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SYNTHESIS AND CHARACTERIZATION OF FERRITES (Fe_3O_4 /$CuFe_2O_4$) - CALCIUM ALGINATE HYBRIDS FOR MAGNETIC RESONANCE IMAGING

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This paper describes the synthesis of Fe_3O_4 and $CuFe_2O_4$ nanoparticles coated with a polysaccharide type biopolymer - calcium alginate (CaA). The obtained hybrid materials have been characterized by X-ray diffraction (XRD), Transmission Electron Microscopy (TEM) and Fourier Transform Infrared spectroscopy (FTIR). The Fe_3O_4- CaA and $CuFe_2O_4$- CaA hybrids under the investigation have an average particles size of about 10 nm and 12 nm respectively, and exhibit superparamagnetic behavior with saturation magnetization values of 81 emu/g and 22 emu/g. The FTIR carboxylate frequencies of both hybrids were shifted to lower values in comparison with those of free polymer indicating the interactions between ferrite particles and calcium alginate. The biological activity tests and MRI investigation results revealed the potential of using the obtained hybrid materials as contrast agents.

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1. Introduction

The interest in nanosized magnetic therapeutic systems has increased due to their excellent biocompatibility, targeting action and subcellular size. The synthesis of biocompatible superparamagnetic hybrids consisting of magnetic oxide nanoparticles and polymers has been intensively studied for their biomedical applications in diagnosis and cancer treatment [1,2,3,4]. Magnetite is the most used magnetic material due to the strong magnetic properties and excellent biocompatibility [5,6]. Together with magnetite, copper ferrite nanoparticles have already been tested in cancer therapy [7]. Based on their magnetic properties the ferrite nanoparticles can be manipulated by an external magnetic field gradient through the human tissue to the tumor cells Thus is possible to diagnose the stage of the cancer development (magnetic resonance imaging, MRI), to destroy the cancerous cells by heating (hyperthermia), to deliver an anticancer drug to a tumor in which the drug release rates would be activated by a magnetic external stimuli. Considering that, the chemotherapy is a non-specific cancer treatment very often used, which leads to a general destruction of healthy cells besides cancerous cells, it is obvious the major potential offered by magnetic nanomaterials for this type of biomedical applications.

Oxide nanoparticles biocompatibilization is made possible through modification of their surface, usually, by coating with biologic molecules like polymers (i.e. dextran, alginate) and phospholipids [8]. This offers many advantages to the final obtained hybrids such as the possibility to link on the target zone (i.e. cancer cells), the increase of the chemical stability in human body fluids and the prevention of the particles agglomeration by providing a steric barrier against the agglomeration [9]. Calcium alginate polysaccharide type biopolymer, composed of (1-4) linked β-

D-mannuronic acid and α-L- guluronic acid monomers, was chosen for providing biocompatibility and targeting action because cancer cells prefer to feed on sugar compounds.

This study presents the synthesis and characterization of two hybrid materials consisting of magnetite or copper ferrite magnetic nanoparticles coated with calcium alginate, which possess superparamagnetic properties and non-toxic effect on *Pseudomonas Aeroginosa*, *Aspergillus Niger*, *Fusarium Oxisporum* and *Candida Scotti* microorganisms. The MRI tests of the Fe_3O_4-CaA and $CuFe_2O_4$- CaA hybrids revealed higher performance images than those obtained for gadolinium contrast agent.

2. Experimental

All reagents were analytical grade. $Fe(C_2O_4)_2{\cdot}2H_2O$, $Cu(CH_3COO)_2$, γ-Fe_2O_3, alginic acid, sodium hydroxide and calcium chloride were purchased from Sigma Aldrich.

