

# MATERIALE HIBRIDE DEAE-CELULOZĂ/Fe<sub>3</sub>O<sub>4</sub>/CEFALOSPORINE PENTRU LIVRAREA ȚINTITĂ A MEDICAMENTELOR DEAE-CELLULOSE/Fe<sub>3</sub>O<sub>4</sub>/CEPHALOSPORINS HYBRID MATERIALS FOR TARGETED DRUG DELIVERY

ALEXANDRU MIHAI GRUMEZESCU<sup>1\*</sup>, ECATERINA ANDRONESCU<sup>1</sup>, ANTON FICAI<sup>1</sup>, CRINA SAVIUC<sup>2</sup>,  
DAN MIHĂIESCU<sup>1</sup>, MARIANA CARMEN CHIFIRIUC<sup>2</sup>

<sup>1</sup>Universitatea Politehnică București, Str. G. Polizu nr. 1, sector 1, cod 011061, București, România

<sup>2</sup>Universitatea București, Aleea Portocalelor nr. 1-3, sector 6, cod 060101, București, România

*The desired pharmacological activity with minimum side effects of different traditional chemical formulations is achieved by enabling the site-specific targeting and controlled release. In this study, magnetic diethylaminoethyl-cellulose (DEAE-Cellulose) has been prepared by magnetite nanoparticles deposition on the DEAE-Cellulose support. The primary method to analyze the surface modification was SEM. DEAE-Cellulose/Fe<sub>3</sub>O<sub>4</sub> was loaded with different antibiotics and their antimicrobial efficacy against bacterial strains belonging to E. coli and S. aureus species were studied. DEAE-Cellulose/Fe<sub>3</sub>O<sub>4</sub>/cephalosporins have been characterized by using FT-IR. Our study indicates that the materials of DEAE-Cellulose/Fe<sub>3</sub>O<sub>4</sub>/cephalosporins have generally increased the activity against tested strains, the antimicrobial effect being not significantly dependent of temperature treatment.*

*Activitatea farmacologică dorită, cu efecte adverse minime a diferitelor formulări chimice tradiționale este obținută prin permiterea eliberării controlate și locale regionale a medicamentelor. În acest studiu, dietilaminoetil-celuloza/Fe<sub>3</sub>O<sub>4</sub> (DEAE-Celuloza/Fe<sub>3</sub>O<sub>4</sub>) a fost obținută prin depunerea nanoparticulelor de magnetită pe suportul de DEAE-Celuloză. Principala metodă pentru analiza modificărilor suprafeței a fost microscopia electronică de baleaj. Dietilaminoetil-celuloza/Fe<sub>3</sub>O<sub>4</sub> a fost încărcată cu diferite antibiotice și eficiența antimicrobiană a materialelor a fost studiată față de tulpini bacteriene care aparțin speciilor E.coli și S.aureus. DEAE-Celuloza/Fe<sub>3</sub>O<sub>4</sub>/cefalosporine au fost caracterizate prin FT-IR. Studiul nostru indică faptul că materialele DEAE-Celuloza/Fe<sub>3</sub>O<sub>4</sub>/cefalosporine au crescut în general activitatea față de tulpinile testate, efectul antimicrobian fiind nesemnificativ dependent de tratamentul termic.*

**Keywords:** Cephalosporins, hybrid material, DEAE-Cellulose, drug delivery, magnetic material, magnetic drug targeting

## 1. Introduction

The objective of drug targeting is to achieve desired pharmacological response at a selected site without undesirable interactions [1]. The magnetic targeted drug delivery system is one of the most attractive strategies of delivering drugs to the area of interest [2]. 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 magnetic field to attract and retain them there [3]. The great problem associated with drug administration is the inability to target a specific area of the body [4]. To reach an acceptable therapeutic level, large doses of the drug must be administered [5]. Only a part of the dose will actually reach the intended disease or organ [6]. These high dosages can cause toxic effects at the non-target disease or organ [7,8]. Targeting specific sites in the body simplifies drug administration procedures, reduces the quantity of drug required to reach therapeutic levels, decreases the concentration of the drug at non-target sites and increase the concentration of the drug at target sites. Incorporating magnetic particles into drug carriers and using an externally applied magnetic field is one way to physically direct these

magnetic drug carrier particles to a site [9]. The main limitation of magnetic drug delivery relates to the strength of the external magnetic field that can be applied to obtain the necessary magnetic gradient to control the residence time of nanoparticles in the desired area or which triggers the drug desorption [10]. As a means to elude the limitations of using external magnetic fields, internal magnets can be located in the vicinity of the target by using minimally invasive surgery. Moreover, the simultaneous use of an external magnetic field and a magnetic implant to direct the magnetic nanoparticles is also a promising strategy [11]. In this context the aims of our study were obtaining, characterization and biological evaluation of novel hybrid material based DEAE-Cellulose/Fe<sub>3</sub>O<sub>4</sub> and different cephalosporins.

