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Magnetic chitosan for drug targeting and *in vitro* drug delivery response

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ABSTRACT

The magnetic targeted drug delivery system is one of the most attractive strategies of delivering drugs to the area of interest. Magnetic drug targeting is based on using magnetic drug carrier particles to selectively deliver drugs to a specific site inside the body by using an external magnet field to attract and retain them there. Our study was focused to the synthesis, characterization and in vitro drug delivery response of magnetic hybrid material based Fe_3O_4/chitosan/cephalosporins (Cefepime, Ceftriaxone, Cefuroxime, Cefoperazone, Cefpirome, Cefaclor). Magnetic materials were characterized by CLSM (Confocal Laser Scanning Microscopy) and µATR-FT-IR (Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy). All these hybrid materials have been prepared in order to develop a magnetic drug delivery system and can be utilized to facilitate the targeted drug delivery of cephalosporins. The hybrid materials are obtained under mild conditions without any organic solvents and surfactants, which are more suitable for pharmaceutical applications.

Keywords: *chitosan, cephalosporins, magnetite, biomaterial, drug targeting, drug delivery*

1. Introduction

Superparamagnetic iron oxide (Fe_3O_4) nanoparticles have attracted researchers in various fields such as physics [1], medicine [2], biology [3] and materials science [4] due to their multifunctional properties such as small size, superparamagnetism and low toxicity [5]. Fe_3O_4 nanoparticles tend to aggregate due to strong magnetic dipole–dipole attractions between particles. Stabilizers such as surfactants [6], or polymeric compounds [7] with some specific functional groups have been used to modify these nanoparticles to increase the stability. In the last decade, magnetic nano- and micro-particles received a lot of attention related to their use in biomedical applications, such as diagnosis, separation and purification of biomolecules, or carriers for drug delivery [8]. Chitosan, poly [b-(1-4)-linked-2-amino-2-deoxy-D-glucose], is a non-toxic, hydrophilic, biocompatible, biodegradable and anti-bacterial [9] product, obtained by partial deacetylation of chitin in alkaline conditions, a natural cationic polyaminosaccharide polymer. Due to its biocompatibility and advantageous functional groups (amino and hydroxyl), chitosan is widely used in medicine (wound dressing material, drug and gene delivery vehicle, candidate for tissue engineering), agriculture, food, biotechnology and

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water treatment [10,11]. In this paper, we report the preparation, characterization and in vitro drug delivery response of novel hybrid materials - Fe_3O_4/Chitosan/cephalosporins.

2. Experimental section

2.1. Synthesis of Fe_3O_4/Chitosan. Fe_3O_4/Chitosan was prepared by chemical co-precipitation of Fe^{2+} and Fe^{3+} ions in aqueous solution of sodium hydroxide. In brief, chitosan was added in aqueous solution of NaOH and then Fe(II) and Fe(III) (in 1:2 molar ratio) were dissolved in ultrapure water and were added drop-wise under constant stirring at 25 °C. Inorganic/organic hybrid material was separated by applying a magnetic field, and washed several times in water and then in ethanol. The purification step was used to remove impurities from Fe_3O_4 nanoparticles synthesis and exces of chitosan. The hybrid material were finally dried at 50° ($chitosan_{50}$) and 100 °C ($chitosan_{100}$). After these, the magnetic hybrid material were prepared for CLSM characterization, as a *wet mount preparation*-like sample, meaning that the suspended chitosan was droped on a microscopical slide, covered with a coverslip and sealed around with polymer adhesive until further analysis.

2.2. Antimicrobial agents. Cephalosporins were chosen from the commercially available class of β-lactams antibiotics: Cefepime, Ceftriaxone, Cefuroxime, Cefoperazone, Cefpirome, Cefaclor.

2.3. Preparation of Fe_3O_4/Chitosan/cephalosporins hybrid material for drug targeting. After drying in oven at 50° and 100°C for 24 h, the magnetic hybrid materials were dispersed in minimum quantity of ultra-pure water and then the cephalosporins were added. Finally it was dried to 40°C for 6 h. The concentration of deposited cephalosporins was 10 %.

