ANTIMICROBIAL STUDIES ON IRON OXIDE NANOPARTICLES IN A SILICA MATRIX

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The aim of this study was to evaluate the antimicrobial activity of iron oxide nanoparticles in a silica matrix (IOTNPs) against gram-positive and gram-negative bacterial strains as well as a fungal strain. The preliminary results presented in this study revealed that the IOTNPs presented antimicrobial properties against gram positive Staphylococcus aureus 6538 and Bacillus subtilis 6683 strains. Also, the results of this study revealed that the presence of physiologic saline solution enhanced the antimicrobial activity of IOTNPs.

Keywords: iron oxide, silica matrix, tetraethylorthosilicate (TEOS), antimicrobial activity

1. Introduction

The continuous progress of science and technology has led to the development of various fields, such as nanoscience and nanotechnology. Nowadays, researchers are focused on creating hybrid nanomaterials in order to combine the properties and functions of the component materials [1]. The past few years have seen a great deal of interest in the development and study of core-shell nanocomposites [1]. Although there are studies focused on the study of

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complex nanocomposites consisting of either more than one core [1,2], more than one shell [3-4] or even no core [5], the majority of the already studied composites are formed of a solid core and a single shell [5-7].

One of the most studied materials in the last few decades have been iron oxide nanoparticles [8]. Due to their non-toxicity and magnetic properties [9-12], it has been proven that iron oxide nanoparticles are good candidates for various applications. Among the many fields that could benefit from the use of iron oxide nanoparticles, biotechnology and data storage have found these nanoparticles as very useful materials with great promise for future development [8]. On the other hand, extensive studies have been conducted on the use of iron oxide nanoparticles in biomedical applications. Researchers have concluded that this kind of materials could be used as magnetic resonance imaging (MRI) agents [8,19], as drug delivery systems [8], or in hyperthermia [8]. Also, it has been proven that they could be used for the separation and purification of biomolecules [8]. However, there are some impediments in using iron oxide nanoparticles for practical applications. Their dispersion abilities in aqueous solutions is limited due to their hydrophobic surface and their surface chemical reactivity can cause more harm than good when used in biomedical applications [8]. Also, when they come in direct contact with biological systems, a biodegradation process occurs, thus losing their original properties [8]. Another worrisome behavior of iron oxide nanoparticles is their tendency to form large aggregates, which leads to an increase of the effective particle size [8]. A solution to most of the problems induced by the characteristic of iron oxide nanoparticles is their encapsulation within a silica shell [8]. The silica shell surface can be easily functionalized, prevents the oxidation of the core nanoparticle while preserving its magnetic properties and it does not have a toxic effect on living organisms [8]. Tetraethyl orthosilicate (TEOS) is a chemical compound used mainly as a crosslinking agent, or as coating for various devices [20]. The aim of this paper is to synthesize and study the antimicrobial activity of a complex compound obtained by encapsulating iron oxide nanoparticles in a silica matrix (IOTNPs). The goal is to create an improved material with properties of the constituent materials.

2. Materials and methods

Ferrous chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$, purity 99.0%), ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$, purity 97%), chlorhidric acid (37%, HCl), ethanol absolute (≥99.8%), and tetraethylorthosilicate (TEOS) with 99.999% purity were purchased from Sigma-Aldrich.

According to Prodan et al. [13] the synthesis of iron oxide nanoparticle in silica matrix was performed as follow: first of all, the tetraethylorthosilicate was mixed with water and ethanol in order to obtain silica xerogel [21]. Then a mixture of ferrous chloride tetrahydrate in 2M HCl and ferric chloride hexahydrate was added drop by drop into silica xerogel and stirred for 1h. Finally, the formed gel based on iron oxide and silica was dried at room temperature [13]. The final product was ground to form a fine powder. The resulting fine powder was then heat treated at 500 °C in an oven, for 24 hours (IOTNPs).

A QUANTA INSPECT F microscope operating at 30 kV accelerating voltage was used for scanning electron microscopy studies. Also, the electron microscope was equipped with an energy dispersive X-ray attachment (EDAX/2001 device).

The iron oxide nanoparticle in silica matrix were investigated by Fourier transform infrared spectroscopy (FTIR) using an SP 100 Perkin Elmer spectrometer with a 4 cm^{-1} spectral resolution. The spectra were recorded in ATR (Attenuated Total Reflectance) in the range of 400-2000 cm^{-1} using a Universal Diamond/KRS-5 accessory.

