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THE ANTI-BACTERIAL ACTIVITY OF MAGNETIC NANOFLUID: Fe_3O_4 /OLEIC ACID/CEPHALOSPORINS CORE/SHELL/ADSORPTION-SHELL PROVED ON *S. AUREUS* AND *E. COLI* AND POSSIBLE APPLICATIONS AS DRUG DELIVERY SYSTEMS

A. S. BUTEICĂ[a], D. E. MIHAIESCU[b1], A. M. GRUMEZESCU[b], B. Ş. VASILE[b], A. POPESCU[c], O. M. MIHAIESCU[d], R. CRISTESCU[e]

[a]*Faculty of Pharmacy, University of Medicine and Pharmacy of Craiova, 66 1 May Avenue, 200638 Craiova, Romania*

[b]*Faculty of Applied Chemistry and Materials Science, "Politehnica" University of Bucharest, 1–7 Polizu Street, 011061 Bucharest, Romania*

[c]*Faculty of Chemistry, University of Craiova, 107 I "Calea Bucureşti" Street nr.107 I, Craiova, Romania*

[d]*Comporter SRL, Rădiţei Street, No 15, Bucharest, Romania*

[e]*National Institute for Lasers, Plasma and Radiation Physics, Atomistilor 409, Bucharest, Romania*

Using this system as active compound carrier, the anti-bacterial activity of the Fe_3O_4/oleic acid/cephalosporins nanoparticles (core/shell/adsorption-shell) on S. aureus and E. coli was tested. The dimensions of Fe_3O_4 nanoparticles were in the 5-20 nm range and they were characterized by High Resolution Transmision Electron Microscopy. The antibacterial activity was observed in both, reference Fe_3O_4/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 targetting 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].

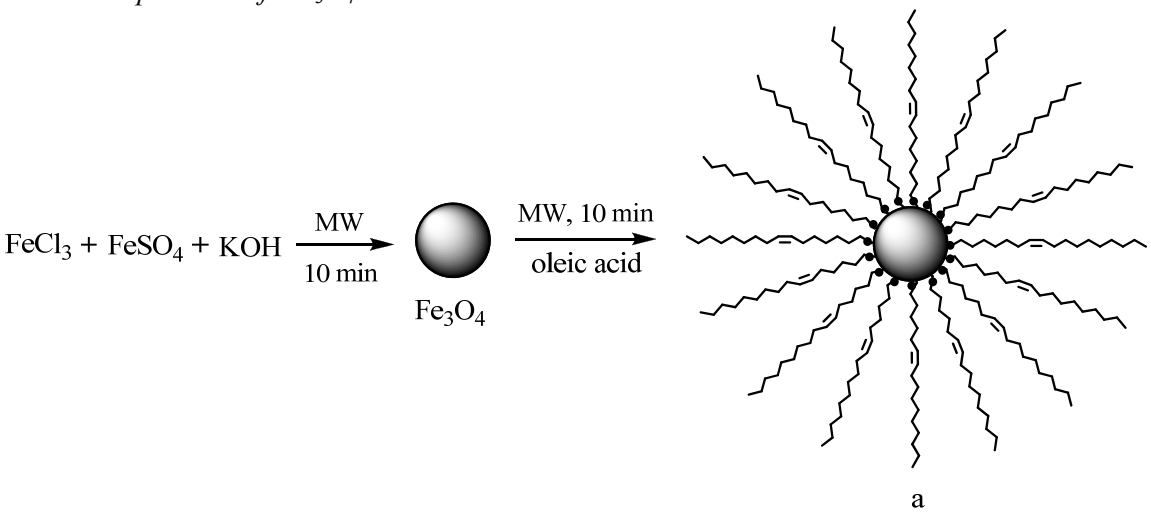
[1] Corresponding author: danmih@usamv.ro

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]. Usualy iron oxides Fe_3O_4 or γ-Fe_2O_3 are synthesized through the co-precipitation of Fe^{2+} and Fe^{3+} 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 (Fe_3O_4) or greigite (Fe_3S_4) [9], or report electrochemical preparation in situ of core-shelled Fe_3O_4 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 Fe_3O_4 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 Fe_3O_4 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 targetting through blood barriers [16]. Oleic acid was used as surfactant for coating the Fe_3O_4 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 Fe_3O_4 / oleic acid core/shell

