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THE CYTOTOXICITY OF (NON)MAGNETIC NANOPARTICLES TESTED ON ESCHERICHIA COLI AND STAPHYLOCOCCUS AUREUS

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This work represents a preliminary study about the Cytotoxicity of (non)magnetic nanoparticles. Antibiograms with nanostructured powder were performed. (Non)magnetic nanoparticles based on C-Fe, C-Cu, C-Al and C-C, were synthesized by plasma processing and analyzed with High-Resolution Transmission Electron Microscopy (HR-TEM). The difusimetric method was used to prove the bacteriostatic activity. This method is easy to perform and allows for obtaining of specific information related to germ sensitivity. Two reference germs were used: Staphylococcus Aureus ATCC 25923 and Escherichia Coli ATCC 25922. Comparing the inhibition zones, it was found that the cytotoxicity effect is related to (non)magnetic nanoparticles.

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1. Introduction

Since the development of nanomaterials used in drug delivery a potential research of applying CNT as nano-carriers for systems, carbon nanotubes (CNT) have been used for transporting and delivering active substances to cells and organs. Due to their low toxicity when they're functionalised [1, 2, 3] and their good biocompatibility and amazing physical, electrical, optical, and mechanical properties, CNT enable development of new classes of materials for technological and biomedical applications.

Most of the recent studies refere to CNT as carriers for anticancer drugs [4,5,6] and involve various technologies, from simple CNT systems, to improved devices, such as CNTs encapsulated in an alginate-poly-L-lysine-alginate (APA) membrane to form a polymeric membrane for protection[7].

Using nanoparticles for targetting and combating infectious strains and resistant strains in particular is mentioned by Rosen and Elman [8], but it is still expected that a more toxicological and pharmacological profile must be obtained in the future researches. In their opinion, the use of this special career system is required in specific challenges for fighting resistant infectious agents due to the property of carbon atoms in the nanometer scale to create nano-channels via cellular membranes, achieving target activity, and also the capacity of the system to minimize toxicity by reducing the dose of therapeutics.

Nowadays metalic nanoparticles including copper, silver, zinc, iron in inorganic or organic

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matrix [9, 10], but also as metal-coomplexes such as Schiff bases [11] or even silver phyto nanoparticles [12] are studied for antibacterial activity and other biological applications. Many numerical models and quantitative parameters are used for evaluation and life-time prediction of antimicrobial systems. The nanoparticle susceptibility constant is suggested as a quantitative parameter for the estimation of antimicrobial activities of silver and copper nanoparticles [13], or measurement of inhibition zone in the disk diffusion tests conducted in plates, and also by determining the minimum growth inhibitory concentrations (MIC) and minimum bactericidal concentration (MBC) of nanoparticles in liquid batch cultures [14].

In this study, citotoxicity of some magnetic and non-magnetic nanoparticles synthesized by plasma processing against some gram-positive and gram-negative germs is reported in order to establish biologic activity of these materials that could be further used as support for other biologic active compounds.

2. Materials and methods

Preparation of (non)magnetic nanoparticles

(Non)magnetic nanoparticles, synthesized by plasma processing, were purified by:

- solvent extraction (successively with benzene, dichloromethane and o-dichlorobenzene);
- inorganic impurities removal (concentrate warm nitric acid/ chlorhidric acid mixture);
- washing (bidistillated water);
- high temperature (>300^{0}C) treatment.

Nanoparticles' characterization

The primary characterization method was HR–TEM (Figure 1).

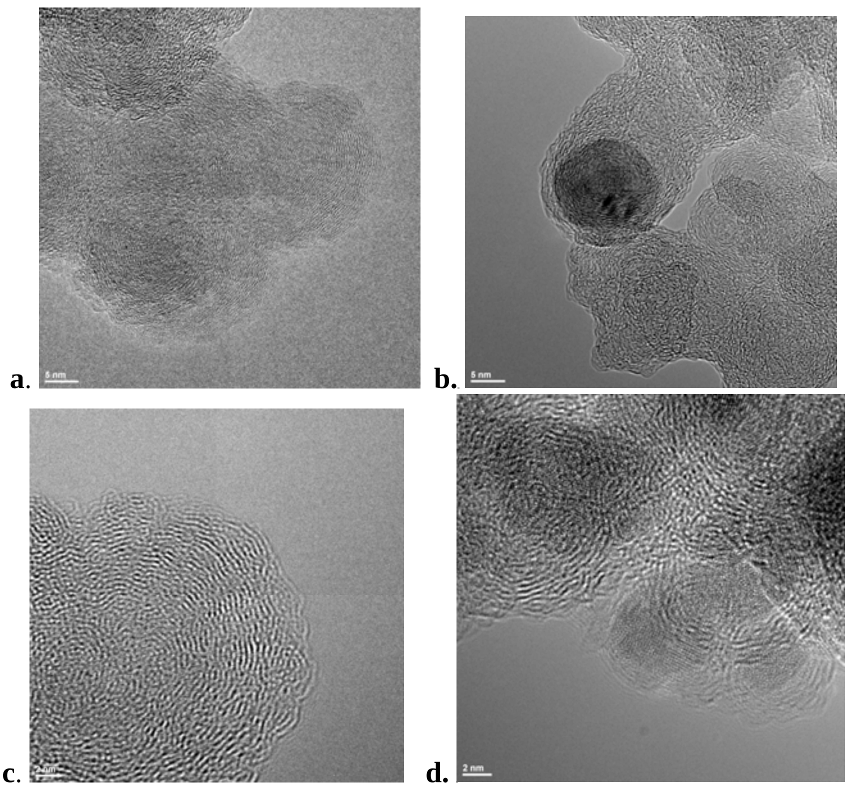

Fig. 1. High-resolution transmission electron microscopy images of magnetic nanoparticles C-Fe/toluene (a - 5 nm, c - 2 nm), C-Fe/benzene-aniline (b – 5 nm, d – 2 nm).

