affinity can be generally regarded as weak. Therefore, its antimicrobial activity is weak when applied alone [9–11]. Chitosan interacts very easily with bacteria and binds to DNA, glycosaminoglycans and most of the proteins thereby enhancing the antimicrobial effect of nanoparticles [12].

Metal oxide nanoparticles (NPs) exhibit outstanding electrical, optical, magnetic, etc. properties that cannot be revealed by their bulk counterparts [13]. Magnetite NPs show many interesting and unique properties. They have been used in various applications, such as catalysis, photomagnetics, magneto-optics, sensors, data storage, ink jet printing, high frequency device, radio frequency, hypothermia, drug delivery systems, magnetic resonance imaging (MRI), medical diagnostics and cancer therapy. Magnetic NPs consisting of magnetite (Fe3O4) possess unique characteristics that make them promising agents for antibacterial applications [14] because the Food and Drug Administration approved that superparamagnetic iron oxide nanoparticles (SPIO NPs) are biocompatible (BC) with human body [15]. This led to a rapid increase in the number of scientific publications devoted to the development of the techniques of uniform and stable Fe3O4 aqueous suspension preparation and further understanding of their properties. These fascinating properties of nanomagnetites strongly depend on size, shape of NPs, their interactions with stabilizers and surrounding media and also on the manner of their preparation [16]. It is interesting to mention that only magnetite particles with size of less than 30 nm have a large surface area and exhibit superparamagnetic properties that make them prone to magnetic fields and they do not become permanently magnetized without an external magnetic field to support them. Therefore the controllable synthesis of nanocrystals is a key challenge to achieve better applied characteristics. Amongst the several experimental routes developed for the synthesis of magnetic IO NPs [17] some require organic solvents and high temperature, conditions which are incompatible with the hydrophilic nature and thermal properties of most of natural polysaccharides. Thus, the polysaccharide-assisted growth of SPIO NPs has been mostly performed using chemical routes that require mild conditions such as the co-precipitation method. This method consists basically in the co-precipitation of a stoichiometric mixture of ferrous and ferric salts in aqueous media under basic conditions and in the absence of oxygen [18]. Thus recently, co-precipitation from the solution of ferrous/ferric mixed salt solution in the alkaline medium has become widely used for preparation of SPIO NPs [19]. The size and shape of the nanoparticles can be controlled by adjusting pH, ionic strength, temperature and nature of the salts [20 – 21]. The addition of chelating organic anions, or polymer surface complexing agents during the formation of magnetite can help control the size of the NPs [22]. Thus, additives play a crucial role in protecting the synthesized particles from rapid flocculation, thus inhibiting the agglomeration of particles [23]. This NPs agglomerate due to the high specific surface area, surface energy and magnetization. One of the best strategies to decrease particles agglomeration and to improve the size distribution and morphology of nanoparticles is coating of the magnetite NPs with a surface active agent such as chitosan. Chitosan with a high content of amino groups (-NH3+), making it possible to form metal complexes to enhance the surface for use in combination with magnetic NPs [24]. Chitosan-coated magnetic NPs with an average diameter of from 14 nm [25] to 30 nm [26] were obtained by co-precipitation.

In the past decade, the emergence of resistance and multiresistance to antimicrobial substances has led to increasing concerns and interests in finding new antimicrobial agents and identifying new strategies for the treatment of infectious diseases [27]. Thus, the development of alternative antibiotics has received considerable attention in the recent years [28, 29]. Therefore, it is necessary to find an alternative treatment without the use of antibiotics for microbial infection that is directed to the site of infection, localized and difficult for bacteria...
to formulate resistance [30]. Along this line, some have hypothesized that reactive oxygen species (ROS) generated by Fe₃O₄ nanoparticles could kill bacteria without harming non-bacterial cells [31]. Specifically, Pareta et al. cultured osteoblasts or bone-forming cells with IO NPs at a concentration of 4.25 mg/ml and found that cell density was greatly enhanced in the presence of IO NPs as compared with cells cultured without NPs [32].

Among Gram-negative bacteria, Pseudomonas aeruginosa (P. aeruginosa) and Escherichia coli (E. coli) are the most frequently involved in infections due to biofilm formation. A worrying feature of biofilm-based infections is represented by their higher resistance to antibiotics and disinfecting chemicals as well as to phagocytosis and other components of the body’s defence system, when compared to planktonic cells [33]. P. aeruginosa is a leading cause of nosocomial infections and is responsible for 10% of all hospital acquired infections [34]. It is also the most common source of burn infections [35]. E. coli is an important pathogen causing 80 to 90% of community-acquired urinary tract infections (UTIs) [36÷38].

