OPEN ACCESS JOURNAL

BIOINTERFACE RESEARCH IN APPLIED CHEMISTRY

ORIGINAL ARTICLE

www.BiointerfaceResearch.com

ISSN 2069-5837

*Volume 1, Issue 4, 2011, 139-144*

**Received: 1.07.2011 / Accepted: 2.08.2011 / Published on-line: 15.08.2011**

# Influence of magnetic MWCNTs on the antimicrobial activity of cephalosporins

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**ABSTRACT**

The purpose of this study was to analyze the antimicrobial activity of cephalosporin antibiotics adsorbed on the surface of magnetic MWCNTs, against microbial cells in suspension and adhered to the substrate, in order to highlight potential improvements of antimicrobial activity parameters of these antibiotics. Magnetic MWCNTs were obtained by plasma processing and characterized by HR-TEM. The need for the discovery of such strategies is imposed by the numerous mechanisms developed by pathogenic microorganisms manage to gain resistance to the majority of currently available antibiotics.

**Keywords:** *magnetic MWCNTs, cephalosporins, antimicrobial activity, nanomaterials*

## 1. Introduction

Carbon nanotubes (CNTs) are one of the new nanomaterials that have excellent optical, electronic, thermal [1], chemical [2], and mechanical [3] properties arising from their unique chemical [4] structure and size [5]. The interest in CNTs is gradually increasing due to their special properties related to electrical conduction and chemical affinity for various chemical species[6]. The applications of these nanomaterials cover a large spectrum, especially in biosciences: drug delivery, nanomedicine [7], cell growth [8] etc. Nanotubes are classified as single-walled nanotubes (SWNTs) and multi-walled nanotubes (MWNTs) and both categories could be functionalized and non functionalized in order to assure good adherence for different substrates [9] as well as improved biocompatibility. Nanopharmaceutical products based on carbon nanotube technologies will require a full understanding of the physical and chemical characteristics of CNTs and their interaction with the biological systems [10]. The application of CNTs as carriers is increased by their propensity to penetrate cells [11]. Cationic functionalized CNTs can be bound to active molecules via stable covalent bonds or supramolecular assemblies based on electrostatic attractions. Two possibilities exist: the more energetically feasible attachment onto the exterior either by covalent or non covalent interactions, and the encapsulation of these molecular assemblies within CNTs [12,13,14,15]. Cephalosporins are antibiotics with a structure and activity similar to penicillins [16]. They are resistant to penicillinase, but susceptible to cephalosporinases. They have a large action spectrum, including Gram-positive (*Staphylococcus sp., Streptococcus sp., Corynebaterium diphteriae*) and

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Gram-negative (*Neisseria gonnorhoeae, Haemophilus influenzae, Escherichia coli, Proteus sp., Klebsiella* sp.) microorganisms.

In this context our aim was to study the influence of magnetic MWCNTs on the antimicrobial activity of cephalosporins from II$^{nd}$, III$^{rd}$ and IV$^{th}$ generation on Gram-positive and Gram-negative bacterial strains with known antibiotic susceptibility patterns.

# 2. Experimental section

**2.1. Synthesis and characterization of magnetic MWCNTs.** Magnetic nanoparticles were obtained by toluene plasma processing [17], and the morphology was determined by transmission electronic microscopy (TEM). The transmission electron images were obtained on finely powdered samples using a TecnaiTM G2F30 S-TWIN high resolution transmission electron microscope (HR-TEM). The microscope was operated in transmission mode at 300kV with TEM point resolution of 2 Å and line resolution of 1 Å [18].

**2.2. Preparation of magntic MWCNTs/Cephalosporins hybrid materials.** The following cephalosporins have been selected for adsorption on the hybrid material: Cefaclor, Cefoperazone, Ceftriaxone, Cefpirome, Cefotaxime and Cefepime. The amount of the antibiotic adsorbed on the nanostructured support was 3 %. In a grinding mortar equipped with a 100 Kgf Nd-Fe-B magnet at the bottom side, the nanostructured material and antibiotic which is to be adsorbed are introduced. The mix is ground with 2 ml of chloroform until the latter completely evaporates.

