

BIOASSAY AND ELECTROCHEMICAL EVALUATION OF CONTROLLED RELEASE BEHAVIOR OF CEPHALOSPORINS FROM MAGNETIC NANOPARTICLES

D. E. MIHAIESCU^a, A. M. GRUMEZESCU^{a*}, A. S. BUTEICA^b,
D. E. MOGOSANU^a, P. C. BALAURE^a, O. M. MIHAIESCU^c, V. TRĂISTARU^a,
B.S. VASILE^a

^a*Faculty of Applied Chemistry and Materials Science, "Politehnica" University of Bucharest, 1-7 Polizu Street, 011061 Bucharest, Romania*

^b*Faculty of Pharmacy, University of Medicine and Pharmacy of Craiova, 66 1 May Avenue, 200638 Craiova, Romania*

^c*Comporter SRL, Bucharest, Romania*

Four antibiotics from the cephalosporin class were used for the evaluation of controlled release behaviour. Plasma processed magnetic nanoparticles (C-Fe) were characterized by HR-TEM. Cephalosporins were deposited on magnetic nanoparticles by adsorption and the antimicrobial activity of cephalosporins and C-Fe/cephalosporins (cephachlor, cefuroxime, cefotaxime, and ceftriaxone) was tested. Two collection strains: *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922, were used for assessing the antimicrobial activity of core/shell type nanoparticles. Controlled release of nanoparticles deposited antibiotics was studied by using a biological assay and an electrochemical approach. The antimicrobial effect and its time dependence of the C-Fe/cephalosporins was studied by diffusimetric method, comparing the growth inhibition zones determined from the functionalised nanoparticles and standardized antibiotic discs. Longer exposure times emphasized the increasing effect. The conductimetric method revealed the controlled release by measuring suspension conductivity at various times. We can conclude that the obtained adsorption shell C-Fe/cephalosporin nanostructures can be used as carriers for the controlled release of cephalosporins.

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1. Introduction

Nanoparticles have a major impact in the medical [1,2] and pharmaceutical area [3,4]. From drug delivery systems to medical implants, their broad application field captured the interest of many researchers. Strategies for biofilms' development [5] and bypassing the antibiotic resistance mechanism of different bacterial strains [6] are being studied worldwide. Carbon nanotubes are used to inhibit different bacterial strains such as *E. coli*, which is one of the most spread etiological agents involved in intestinal infections [7].

Kang S. *et al.* (2007) [8] have evaluated the toxicity of four types of carbon nanomaterials: single-walled carbon nanotubes, multi-walled carbon nanotubes, C60 nanoparticles in aqueous phase and colloidal graphite, using gram positive and gram negative bacteria. Single-wall nanotubes (SWNT) shows inhibition for *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus epidermis* monocultures, as well as for different microbial communities of river waters and wastewater effluents. The bacteriostatic activity was demonstrated to be time dependent, longer exposure times emphasizing the monoculture toxicity with initial tolerance for SWNT's.

* Corresponding author: grumezescu@yahoo.com

Kang S. *et al.* (2007–2009) [9, 10, 11] evaluated the antimicrobial activity of highly purified single-walled nanotubes, demonstrating their capacity as building blocks for antimicrobial materials. By using single-walled carbon nanotubes (SWNT) with a narrow diameter distribution, the cell membrane damage resulted from the direct contact with the SWNT aggregates was observed. Jia G. *et al.* [12] evaluated a cytotoxicity test protocol for single-walled nanotubes, multi-walled nanotubes (with a diameter ranging from 10 to 20 nm) and fullerenes (C60). The high cytotoxicity of MWNT in the alveolar macrophage (AM) after a six hours *in vitro* exposure was observed. The cytotoxicity was increased up to 35% as the multi-walled nanotubes dosage increased with 35 $\mu\text{g}/\text{cm}^2$. Up to a 226 $\mu\text{g}/\text{cm}^2$ dosage, no significant cytotoxicity was observed for C60. The cytotoxicity seems to follow a mass sequence basis: SWNT > MWNT10 > quartz > C60. Carbon nanotubes with various geometrical structures present a quite different cytotoxicity and *in vitro* bioactivity, although these properties might not be exactly reflected in the comparative *in vitro* toxicity. Nepal D. *et al.* [13] developed single-walled carbon nanotubes coatings, with controlled morphology, by using the layer-on-layer assembly. These showed clearly a high antimicrobial activity, the thickness being controlled up to 1.6 nm and the nanotubes orientation with an oriented air stream. This unique mixture of multifunctionality and vertical and lateral control of the assembly process [9, 14] is a significant advance in the development of macro scale assemblies with combined attributes of SWNT and natural materials [15, 16]. In our study, bioassay and electrochemical evaluation of controlled release behavior of cephalosporins from magnetic nanoparticles was tested and compared.