The antimicrobial activity of the IOTNPs was determined against ATCC reference and clinical microbial strains. In order to evaluate the antimicrobial activity of the nanoparticles, 0.5 McFarland microbial suspensions of gram-positive (*Staphylococcus aureus* 6538, *Bacillus subtilis* 6683, *Enterococcus faecium* E5), gram-negative (*Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 27853) bacterial strains as well as a fungal strain (*Candida albicans* 393) developed on solid media were used in our experiments. The antibacterial activity was assessed on a Mueller-Hinton Agar (MHA) medium and the antifungal activity was evaluated using a Yeast Peptone Glucose (YPG) medium. The qualitative screening of the antimicrobial activity of the IOTNPs was performed using an adapted Kirby-Bauer disk diffusion susceptibility test [22,23]. Sterile disks (6 mm diameter) were impregnated with a known concentration of IOTNPs powder solubilized in dimethyl sulfoxide (DMSO) and then placed on the seeded agar plates. In order to better understand the antimicrobial activity of those types of nanoparticles, a medium containing various concentrations of 0.9% NaCl solution from Innaxon, (physiologic saline solution-PSS) was also used. To evaluate the antimicrobial activity of each sample in the presence of PSS, a 10%, 30% or 50% PSS solution was added to the sterile disks impregnated with IOTNPs powder solubilized in dimethyl sulfoxide (DMSO) before their placement in the inoculated agar plates. All samples were tested in triplicate.

3. Results and discussions

Nowadays, the occurrence of antibiotic resistant bacterial strains is considered to be a major health problem worldwide. Therefore, the need for developing new and improved antimicrobial compounds or to find alternative solutions to antibiotics in the treatment of infections is a major concern amongst scientific communities and also pharmaceutical companies. Recently, as an alternative to antibiotics in the prevention or treatment of microbial infections various types of nanoparticles have been proposed [24].

The SEM images of IOTNPs at various magnifications and the size distribution are presented in the Fig. 1.

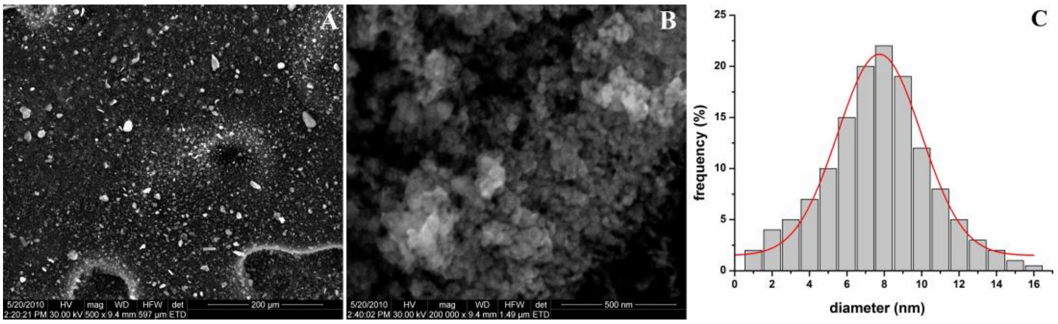

Fig. 1 SEM images of IOTNPs at various magnifications: (A) 500x and (B) 200000x. The size distribution of IOTNPs (C).

SEM images (at various magnifications) of IOTNPs revealed that the particles have nanometric dimensions and exhibit a spherical morphology (Fig. 1). More of that, the micrograph shows particles homogeneous in shape with sizes from 1 to 16 nm. Based on the SEM images, the size distribution was made. It can be seen that the average particle size is 7.8 ± 0.8 nm (Fig. 1C).

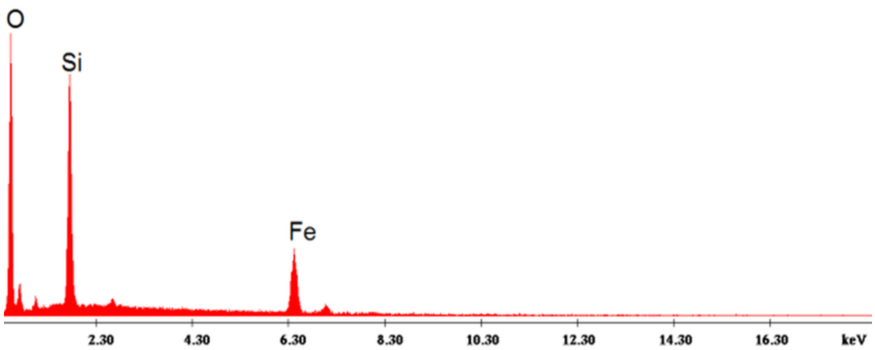

Fig. 2 EDAX spectrum of IOTNPs.