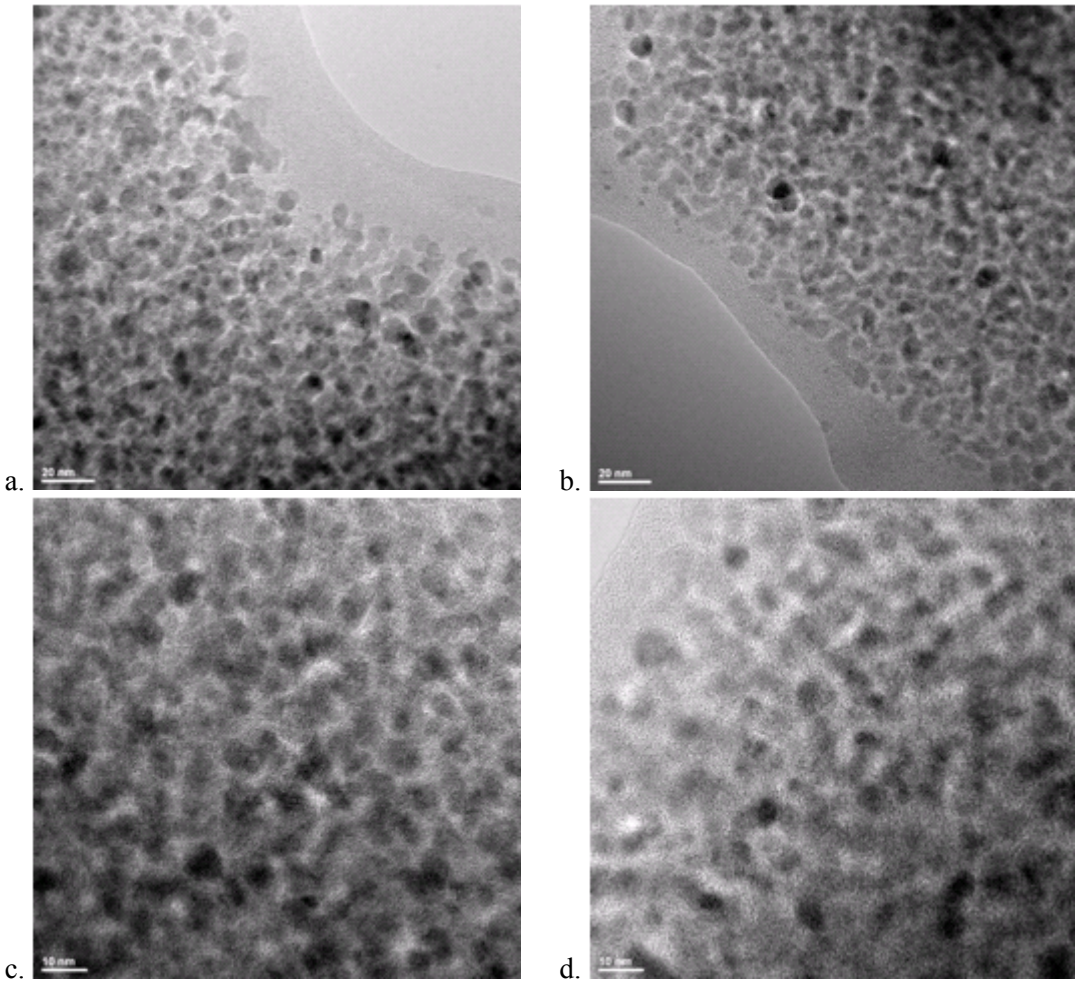


Scheme 1. a Fe_3O_4 / oleic acid - core/shell

Magnetic Fe based nanofluid has been synthesized by Massart method using $FeCl_3$ and Fe^{2+} 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_3O_4 /oleic acid-core/shell

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

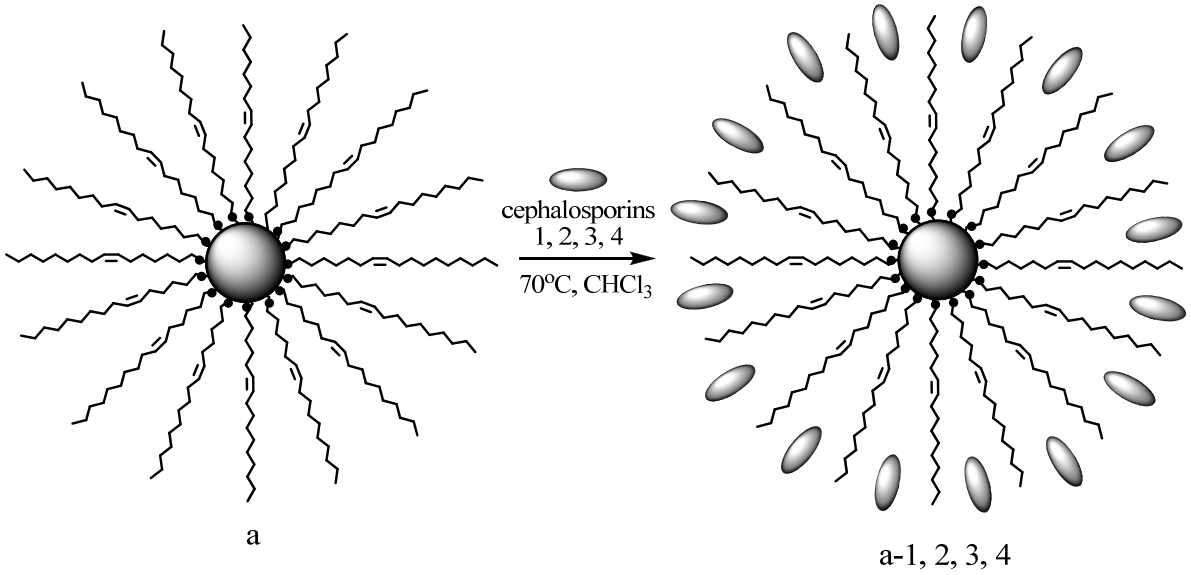


High-resolution transmission electron microscopy images of nanofluids Fe_3O_4 (*a,b* - 20 nm, *c,d* - 10 nm)