In the present work, we describe a versatile, effective and one-step technique for the preparation of well-dispersed aqueous SPIO colloid suspensions through co-precipitation method. CTS playing the role of a protecting and templating agent leads to the formation of SPIO/CTS BCNC without using any chemical surface active agent. The synthesis route is environmentally friendly because of using non-toxic chemicals and no heat treatment. The SPIO/CTS BCNC could exist in the form of very stable aqueous dispersion and exhibit an excellent antibacterial activity against gram-negative bacteria P. aeruginosa and E. coli, thus SPIO/CTS BCNC is used as bacteria-resistant coating and anti-infective application for biomedical devices.

**Experimental details**

**Materials**

All reagents in this article were analytical grade and used as received without further purification. The following materials were used to prepare the SPIO/CTS BCNC: CTS (Aldrich, low MW, Brookfield viscosity 20 cps), FeCl₂·4H₂O, FeCl₃·6H₂O, pure acetic acid, ammonium hydroxide 25% solution (Merck) and 25% aqueous tetramethylammonium hydroxide solution (TMAOH) from Aldrich. Water used in the experiments was double distilled deionized (DD-water).

**Preparation of SPIO/CTS BCNC**

For a typical procedure, predetermined amount CTS dissolved in a 100 ml 1% acetic acid aqueous solution, then 17.4 ml of this solution, 0.2 ml of 1 M FeCl₂, and 0.4 ml of 1 M FeCl₃ were mixed to obtain an iron chlorides solution in 0.05%(w/v) CTS concentration. The mixture under vigorous stirring by N₂ bubbles using a pipette was entered into the mixed solution at room temperature. The mixture was maintained at pH = 6.9 by adding dropwise from burette a 0.8 M ammonium hydroxide solution. Slow rate addition is an important and critical factor, leading to a uniform and black stable aqueous colloidal suspension. This product was centrifuged several times in DD-water and then in ethanol and dried in vacuum at 70°C. The solid form of SPIO/CTS BCNC can be easily suspended in DD-water and studied for characterization. The same procedure was carried out by adding TMAOH ((CH₃)₄NOH) instead of CTS as the surfactant to produce bare SPIO NPs and purified as mentioned above and used in antibacterial studies for comparison.

**2.3. Antibacterial Tests**

The antibacterial activity was tested against two pathogenic strains of gram-negative bacteria P. aeruginosa (ATCC 27853) and E. coli (ATCC 25922). Each of the two pathogenic strains bacteria were cultured into a Muller Hinton agar (MH) nutrient medium with pH 7.3, in which they have good growth. The culture medium was incubated for 24 h at 37°C. After that, a No. 0.5 McFarland Standard suspension sample, which is equal to 1.5 × 10⁸ Colony-Forming Units per ml (CFU.ml⁻¹), was provided. The standard dilution micromethod was applied in performing the antibacterial activity tests on MH plates according to the previous report [1].

Aqueous dispersions of SPIO or SPIO/CTS BCNC of varying concentrations were prepared from initial magnetite or magnetite/CTS colloidal solution. To get a uniform distribution, the nutrient MH medium was heated to 50°C. Next, 10 ml of each SPIO or SPIO/CTS BCNC solution was added into Petri plates containing 25 ml of nutrient MH medium. Total volume in each Petri plate was kept at 35 ml and the mixing solution was solidified with MH after 15 min. After that 100 μl of a suspension of bacteria was pipetted and spread on the surface of MH medium containing SPIO or SPIO/CTS BCNC. The Petri plates were incubated at 37°C for 24 h in a shaking incubator (150 rpm) to encourage bacterial cell growth. All the obtained results were compared with each of bacteria growth intensity tests on the MH plate in the absence of SPIO or SPIO/CTS BCNC. In order to quantitatively determine the bactericidal activity of SPIO or SPIO/CTS BCNC we made a calculation of the number of CFU which has been grown after addition of bacteria suspension of less concentration (103 or 104 CFU) in MH medium mixed with SPIO and SPIO/CTS BCNC colloids of different concentrations. Control sample test was also conducted for the comparison. All experiments were performed under sterile conditions and in triplicate. The percentage reduction ratio of the bacteria for quantitative antibacterial evaluation has been expressed as:

\[
R = \frac{A - B}{A} \times 100\%
\]

where R is the percentage reduction ratio, A is the number of bacterial colonies in the Petri plates without SPIO or SPIO/CTS BCNC and B is the number of colonies in the Petri plates containing SPIO or SPIO/CTS BCNC.