In Fig. 2 is presented the EDAX spectrum of IOTNPs. This analysis revealed that the main constituent elements of the IOTNPs are Fe, Si and O.

The FTIR spectrum of IOTNPs recorded in Attenuated Total Reflectance in the range of 450-2000 cm^{-1} is shown in Fig. 3.

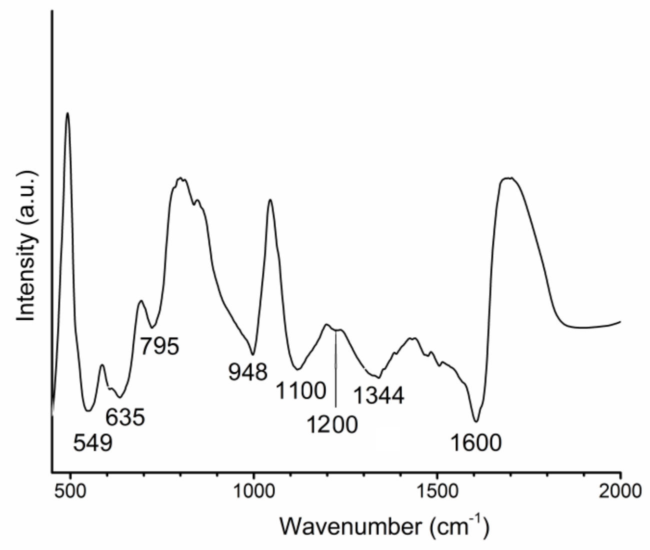

Fig. 3 Fourier transform infrared spectrum of IOTNPs recorded in Attenuated Total Reflectance in the range of 450-2000 cm^{-1}.

The main characteristic vibrational bands of a SiO_2 can be seen at 1200 cm^{-1} and 1100 cm^{-1} and were assigned to the Si-O-Si bending mode (ν_{as}) while the band at 948 cm^{-1} was assigned to the Si-O(H) [25]. The vibrational bands specific to CH_3 groups bonded at silicon atoms were observed at 785 cm^{-1} and 1344 cm^{-1} [26,27]. The band observed at 795 cm^{-1} was assigned to the Si-O-Si bending mode (ν_s) while the band at 1344 cm^{-1} was assigned to the ν_s CH_3. The band at 780 cm^{-1} characteristic vibration due to EtOH [28] and C-H bending mode (δ) are stacked with the band characteristic to Si-O-Si bending mode (ν_s). The band observed at 1344 cm^{-1} was assigned to the CH_3 (ν_s) [29]. The ν Fe-O vibrational band at 530 cm^{-1} is hidden by the overlapping with the characteristic vibration band of Si-O-Si from cyclic tetramers observed at around 540 cm^{-1}. The adsorption band of O-H-O due to the absorbed water appears at 1600 cm^{-1} [30,31]. The results of IR spectra for the prepared IOTNPs samples are presented in the Table 1.

The objective of this study was to investigate the antibacterial and antifungal activity of IOTNPs on gram-positive (*Staphylococcus aureus 6538, Bacillus subtilis 6683, Enterococcus faecium* E5), gram-negative (*Escherichia coli ATCC 8739, Pseudomonas aeruginosa ATCC 27853*) strains and a fungal strain (*Candida albicans 393*).

Table 1

Results of IR spectra for the IOTNPs materials

No.	Wave number (cm^{-1})	Assignment
1	530	ν Fe-O
2	540	ν Si-O-Si from cyclic tetramers
3	780	EtOH δ C-H
4	795	ν_s Si-O-Si
5	948	ν_{as} Si-O(H)
6	1100	ν_{as} Si-O-Si
7	1200	ν_{as} Si-O-Si
8	1600	O-H-O
9	1344	ν_s CH_3

The antimicrobial activity was evaluated in triplicate by measuring the diameters of complete inhibition zones formed around the sterile disks (6 mm diameter) impregnated with a known concentration of IOTNPs powder solubilized in DMSO and various concentrations of PSS. The results of the qualitative screening of the antimicrobial activity of IOTNPs against gram-positive, gram-negative strains and also a fungal strain are presented in Table 2. The qualitative antimicrobial activity of the tested compounds was performed using stock solutions of 1 mg/mL obtained in DMSO without the addition of 10%, 30% and 50% serum solutions. It was revealed the efficiency of IOTNPs against *Staphylococcus aureus 6538*, *Bacillus subtilis 6683* and *Candida albicans 393* strains (+) without having any effect on *Enterococcus faecium E5* and *Escherichia coli ATCC 8739* strains (-).

