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In vitro efficacy of antibiotic magnetic dextran microspheres complexes against *Staphylococcus aureus* and *Pseudomonas aeruginosa* strains

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ABSTRACT

This paper describes the direct precipitation of magnetite on the surface of dextran as a method to improve the efficiency of this polymer in delivering antibiotics in active forms. FT-IR, SEM, XRD and *in vitro* biological assay were used to characterize the structure, composition, and the capacity of the magnetic polymeric microspheres to improve the antimicrobial activity of some antistaphylococcal and apntipseudomonal drugs. Our results demonstrated that the magnetic dextran microspheres could be used as macromolecular carriers for large-spectrum antibiotics, particularly for those with small, polar molecules belonging to penicillins, aminoglycosides, rifampicines and quinolones classes. The obtained results are suggesting that the size and the electric charge of the active drugs are influencing the specific interactions between the drug carrier and the active substance.

Keywords: magnetic microspheres, antimicrobial activity, *S. aureus, P aeuruginosa*

1. INTRODUCTION

In the last years, magnetic nanoparticles have found an increasing interest in biomedical and engineering applications [1,2,3]. The reactivity of iron oxide particles has been shown to greatly increase as their dimensions are reduced, and they may undergo rapid biodegradation when they are directly exposed to the biological system [4,5]. Magnetic materials are used for delivering the drug carrier, represented by inorganic materials (ussualy magnetite, maghemite, cobalt ferrite, chromium dioxide) [6, 7] or polymers [8, 9, 10], to the specific site. Thermal stability, resistance to solvents and microbial attack, ease of manufacture and excellent shelf life make inorganic materials ideal supports. But they have limited functional groups for selective binding [11,12]. The substances with biological activity are thus connected to the magnetite core through an organic or polymeric shell. The shells are biocompatible in general [13] or possess active groups which can be conjugated to biomolecules [14]. Dextran is a polysaccharide and has many significant biological advantages such as being biodegradable, biocompatible and bioactive. Dextran is water soluble, inert in biological systems and does not influence cell viability [15,16]. This paper describes the direct precipitation of magnetite on the surface of dextran as a method to improve the efficiency of dextran in delivering antibiotics in active forms. FT-IR, SEM, XRD and *in vitro* biological assay were used to characterize

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the structure, composition, and capacity of the magnetic microspheres to improve the activity of some usual antibiotics against *S. aureus* and *P. aeruginosa* reference strains.

2. EXPERIMENTAL SECTION

2.1. Preparation of magnetic microspheres. Magnetic iron oxide particles are usually prepared by wet chemical precipitation from aqueous iron salt solutions by means of alkaline media, like NH_3 [17]. In the present paper, magnetic microspheres were prepared by a modified precipitation method. Five grams of dextran were dispersed in a known volume of distilled-deionized water, corresponding to a 1.00% (w/w) solution, under stirring at room temperature. Then, 8 mL of a basic aqueous solution consisting of 28% NH_3 were added to dextran solution. After these, 200 mL $FeSO_4/FeCl_3$ (1.25/0.625-w/w) were dropped under permanent stirring up to pH = 8, leading to the formation of a black precipitate. The product was repeatedly washed with methanol and subsequently dried in oven at 60 °C until reaching a constant weight.

2.2. FT-IR analysis. A Nicolet 6700 FT-IR spectrometer (Thermo Nicolet, Madison, WI) connected to the 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 (25^oC). 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 rationed 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. Scanning Electron Microscopy. The magnetic microspheres were assessed by SEM analysis that was performed on a HITACHI S2600N electron microscope, at 15 keV, in primary electrons fascicle, on samples covered with a thin silver layer.

2.4. X-Ray Difractometry. 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.

2.5. The assay of the antimicrobial activity of the magnetic microspheres. An adapted diffusion method was used in order to assess the potentiator effect of the magnetic microspheres on the antimicrobial actitivity of VA (vancomycin), DA (clindamycin), AZM (azithromycin), OX (oxacyllin), SXT (trimethoprim/sulfamethoxazole), RA (rifampicin), OFX (ofloxacin), TE (tetracycline), P (penicillin), CIP (ciprofloxacin), GM (gentamicin), TZP (piperacillin/tazobactam), FEP (cefepime), ATM (aztreonam), CAZ (ceftazidim) and PRL (piperacillin) against *S. aureus* ATCC 25923 and *P. aeruginosa* ATCC 27853 strains. The tested antibiotics have been chosen according to CLSI reccomandations. 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 magnetic micropsheres were spoted over the antibiotic disks. The plates were incubated 24h at 37°C, and the inhibition zones diameters for each antibiotic, after the addition of the tested microsphere suspensions were quantified and compared with the growth inhibition zones obtained for the respective antibiotics.