The hybrid materials were obtained in a single step method: 100 mL of 0.01M $FeC_2O_4{\cdot}2H_2O$ or 100 mL of 0.01M $Cu(CH_3COO)_2$ aqueous solution and γ-Fe_2O_3 nanoparticles in 1:1 molar ratio were mixed under reflux for 5 days at 100^0C. Then, 50 mL of 1% sodium alginate aqueous solution was added drop wise into the reaction mixture and the suspension was kept at 60^0C. After 7 days, 50 mL of 0.5% $CaCl_2$ solution was added into the mixture and the suspension was kept at 80^0C for 6 h. The two obtained hybrids (Fe_3O_4- CaA and $CuFe_2O_4$- CaA) were purified by repeatedly washing with water and ethanol.

Characterization of magnetite and copper ferrite powders was done by X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR). The Fe_3O_4- CaA and $CuFe_2O_4$- CaA hybrids were characterized by atomic absorption spectroscopy (AAS), XRD, FTIR and Transmission electron microscopy (TEM). AAS measurements were carried out on ANALYTIK JENA Atomic Absorption Spectrophotometer Contra AA7. X-ray diffraction was performed on a Bruker AXS diffractometer type D8 ADVANCE with Cu Kα radiation. FTIR spectra were done on a Testscan Shimadzu 8000 spectrophotometer (KBr pellet technique). Transmission electron microscopy (TEM) images of the magnetic hybrid materials were obtained by using a Philips CM 20 transmission electron microscope. The magnetic measurements of the hybrid materials samples were done as a function of magnetic field using a VSM Lake Shore 7300 magnetometer. The disk diffusion and MIC (minimum inhibitory concentration) methods were used to evaluate the biological activity of the prepared hybrids against three bacterial and one fungal cultures *Pseudomonas Aeroginosa* (as gram positive bacteria) *Staphylococcus Aureus* (as gram positive bacteria), *Escherichia Coli* (as gram negative bacteria) and *Candida Albicans* (as gram positive fungus). The qualitative disc diffusion method was described elsewhere [10]. MIC is the lowest concentration that will inhibit the growth of the microorganisms and is used by diagnostic laboratories to determine *in vitro* activity of compounds. The Eppendorf tubes with Mueller Hinton broth for bacteria and Sabouraud glucose broth for fungi containing the samples are incubated at 37°C for 48 hours. The performance of the obtained hybrid materials as contrast agent in solution have been measured by MRI tomography.

3. Results and discussion

The ferrites contents of the hybrids, determined by using atomic absorption spectroscopy, were 82% magnetite for Fe_3O_4- CaA and 80% copper ferrite for $CuFe_2O_4$- CaA.

The XRD patterns of the uncoated powders depicted in Fig.1a and b have proved the formation of Fe_3O_4 and $CuFe_2O_4$ with the expected inverse spinel structure. The magnetite power has cubic symmetry, whereas copper ferrite has tetragonal symmetry.

The XRD patterns of magnetite - calcium alginate and copper ferrite-calcium alginate hybrids present beside the characteristic peaks of ferrites, peaks assigned to calcium alginate biopolymer indicating the coating of ferrite nanoparticles with biopolymer (Fig.1.d and e). The average crystallite size calculated by Scherrer formula for uncoated magnetite and copper ferrite is 9 nm and 11 nm respectively.

