THE ANTI-BACTERIAL ACTIVITY OF MAGNETIC NANOFLUID: Fe₃O₄/OLEIC ACID/CEPHALOSPORINS CORE/SHELL/ADSORPTION-SHELL PROVED ON S. AUREUS AND E. COLI AND POSSIBLE APPLICATIONS AS DRUG DELIVERY SYSTEMS


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Using this system as active compound carrier, the anti-bacterial activity of the Fe₃O₄/oleic acid/cephalosporins nanoparticles (core/shell/adsorption-shell) on S. aureus and E. coli was tested. The dimensions of Fe₃O₄ nanoparticles were in the 5-20 nm range and they were characterized by High Resolution Transmission Electron Microscopy. The antibacterial activity was observed in both, reference Fe₃O₄/oleic acid shell nanoparticles and adsorption shell cephalosporins case. These nanofluids may be used in drug delivery systems.

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1. Introduction

Using magnetic nanoparticles for biological and medical purposes is one of the challenges of the last decade. Magnetic iron-based inorganic nanostructured materials have been synthetized and tested for various applications in medicine: as imaging agents, as heat mediators in hyperthermia treatments, in tissue repair, immunoassay, detoxification of biological fluids, cell separation, as magnetic guidance in drug delivery, etc. The advantages of using these materials come from their magnetic properties, but also from a higher surface per volume ratio, that provides higher sensitivity, better targeting and improvement of the colloidal stability of the nanostructures [1].

One of the most challenging subject of study in nanomaterials science and technology is the synthesis of magnetic nanoparticles. Special properties of the nanoparticles required for biomedical applications [2,3] imply a precise control of particle size, shape, dispersion and conditions that affect these properties. Coating nanoparticles with natural or synthetic polymers or surfactants is a method that provide stability of the ferrofluid colloidal suspensions. Use of surfactants such as: decanoic acid, oleic acid, hexaldehyde or sodium carboxymethylcellulose leads to highly dispersed and high quality nanoparticles with good biocompatibility and smaller particle size [4].

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Coated nanoparticles are important to be obtained for their lower toxicity due to the presence of the biocompatible coating, and also due to the lower adsorption sites for proteins, ions and other components in medium [5]. Usually iron oxides Fe₃O₄ or γ-Fe₂O₃ are synthesized through the co-precipitation of Fe²⁺ and Fe³⁺ aqueous salt solutions [6], by addition of a base. Properties of nanoparticles: size, shape and composition, are influenced by the type of salt, pH, ions ratio and ionic strength of the medium [7]. Other methods use magnetotactic bacteria (MTB) [8] that are able to internalize Fe and convert it into magnetic nanoparticles, in the form of either magnetite (Fe₃O₄) or greigite (Fe₃S₄) [9], or report electrochemical preparation in situ of core-shelled Fe₃O₄ nanoparticles [10]. Sun et al. [11,12] developed a thermal decomposition method that uses a Fe/acac salt, 1,2-hexadecanediol, oleic acid, oleylamine, and biphenyl ether mixture to obtain Fe₃O₄ nanoparticles that are further used for silver coating in order to improve bacterial activity and paramagnetic properties of the nanostructures [13]. A system that uses a combination of magnetron sputtering and gas–aggregation techniques produces Fe nanoclusters of variable controlled mean size (diameters from 2 to 100nm) and high magnetic moments for biomedical applications [14]. Magnetic liposomes based on Fe₃O₄ could be heated at certain temperature in a few minutes and during this time encapsulated anticancer drugs are massively released and strong anticancer effect are produced [15].

In this study, new magnetic core-shelled iron based nanomaterials were obtained, adapting the Massart method in order to improve colloidal dispersion. and to control particles size. Small size of nanoparticles was also on purpose, due to the possibility of targeting through blood barriers [16]. Oleic acid was used as surfactant for coating the Fe₃O₄ nanoparticles, followed by adsorption-coating with four different cephalosporins. The bacterial activity was tested on two different germs: Escherichia Coli and Staphilococcus Aureus.

2. Materials and methods

Preparation of Fe₃O₄ / oleic acid core/shell

Magnetic Fe based nanofluid has been synthesized by Massart method using FeCl₃ and Fe²⁺ salts with oleic acid as the surfactant (scheme 1), under microwave conditions. All the materials were reagent grade and used without further purification. Double distilled, de-ionized water was used as a solvent.
Characterization of Fe$_3$O$_4$/oleic acid-core/shell

Transmission Electron Microscope (TEM) confirmed the formation of nanofluids in the range of 5-20 nm.