In vitro antibacterial activity of the solid samples was evaluated using disc diffusion method with MH, and a determination of inhibition zone diameter in millimeters (mm) which conforms to the recommended standards of the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Read zone edges as the point showing no growth viewed from the back of the plate against a dark background illuminated with reflected light [39]. The antibacterial activity of SPIO and SPIO/CTS BCNC thin films was scrutinized against each of bacteria, P. aeruginosa (ATCC 27853) and E. coli (ATCC 25922). In order to recover the lyophilized culture, the desired culture contained in the plastic bead was aseptically transferred into a tube containing 5 ml of nutrient broth and maintained in an incubator at 37°C for 24 hours for bacteria and 25°C for 72 hours for thin films. The initial concentration of the cultures was No. 0.5 McFarland Standard suspension sample for each of the bacteria, and this was determined using the solid agar plate test. Cellulose plates (disc samples diameter of 6 mm) coated with CTS, SPIO or SPIO/CTS BCNC thin films were sterilized by dipping them in ethanol for 15 minutes and placed on the surface of MH which was seeded by 1.0 ml of microorganism culture. The plates were inoculated at 37°C for 24 hours. The inhibition zone diameters of the film specimen were measured against each of bacteria, E. coli and P. aeruginosa, and the average of triplications was recorded. In this study a disk containing ciprofloxacin (CIP) standard antibiotics placed on the surface of MH was used for comparison.
Characterization Methods

The intensity of bacterial growth on MH plates with CTS, bare SPIO and SPIO/CTS BCNC of variable concentration was monitored using an Olympus CX 31 naked eye microscope. Fourier transform infrared spectroscopy (FTIR) was obtained by a BOMEM, MB-Series FTIR system, which operated from 400 cm⁻¹ to 4000 cm⁻¹ at room temperature. The samples were prepared by placing a drop of a magnetite aqueous colloidal solution on KBr which was dried at room temperature.

Scanning Electron Microscope (SEM), LEO-435-VP SEM machine working at EHT 18 kV of accelerating voltage was used to characterize mean particle size and morphology of the nanocomposite powder. The transmission electron microscopy (TEM) investigation for observing the morphology and analyzing the size of the Fe₃O₄ particles in NC was carried out by a Philips CM100 electron microscope working at HT 100 kV. The mean particle size of the colloidal magnetite dispersions was determined by image analysis of the TEM micrographs. Samples for TEM measurement were prepared by placing a drop of magnetite aqueous colloidal solution on carbon-coated copper grid and dried at room temperature. X-ray diffraction pattern (XRD) of the magnetite crystals was obtained from the films deposited on glass by dropping magnetite colloidal solution and 2θ range of 10-80° by a Philips, X'pert, Cu Kα radiation (λ = 0.154 nm) source operated at 30 mA and 40 kV. The scan speed of 0.05 °s⁻¹ was used at room temperature. The nanoparticles always exhibit good crystallinity. The strongest peaks of Fe₃O₄/CTS BCNC corresponding to (3 1 1) were then investigated to evaluate the crystallinity of the sample. The mean nanocrystal size L was determined from the broadening β of the most intense line in the XRD pattern. The size was calculated for nanocrystal, based on the Scherrer equation:

\[ L = \frac{k\lambda}{\beta \cos \theta} \]

where \( \lambda \) is the radiation wavelength, \( k = 0.90 \) and \( \beta \) is the Bragg angle [40].

The magnetic properties of dried NPs were obtained using alternating gradient force magnetometer (AGFM, MDK Corporation) at room temperature.

Results and discussion

FTIR Chemical Analysis

The FTIR spectra of CTS and SPIO/CTS BCNC are shown in Figure 1. The spectrum for pure CTS clearly indicates that the observed absorption peaks correspond to the characteristics of chemical bonds present in CTS. The main bands appearing in that spectrum were due to stretching vibrations of OH groups in the range of from 3750 cm⁻¹ to 3000 cm⁻¹, which are overlapped to the stretching vibration of N-H and C-H bond in -CH₂ (2922 cm⁻¹) and -CH₃ (2875 cm⁻¹) groups, respectively [41].