## 2. Materials and Methods

### 2.1. Preparation of DEAE-Cellulose/Fe<sub>3</sub>O<sub>4</sub>

DEAE-Cellulose/Fe<sub>3</sub>O<sub>4</sub> was prepared by chemical precipitation of Fe<sup>2+</sup> and Fe<sup>3+</sup> ions in aqueous solution of sodium hydroxide. In brief, 2 g of DEAE-Cellulose was added in 500 mL aqueous solution of NaOH (2%) and then Fe(II) and Fe(III) (in 1:2 molar ratio) were dissolved in 500 mL ultra-

\* Autor corespondent/Corresponding author,  
Tel. +4021 402 39 60, E-mail: grumezescu@yahoo.com

pure water and were added drop-wise under constant stirring at 25°C. Organic/inorganic 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<sub>3</sub>O<sub>4</sub> nanoparticles synthesis. The hybrid material were finally dried at 50° (DEAE-C<sub>50</sub>/Fe<sub>3</sub>O<sub>4</sub>) and 100 °C (DEAE-C<sub>100</sub>/Fe<sub>3</sub>O<sub>4</sub>).

## 2.2. Antimicrobial agents

Cephalosporins were chosen from the commercially available class of β-lactams antibiotics: *cefuroxime* (second-generation), *cefotaxime* (third-generation), *cefoperazone* (third generation), *ceftriaxone* (third-generation), *cefepime* (fourth-generation), *cefaclor* (*second generation*).

## 2.3. Preparation of DEAE-Cellulose/Fe<sub>3</sub>O<sub>4</sub>/cephalosporins hybrid material as a magnetic drug delivery system

After drying in oven at 50° and 100°C for 24 h, the magnetic hybrid materials were dispersed in chloroform and then the cephalosporins were added. The mixture was grounded for 10 min until complete evaporation of chloroform. This step is repeated three times for a uniform dispersion of cephalosporins in the structure of hybrid material. Finally the minimum quantity of ultra-pure water was added to form a paste. It was dried to 40°C for 6 h. The concentration of deposited cephalosporins was 10 %.

## 2.4. Scanning electron microscopy (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 [12].

## 2.5. 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<sup>-1</sup> by co-adding 32 scans and at a resolution of 4 cm<sup>-1</sup> 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.

## 2.6. Qualitative antimicrobial screening as drug delivery response

Qualitative screening of the susceptibility of the microbial strains to DEAE-Cellulose/Fe<sub>3</sub>O<sub>4</sub>/cephalosporins has been accomplished through an adapted diffusimetric method, on Mueller Hinton (MH) solid medium [13,14]. In this purpose, a 0.5 McFarland standard adjusted microbial suspensions were obtained from *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 strains grown on solid medium and were further used for Petri plates seeding. Then, 5 μl from a stock solution of the tested product, containing 30 μg/mL of antibiotic were distributed in spots on the surface. The result reading was performed by measuring the bacterial growth inhibition zones diameters around the spots. The used solvent, dimethyl sulfoxide (DMSO) [15,16], was comparatively tested for its potential antimicrobial activity.

## 3. Results

### 3.1. SEM analysis

The scanning electron microscopy was used to characterize pure DEAE-Cellulose (Figure 1-a,b) and DEAE-Cellulose/Fe<sub>3</sub>O<sub>4</sub> systems obtained at 50 °C (figure 1-c,d) and 100 °C (Figure 1-e,f) respectively. As a prime observation it can conclude that magnetite nanoparticles can be well visualized in both magnetic systems and form agglomerates on the DEAE-Cellulose fibers (Figure 1-g,h).