2.4. Characterization of magnetic chitosan hybrid material.

2.4.1. CLSM characterization. The surface morphology of chitosan was carried out before and after magnetite coating by using Leica microscope (TCS-SP CSLM), equipped with PL FLUOTAR (40X NA0.5, electronic zoom 1) and an He-Ne laser tuned on 633 nm wavelength. Samples were comparatively visualized, in reflection and transmition mode. Leica software was used for surface topography.

2.4.2. FT-IR analysis. 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 (25°C). 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.

2.5. In vitro drug delivery response. *Escherichia coli* ATCC 259922 and *Staphylococcus aureus* ATCC 25923 references bacterial strains were used in the study. The inocula were obtained from 18 hrs microbial cultures on solid media and adjusted to 0.5 McFarland standard. Qualitative screening of the susceptibility of different microbial strains to magnetic chitosan/cephalosporins has been accomplished through an adapted diffusion method, on Mueller Hinton agar medium [12]. In this purpose, 5 µl from a stock solution of the tested product, containing 30 µl of antibiotic were distributed in spots on previously seeded Petri plates. The result reading was performed by measuring the bacterial growth inhibition zones diameters around the spots. The used solvent, dimethyl sulfoxide (DMSO) [13], was comparatively tested for its potential antimicrobial activity.

3. Results section

3.1. CLSM characterization of hybrid inorganic/organic material. Qualitative analysis of hybrid material provides a spatial exploration of microparticles in both transmission and reflection mode which revealed the chitosan specific shape and the boundary edges containing antibiotic functionalized ferrite with no semnificatively differences between samples with different termic treatment (figure 1).