The characteristic absorption bands appearing at 1659 cm⁻¹ and 1602 cm⁻¹ are due to the absorption by amide I (the asymmetric stretching vibration of the amide carbonyl groups) and amide II (the N-H in-plane bending vibration in the amide groups), respectively [42]. Bending vibrations of methylene and methyl groups were also visible at 1383 cm⁻¹ and 1425 cm⁻¹, respectively [41]. Absorption in the range of from 1160 cm⁻¹ to 1000 cm⁻¹ has been attributed to vibrations of CO group [43] with the distinct absorption peak at 1162 cm⁻¹ in the region of C–O–C stretching vibration for ether groups and skeletal vibration of the glucosamine residue [44 – 47]. The bands near 1080–1029 cm⁻¹ are attributed to \( \nu_{OC} \) of the ring COH, COC and CH₂OH [48]. The small peak at ~890 cm⁻¹ corresponds to wagging of the saccharide structure of chitosan [49 – 50].

However, the band has shifted from 1162 cm⁻¹ to 1064 cm⁻¹ in the spectra of the SPIO/CTS NPs. The same occurs from 1383 cm⁻¹ and 1425 cm⁻¹ to 1400 cm⁻¹. On the other hand the amide absorptions in the range from 1650 cm⁻¹ to 1640 cm⁻¹ became relatively larger than those in the pure CTS spectra. It seems that this phenomenon is due to the interactions between the oxygen atom of the Fe₃O₄ nanoparticles and the hydrogen atom in the amino group of CTS and formation of strong hydrogen bonding, which is the reason of the larger amide absorption band in the spectra of SPIO/CTS NPs as shown in Figure 1. Note that hydroxyl group of CTS didn’t bond to the nanoparticles, without any shift or change as seen in the spectra of CTS and SPIO/CTS NPs. Therefore positive partial charge of hydrogen atom in polar ²⁹O–H¹ bond in CTS may be the cause of existence of positive charge on the surface of NPs [51]. Due to the Coulomb repulsion between these positively charged particles, the black stable aqueous colloidal suspension was formed in our experiment without any surface active agent. Schematic representation of the Fe₃O₄/chitosan core-shell and hydrogen bonding formation is shown in Figure 2.

![Fig. 2. Schematic representation of the Fe₃O₄/chitosan core-shell and hydrogen bonding formation.](image-url)
Y. Wang et al. indicated that the main peak at 399.5 eV in X-ray photoelectron spectroscopy spectra of SPIO/CTS composite prepared by him and co-workers was attributed to the amino groups that were involved in hydrogen bond (NH\(_2\)-O) \[52\]. The two distinct absorption peaks at 583 and 477 cm\(^{-1}\) are attributed to the vibrations of Fe\(^{2+}\)-O\(^2-\) and Fe\(^{3+}\)-O\(^2-\), respectively. The sharp and high intense peak appearing at 583 cm\(^{-1}\) demonstrates the high degree of crystallinity of the Fe\(_3\)O\(_4\) nanoparticles. The characteristic absorption bands therefore confirm the presence of spinel structure Fe\(_3\)O\(_4\) \[53\].

**Morphology of the Fe\(_3\)O\(_4\)/CTS BCNC**

**SEM images**

The microstructure and morphological studies of synthesized Fe\(_3\)O\(_4\) particles contained in colloid nanocomposite were analyzed by SEM as shown in Figure 3. Further analysis of the SEM image of synthesized SPIO/CTS NPs, showed a uniform and highly dense SPIO/CTS NPs which are homogeneous sized Fe\(_3\)O\(_4\) nanoparticles with nearly spherical shape with diameter of average value 22.0 nm, which is in agreement with the results of the XRD and TEM analysis.

![Fig. 3. Typical SEM images of synthesized SPIO/CTS NPs. Some of the fine particles are indicated by the white arrows](image)

**TEM images**

The microstructure of Fe\(_3\)O\(_4\) particles contained in colloid NC was analyzed by TEM as shown in Figure 4. The Fe\(_3\)O\(_4\)/CTS NPs are nearly spherical which was confirmed by scanning electron microscopy. All the NPs were well separated and no agglomeration was noticed and exhibiting inverse spinel facets, which is consistent with the important peaks measured in the XRD spectra. After the examination of micrographs, we measured a diameter distribution in the range of 9-32 nm, with an average value 22.0 nm (SD ca. 7.8 nm) for the particles in the nanocomposites as shown (inset) in Figure 4. No aggregation or precipitation of NPs in an aqueous environment was observed during storage, as a result of the electrostatic repulsion between the positively charged NPs.