Fig. 1. HR-TEM images of nanofluids

Preparation of Fe_3O_4 /oleic acid/cephalosporins core-shell/adsorption-shell

The core-shell magnetic nanoparticles/oleic acid are dried for 24 hours at 105ºC (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. Fe_3O_4 / oleic acid - core-shell, a-1, 2, 3, 4. Fe_3O_4 / oleic acid/cephalosporins - core-shell/adsorption-shell

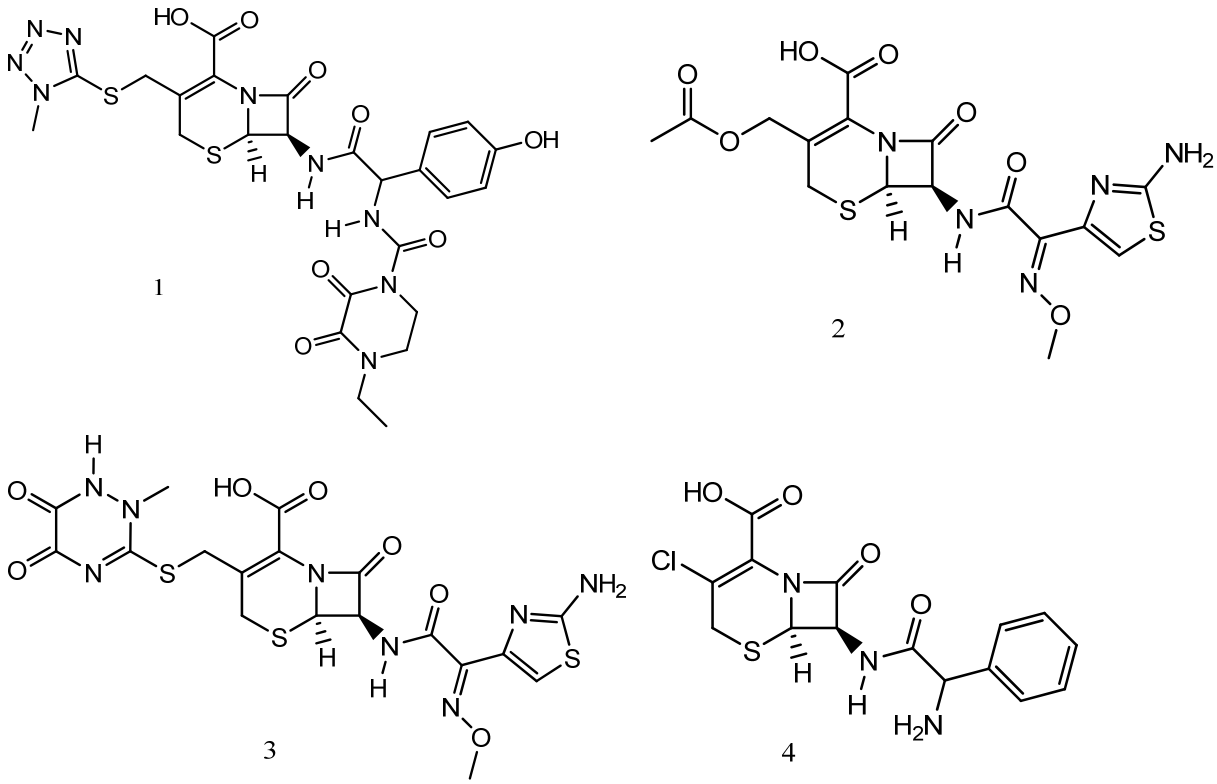


Fig. 2. Cephalosporins used as adsorption-shell: 1. Cefoperazone, 2. Cefotaxime, 3. Ceftriaxone, 4. Cephachlor

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

Cephalosporins	**Inhibition zone diameter [mm] on *Escherichia coli***	**Inhibition zone diameter [mm] on *Staphylococcus aureus***
Cefoperazone	23	27
Cefotaxime	29	26
Ceftriaxone	30	23
Cephachlor	24	29

Table 2. Inhibition zone diameter on E. coli and S. Aureus on Fe_3O_4 /oleic acid (core/shell)

Nanofluids	**Inhibition zone diameter [mm] on *Escherichia coli***	**Inhibition zone diameter [mm] on *Staphylococcus aureus***
Fe_3O_4-oleic acid core/shell	15	12

Table 3. Inhibition zone diameters of cephalosporins extra-shelled Fe_3O_4

Cephalosporins adsorption-shelled Fe_3O_4	**Inhibition zone diameter [mm] on *Escherichia coli***	**Inhibition zone diameter [mm] on *Staphylococcus aureus***
Cefoperazone	22	22
Cefotaxime	25	28
Ceftriaxone	26	28
Cephachlor	19	22

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 targetting certain organs like the brain and kidney.

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

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