2. Materials and methods

2.1 Microbial strains.

Two collection strains: *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922, were used for assessing the antimicrobial activity of core/shell type nanoparticles. 0.5 McFarland bacterial suspensions were obtained from 15-18h cultures grown on solid medium.

2.2 Reagents.

All of the analytical grade solvents and reagents were purchased from Merck (Darmstadt, Germany). Standardised antibiotic disks (30 $\mu\text{g}/\text{mL}$) from HiMedia Laboratories were used for bioassay studies. The tested antibiotics (were purchased as injectable powder) belong to the second and third generation of cephalosporins: cephachlor, cefuroxime, cefotaxime, and ceftriaxone.

2.3 Synthesis and characterization of magnetic nanoparticles

Magnetic nanoparticles, C-Fe (toluene) and C-Fe (benzene/aniline), obtained by toluene and benzene/aniline mixture plasma processing, were purified as following: ▪ solvent extraction (successively with benzene, dichloromethane and *o*-dichlorobenzene); ▪ inorganic impurities removal (concentrate warm nitric acid / hydrochloric acid mixture); ▪ washing (ultra-pure water); ▪ high temperature (>300 $^{\circ}\text{C}$) treatment. The primary characterization method was HR-TEM (Figure 1, 2).

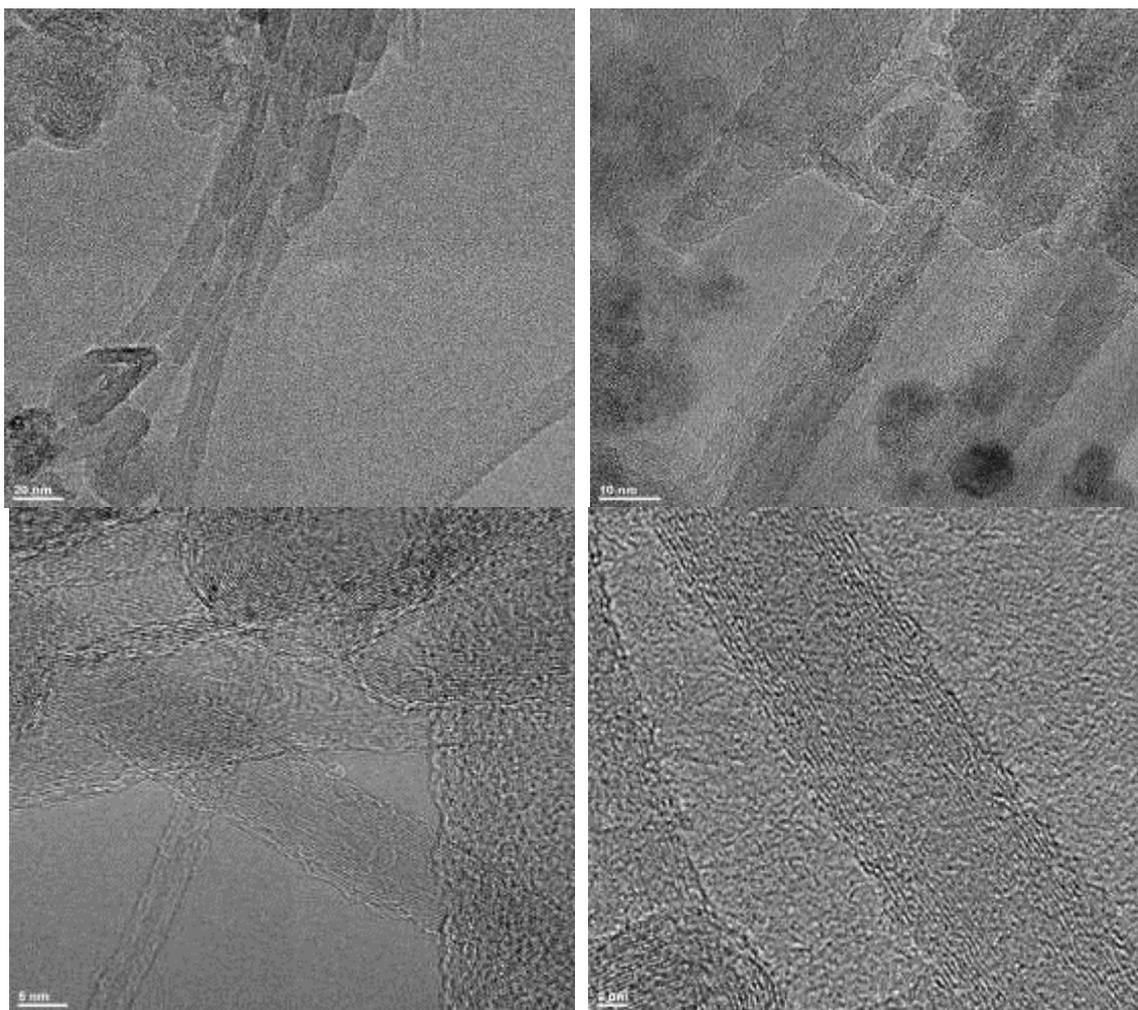


Fig. 1. HR-TEM images of magnetic nanoparticles: C-Fe (Toluene)

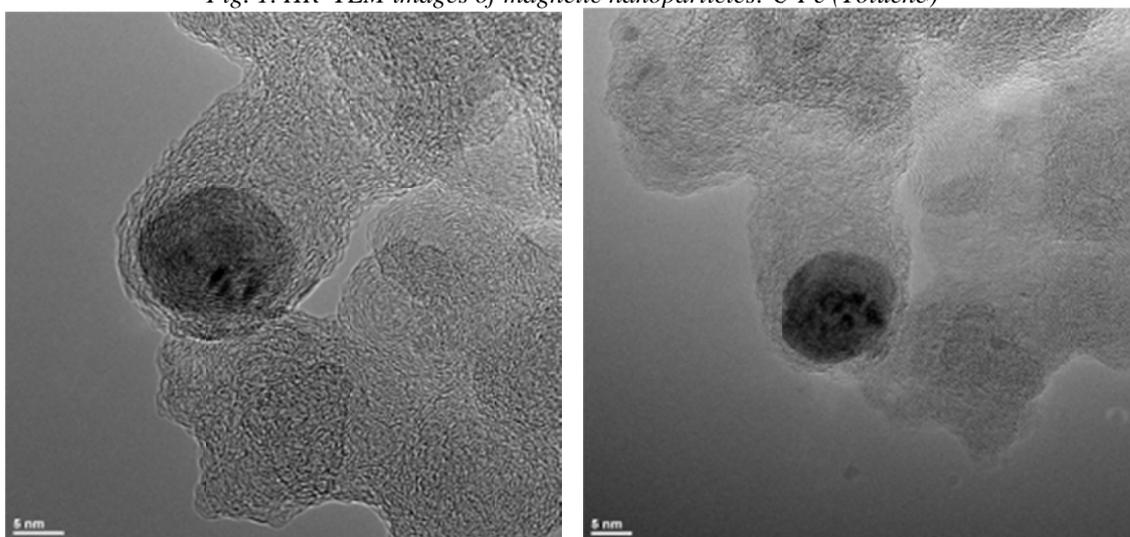


Fig. 2. HR-TEM images of magnetic nanoparticles: C-Fe (Benzene/Aniline)

2.4 Preparation of C-Fe/cephalosporins - core/shell

Four antibiotics from the cephalosporin class have been selected: cephachlor, cefuroxime, cefotaxime and ceftriaxone. The cephalosporins deposited on magnetic nanostructures with 0.3%

active substance concentration, were prepared as following: the nanostructured powder was mixed with 0.3% cephalosporin (related to powder mass) and milled until homogenization. Water was added under stirring and the mixture was homogenised for 30 min; finally, the drying step was performed at 50°C for 24h [17, 18].