**X-ray Diffraction Analysis**

Another question of interest in the synthesis of nanoparticles is their crystalline nature and the most common facets. It is also important for demonstration that we predominantly have SPIO NPs in the colloid. Figure 5 shows the XRD pattern of the SPIO/CTS BCNC. The diffractogram exhibits seven distinct diffraction peaks at 2\(\theta\) values of 30.3°, 35.5°, 43.2°, 53.5°, 57°, 62.7° and 74.5° which correspond to the (220), (311), (400), (422), (511), (440) and (533) crystallographic planes of the inverse spinel magnetite crystal. They matched the diffraction peaks for pure magnetite (Fe\(_3\)O\(_4\)) from the reference database (JCPDS File No. 19–629). The average crystallite size was evaluated by diffraction line broadening (d\(^{11}\)) using the Scherrer equation. The average size of Fe\(_3\)O\(_4\) crystallites obtained from nanocomposite was 21 nm. Although the Scherrer formula always tends to underestimate the real crystallite size, this value is very close to the TEM result and consequently, each particle should be a single crystal \[54\]. Lattice constant of Fe\(_3\)O\(_4\) is 8.378, which is matching with \(a=8.384\) of the standard (JCPDS 75 – 0033). XRD patterns indicate that synthesized magnetite NPs are perfectly crystalline and exhibit some predominant facets.

![Fig. 5. XRD pattern of the SPIO/CTS crystallites](image)

**Magnetic Results**

The magnetic properties of dried NPs were obtained using alternating gradient force magnetometer (AGFM, MDK Corporation) at room temperature between -10k to 10kOe. The results clearly showed SP behavior of the NPs as shown in Figure 6. The saturation magnetization (Ms) value of the bulk phase of magnetite is about 90-92 emu/g, \[55-56\] but here, the Ms value is 65 emu/g. The difference can be most likely attributed to several factors including the finite size effect and large surface-to-volume ratio, the spin canting effect found at a grain boundary, the incomplete crystallization of magnetite particles \[57\] and the existence of coated materials \[58\], all of which may lead to decrease in the effective magnetic moment. As expected, because of the CTS coating, saturation magnetization of nanoparticles was lower than that of bulk phase magnetite. According to the AGFM results as it is seen in Figure 6, the negligible coercivity of SPIO NPs showed properties of SP materials. The high
magnetization and SP properties are highly desirable for biomedical applications because larger magnetic particles form aggregates after exposure to a magnetic field [59].

![Graph](image)

**Fig. 6.** Magnetization curve of SPIO/CTS nanoparticles as measured by AGFM at room temperature. The results clearly showed superparamagnetic behavior of the nanoparticles

### 3.5. Antibacterial Results

Parallel experiments were performed on the antibacterial activity of the obtained bare SPIO NPs and SPIO/CTS BCNC against each of the bacteria. For qualitative assessment of antibacterial activity of NPs, MH plates containing SPIO NPs and SPIO/CTS BCNC with different concentrations were inoculated with bacterial suspension (1.5 × 10^8 CFU). The results showed the observations of bacteria grown on MH plates after 24 hours at different concentrations of the SPIO NPs and SPIO/CTS BCNC ranging from 0, 75, 125, 175, and 225 μg.ml⁻¹. For control sample (0 μg.ml⁻¹ SPIO, 0.05% w/v CTS in 1% acetic acid solution), the bacteria were grown easily, but there was no evident bacteria growth on the agar plates after 24 hours at different concentrations of the SPIO NPs and SPIO/CTS BCNC.

The more experiments showed the minimal inhibitory concentration (MIC) value of SPIO NPs against P. aeruginosa and E. coli are 70 μg.ml⁻¹ and 90 μg.ml⁻¹ and SPIO/CTS BCNC against P. aeruginosa and E. coli are 40 μg.ml⁻¹ and 45 μg.ml⁻¹ respectively. In our experiments, the MIC value for control sample were found to be 1000 μg.ml⁻¹ (CTS, 0.1% w/v) for E. coli [60-62] and 1700 μg.ml⁻¹ (CTS, 0.17 % w/v) for P. aeruginosa [61]. As mentioned before, the antimicrobial activity of CTS is weak when applied alone [9-11].