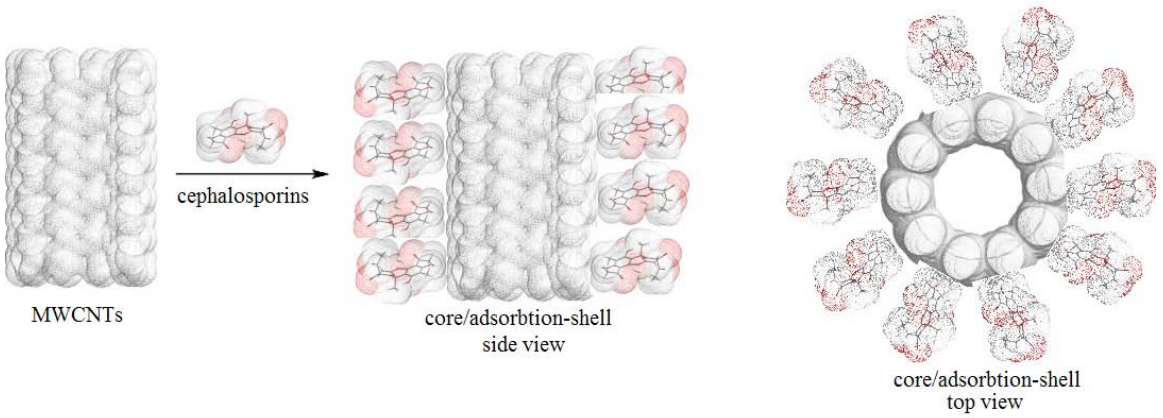



**Figure 1.** Ilustrative representation of cephalosporins absorbed on the surface of magnetic MWCNTs

**2.3. Evaluation of the antimicrobial activity of cephalosporins and MWCNTs/cephalosporins**

**2.3.1. Bacterial strains.** Bacterial strains used in the study were selected from the collection of the Laboratory of Microbiology-Botany Department, and belong to the following species: *Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Bacillus subtilis.*

**2.3.2. Qualitative screening of the antimicrobial activity.** Qualitative screening of the susceptibility of different microbial strains to magnetic MWCNTs/cephalosporins has been accomplished through a adapted diffusion method, on Mueller Hinton solid medium [19, 20]. In this purpose, 5 µl from a stock solution of the tested product, containing 30 µl of antibiotic were distributed in spots on Petri plates. The results' reading was performed by measuring the bacterial growth inhibition zones' diameters around the spots. The used solvent, dimethyl sulfoxide (DMSO) [21], was comparatively tested for its potential antimicrobial activity.

**2.3.3. Quantitative assay of the antimicrobial activity.** Quantitative testing of antimicrobial activity of hybrid nanosystems and the establishment of MIC (minimum inhibitory concentration) was determined by microdilution technique in liquid medium (Mueller Hinton broth), using 96 multiwell plates [22]. Twofold serial microdillutions were achieved in 200 µl medium, the dilution

range varying, depending on the tested antibiotic and the bacterial strain, in accordance with CLSI breakpoints (CLSI, 2010). Subsequently, the wells were seeded with 50 ml of each bacterial suspension, adjusted at MacFarland standard 0,5 [23]. Positive and negative controls were used. After incubating the plates at 37oC for 24 hours, the results were macroscopically assessed for bacterial groth, MIC corresponding to the well with clea content, thus without no visible microbial growth.

# 3. Results section

**3.1. HR-TEM analysis.** The multiple graphitic walls of the CNTs were clearly visible in TEM image, which showed that diameter of the tube not exceeding 15 nm (Fig. 2).

