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Water soluble magnetic biocomposite with potential applications for the antimicrobial therapy

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ABSTRACT

The objective of this work was to synthesize a water soluble carrier comprised of magnetite coated with chitosan for active delivery of antibiotics. The biocomposite was characterized by FT-IR, XRD and SEM. The water soluble carrier enhanced the activity of beta-lactam antibiotics (penicillins, cephalosporins) recommended for the assessment of *in vitro* susceptibility of *Escherichia coli* and *Pseudomonas aeruginosa* strains.

Keywords: *biocomposite,, magnetite, antibiotic drug carrier, E. coli, P. aeruginosa.*

1. INTRODUCTION

The multidisciplinary area of nanobiotechnology involves primarily nanoscience, especially materials science, biology, biomedicine and biotechnology. In literature many studies are focused on the biomedical applications of magnetite nanoparticles, such as in biosensors [1], drug delivery [2,3,4], drug targeting [5], antitumoral treatments [6], stabilization of essential oils [7], magnetic resonance imaging [8,9], inhibition of biofilm development [10,11], antimicrobial therapy [12,13,14], or wound healing [15]. Also, magnetite nanoparticles have shown great potential for sorption of heavy metals in contaminated soil and water because of the small particle size, large specific surface area, and high sorption capacity and affinity [16,17]. Magnetite nanoparticles have a natural tendency to interact with each other and form aggregates, due to the magnetic attractive forces between the magnetic particles and to the Van der Walls long range attractive forces between the particles. These interactions cause precipitation. To counteract these interactions and promote the stability of the colloidal solution, creating a steric hindrance between the particles is required and could be achieved by electrostatic repulsion between the particles formed by coating the particles with polymers [18]. The homogeneous dispersion of magnetite nanoparticles with orientation structure in polymer matrix became a key problem for obtaining high-performance hybrid materials [19]. Here we report a simple method to prepare water soluble biocomposites, which have been furthermore tested for their biological activities. They proved to be efficient macromolecular carriers for antibiotics, as shown by the improvement of some currently used antibiotics activity against Gram-negative bacterial strains.

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2. EXPERIMENTAL SECTION

2.1. Materials. Ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$), ferric chloride ($FeCl_3$), ammonia (NH_3), methanol (CH_3OH) and chitosan (CS, 85 % deacetylated) were purchased from Sigma-Aldrich.

2.2. Fabrication. Magnetic iron oxide particles are usually prepared by wet chemical precipitation from an aqueous iron salt solution by means of alkaline media, like HO^- or NH_3 [20,21,22]. One gram of chitosan powder was added into 100mL acetic acid aqueous solution with a concentration of 2% under vigorous stirring for 15 minutes, and then 400mL of magnetite precursor containing $FeCl_3$ and $FeSO_4x7H_2O$ solution with the ratio of $Fe^{3+}/Fe^{2+}= 2$ was added into the chitosan solution. The mixture was stirred and dropped into the 200mL of NH_3 solution (2%) leading to the formation of a black precipitate. The product was repeatedly washed with ultrapure water and methanol and subsequently dried in oven at 60°C until reaching a constant weight. In order to obtain the water soluble magnetic biocomposite, one gram of it was grounded in the presence of 10mL of ultrapure water and 10μL acetic acid 1N. The result is plotted in figure 1.

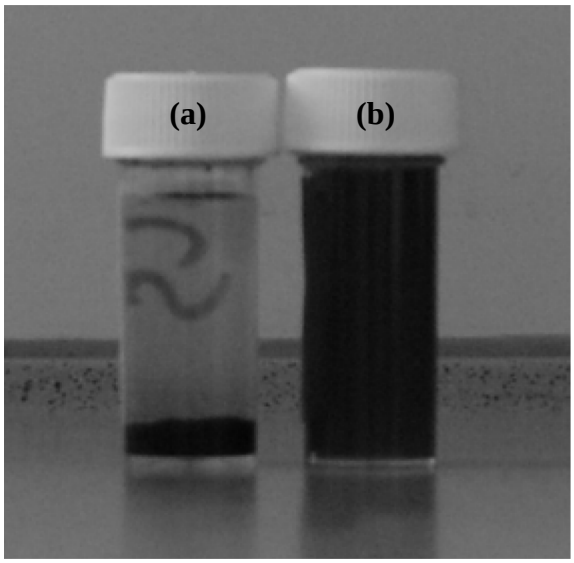

Figure 1: Images of precipitated product in basic media (a) and water soluble biocomposite (b)