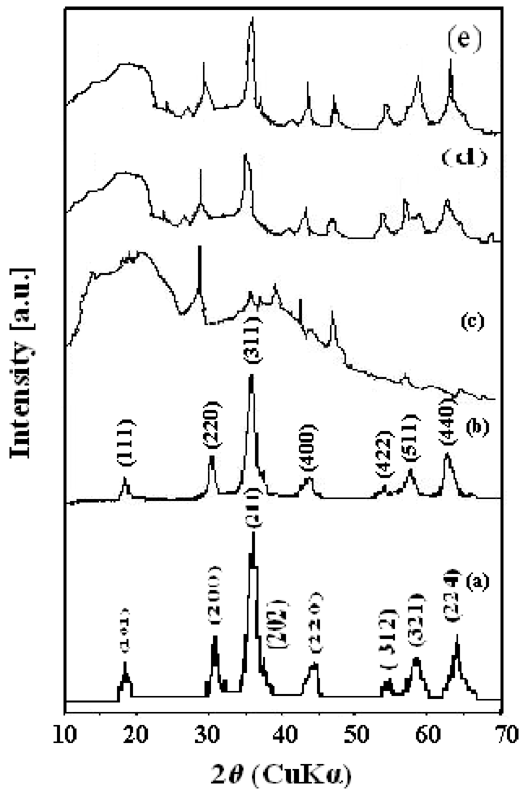


Fig. 1. XRD patterns of: (a) copper ferrite, (b) magnetite, (c) calcium alginate, (d) Fe_3O_4-CaA and (e) $CuFe_2O_4$- CaA hybrids

The interactions between ferrite nanoparticles and calcium alginate were studied by FTIR spectroscopy. Tabel 1 shows the most relevant FTIR spectral bands of the ferrites nanopowders, calcium alginate biopolymer and prepared hybrids.

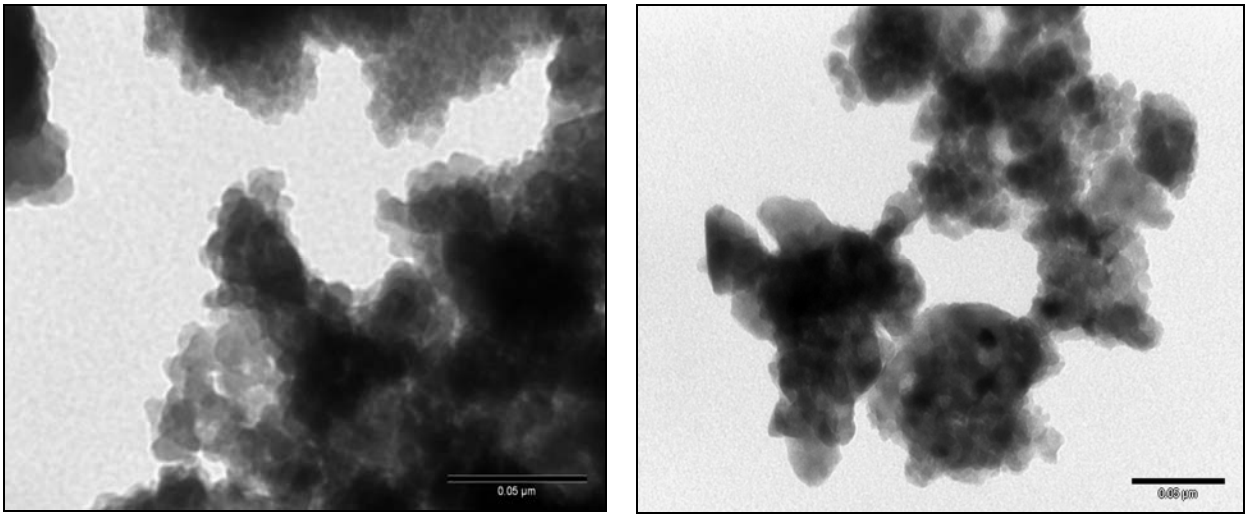
The FTIR spectra of the ferrites present a broad band centered at approximately 3450 cm^{-1} of –OH stretching vibration (vOH) attributed to the surface hydroxyl groups that could interact with the carboxylate binding site of the calcium alginate .