From the SEM images DEAE-Cellulose fibers with lengths of 100-150 μm and diameters of 10-20 μm can be observed. The SEM image (Figure 1-b) of one individual macrofibril shows that the surface of untreated DEAE-C is almost free of trenches, but there are obvious boundary edges in different regions, compared with (Figure 1-d) and (Figure 1-f), where the deposition of Fe<sub>3</sub>O<sub>4</sub> was observed. It is important to mention that the thermal treatment used for the synthesis of the DEAE-Cellulose/Fe<sub>3</sub>O<sub>4</sub> systems do not affect the fibers morphology and are in good agreement with the FT-IR analysis.

### 3.2. FT-IR characterization

Figures 2 and 3 shows band intensities in the infrared spectra of DEAE-Cellulose, DEAE-Cellulose/Fe<sub>3</sub>O<sub>4</sub> dried at 50°C and respectively at 100°C and with cephalosporins loaded.

In addition, there are many well-defined peaks in the “fingerprint” region between 1200 and 800 cm<sup>-1</sup>. The “fingerprint” region of the spectra from the reference (1) and modified DEAE-Cellulose (2, 3) regions shows clear no differences after reaction by comparing the spectral characteristics of all types of samples as well as the band assignments can be ascertained.

To evidence the deposition of cephalosporins on the surface of DEAE-Cellulose/ /Fe<sub>3</sub>O<sub>4</sub>