2.3. Characterization

2.3.1. FT-IR. A Nicolet 6700 FT-IR spectrometer (Thermo Nicolet, Madison, WI) connected to software of the OMNIC operating system (Version 7.0 Thermo Nicolet) was used to obtain FT-IR spectra of hybrid materials. The samples were placed in contact with attenuated total reflectance (ATR) on a multibounce plate of ZnSe crystal at controlled ambient temperature (25oC). FT-IR spectra were collected in the frequency range of 4,000–650 cm-1 by co-adding 32 scans and at a resolution of 4 cm^{-1} with strong apodization. All spectra were ratioed against a background of an air spectrum. After every scan, a new reference air background spectrum was taken. The plate was carefully cleaned by wiping with hexane twice followed by acetone and dried with soft tissue before filling in with the next sample. The spectra were recorded as absorbance values at each data point in triplicate.

2.3.2. XRD. X-ray diffraction analysis was performed on a Shimadzu XRD 6000 diffractometer at room temperature. In all the cases, Cu Kα radiation from a Cu X-ray tube (run at 15 mA and 30 kV) was used. The samples were scanned in the Bragg angle 2θ range of 10-80.

2.3.3. SEM. SEM analysis was performed on a HITACHI S2600N electron microscope, at 25 keV, in primary electrons fascicle, on samples covered with a thin silver layer.

2.4. Assessment of the biocomposite influence on the activity of some currently used antibiotics. An adapted diffusion method according to recently published papers [23, 24, 25, 26] was used in order to assess the potentiating effect of the biocomposite on the antimicrobial activity of piperacillin-tazobactam (TZP), cefepime (FEP), piperacillin (PIP), imipenem (IPM), gentamicin (CN), ceftazidime (CAZ) against *Pseudomonas. aeruginosa* ATCC 27853 and cefazolin (KZ), cefaclor (CEC), cefuroxime (CXM), ceftriaxone (CRO), cefoxitin (FOX), trimethoprim/ sulfamethoxazole (SXT) against *Escherichia coli* ATCC 25922 strains. The tested antibiotics have been chosen according to CLSI recommendations. Standardized antibiotic discs have been placed on the Mueller Hinton agar medium distributed in Petri dishes previously seeded with a bacterial inoculum with a density corresponding to the 0.5 McFarland standard. Five µL of the stock solutions of the dispersed biocomposite were spotted over the antibiotic disks. The plates were incubated 24 h at 37 °C, and the growth inhibition zone diameters for each antibiotic, after the addition of the tested biomaterial suspensions were quantified and compared with the growth inhibition zones obtained for the respective antibiotics alone.

3. RESULTS SECTION

The use of nanoparticles in the development of delivery systems for active molecules, including drugs has been studied extensively over the past decade [27]. Nanostructures based on an inorganic core of iron oxide, such as magnetite (Fe_3O_4) or maghemite (γ-Fe_2O_3), coated with a polymer such as dextran, chitosan (CS), poly(ethylenimine) (PEI), poly(ethylene glycol) (PEG), or copolymers, such as (PEI-PEG-CS) were obtained to enhance the nanoparticles bioavailability and their capture in capillary tissue [28, 29, 30]. The objective of this work was to synthesize a carrier comprised of magnetite nanoparticles coated with chitosan for active delivery of antibiotics.

FT-IR spectrum of the water soluble biocomposite is plotted in Figure 1b. In order to compare the chitosan bands with the bands of the obtained biocomposite, a spectrum of pure chitosan was also recorded (figure 1a).