3. RESULTS SECTION

The XRD pattern (figure 1) of the synthesized composite material proves the formation of Fe_3O_4; the most important peaks of magnetite being centered at 2θ = 30.08, 35.43, 43.05, 53.41, 56.94 and 62.62°. Based on the lack of additional peaks it can assume that magnetite is the unique crystalline phase from the composite material.

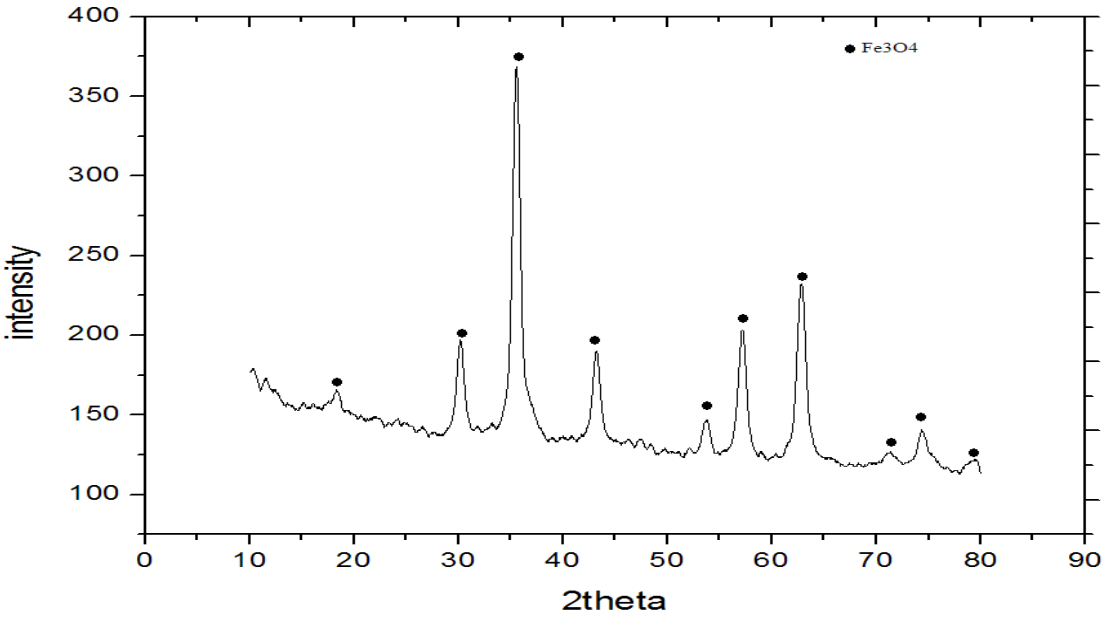

Figure 1: XRD pattern of dextran/Fe_3O_4

FT-IR spectrum of dextran and magnetic microspheres are presented in Figure 2. The absorbance band in the region of 3300 cm^{-1} was due to the hydroxyl stretching vibration of the polysaccharide [18]. The band in the region of 2915 cm^{-1} was due to C–H stretching vibration and the one in the region of 1635 cm^{-1} to carboxyl group [19]. The main characteristic bands found in the spectra of dextran at 1003 cm^{-1} are due to valent vibrations of C–O and C–C bonds and deformational vibrations of the CCH, COH and HCO bonds. The presence of the peak at 1003 cm^{-1} is due to the great chain flexibility present in dextran around the α(1→6) glycosidic bonds as shown by Shingel [20]. FT-IR spectral analysis of magnetic microspheres confirmed the all above mentioned data.