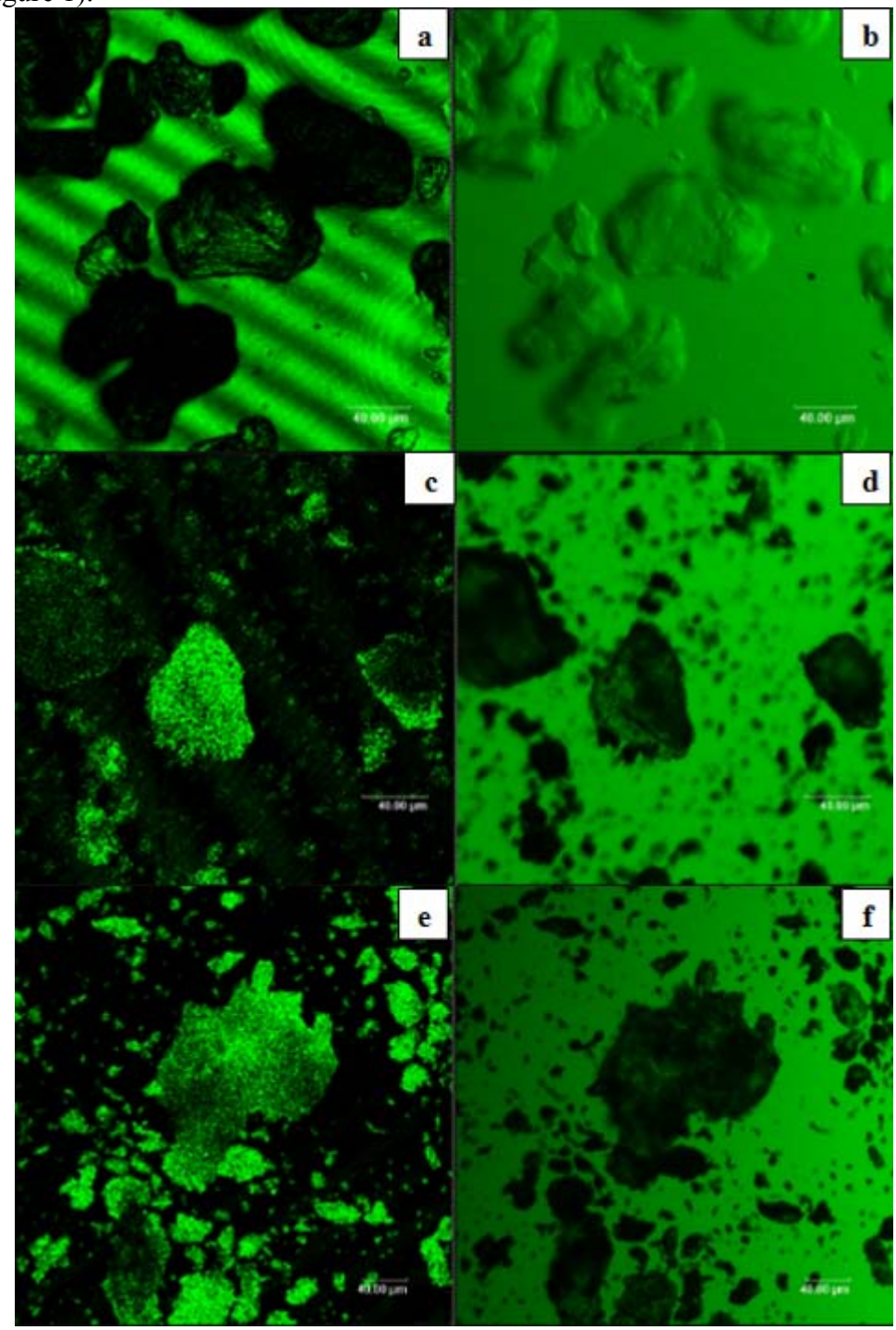


Figure 1. Hybrid inorganic/organic material - Chitosan sample(a, b), Fe_3O_4/Chitosan$_{50}$(c, d), Fe_3O_4/Chitosan$_{100}$(e, f)

3.2. FT-IR characterization. Characteristic peaks assignment of Chitosan and Fe_3O_4/Chitosan are: 3354 cm^{-1} (O-H stretch overlapped with N-H stretch), 2921 and 2867 cm^{-1} (C-H stretch), 1640 cm^{-1} (amide II band, C-O stretch of acetyl group), 1592 cm^{-1} (amide II band, N-H stretch) 1485–1380 cm^{-1} (asymmetric C-H bending of CH_2 group) and 1035 cm^{-1} (bridge O stretch) of glucosamine residue.

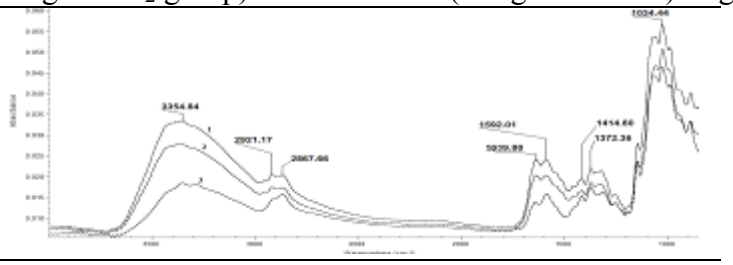


Figure 2: FT-IR spectra of Chitosan (1), Fe_3O_4/Chitosan$_{50}$ (2) and Fe_3O_4/Chitosan$_{100}$

To evidence the deposition of cephalosporins on the surface of Fe_3O_4/Chitosan, FT-IR spectra were plotted in Figure 2 and 3. The changes in area of the bands and many peaks in the "fingerprint" region between 1600 and 1200 cm^{-1} was observed. The "fingerprint" region of the spectra from the reference (1) and modified Chitosan$_{100}$/cephalosporins (2-7) regions shows clear differences after deposition of cephalosporins.