The mechanism of CTS underlying the inhibition of bacterial growth, is thought to be that the cationically charged amino-group may combine with anionic components such as N-acetylmuramic acid, sialic acid and neuraminic acid, on the cell surface, and may suppress bacterial growth by impairing the exchanges with the medium, chelating transition metal ions and inhibiting enzymes [61]. As mentioned above, when the magnetite in nanocomposite content is even 40 μg.ml⁻¹ and 45 μg.ml⁻¹ for P. aeruginosa and E. coli respectively, a complete inhibition in bacteria growth was observed. At these concentrations and above, there was no evident bacteria growth. These results have demonstrated that an optimal condition for effective inhibition in bacteria growth in the present work were found at SPIO/CTS BCNC concentrations of 40 μg.ml⁻¹ and 45 μg.ml⁻¹ for P. aeruginosa and E. coli, respectively. It is interesting to note that the Fe₃O₄ colloid concentration sufficient for inhibition of bacteria growth in our work is noticeably lower than that described in the reports of the other workers [63 – 66].

This enhanced antibacterial potency is likely due to greater stability of our SPIO/CTS BCNC in aqueous medium because that CTS protected the SPIO NPs from aggregation. The excellent antibacterial activity over each of bacteria even under low Fe₃O₄ loading observed for the obtained colloidal solutions of SPIO/CTS BCNC makes them very ideal for cost effective highly antibacterial solution with long lasting effect in green industrial applications. The colloidal solutions of Fe₃O₄/CTS BCNC were found to exhibit a high antibacterial activity against gram-negative bacteria P. aeruginosa and E. coli on MH plates after 24 h at different concentrations of the magnetite nanoparticles. Therefore SPIO/CTS BCNC could be suitable for antimicrobial applications and biomedical devices.

Based on the results gathered from the agar disc diffusion test, all the SPIO/CTS BCNC and SPIO NPs films were found to exhibit a significant inhibition activity against P. aeruginosa (ATCC 27853) and E. coli (ATCC 25922). Comparison of inhibition zone diameter test for CIP (5 μg.disk⁻¹) as a standard antibiotics, CTS (0.05 wt%) as a control sample, SPIO/CTS BCNC and SPIO NPs that have content of 116 μg Fe₃O₄.disk⁻¹ on MH plate against P. aeruginosa bacterium are seen in Figure 7. Meanwhile, the inhibition zones diameters surrounding the samples were formed, with the average diameter of 16±1 mm and 12 ±1 mm against P. aeruginosa, and E. coli respectively suggesting an antimicrobial activity of SPIO/CTS BCNC. The results are 13 ±1 mm and 10 ±1 mm against P. aeruginosa, and E. coli, respectively for SPIO NPs. It is important to note that the antibacterial efficiencies against SPIO/CTS BCNC films indicate that SPIO/CTS BCNC is responsible for the antibacterial activity in the polymer NC, and this activity is strong. A similar inhibition zone diameter is not found around the CTS (0.05% which is below MIC value) film as a control against any of the bacteria using this disc. As mentioned, control experiments were also carried out in the presence of known standard antibiotics (CIP) for comparison as shown in Figure 7. The obtained results are summarized in Table 1.

![Image](image)