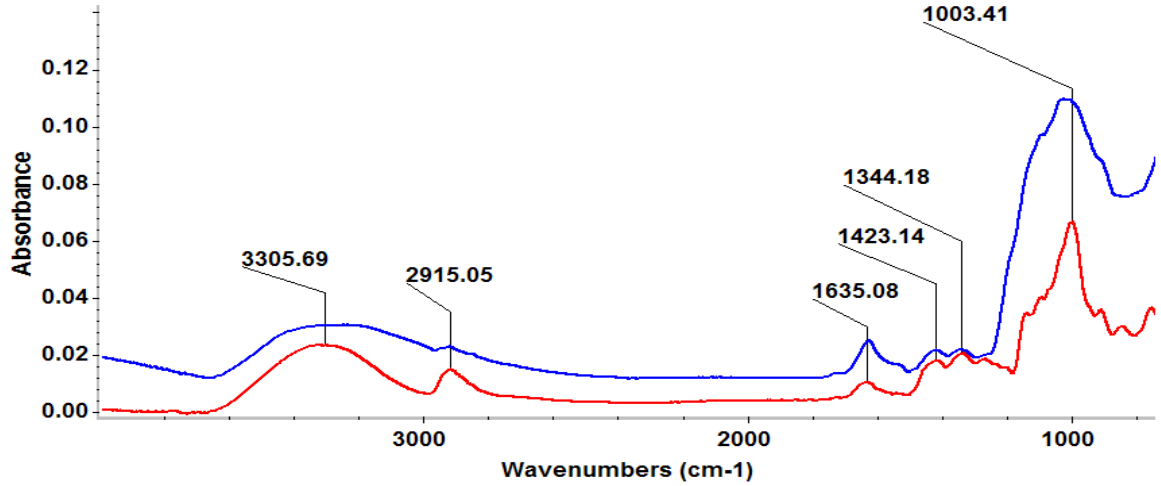

Figure 2: FT-IR spectrum of dextran (red line) and magnetic microspheres (blue line)

The morphology and size of the magnetic microspheres were investigated using SEM (Fig. 1 a, b, c). The magnetic microspheres are well shaped spheres with a rather rough surface. Fig. 1(d) shows the

surface of the microspheres, and it can clearly be seen that Fe_3O_4 particles have been deposited on the surface of the microspheres. The size of the magnetic microspheres ranges between 50 and 80 µm.