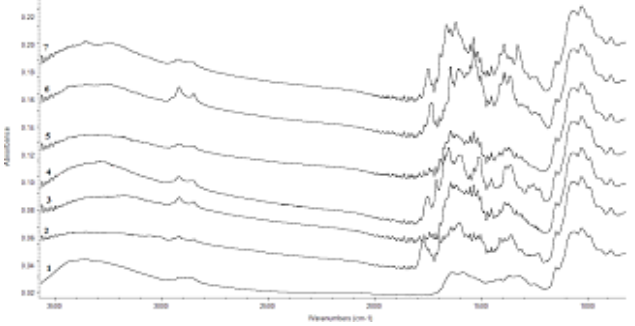


Figure 2: FT-IR spectra of Fe_3O_4/Chitosan$_{50}$ (1), Fe_3O_4/Chitosan$_{50}$/cefaclor (2), Fe_3O_4/Chitosan$_{50}$ /cefepime (3), Fe_3O_4/Chitosan$_{50}$/cefoperazone (4), Fe_3O_4/Chitosan$_{50}$/cefpirome (5), Fe_3O_4/Chitosan$_{50}$/ceftriaxone (6), Fe_3O_4/Chitosan$_{50}$/cefuroxime (7)

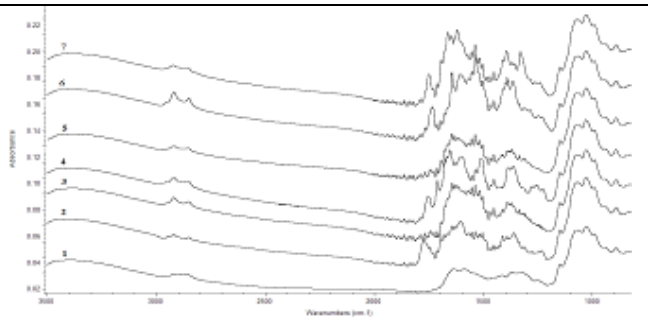
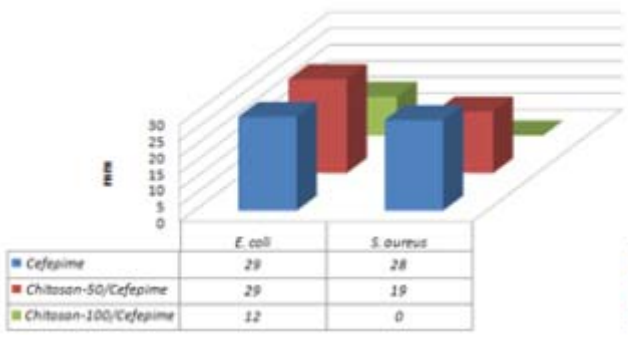
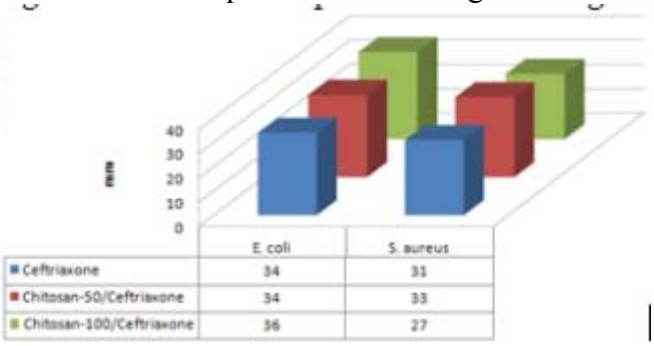


Figure 3: FT-IR spectra of Fe_3O_4/Chitosan$_{100}$ (1), Fe_3O_4/Chitosan$_{100}$/cefaclor (2), Fe_3O_4/Chitosan$_{100}$ /cefepime (3), Fe_3O_4/Chitosan$_{100}$/cefoperazone (4), Fe_3O_4/Chitosan$_{100}$/cefpirome (5), Fe_3O_4/Chitosan$_{100}$/ceftriaxone (6), Fe_3O_4/Chitosan$_{100}$/cefuroxime (7)

3.3. *In vitro* drug delivery response. The antimicrobial effect of tested cephalosporins was quantifyble in all tested variants as inhibition zones around the spots (figure 4). Significantly higher activity eas noticed for the hybrid material (chitosan/ second generation cefuroxime) as compared to the respective antibiotic alone. A slight increase in antimicrobial activity both agaist *E. coli* and *S. aureus* was observed for cefoperazone and ceftriaxone, when incorporated in chitosan. For the rest of antibiotics the results were similar or decreased as compared to those obtained for the respective cephalosporins alone (figure 4), probably due to their slow delivery and diffusion rates through culture medium. No significant differences concerning the antimicrobial effect were observed between materials obtained after different heating treatments at the qualitative screening.