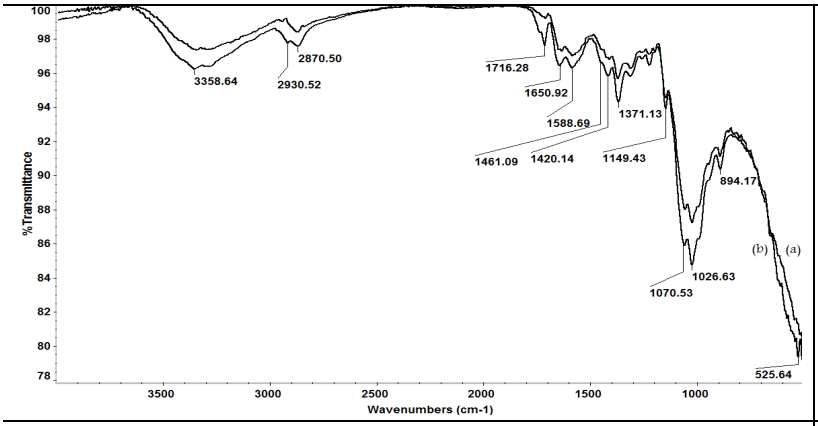

Figure 2: FT-IR spectra of chitosan (a) and fabricated water soluble biocomposite (b)

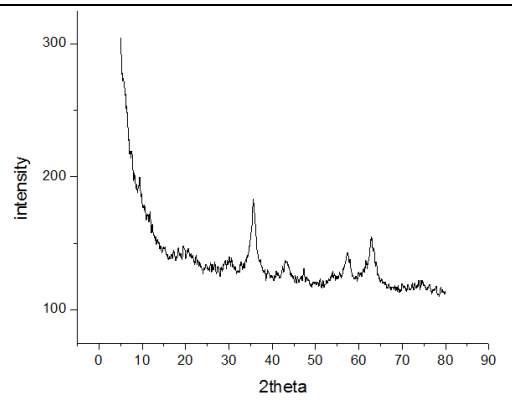

Figure 3: XRD pattern of fabricated water soluble biocomposite

The main bands appearing in the spectrum of chitosan powder are due to stretching vibrations of OH groups in the range from 3600 cm^{-1} to 3000 cm^{-1} and C–H bond in $–CH_2$ (2930 cm^{-1}) and $–CH_3$ (2870 cm^{-1}) groups, respectively. Bending vibrations of methylene and methyl groups were also visible at 1371 cm^{-1} and 1460 cm^{-1}, respectively [31]. The range of 1680–1480 cm^{-1} was related to the vibrations of carbonyl bonds (C=O) of amide group C(=O)NHR (secondary amide, 1660 cm^{-1}) and to the vibrations of amine group NH_2, 1588cm^{-1} [32]. The band located near 1149 cm^{-1} is

related to asymmetric vibrations of C-O in oxygen bridge. The bands near 1080–1026 cm^{-1} are attributed to CO of the ring COH, COC and CH_2OH [33].

The crystalline property of the sample was investigated by powder X-ray diffraction (XRD). The XRD pattern of the sample is shown in Figure 3. The diffraction peaks appeared at 2theta = 35.71^o, 43.31^o, 57.61^o and 62.81^o in the water soluble biocomposite correspond to the scattering from (311), (400), (511) and (440) plane of magnetite lattice, respectively. The iron oxide existing in the fabricated biocomposite was identified as magnetite. XRD results are in agreement with reported literature [34,35].