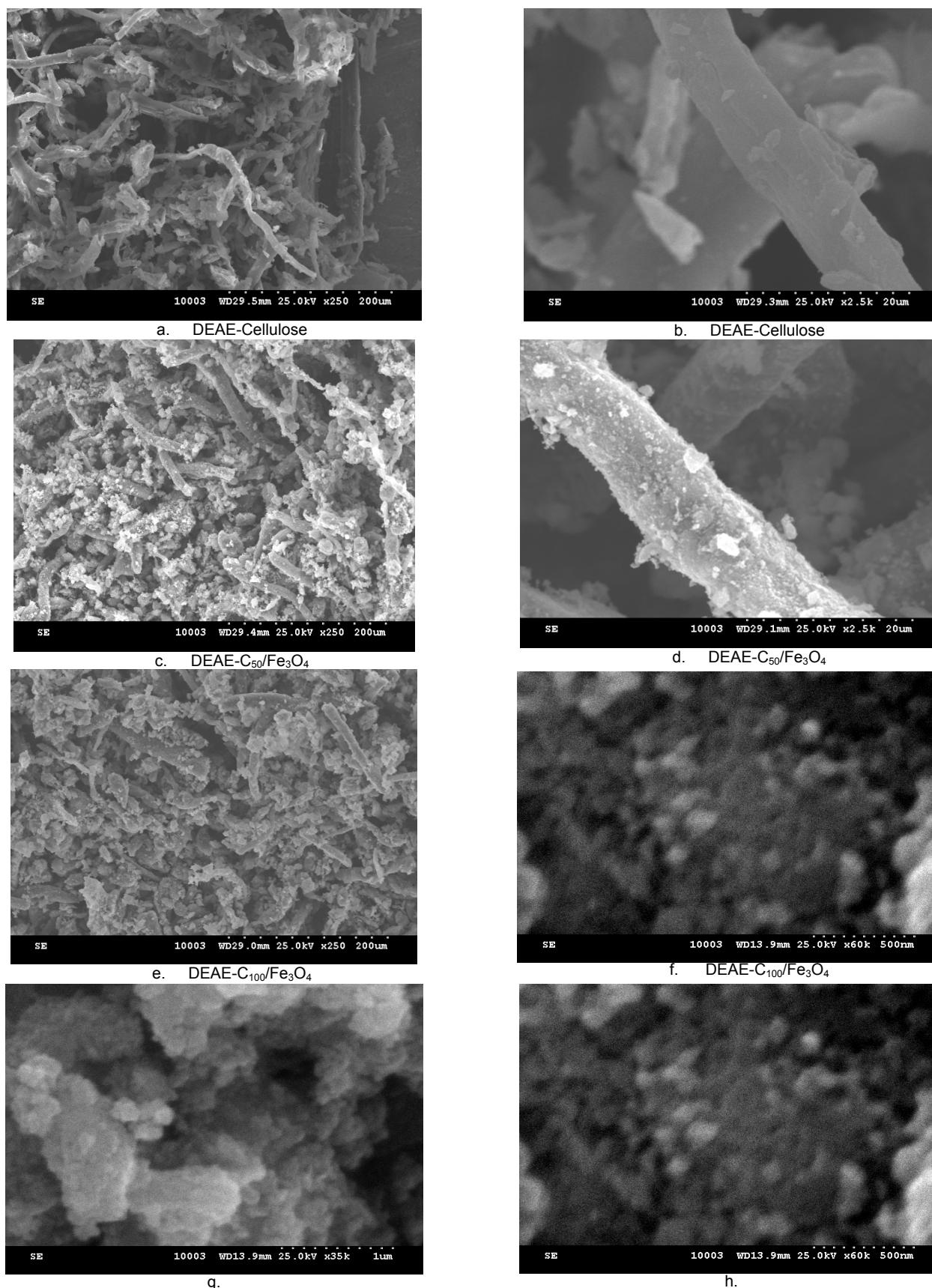


Fig. 1 - SEM micrographs of DEAE-Cellulose, DEAE-C<sub>50</sub>/Fe<sub>3</sub>O<sub>4</sub> and DEAE-C<sub>100</sub>/Fe<sub>3</sub>O<sub>4</sub> / Micrografii SEM ale DEAE-Celuloză, DEAE-C<sub>50</sub>/Fe<sub>3</sub>O<sub>4</sub> și DEAE-C<sub>100</sub>/Fe<sub>3</sub>O<sub>4</sub>

FT-IR spectra were plotted in Figure 2 and 3. The changes in area of the bands and many peaks in the “fingerprint” the reference (1, 2) and modified

region between 1600 and 1200 cm<sup>-1</sup> was observed.

The “fingerprint” region of the spectra from

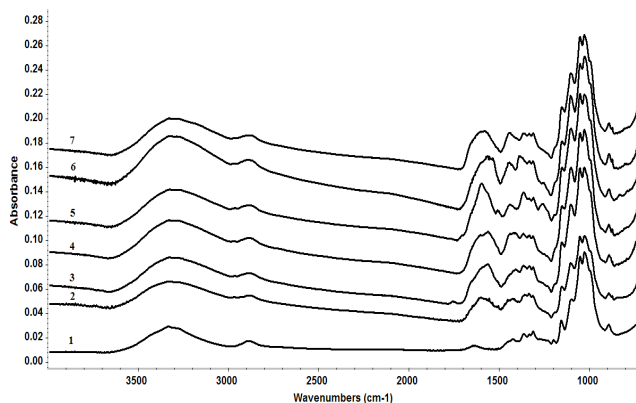


Fig. 2 - FT-IR spectra of DEAE-Cellulose (1), DEAE-C<sub>50</sub>/Fe<sub>3</sub>O<sub>4</sub> (2), DEAE-C<sub>50</sub>/Fe<sub>3</sub>O<sub>4</sub>/cefuroxime (3), DEAE-C<sub>50</sub>/Fe<sub>3</sub>O<sub>4</sub>/cefotaxime (4), DEAE-C<sub>50</sub>/Fe<sub>3</sub>O<sub>4</sub>/cefoperazone (5), DEAE-C<sub>50</sub>/Fe<sub>3</sub>O<sub>4</sub>/ceftriaxone (6), DEAE-C<sub>50</sub>/Fe<sub>3</sub>O<sub>4</sub>/cefepime (7) / Spectrele FT-IR pentru DEAE-Celuloză(1), DEAE-C<sub>50</sub>/Fe<sub>3</sub>O<sub>4</sub>(2), DEAE-C<sub>50</sub>/Fe<sub>3</sub>O<sub>4</sub>/cefuroxim (3), DEAE-C<sub>50</sub>/Fe<sub>3</sub>O<sub>4</sub>/cefotaxim (4), DEAE-C<sub>50</sub>/Fe<sub>3</sub>O<sub>4</sub>/cefoperazonă (5), DEAE-C<sub>50</sub>/Fe<sub>3</sub>O<sub>4</sub>/ceftriaxona (6), DEAE-C<sub>50</sub>/Fe<sub>3</sub>O<sub>4</sub>/cefepim (7)