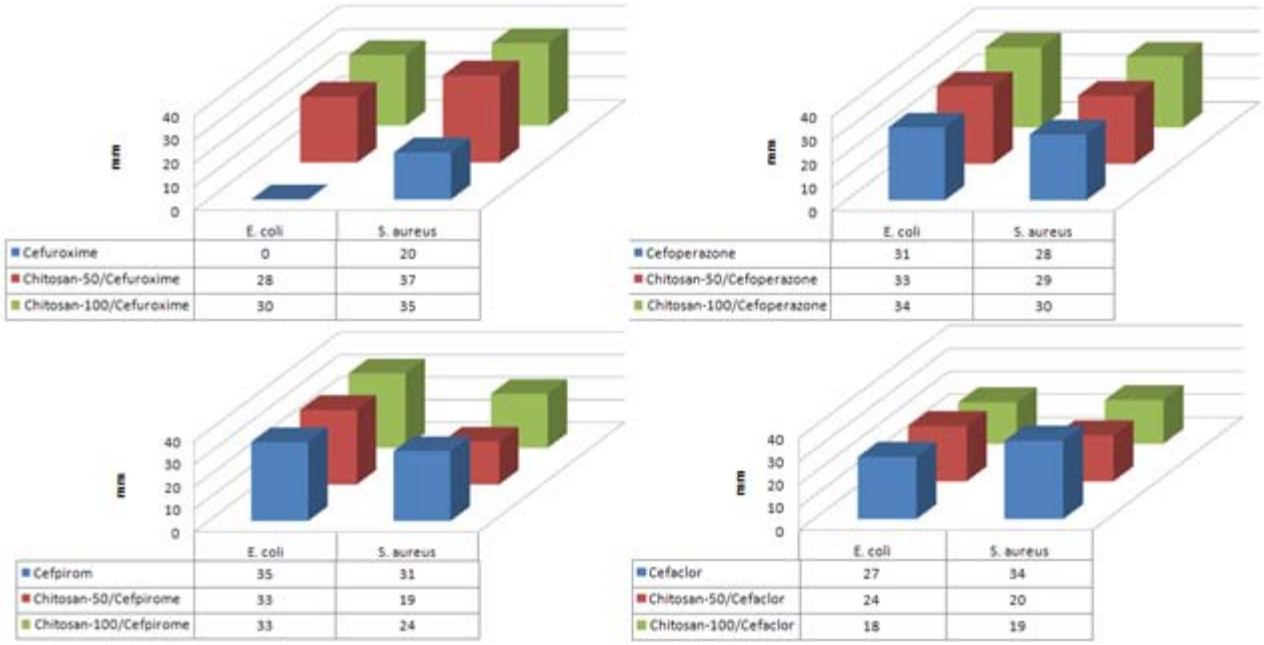


Figure 4: *In vitro* drug delivery response

4. Conclusions

Chitosan has the desired properties for safe use as a pharmaceutical excipient. This has prompted accelerated research activities worldwide on chitosan micro and nanoparticles as drug delivery vehicles. Chitosan/Fe_3O_4/Cephalosporins have been prepared in order to develop a magnetic drug delivery system and can be utilized to facilitate the targeted drug delivery of cephalosporins. All these hybrid materials are obtained under mild conditions without any organic solvents and surfactants, which are more suitable for pharmaceutical applications. The preparation of magnetic chitosan bead by chemical co-precipitation method has been investigated, and the FT-IR and CLSM has been used for ensuring the structure of the magnetic chitosan/cephalosporins bead. The surface morphology of the magnetic chitosan particles showed that the magnetite nanoparticles conglomerated in a compact fashion.

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