**Fig. 7.** Comparison of inhibition zone diameter test for (a) CIP (5 μg.disk⁻¹), CTS (0.05 wt %) and SPIO/CTS BCNC has content of 116 μg Fe₃O₄.disk⁻¹ on MH plate against P. aeruginosa bacterium and (b) CIP (5 μg.disk⁻¹), CTS (0.05 wt %) and bare SPIO has content of 116 μg Fe₃O₄.disk⁻¹ on MH plate against P. aeruginosa bacterium
There are several factors that caused the presently studied SPIO nanoparticles to be bactericidal. The main mechanism by which antibacterial drugs and antibiotics work is via oxidative stress generated by ROS [67]. ROS, including superoxide radicals (O$_2^-$), hydroxyl radicals (OH$^-$), hydrogen peroxide (H$_2$O$_2$), and singlet oxygen ($^1$O$_2$), can cause damage to proteins and DNA in bacteria [68]. Park et al. also demonstrated an antibacterial activity of silver nanoparticles to be bactericidal. The main mechanism by which these agents may find potential applications in antimicrobial treatments is via oxidative stress and because of ROS generation [69]. In this case, metal oxide Fe$_3$O$_4$ could be the source that created ROS leading to the inhibition of _P. aeruginosa_ and _E. coli_. A similar process was described by Keenan et al. in which Fe$^{3+}$ reacted with oxygen to create hydrogen peroxide [70]. This H$_2$O$_2$ consequently reacted with ferrous ions via the Fenton reaction and produced hydroxyl radicals which are known to damage biological macromolecules [56, 71]. Other research has demonstrated that the small size of nanoparticles can also contribute to bactericidal effects. For example, Lee et al. reported that the inactivation of _E. coli_ by zero-valent iron nanoparticles [72] could be because of the penetration of the small particles with sizes ranging from 10 to 80 nm into _E. coli_ membranes. Nano-Fe$_3$O$_4$ could then react with intracellular oxygen, leading to oxidative stress and eventually causing disruption of the cell membrane. However, the reduction of bactericidal activity of nano-Fe$_3$O$_4$ by oxidative corrosion will limit its application [72]. Several other studies on ZnO and MgO nanoparticles also concluded that antibacterial activity increased with decreasing particle size [56, 73 – 75].

### Table I

<table>
<thead>
<tr>
<th>Strain</th>
<th>Control sample (0.05% CTS solution)</th>
<th>Fe$_3$O$_4$ (116 μg Fe$_3$O$_4$ disk$^{-1}$)</th>
<th>Fe$_3$O$_4$/CTS (116 μg Fe$_3$O$_4$ disk$^{-1}$)</th>
<th>Standard antibiotics (5 μg CIP disk$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. aeruginosa</em></td>
<td>0</td>
<td>13±1</td>
<td>16±1</td>
<td>36±1</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>0</td>
<td>10±1</td>
<td>12±1</td>
<td>36±1</td>
</tr>
</tbody>
</table>

4. Conclusions

A one-pot co-precipitation method to produce a stable aqueous suspension of dispersed highly antibacterial and superparamagnetic Fe$_3$O$_4$ core-shell nanostructures in the presence of low MW CTS and Fe$_3$O$_4$/CTS BCNC on _P. aeruginosa_ and _E. coli_. The magnetic properties of these nanostructures were successfully utilized to isolate them from the medium by means of an external magnetic field and preventing from contamination of the environment during waste disposal.

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**Literature**

Dokonczenie ze strony 25

ItraPol i LutaPol Polskim Produktem Przyszłości

Nowe leki opracowane i produkowane w Narodowym Centrum Badań Jądrowych (NCBJ) – ItraPol i LutaPol zwyciężyły w konkursie „Polski Produkt Przyszłości”. Ich zastosowanie w medycynie nuklearnej przyczyni się do skuteczniejszego zwalczania nowotworów i poprawy jakości życia pacjentów. Pierwsze partie są już teraz w fazie produkcji.

Podczas trwającego trzy lata projektu badawczego o wartości 7,8 mln PLN, dofinansowanego z Programu Innowacji Gospodarki Europejskiego Funduszu Rozwoju Regionalnego, naukowcy NCBJ udowodnili, że dotychczas produkowane, w badawczych reaktorach jądrowych na niewielkiej skalę izotopy, w połączeniu z substancjami czynnymi (takimi jak białka) tworzą niezwykle skuteczne możliwości leczenia guzów nowotworowych. Uzyskane wyniki wykorzystali przy opracowywaniu nowych technologii otrzymywania Itru-90 i Lutetu-177. Gwarantują one wysoką aktywność właściwą (wpływającą bezpośrednio na efektywność radioterapii) oraz wysoką czystość chemiczną (ni-skoi poziomu zanieczyszczeń jonami innych pierwiastków) jako również radionuklidową. Uruchomiona w 2013 r. nowa linia technologiczna pozwoliła na wyprodukowanie pilotażowych partii ItraPolu i LutaPolu. Obecnie trwa produkcja nowych leków w skali przemysłowej. Dostawcy z tej powodzeniu w części zapotrzebowania krajowe. Szacuje się, że co najmniej 50% udziału w produkcji będzie stanowił eksport.


X Konkurs „Forum Akademickiego” rozstrzygnięty


Dokonczenie na stronie 35