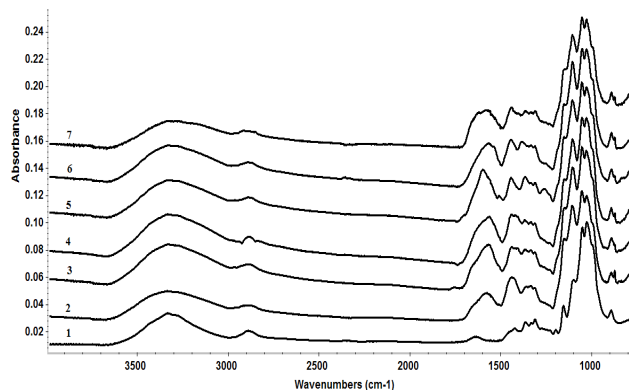


Fig. 3 - FT-IR spectra of DEAE-Cellulose (1), DEAE-C<sub>100</sub>/Fe<sub>3</sub>O<sub>4</sub> (2), DEAE-C<sub>100</sub>/Fe<sub>3</sub>O<sub>4</sub>/cefuroxime (3), DEAE-C<sub>100</sub>/Fe<sub>3</sub>O<sub>4</sub>/cefotaxime (4), DEAE-C<sub>100</sub>/Fe<sub>3</sub>O<sub>4</sub>/cefoperazone (5), DEAE-C<sub>100</sub>/Fe<sub>3</sub>O<sub>4</sub>/ceftriaxone (6), DEAE-C<sub>100</sub>/Fe<sub>3</sub>O<sub>4</sub>/cefepime (7) / Spectrele FT-IR pentru DEAE-Celuloză(1), DEAE-C<sub>100</sub>/Fe<sub>3</sub>O<sub>4</sub>(2), DEAE-C<sub>100</sub>/Fe<sub>3</sub>O<sub>4</sub>/cefuroxim (3), DEAE-C<sub>100</sub>/Fe<sub>3</sub>O<sub>4</sub>/cefotaxim (4), DEAE-C<sub>100</sub>/Fe<sub>3</sub>O<sub>4</sub>/cefoperazonă,(5), DEAE-C<sub>100</sub>/Fe<sub>3</sub>O<sub>4</sub>/ceftriaxonă(6), DEAE-C<sub>100</sub>/Fe<sub>3</sub>O<sub>4</sub>/cefepim (7)

DEAE-C<sub>100</sub>/Fe<sub>3</sub>O<sub>4</sub>/cephalosporins (3-7) regions shows clear differences after deposition of cephalosporins.

### 3.3. Qualitative antimicrobial screening as drug delivery response

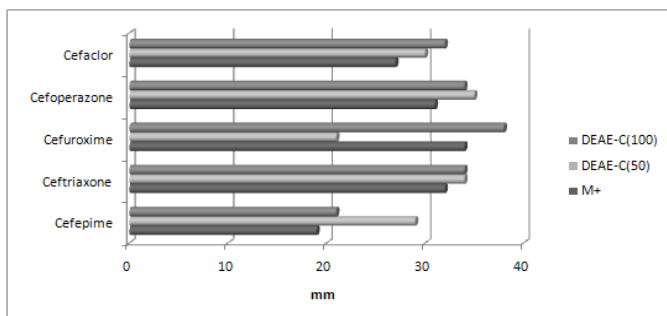


Fig. 4 - Antimicrobial efficacy against *E. coli*, expressed in diameter of the growth inhibition zone diameter (mm) (M<sup>+</sup> = Antibiotic control) / Eficiența antimicrobiană asupra *E. coli*, exprimată în diametrul zonei de inhibiție a creșterii (mm) (M<sup>+</sup> = Antibiotic de control).

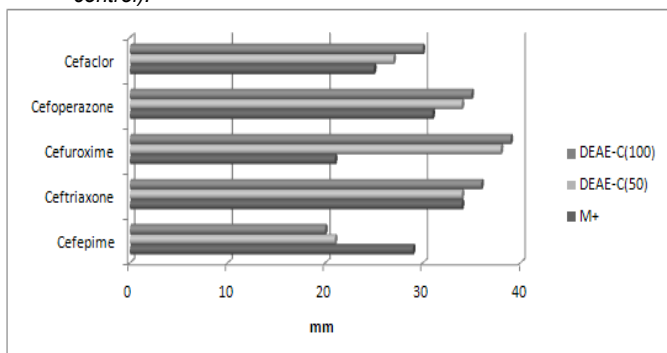


Fig. 5 - Antimicrobial efficacy against *S. aureus*, expressed in diameter of the growth inhibition zone diameter (mm) (M<sup>+</sup> = Antibiotic control) / Eficiența antimicrobiană asupra *S. aureus*, exprimată în diametrul zonei de inhibiție a creșterii (mm) (M<sup>+</sup> = Antibiotic de control).