Table 2

Results of the qualitative screening of the antimicrobial activity of IOTNPs against different gram-positive and gram-negative bacteria, as well as fungal strains

Sample / **Microbial Strains**	IOTNPs	IOTNPs+10% PSS	IOTNPs+30% PSS	IOTNPs+50% PSS
S. aureus 6538	+	+	+	+
B. subtilis 6683	+	+	+	+
E. faecium E5	-	-	-	±
E. coli ATCC 8739	-	-	-	±
P. aeruginosa ATCC 27853	-	±	±	±
C. albicans 393	+	+	+	+

On the other hand, the results suggested that the antimicrobial activity of IOTNPs was enhanced by the addition of PSS. In consequence, when on the sterile disk was added a 10% solution of physiologic saline solution, *Pseudomonas aeruginosa ATCC 27853* showed signs of being susceptible to

IOTNPs (±). Similar results were obtained in the case of *Enterococcus faecium E5* and *Escherichia coli ATCC 8739* strains when the concentration of PSS was 50%.

Obviously, the inhibition diameter zone has an important role in the preliminary evaluation of the bactericidal effect of different compounds against various strains. The inhibition zone plays a major role in assessing whether a microorganism is susceptible or not to a compound. Hence, in this study we have also evaluated the inhibition zone diameter (mm) of the tested microbial strains in the presence of IOTNPs and in the presence of IOTNPs with 10%, 30% and 50% PSS. Table 3 presents the inhibition zone diameter obtained in the case of gram-positive (*Staphylococcus aureus 6538*, *Bacillus subtilis 6683*, *Enterococcus faecium E5*), gram-negative (*Escherichia coli ATCC 8739, Pseudomonas aeruginosa ATCC 27853*) strains and a fungal strain (*Candida albicans 393*) in the presence of IOTNPs with and without addition of PSS.

Table 3

Inhibition zone diameter (mm)

Sample / **Microbial Strains**	IOTNPs	IOTNPs +10% PSS	IOTNPs +30% PSS	IOTNPs +50% PSS
S. aureus 6538	10±1	10±1	12±1	14±1
B. subtilis 6683	10±1	10±1	12±1	14±1
E. faecium E5	0	0	6.3±1	7±1
E. coli ATCC 8739	0	0	6.3±1	7±1
P. aeruginosa ATCC 27853	0	6.2±1	6.5±1	7±1
C. albicans 393	10±1	12±1	12±1	15±1

According to the results presented in Table 3, the bactericidal effect of the IOTNPs was enhanced due to the presence of serum.

It could be observed (Table 3) that the inhibition zone diameter obtained for *Staphylococcus aureus 6538* and *Bacillus subtilis 6683* increased when the concentration of serum was 50%. Also, the presence of the serum induced an increase of the microbial inhibitory effect of the IOTNPs against *C. albicans.* The inhibition zone diameter increased from 10±1 (mm) to 15±1 (mm) in the presence of a 50% physiologic saline solution.

4. Conclusions

Our studies have confirmed that the silica matrix plays a significant role in the structural, morphological and biological properties. The IOTNPs were characterized from morphological and structural point of view using SEM and FTIR analysis. SEM and EDAX analysis revealed that nanoparticles present a spherical morphology and the main constituent elements of the IOTNPs are Fe, Si and O. The results presented in this study revealed that the IOTNPs exhibit antimicrobial properties against gram positive *Staphylococcus aureus 6538* and *Bacillus subtilis 6683* strains. The inhibitory effect against the fungal strain *C. albicans* was also evidenced. Therefore, it can be concluded that IOTNPs could be considered as potential new antimicrobial products. In addition, the influence of physiologic saline solution on the antimicrobial activity of IOTNPs was evaluated. The results highlighted that the presence and concentration of the PSS influenced the antimicrobial activity of IOTNPs. The present research offers new potential opportunities regarding the development of new antimicrobial compounds.

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