2.5 Design of core/shell interaction

Molecular modeling is frequently used in organic synthesis, especially in the nanotechnological area, due to both development of the mechanical-molecular calculus methods and the spectacular evolution of the computation systems that allows advanced mechanic molecular calculus, excluding as much as possible the simplifying hypotheses. The molecule orientation in the adsorption process is expected to be accomplished so that the non-polar part interacts with the non-polar shell of the nanoparticle, and the polar part will be oriented toward the exterior. This geometry allows interactions with other molecules, possibly yielding secondary shells, supposition that can be correlated with experimental controlled release observations. Therefore, the molecular modeling methods allowed the development of some operational models that are able to sustain further experimental data processing.

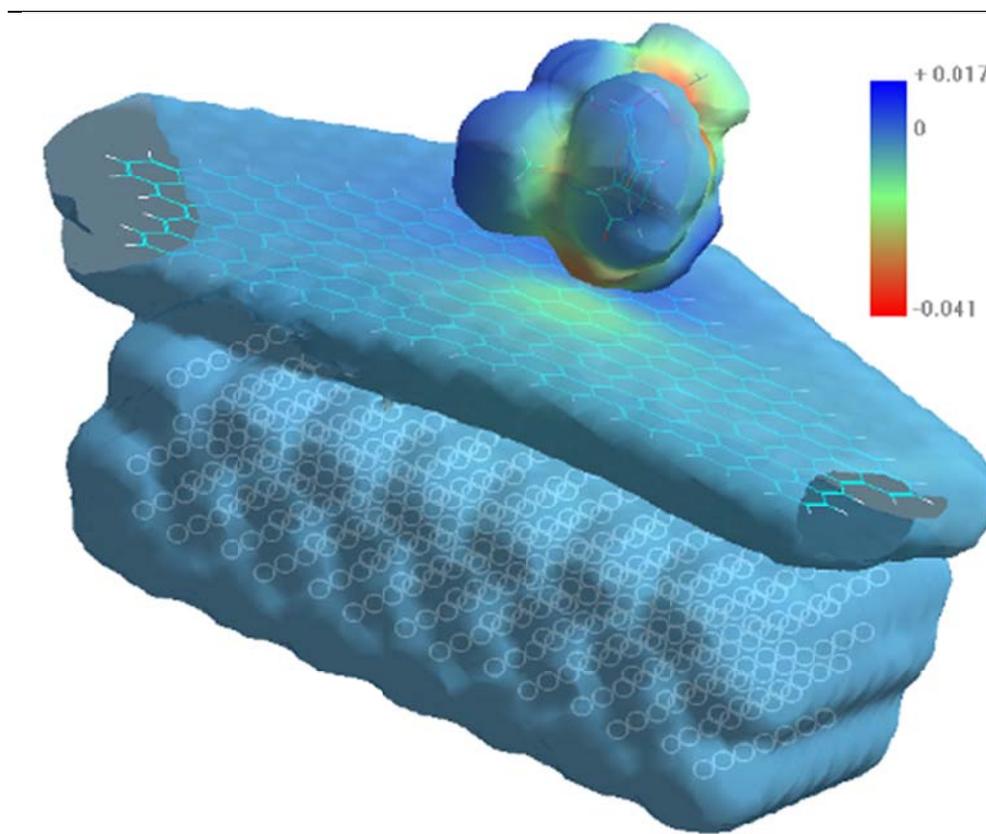


Fig. 3. Interaction model of core/shell - graphene surface/cephalosporin

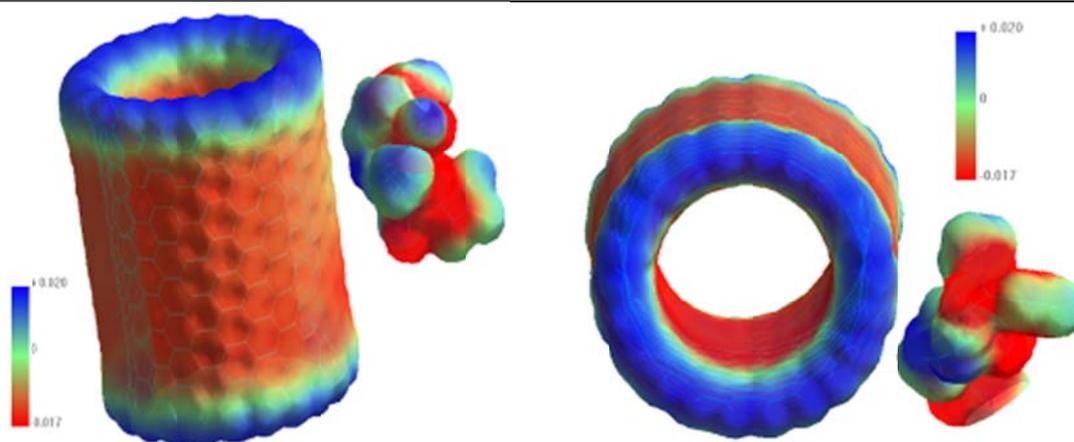


Fig. 4. Interaction model (spatial charge distribution visualization) of core/shell - carbon nanotubes/cephalosporin

2.6 Controlled cephalosporin release from the magnetic nanoparticles

2.6.1 Qualitative bioassay for controlled release of cephalosporin loaded nanoparticles