Determination of antibacterial activity

The difusimetric method was used to determine the antibacterial activity. This surface method is easy to perform and allows obtaining of specific information on germ sensitivity, towards a number of antibiotics, also about minimum inhibitory concentration and the minimum bactericidal concentration. This method gains data about the inhibitor and the bactericide efficiency level.

(Non)magnetic nanoparticles were used for disks placed on the gelose surface, seeded with the researched germ. Inhibition zones can be observed due to (non)magnetic nanoparticles gelose diffusion, related to a logarithmic profile. In order to obtain a double inhibition zone, the disks concentration has to be 4 times higher or the researched germ has to be 4 times more sensitive.

When the minimum inhibitory concentration is surpassed (exceeded), the seeded germ does not grow in the (non)magnetic nanoparticles concentration area. This concentration corresponds to the inhibition zone limit. The indirect determination of minimum inhibitory concentration is based on the linear relationship between the inhibition zone dimension and the minimum inhibitory concentration. Therefore, the minimum inhibitory concentration – micrograms per milliliters – and the dimension – in millimeters – of the inhibition zones were determined simultaneously on a large number of germs.

The found dots were placed on semi logarithmic diagram with the diameters of the inhibition zones marked on the arithmetic scale and the minimum inhibitory concentration on the logarithmic one. The regression curve drawn through these dots represents the inhibition diameter.

Following these diagrams, one for each active compound, the minimum inhibitory concentration starting from the dimension of the inhibition zone was determinate, using standard disks, toward a certain germ.

Antibiograms with (non)magnetic nanoparticles were performed. The micro pills used were procured from HiMedia Laboratories.

The micro pills are strictly standardized, and the antibiotic load was in conformity with the standards established by NCCLS USA. In order to perform the antibiograms by difusiometric method, we proceeded as follows: (non)magnetic nanoparticles impregnated micro pills were deposed on the surface of a Mueller Hinton nutritive gelose medium, poured in a Petri dish and uniformly seeded with a bacterial suspension. To be noted that the bacterial suspension consisted of Staphylococcus Aureus and Escherichia coli was in its exponential growing stage. Two reference germs were seeded: Staphylococcus Aureus ATCC 25923 and Escherichia Coli ATCC 25922. The micro pills were taken out of the refrigerator 1-2 hours before their usage in order to thermal stabilization. The minimum distance between the micro pills and the edge of the Petri dish was 15 mm and between the centers of two neighboring disks was 30 mm.

The antibiograms were incubated at 37 °C for 24 hours. The next day the antibiograms results were interpreted by diameter measurements of the complete inhibition zones, around each micro pill with nanostructures powder. Based on the same principle, the difusimetric method was used to perform the antibiograms with (non)magnetic nanoparticles, that were deposed in the same concentrations. The growing medium diameter was correlated to those of the micro pills for a fair assessment of the inhibition zone diameters.

3. Results and discussion

The diameters of inhibition zones (mm) obtained for the use of (non)magnetic nanoparticles are presented in Table I.

The increased inhibition zone diameter for C-Cu particles is due probably to the enhanced transmembrane Cu ion related to the interaction of the no polar carbon surface with the phospholipidic membrane of the bacterial cell.

Table 1. Inhibition zone diameter on (non)magnetic nanoparticles.

(non)magnetic nanoparticles	Inhibition zone diameter [mm] on Escherichia coli	Inhibition zone diameter [mm] on Staphylococcus Aureus
C-C(hexane)	Negative	6
C-Cu(hexane)	10	11
C-Al(hexane)	Negative	Negative
C-Fe(hexane)	Negative	8
C-Fe(toluene)	Negative	Negative
C-Fe(benzen-aniline)	7	7

4. Conclusions

Using the difusiometric method, the Cytotoxicity of (non)magnetic nanoparticles on the E. Colis and S. Aureus bacterium was proved.

The correlation of the inhibited areas with the nanoparticles synthesis path (example C-Fe/hexane or C-C/hexane) shows a strong dependence to the metal type and reagent polarity. The two germs have different behaviors: E. Coli is not sensible, but S. Aureus is very sensible. Another different behavior, related to C-Fe nanoparticles shows different Cytotoxicity for the two germs (Table 1). Using two different reaction environment (hexane, toluene or benzene/aniline) in the plasma process, the resulted nanoparticles present a distinct structure (Figure 1), confirmed with the HR-TEM analysis.

We prove a specific bactericidal activity of the pure nanostructurated support, materials that are used in previously studies only as support for the biologic active compounds. Also an incorporated metal type dependency was proved and will be the subject of further studies.

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