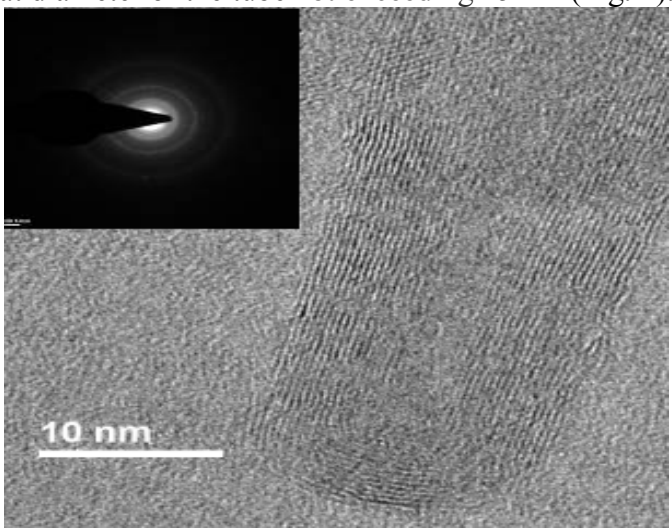
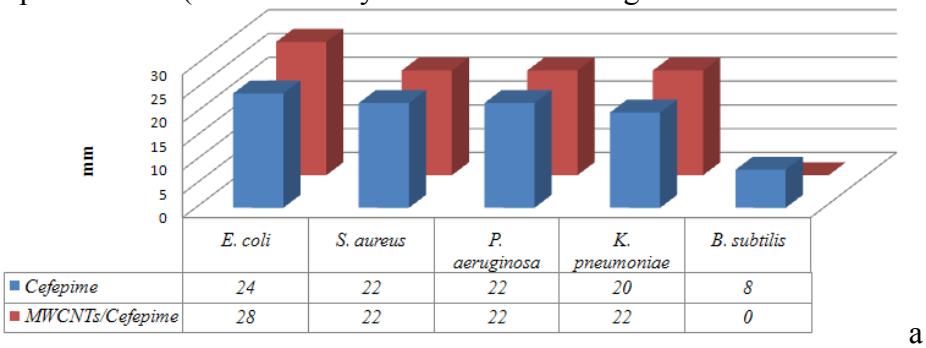
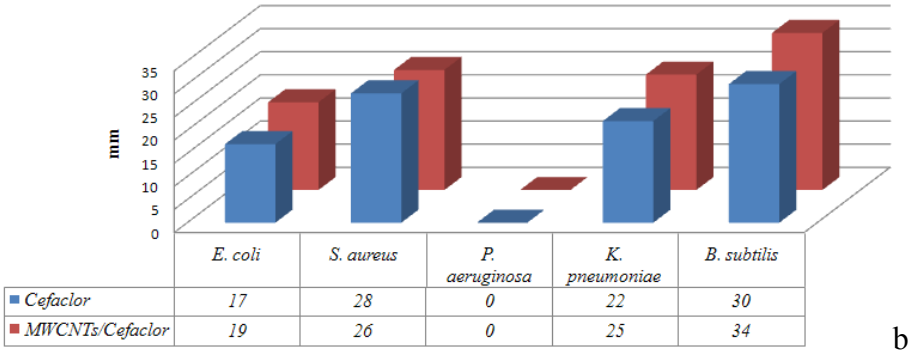