The difusimetric method [19] (disc diffusion technique) was used to determine the antibacterial activity of functionalized nanoparticles. The tested compounds diffuses through the medium and a inverse ratio correlation was established between the diffusion diameter and the antibiotic concentration. The inhibition zone is linearly correlated with the antibiotic concentration, which represents the MIC (minimum inhibitory concentration) value. Both positive (antibiotic discs) and negative control (nanoparticles loaded discs) were used. The antibiotics were tested separately or in pair association. Same concentration of active compound for reference and C-Fe/cephalosporin nanostructures were used for discs placed on the agar plates, previously seeded with 0.5 McFarland inoculums of each strain. 5-10 μ L from suspended core/shell type nanoparticles were used in order to fill sterilised filter paper disks. The plates were incubated for 24h at 37°C and thereafter the inhibition zones were measured.

2.6.2 Electrochemical assay for controlled release of cefalosporines from nanoparticles

The cephalosporin controlled release dynamics from the nanostructured system was studied using a conductometric method (Denver Instruments 220 conductometer, OIML 1408 μ S/cm KCl reference material). The analytical instrument was linked to PC via RS232 interface using 2Hz data acquisition frequency. Data processing was performed by Microsoft Excel using spline interpolation. The samples were introduced in the working vessel at 25°C under continuous stirring. Experimental data proves a larger retention for the nanostructured support adsorption (for all tested compounds) related to reference sample.