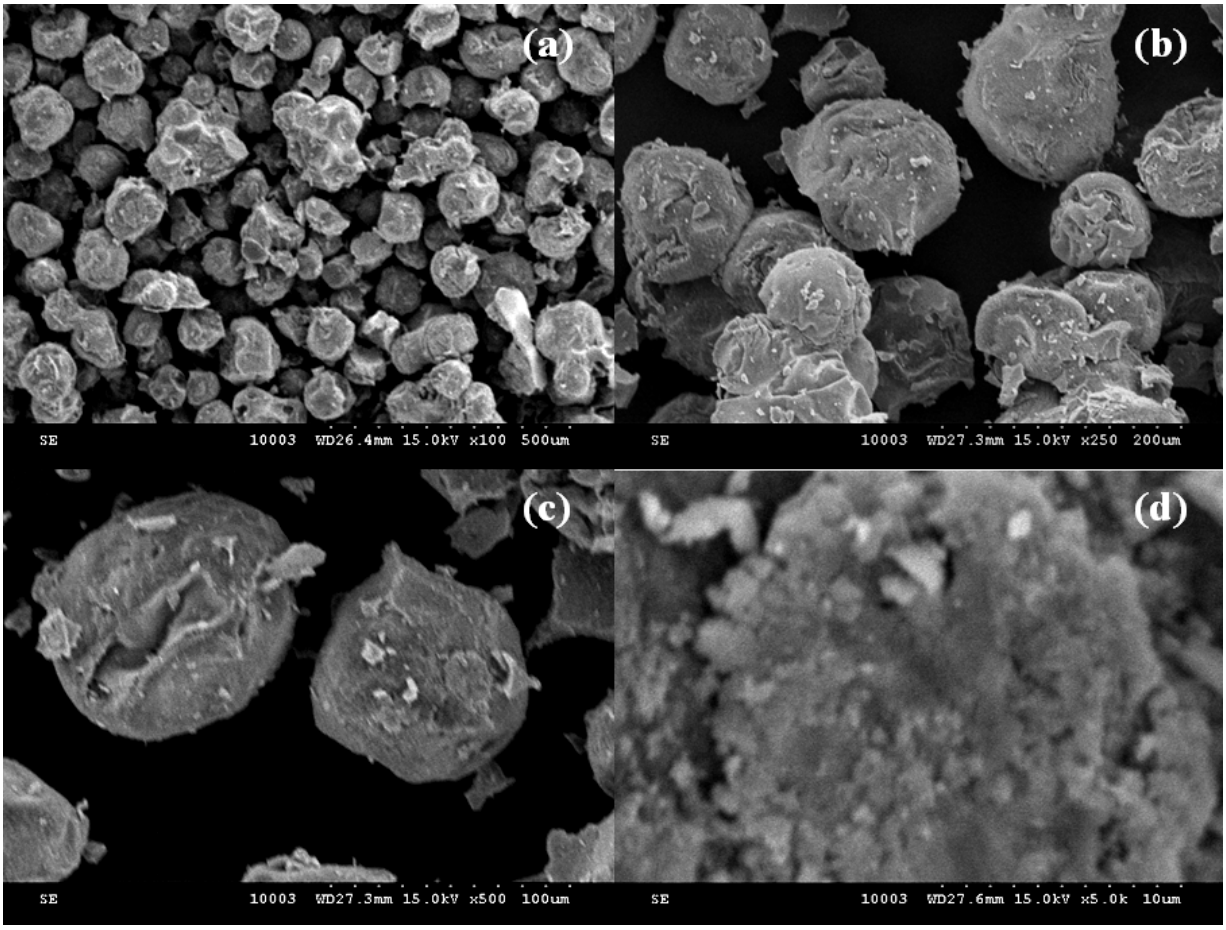

Figure 3: SEM micrographs of magnetic microspheres

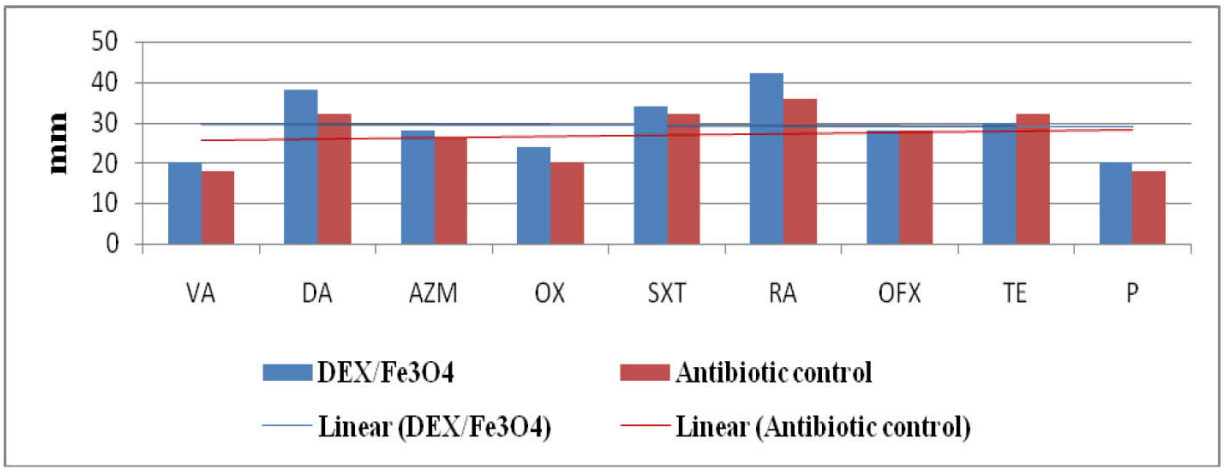

Figure 4: The growth inhibition zone diameters (mm) obtained for the tested antibiotics in the presence of DEX/Fe_3O_4 on the *S. aureus* ATCC 25923 strain

The magnetic dextran microspheres slighlty improved the antimicrobial activity of the majority of anti-staphylococcal drugs, excepting ofloxacin for which the zone diameter was not changed and tetracycline, whose activity was slighlty decreased by the addition of magnetic dextran microspheres (figure 4). The most significant improvement of the antimicrobial activity, highlighted by the

enlargement of the growth inhbition zones with 20% was obtained for clindamycin and rifampicin. These results are demonstrating the specific interaction of the proposed delivery system with different antibiotics, and the necessity to develop „personnalized" drug carriers for different therapeutic substances.