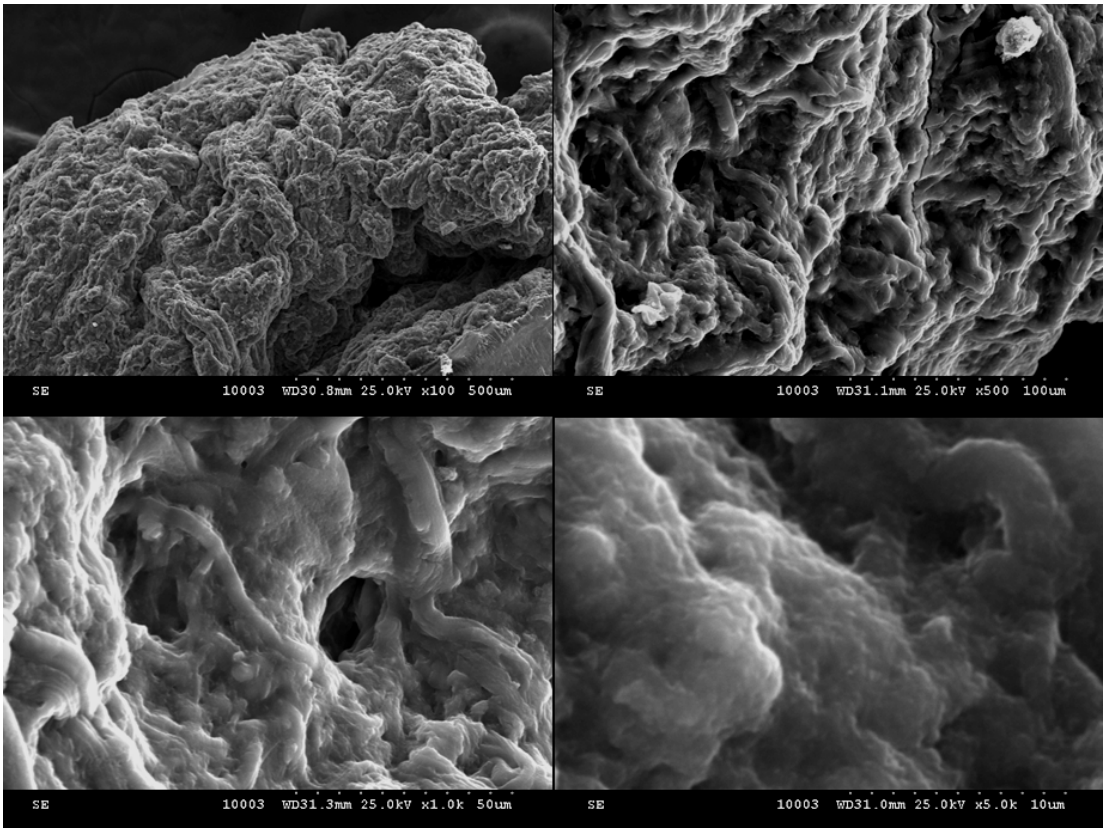

Figure 4: SEM micrographs of fabricated water soluble biocomposite

The SEM micrographs of water soluble biocomposite exhibited a microporous structure. Magnetite was successfully integrated in to the polymer matrix with no visible agglomerate formation at low particle amounts. SEM images indicated that the fibers are oriented randomly in all direction with the thickness always small. The pore dimensions are non-uniform with thin walls and are randomly dispersed in the polymer matrix.

When a foreign particle, especially one with a highly hydrophobic surface, enters the bloodstream it is opsonized and removed by the body's reticulo-endothelial system [36]. In order to avoid the rapid elimination of hydrophobic nanoparticles from the body, they could be stabilized by adding a layer of hydrophilic material [37]. Chitosan is a hydrophilic amino-saccharide polymer that can be used to coat the iron particles and increase their circulation time in the bloodstream [38].

In this study we have assessed the potential of the soluble biopolymers based on magnetite and chitosan to improve the antimicrobial activity of the antibiotics currently used for the treatment of Gram-negative infections.

In case of antibiotics recommended to be tested for *E. coli*, the obtained biocomposite improved the activity of first (KZ) and second generation (CEC, CXM) cephalosporins (Figure 5), while in case of

P. aeruginosa, enlarged bacterial growth inhibition diameters were registered for anti-pseudomonal penicillins (TIC), beta-lactam and beta-lactamase inhibitors associations (TZP) and fourth generation cephalosporins (FEP) (Figure 6).