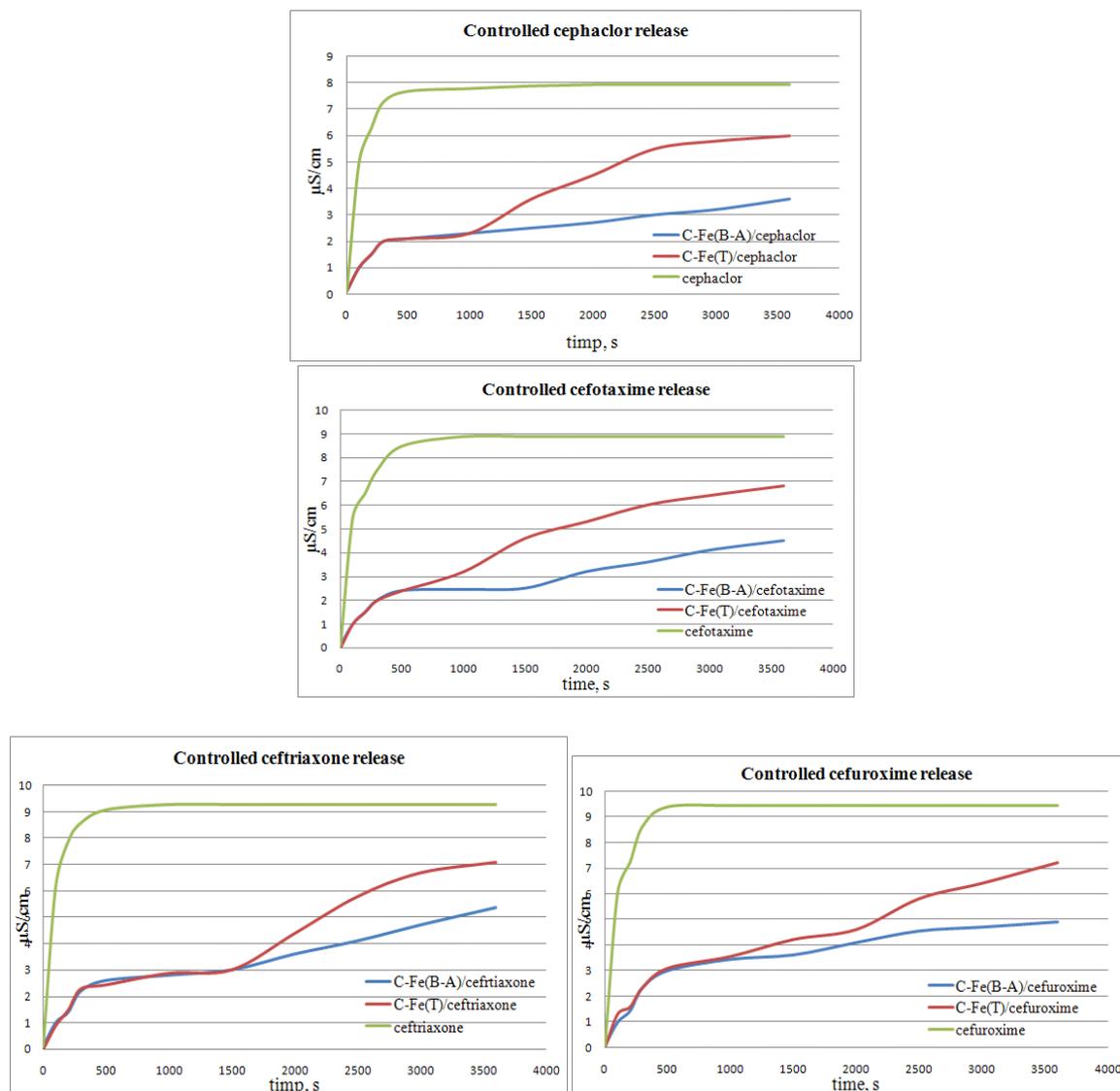


Fig. 5. Cephalosporins release profile from magnetic nanoparticles

3. Results and discussion

3.1 Antimicrobial activity

The growth inhibition zones for the two tested strains are presented in fig.no. 6 and 7, showing the strains' sensitivity of the tested core/shell materials. The inhibition zones diameters raise as the exposure time to loaded nanoparticles increases. This dynamic of growth inhibition zones exhibited the controlled release of antibiotic, which gradually diffuses in culture medium.

3.2 Controlled release profile of Cephalosporins from magnetic nanoparticles

The release profile (fig.no.5) shows lower release levels from C-Fe/B-A support, related to higher surface polarity (by nitrogen inclusion), for all tested compounds. Also, C-Fe/B-A and C-Fe/T support shows significant lower release levels in comparison with reference sample (much over 3600s partial release time in comparison with 500s maximum release time for the reference material).

Secondary (multi layer) adsorption shells hypothesis, related to nanoparticles' covering capacity surpass, could explain the front higher release levels (close to the reference material solvation profile) and the further 2 - 3 release "peaks" over the 3600s acquisition time. Slight changes in the release profile could be associated with some small changes of the experimental parameters, but the large release "peaks" would involve changes in the release mechanism like successive solvation of active compound adsorption layer.

In conclusion, the cephalosporins' gradual release profile, from C-Fe/B-A and C-Fe/T nanostructured support in aqueous solution, proves differentiate release levels related to support structural differences, significant lower amount of released compound versus reference and significant time dependent release mechanism changes.