The FTIR spectra Fe_3O_4- CaA and $CuFe_2O_4$- CaA hybrids present the characteristic band of carboxylate ions shifted to higher frequencies (1650 cm^{-1} and 1646 cm^{-1} respectively) in comparison with that of free biopolymer (1596 cm^{-1}) indicating interactions of Fe-OH.....OOC or Fe....OOC type. The FTIR spectra of the obtained hybrids indicate in the region 400-1000 cm^{-1} characteristic bands for spinels. Thereby, in FTIR spectra of the prepared hybrids the metal-oxygen vibration band in the octahedral sites is placed at around 600 cm^{-1}, whereas the metal-oxygen vibration band in the tetrahedral sites is found at 480 cm^{-1}. In the case of FTIR spectra of uncoated ferrites, the metal-oxygen bands are at lower frequencies than those of the corresponding hybrids.

Tabel 1. The most relevant FTIR spectral bands of ferrites, calcium alginate and *corresponding hybrids.*

Calcium alginate (cm^{-1})	Fe_3O_4 (cm^{-1})	$CuFe_2O_4$ (cm^{-1})	Fe_3O_4- CaA (cm^{-1})	$CuFe_2O_4$- CaA (cm^{-1})	Assignments
3440	3450	3452	3462	3458	ν_{O-H}
1630	-	-	1650	1646	$\nu_{as\ (CO)}$
1429	-	-	1456	1444	$\nu_{s\ (CO)}$
-	482	470	490	480	$\nu_{M(-O}$ ν_{M-O}
-	580	600	600	620	ν_{M-O}

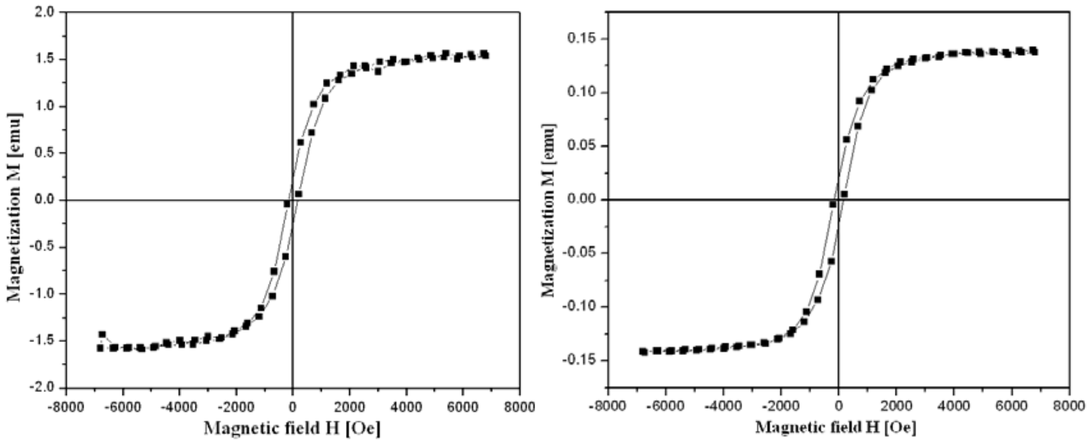
TEM images of the obtained hybrids are presented in Figs. 2.a and b. It could be observed that the magnetite and copper ferrite nanoparticles are coated by calcium alginate. The mean particles size of Fe_3O_4- CaA and $CuFe_2O_4$- CaA hybrids determined by considering around 150 particles of the TEM images is 10 nm and 12 nm, respectively. This aspect is in agreement with the mean crystallite size determined from XRD analysis. The small particle sizes of the obtained hybrids could be explained by stopping the ferrite particles growth after coating with calcium alginate. The spherical shaped hybrid particles are monodispersed suggesting that the using of biopolymer is an effective way of controlling agglomeration and the particles size.



(a) (b)

Fig.2. TEM imagines of Fe_3O_4- CaA (a) and $CuFe_2O_4$- CaA (b) hybrids

The magnetization versus magnetic field plots (M–H hysteresis loop) at 298 K, for Fe_3O_4- CaA and $CuFe_2O_4$- CaA hybrids are shown in Figs. 3. a and b. The weak hysteresis is probably assigned to the small size of the ferrite particles having a single magnetic domain. The saturation magnetization values obtained considering the ferrites percentage of the hybrids were found to be 81 emu/g for magnetite based hybrid and for 22 emu/g for copper ferrite based hybrid lower than those of the corresponding "*bulk*" ferrites.