![High-resolution transmission electron microscopy images of nanofluids Fe$_3$O$_4$](image)

**Fig. 1. HR-TEM images of nanofluids**

Preparation of Fe$_3$O$_4$/oleic acid/cephalosporins core-shell/adsorption-shell

The core-shell magnetic nanoparticles/oleic acid are dried for 24 hours at 105°C (after the preliminary CHCl$_3$ dispersion) and then dispersed alternately in chloroform with *Cephoperazone, Cefotaxime, Ceftriaxone* and *Cephaclor*. The cephalosporin deposition concentration on nanoparticles was 0.3%. The nanofluid was dried and redispersed. This procedure was repeated 3 times for a maximum yield.
Scheme 2. a. $\text{Fe}_3\text{O}_4 \text{ / oleic acid - core-shell, a-1, 2, 3, 4. Fe}_3\text{O}_4 \text{ / oleic acid/cephalosporins - core-shell/adsorption-shell}$

Fig. 2. Cephalosporins used as adsorption-shell: 1. Cefoperazone, 2. Cefotaxime, 3. Ceftriaxone, 4. Cephachlor

Determination of anti-bacterial activity

The qualitative antibiogram interpretation process follows international standards. Currently accepted standard in most countries (including Romania) is the U.S. standard, developed by CLSI (Clinical Laboratory Standards Institute) [17].

Achievement standard CLSI antibiograms: *Incolumum preparation:* suspensions were made from 2-3 colonies isolated in physiological serum; the suspension turbidity was either
nephelometric controlled or by comparison with standard tubes (that contain latex particles/barium sulfate suspensions with determined turbidity); *Seeding*: a proper medium was chosen according to the tested bacterial species Mueller-Hinton for the majority of bacteria: a cotton ball was introduced in the bacterial suspension, and then squeezed in order to eliminate the liquid excess. The entire surface of the medium was cleaned up three times; *Antibiotic micro pill deposition*: the antibiotics were chosen depending on the bacterial species; the antibiotic disks were deposed at a distance of 1.5 cm from the edge of the Petri dish and 3 cm from each other (maximum 12 disks can be deposed); 15 minutes at room temperature; *Incubation*: depending on the bacterial species: in normal atmosphere, 35°C, 20-24 hours; *Interpretation*: a confluent bacterial culture appeared; inhibition zones appeared around the micro pills (the lack of the bacterial growth); the diameters of the inhibition zones were read, taking into account the used antibiotic, the quantity of the antibiotic in the pill and the tested bacterial species. The experimental diameters were compared with the standard ones.

### 3. Results and discussion

The ferrofluid alone presented a bacteriostatic activity on E. coli and S. aureus (Table II) bacteria. It was observed that, for the same time interval, the inhibition zone diameters for cephalosporins (Table I) was higher than the ones for the cephalosporin-nanofluid (Table 3). This leads to the conclusion that the nanofluid acts as a carrier for the antibiotic. However, the exception was that ceftriaxone and cefotaxime presented a higher inhibition zone in the presence of the ferrofluid than without it.

#### Table 1. Inhibition zone diameter on E. coli and S. aureus

<table>
<thead>
<tr>
<th>Cephalosporins</th>
<th>Inhibition zone diameter [mm] on <em>Escherichia coli</em></th>
<th>Inhibition zone diameter [mm] on <em>Staphylococcus aureus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefoperazone</td>
<td>23</td>
<td>27</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>29</td>
<td>26</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>30</td>
<td>23</td>
</tr>
<tr>
<td>Cephachlor</td>
<td>24</td>
<td>29</td>
</tr>
</tbody>
</table>

#### Table 2. Inhibition zone diameter on E. coli and S. Aureus on Fe₃O₄/oleic acid (core/shell)

<table>
<thead>
<tr>
<th>Nanofluids</th>
<th>Inhibition zone diameter [mm] on <em>Escherichia coli</em></th>
<th>Inhibition zone diameter [mm] on <em>Staphylococcus aureus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe₃O₄-oleic acid core/shell</td>
<td>15</td>
<td>12</td>
</tr>
</tbody>
</table>

#### Table 3. Inhibition zone diameters of cephalosporins extra-shelled Fe₃O₄

<table>
<thead>
<tr>
<th>Cephalosporins adsorption-shelled Fe₃O₄</th>
<th>Inhibition zone diameter [mm] on <em>Escherichia coli</em></th>
<th>Inhibition zone diameter [mm] on <em>Staphylococcus aureus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefoperazone</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>25</td>
<td>28</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>26</td>
<td>28</td>
</tr>
<tr>
<td>Cephachlor</td>
<td>19</td>
<td>22</td>
</tr>
</tbody>
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4. Conclusions

Microwave processing in nanoparticle synthesis leads to high dispersion yields of nanoparticles and small particle size.

Using adsorption process as secondary shell generation process is a convenient way to obtain drug carriers, but the coverage rate is related to active compound structure and polarity.

The bacteriostatic activity on E. Coli and S. Aureus was proved on reference and adsorption shelled nanoparticles, the exceptions being associated with shell-shell interfacial interactions, poor coverage of the adsorption shell or to a low delivery rate of the active compound.

The small size of the particles make possible the delivery of the antibiotic when targeting certain organs like the brain and kidney.

The prepared magnetic nanoparticles can be used for further studies and applications as drug delivery systems.

References