**Figure 2.** HR-TEM images of magnetic MWCNTs

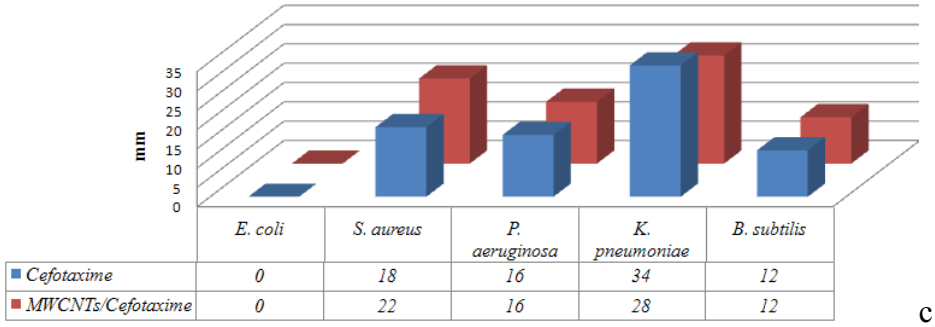
**3.2. Qualitative testing results of susceptibility of bacterial strains to the tested cephalosporins, as well and embedded in NTs.** The qualitative screening revealed that the antibacterial activity of hibrid nanosystems was improoved or at list similar on Gram-negative strains comparing with the standard cefalosporine discs (as revealed by the increase in the growth inhibition diameter).




a

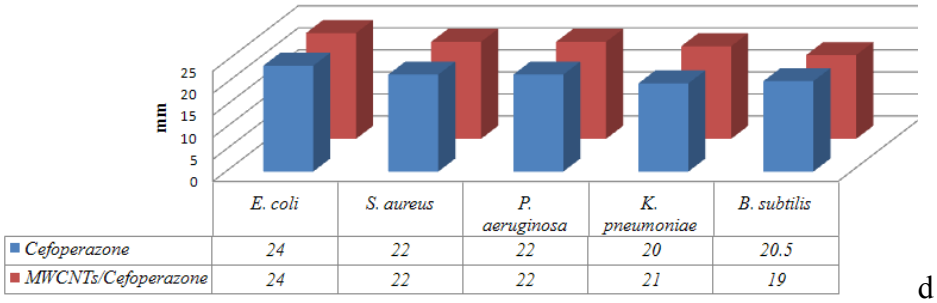



b



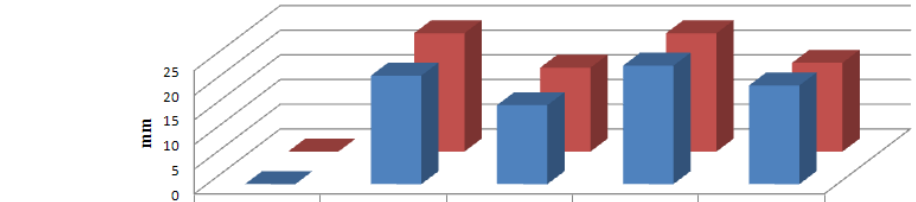

| | E. coli | S. aureus | P. aeruginosa | K. pneumoniae | B. subtilis |
|---|---|---|---|---|---|
| Cefotaxime | 0 | 18 | 16 | 34 | 12 |
| MWCNTs/Cefotaxime | 0 | 22 | 16 | 28 | 12 |

c




| | E. coli | S. aureus | P. aeruginosa | K. pneumoniae | B. subtilis |
|---|---|---|---|---|---|
| Cefoperazone | 24 | 22 | 22 | 20 | 20.5 |
| MWCNTs/Cefoperazone | 24 | 22 | 22 | 21 | 19 |