(a) (b)

Fig.3. Magnetization vs. magnetic field of Fe_3O_4- CaA (a) and $CuFe_2O_4$- CaA (b) hybrids

Table 2. Magnetic properties information for obtained hybrids.

No.	Sample	Mass [g]	Moment [emu]	M_S [emu/g]
a	Fe_3O_4- CaA	0,0194	1,58	81,44
b	$CuFe_2O_4$- CaA	0,0064	0,141	22,03

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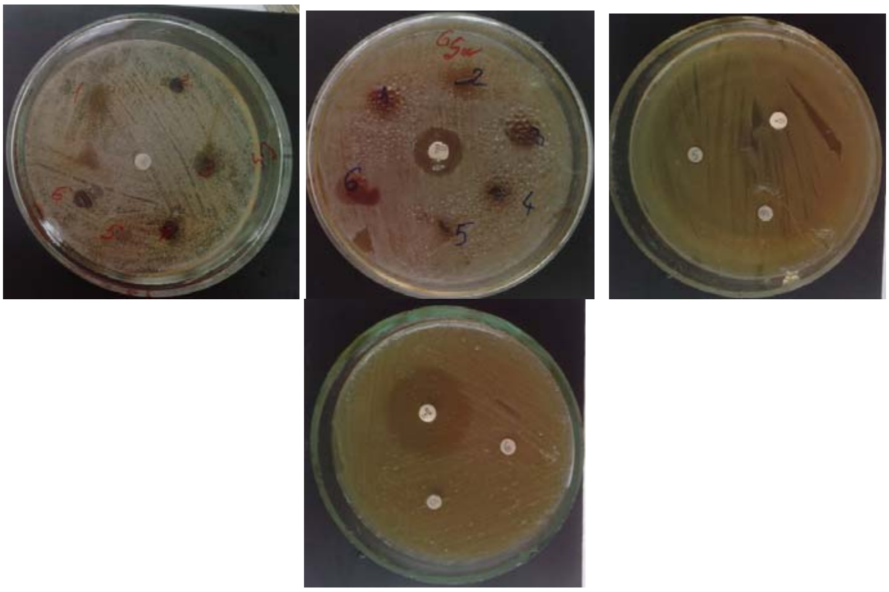
Superparamagnetic behavior (i.e. no hysteresis upon a switching field) is preferred for MRI applications. The superparamagnetic behavior is characteristic for smaller magnetic nanoparticles with the sizes below 20 nm [11].

Biological tests

The biological activity of Fe_3O_4- CaA and $CuFe_2O_4$- CaA hybrids determined by using the disc diffusion and MIC method against *Escherichia Coli*, *Pseudomonas Aeroginosa*, *Staphylococcus Aureus* and *Candida Scotti* revealed no inhibitory effects on the microorganisms growth.

In the case of disc diffusion method, the experiments were conducted in comparison with standard antibiotic effect by measuring inhibition zone diameters and the experimental results are presented in Fig.4. For MIC determination the concentrations range of the tested hybrid was 1000-3000 μg/mL.



(a) (b) (c) (d)

Fig. 4. Pictorial diffusion spots of Candida Scotti (a) and Staphylococcus Aureus (b) for 1,2,3 Fe_3O_4- CaA sample and 4,5,6 $CuFe_2O_4$- CaA sample; Pseudomonas Aeroginosa (c) and Escherichia Coli (d) for 1 Fe_3O_4- CaA and 2 $CuFe_2O_4$- CaA.

MRI tests

In magnetic resonance imaging (MRI) technique, intrinsic differences between tissues are often too small to provide distinguishable relaxation times for protons. For this reason, the contrast agents are often used. They accelerate the T1 and T2 relaxation processes of water protons in their surroundings. The samples used for MRI tests were:

P1 – Gadolinium contrast agent.