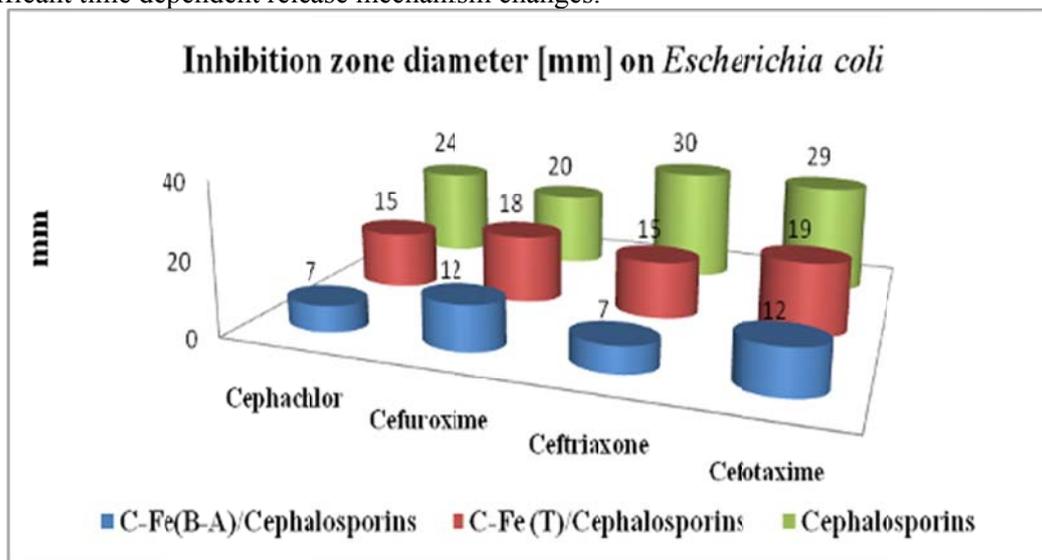


Fig. 6. Growth inhibition zones diameter for *E. coli*

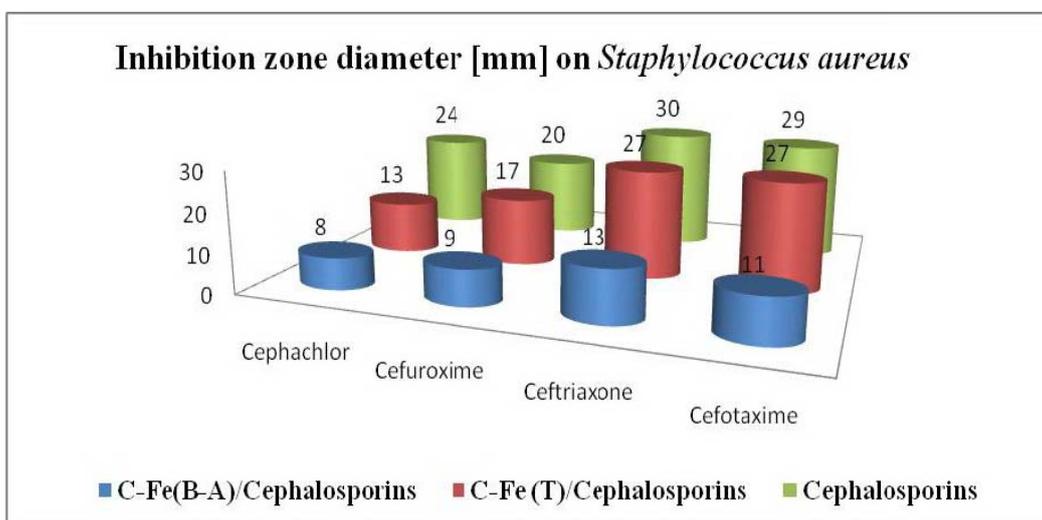


Fig. 7. Growth inhibition zones diameter for *S. aureus*

4. Conclusions

Two different magnetic nanostructured materials were synthesized by plasma processing, purified and characterized by HR-TEM. 4 β -lactamic antibiotics were deposited by adsorption and the related release profiles were acquired by conductometric method. The difusimetric method was used for antibacterial activity testing and a time dependent growth inhibition pattern was observed for all tested materials.

The gradual release profile, from C-Fe/B-A and C-Fe/T nanostructured support in aqueous solution, proves differentiate release levels related to support structural differences, significant lower amount of released compound versus reference and significant time dependent release mechanism changes. Due to the possibility of a stronger interaction between the active compound and nitrogen doped nanoparticles, we concluded that C-Fe/ benzene-aniline nanoparticles prove a better release of the active compound than C-Fe/toluene ones. The antimicrobial effect of active substances of C-Fe magnetic nanoparticles was proved on *Staphylococcus aureus* and *Escherichia coli*, using a Mueller-Hinton medium, by comparison of the inhibition diameter. The conductometric profile sustain also the antibiogram interpretation.

Acknowledgements

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