d



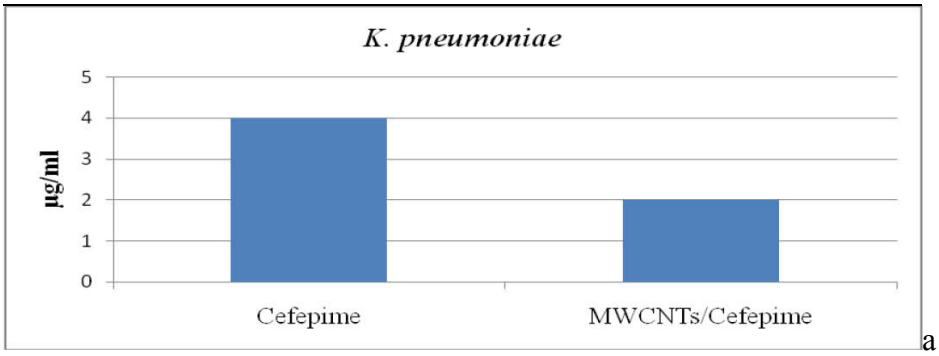

| | E. coli | S. aureus | P. aeruginosa | K. pneumoniae | B. subtilis |
|---|---|---|---|---|---|
| Ceftriaxone | 0 | 22 | 16 | 24 | 20 |
| MWCNTs/Ceftriaxone | 0 | 24 | 17 | 24 | 18 |

e

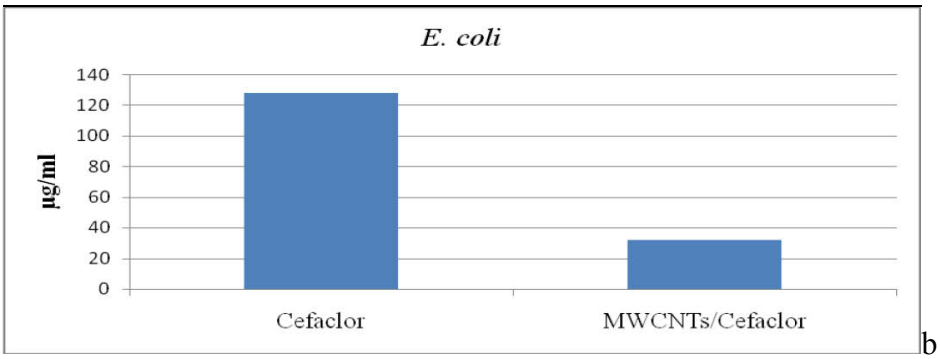
**Figure 3 (a-e)**. The comparative graphic representation of the growth inhibition zone diameter values obtained for cefepime (a), cefaclor (b), cefotaxime (c), cefoperazone (d) and ceftriaxone (e) as well and respectively, embedded in magnetic MWCNTs on different bacterial strains

Improved activity was obtained for cefepime and cefaclor against *E. coli* and K. *pneumoniae*, for ceftriaxone against *K. pneumoniae* and for cefoperazone on *P. aeruginosa*. Concerning the Gram-positive strains, cefotaxime activity on S. *aureus* and cefaclor's on *B. subtilis* were enhanced by the hybrid nanosystems (Fig. 3 a-e).

**3.3. Quantitative analysis (MIC) of antimicrobial activity.** The quantitative assay confirmed the qualitative screening results, significantly decreased MIC values being achieved for cefaclor loaded hybrid nanosystem on *E. coli* strain, from 128µg/mL to 32µg/mL. The MIC value slightly decreased for cefepime charged nanosamples, from 4µg/mL to 2µg/mL (Fig. 4 a, b).




a



b

**Figure 4.** The comparative graphic representation of the MIC values obtained for cephalosporins as well and respectively, embedded magnetic MWCNTs on K. pneumoniae (a) and E. coli (b) bacterial strains

# 4. Conclusions

Magnetic MWCNTs significantly improved the antimicrobial activity of cephalosporin antibiotics adsorbed onto their surface in a specific manner, dependent on the tested microbial strain and the tested antibiotic, demonstrating that this type of nanosytems could be used not only as drug delivery systems, but also as active potentiators of the antimicrobial activity of different substances. However, indepth studies are reuquired in order to confirm these results on a large number of strains and to elucidate the interactions established between the active substances and the carrier system on one side, and between the hybrid nanosystem and the target cell on the other one.

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**Acknowledgements:** The results presented in this paper have been obtained with the financial support obtained from the Human Resources Project no. 135/2010 (Contract no. 76/2010) and PN2 42150/2008.