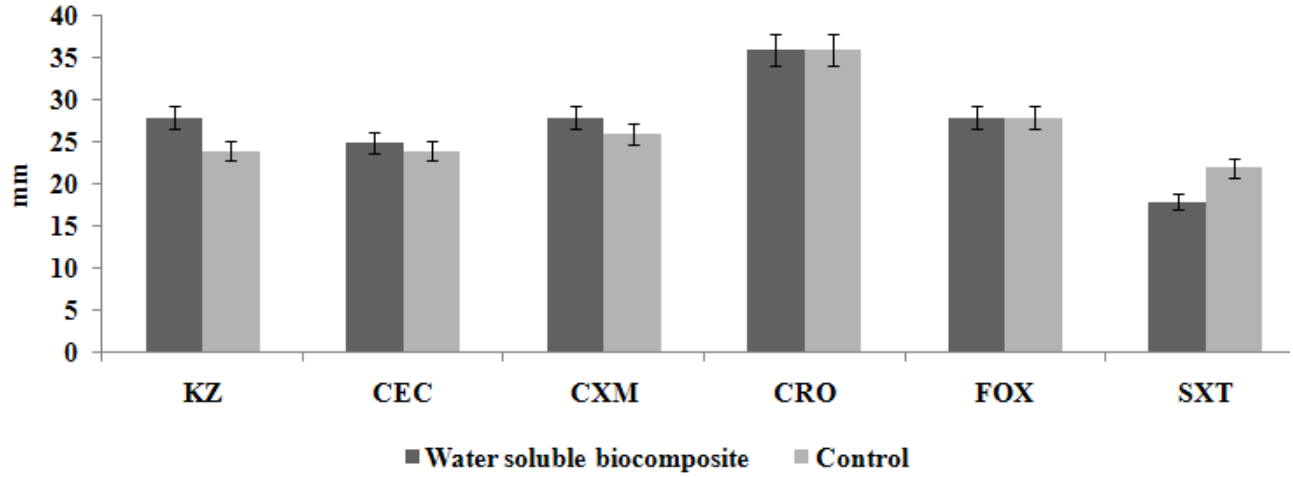

Figure 5: The growth inhibition zone diameters (mm) obtained for the tested antibiotics in the presence of water soluble biocomposite on the *E. coli* ATCC 25922 strain

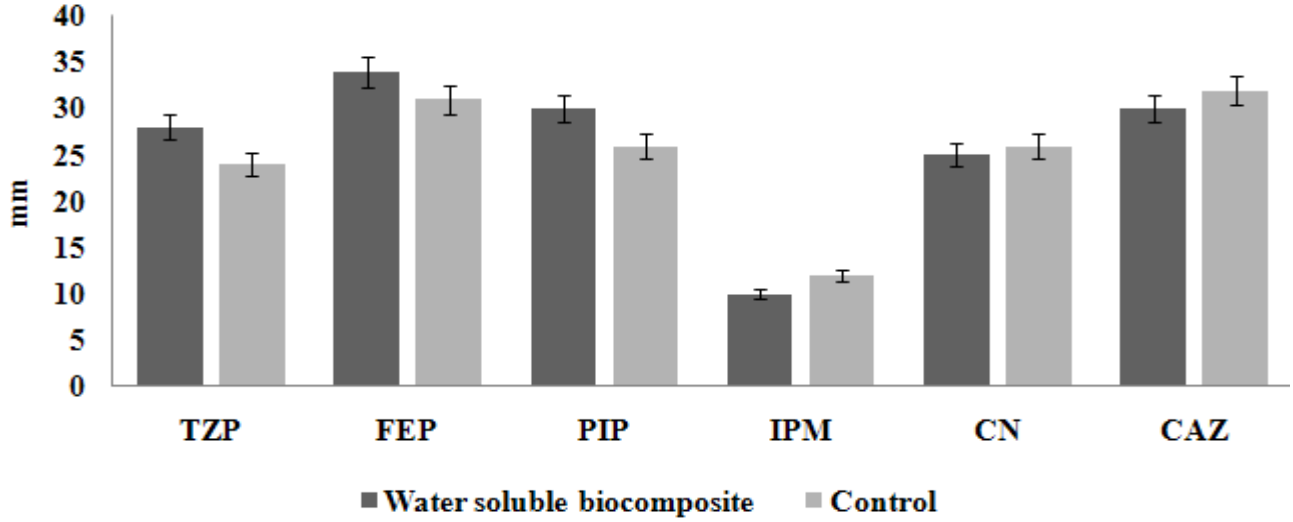

Figure 6: The growth inhibition zone diameters (mm) obtained for the tested antibiotics in the presence of water soluble biocomposite on the *P. aeruginosa* ATCC 27853 strain

However, the antimicrobial activity of SXT, IMP, CN and CAZ was slightly reduced in the presence of the obtained water soluble biocomposite, demonstrating that the potentiating effect of the proposed carrier on the antibiotics activity is based on specific interactions between the carrier shells and different antimicrobial structures.

4. CONCLUSIONS

The obtained water soluble biocomposite based magnetite and chitosan enhanced the activity of the majority from the tested beta-lactam antibiotics (penicillins, cephalosporins) recommended for the assessment of the *in vitro* susceptibility of *Escherichia coli* and *Pseudomonas aeruginosa* strains, and slightly reduced the efficiency of other antibiotics, demonstrating the existence of specific interactions established between the biocomposite components and different antimicrobial structures.

5. ACKNOWLEDGMENT

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