All measured diameter of inhibition for tested hybrid materials were superior for the antibiotic positive control, excepting DEAE-C<sub>50</sub>/100/Fe<sub>3</sub>O<sub>4</sub>/cefepime against *S. aureus* and DEAE-C<sub>50</sub>/Fe<sub>3</sub>O<sub>4</sub>/cefuroxime against *E. coli*. The results were not significantly dependent on the temperature treatment, excepting DEAE-C<sub>50</sub>/100/Fe<sub>3</sub>O<sub>4</sub>/cefuroxime and cefepime for *E. coli*.

The most efficient was DEAE-C<sub>50</sub>/Fe<sub>3</sub>O<sub>4</sub>/cefepime against *E. coli* and DEAE-C<sub>100</sub>/Fe<sub>3</sub>O<sub>4</sub>/cefuroxime against *S. aureus*. Similar patterns of antimicrobial effect of DEAE-C<sub>50</sub>/Fe<sub>3</sub>O<sub>4</sub>/antibiotic and DEAE-C<sub>100</sub>/Fe<sub>3</sub>O<sub>4</sub>/antibiotic against the tested strains were observed, excepting DEAE-C<sub>50</sub>/Fe<sub>3</sub>O<sub>4</sub>/cefuroxime and DEAE-C<sub>100</sub>/Fe<sub>3</sub>O<sub>4</sub>/cefuroxime against *E. coli*, probably due to specific interaction mechanisms between the hybrid material and the bacterial target, represented by the cell wall. There are a lot of studies reporting that different types of nanoparticles, such as those containing Ag, CuO and ZnO attack bacterial membranes, by collapsing the plasma membrane potential and decreasing the ATP [17,18].

### 4. Conclusions

In the present study, we report the synthesis, characterization and *in vitro* drug delivery response of magnetic DEAE-Cellulose/Fe<sub>3</sub>O<sub>4</sub> systems which act as drug targeting of five cephalosporins. The primary method to analyze the surface modification of hybrid material was SEM and to evidence the deposition of cephalosporins on the DEAE-Cellulose/Fe<sub>3</sub>O<sub>4</sub>, FT-IR measurements were performed. The SEM reveals that magnetite

particles form agglomerates on the DEAE-Cellulose fibers however substantial modifications of the DEAE-Cellulose fiber's morphology can't be highlighted. The "fingerprint" region of the FT-IR spectra from the reference and DEAE-Cellulose/Fe<sub>3</sub>O<sub>4</sub>/cefalosporins regions shows clear differences after deposition of cephalosporins. The effect of hybrid magnetic materials based on traditional antibiotic formulations on the antibacterial properties of different cephalosporins was investigated against *S. aureus* and *E. coli* bacterial strains by a diffusimetric method. The tested magnetic hybrid materials generally increased the activity against tested strains, probably due to the morphological and/or physiological changes induced in the plasma membrane by the nanoparticles, as well as to a better adsorption of the small size nanoparticles, the antimicrobial effect being not significantly dependent of temperature treatment.

**Acknowledgment**

This research was financed by the European Funding Program POSDRU 107/1.5/S/80765 and by the Human Resources Project no. 135/2010 Contract no. 76/2010).

**REFERENCES**

1. S. Sershen, and J. West, Implantable polymeric systems for modulated drug delivery, *Advanced Drug Delivery Review*, 2002, **54**, 1225.
2. R. Asmatulu, M.A. Zalich, R.O. Claus, and J.S. Riffle, Synthesis, characterization and targeting of biodegradable magnetic nanocomposite particles by external magnetic fields, *Journal of Magnetism and Magnetic Materials*, 2005, **292**, 108.
3. M. O. Avile's, A. D. Ebner, and J. A. Ritter, In vitro study of magnetic particle seeding for implant-assisted -magnetic drug targeting, Seed and magnetic drug carrier particle capture, *Journal of Magnetism and Magnetic Materials*, 2009, **321**, 1586
4. J. A. Ritter, A.D. Ebner, K. D. Daniel, and K. L. Stewart, Application of high gradient magnetic separation principles to magnetic drug targeting, *Journal of Magnetism and Magnetic Materials*, 2004, **280**, 184.
5. B. Ganter, S.Tugendreich, C. I. Pearson, E. Ayanoglu, S. Baumhueter, K. A. Bostian, L. Brady, L. J. Browne, J. T. Calvin, G. J. Day, N. Breckenridge, S. Dunlea, B. P. Eynon, L. M. Furness, J. Ferng, M. R. Fielden, S. Y. Fujimoto, L. Gong, C. Hu, R. Idury, *et al.*, Development of a large-scale chemogenomics database to improve drug candidate selection and to understand mechanisms of chemical toxicity and action, *Journal of Biotechnology*, 2005, **119** (3), 219.