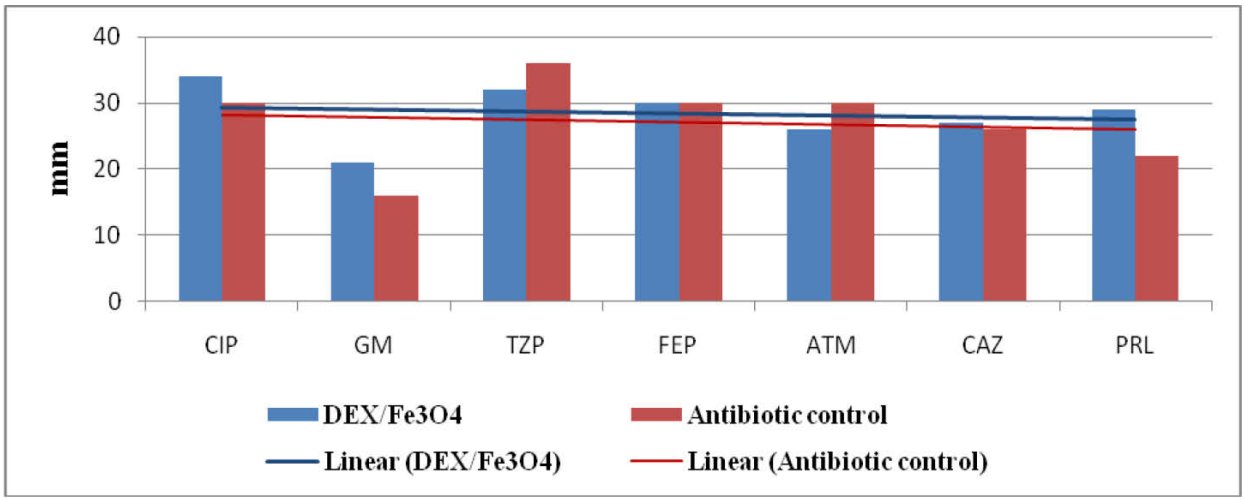

Figure 5: The growth inhibition zone diameters (mm) obtained for the tested antibiotics in the presence of DEX/Fe_3O_4 on the *P. aeruginosa* ATCC 27853 strain

Concerning the antipseudomonal drugs, an increased antimicrobial activity in the presence of the magnetic dextran nanospheres was noticed for ciprofloxacin, gentamycin and piperacillin, for which the zone inhibition diameters have significanlty increase with 4, 5 and repectively 7 mm (Fig. 5). Surprinsingly, although the piperacillin was the most improved antibiotic, the microspheres suspension inhibited the activity of piperacillin, in combination with tazobactam, these results suggesting that the molecular and the antibiotic conformation are influencing the specific interactions between the drug carrier and the active substance.

Taken together, the antibiotics positively influenced in their biological activity by the magnetic dextran microspheres suspension were: clindamycin, rifampicin, ciprofloxacin, gentamycin and piperacillin. The majority of these antibiotics are small, polar molecules with large-spectrum antimicrobial activity. Out of these antibiotics, clindamycin, rifampicin and ciprofloxacin are also acting against intracellular pathogens, which are difficult to destroy and leaving the host cells intact. One of the major challenges of drug delivery research field is to design conjugates able to target and to deliver into the infected cells in an active form [21,22]. Dextran microspheres could act as macromolecular carriers for the small molecules antibiotics and induce the endocytosis of the drug by the target cell via a specific receptor and, following this first step, subcellular distribution of the drug to sites where the microbial cells are localized. However, in order to demonstrate this hypothesis, the present results, proving the *in vitro* efficacy of the different antibiotics associated with magnetic dextran must be completed with the *in vivo* assessment of the efficacy of these conjugates against intracellular bacterial pathogens.

4. CONCLUSIONS

Our results demonstrated that the magnetic dextran microspheres could be used as macromolecular carriers for large-spectrum antibiotics, especially for those with small, polar molecules belonging to penicillins, aminoglycosides, rifampicines and quinolones classes. The

obtained results are suggesting that the size and the electric charge of the active drugs are influencing the specific interactions between the drug carrier and the active substance.

5. ACKNOWLEDGMENT

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