P2 - $CuFe_2O_4$ nanoparticles coated with calcium alginate polymer.

P3 – Fe_3O_4 nanoparticles coated with calcium alginate polymer.

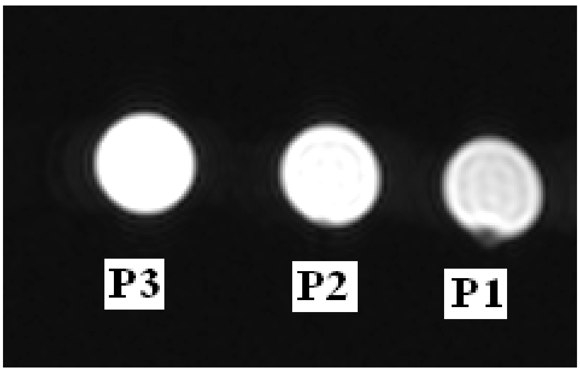


Fig.5. T2 weighted transversal images (A spin-spin relaxation MRI images) of gadolinium contrast agent (P1), copper ferrite- calcium alginate hybrid (P2) and magnetite- calcium alginate hybrid (P3).

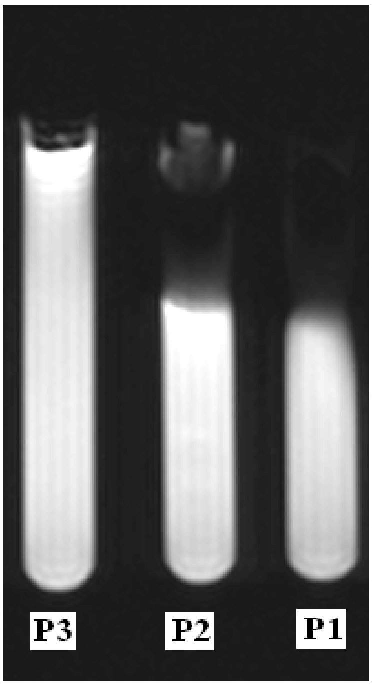


Fig.6. T2 weighted coronal images (A spin-spin relaxation MRI images) of gadolinium contrast agent (P1), copper ferrite- calcium alginate hybrid (P2) and magnetite- calcium alginate hybrid (P3).

The Figs. 5 and 6 show that Fe_3O_4- CaA and $CuFe_2O_4$- CaA hybrids present a higher image contrast than that of the gadolinium (MRI standard contrast agent).

The calcium alginate component of the hybrid materials, that protects the ferrites core from aqueous environment, generates a distance between the water protons and ferrites core. The interaction of the superparamagnetic core with water protons is made through a magnetic dipole coupling, calculated as $1/d^3$ (where d is the distance between the magnetic core and the water protons). If the polymer coat is very large, the interaction through magnetic dipole coupling disappears. In order to a strong interaction, the distance between the magnetic core and the water protons have to be reduced [12]. This approach was used in the present study.

4. Conclusions

Two hybrid materials based on ferrites (Fe_3O_4 and $CuFe_2O_4$) and calcium alginate biopolymer were obtained in a single-step procedure. Coating of ferrite nanoparticles with calcium alginate has been done successfully as indicated by the FTIR, XRD and TEM analyses. TEM images indicated for Fe_3O_4- CaA and $CuFe_2O_4$- CaA hybrids the average particles size of 10 and 12, respectively. The tests on four different microorganisms, *Escherichia coli*, *Pseudomonas aeroginosa*, *Staphylococcus aureus* and *Candida Scotti, respectively* revealed the non-toxic activity of the as-prepared hybrids. The MRI images indicate a higher performance as contrast agents of copper ferrite -calcium alginate and magnetite calcium alginate hybrids in comparison with standard gadolinium contrast agent.

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