6. K. Sugano, Fraction of a dose absorbed estimation for structurally diverse low solubility compounds, *International Journal of Pharmaceutics*, 2011, **405**, (1-2), 79.
7. S. Dhanasingh, Mallesha, and J. J. Hiriyannaiah, Preparation, characterization and antimicrobial studies of chitosan/silica hybrid polymer, *Biointerface Research in Applied Chemistry*, 2011, **1** (2), 48.
8. P. Torchilin, Drug targeting, *European Journal of Pharmaceutical Sciences*, 2000, **11**(2), 81.
9. J.L. Arias, V. Gallardo, S.A. Gomez-Lopera, R.C. Plaza, and A.V. Delgado, Synthesis and characterization of poly(ethyl-2-cyanoacrylate) nanoparticles with a magnetic core, *Journal of Controlled Release*, 2001, **77**, 309.
10. Q.A. Pankhurst, J. Connolly, S.K. Jones, and J. Dobson, Applications of magnetic nanoparticles in biomedicine, *Journal of Physics. D: Applied Physics*, 2003, **36**, 167.
11. R. Ferna'ndez-Pacheco, C. Marquina, J.G. Valdivia, M. Gutie'rrez, M.S. Romero, R. Cornudella, A. Laborda, A. Viloria, T. Higuera, A. Garcí'a, J.A. Garcí'a de Jalo' n, and M.R. Ibarra, Magnetic nanoparticles for local drug delivery using magnetic implants, *Journal of Magnetism and Magnetic Materials*, 2007, **311** (1), 318.
12. E. Crăciun, A. Ioncea, I. Jitaru, M. Ghiurea, and O. Oprea, Organo-inorganic nanostructured coating materials, *Romanian Journal of Materials*, 2011, **41** (1), 64.
13. C. Saviuc, A. M. Grumezescu, A. Holban, C. Chifiriuc, D. Mihăiescu, and V. Lazar, Hybrid nanostructured material for biomedical applications, *Biointerface Research in Applied Chemistry*, 2011, **1** (2), 64.
14. C.Saviuc, A. M.Grumezescu, A. Holban, C. Bleotu, C. Chifiriuc, P. Balaure, and V. Lazăr, Phenotypical studies of raw and nanosystem embedded *Eugenia carryophyllata* buds essential oil antibacterial activity on *Pseudomonas aeruginosa* and *Staphylococcus aureus* strains, *Biointerface Research in Applied Chemistry*, 2011, **1** (3), 111.
15. C. Saviuc, A. M. Grumezescu, E. Oprea, V. Rădulescu, L. Dascălu, M. C. Chifiriuc, M. Bucur, O. Banu, and V. Lazăr, Antifungal activity of some vegetal extracts on *Candida* biofilms developed on inert substratum, *Biointerface Research in Applied Chemistry*, 2011, **1** (1) , 15.
16. A. M. Grumezescu, E. Ilinca, C. Chifiriuc, D. Mihăiescu, P. Balaure, V. Trăistar, and G. Mihăiescu, Influence of magnetic MWCNTs on the antimicrobial activity of cephalosporins, *Biointerface Research in Applied Chemistry*, 2011, **1** (4), 139.
17. P.Gajjar, B.Pettee, D. W. Britt, W.Huang, W. P. Johnson, and A. J. Anderson, Antimicrobial activities of commercial nanoparticles against an environmental soil microbe, *Pseudomonas putida* KT2440', *Journal of Biological Engineering*, 2009, **(3)**, 9.
18. Lok, C.M. Ho, R. Chen, Q.Y. He, W.Y. Yu, H. Sun, P.K. Tam, J.F. Chiu, and C.M. Che, Proteomic analysis of the mode of antibacterial action of silver nanoparticles, *J. Proteome Res.*, 2006, **5** (4), 916.

\*\*\*\*\*