BULETINUL INSTITUTULUI POLITEHNIC DIN IAȘI
Publicat de
Universitatea Tehnică „Gheorghe Asachi” din Iași
Volumul 65 (69), Numărul 4, 2019
Secția
ELECTROTEHNICĂ. ENERGETICĂ. ELECTRONICĂ

DEVELOPMENT AND CHARACTERISATION OF NOVEL NANO-CONTRAST PARTICLES FOR MEDICAL IMAGING

BY

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Received: July 30, 2019
Accepted for publication: September 18, 2019

Abstract. The paper describes the laboratory development of new contrast agents for both MRI and THz imaging, starting from gadolinium oxide and superparamagnetic iron oxide composites, based on carboxymethylcellulose, with larger molecular weight and improved features. Transmission Electron Microscopy (TEM) analysis of the functionalized metallic oxides revealed the presence of the carboxymethylcellulose in the proximity of the inorganic component of the system, with a homogenous distribution. In addition, the functionalization process allowed the inorganic particles to be fully disaggregated in the presence of the considered polymer. The functionalized metallic oxide particles had dimensions resulting in an average size of 9,…, 11 nm. The nano-composites presented a greater optical-electromagnetic activity, especially in the wavelength range of 250 nm. Preliminary tests for bio-compatibility demonstrated an acceptable tolerability of functionalized nanoparticles with a degree of over 70%, which may suggest a good perspective for further in vivo experiments.

Keywords: nano-contrast particles for medical imaging; superparamagnetic oxides; carboxymethylcellulose based composites; optical-dielectric tests.

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1. Introduction

Nanotechnology offers the possibility of innovation in the field of medical imaging, by creating new contrast agents in order to increase the image resolution in regions where normal or pathological tissues coexist. Although this area of research is still in its infancy, the preliminary results described by literature are positive and allow new horizons for the clinical application of MRI and THz medical images, e.g. the research results of Oh S.J. and his team in the field of THz molecular imaging in vitro and in vivo (Oh *et al*., 2008, 2009, 2011). Initially, gold hydroxyapatite nanocomposites and gold nanorods (GNRs) have been studied and it has been shown that such contrast agents can increase sensitivity and improve the contrast of cancerous tissues. Other research groups *e.g*. (Lee *et al*., 2008; Stelianou & Talias, 2013; Hellebust & Richards-Kortum, 2012; Dong *et al*., 2011; Yousafa *et al*., 2012; Zhuxian & Zheng-Rong, 2013) studied magnetic and gadolinium oxide nanoparticles as THz and MRI contrast agents, as their interaction with e.g. THz waves is very strong (power absorption is about 3 orders higher than water). Also, gadolinium diethylene glycol penta-acetic acid (Gd-DTPA) received attention because it can be targeted to a particular cell by attaching an antigen or antibody, and demonstrated low toxicity, but it is easily eliminated by renal excretion due to its low molecular weight. Therefore, new gadolinium oxide or superparamagnetic iron oxide composites, with larger molecular weight and improved bio-compatibility must be developed to serve as contrast agents for tumor diagnosis, using both MRI and THz imaging. In addition, such composites may be additionally used in cell distributions monitoring, cell tracking, and other biological applications, i.e. monitoring of drug delivery processes.

2. Development of Gadolinium Oxide and Superparamagnetic Iron Oxide Composites with Increased Molecular Weight

The development of superparamagnetic iron oxide composites was based on tailored magnetite powder, obtained at laboratory level by co-precipitation method. The process consisted in adding a basic solution (NH_4OH 25%) to a solution containing iron (II) and iron (III) ions ($FeCl_3 \times 6H_2O$ and $FeCl_2 \times 4H_2O$) in stoichiometric proportions until a certain pH value is reached in a controlled atmosphere (constant flow rate of N_2 into the system). The main advantages are: simple and fast preparation of compounds; the reproducibility of the chemical composition; good control over the particle size distribution through the co-precipitation process parameters. It should be noted that temperature is an important factor in the co-precipitation process, determining the primary size of the crystallites, the specific surface and phases. The experimental setup used for magnetite synthesis is shown in Fig. 1. The solid product was separated by magnetic decantation, washed with distilled water to remove the excess of basic solution and dried in the oven at 50°C for 12 hours.

The obtained superparamagnetic iron oxide was characterized by X-ray diffraction (XRD) – to verify the success of the synthesis process – and by transmission electron microscopy (TEM) - to determine the particle size. All the diffraction maxima, as in Fig. 2 can be identified and indexed according to JCPDS card no. 19-0629 (Iyengar *et al.*, 2014), *i.e.* corresponding to the plans (2 2 0), (3 1 1), (2 2 2), (4 0 0), (4 2 2), (5 1 1), (4 4 0) , (6 2 0), (5 3 3), fact which confirms the formation of spinel structure with a very high degree of crystallinity. The morphology of the magnetite particles was studied by TEM (Fig. 3), and it was found that magnetite nanoparticles present a regular shape, almost spherical, with dimensions resulting in an average size of 9.42 nm.

Fig. 1 – Experimental setup for synthesis of Fe_3O_4

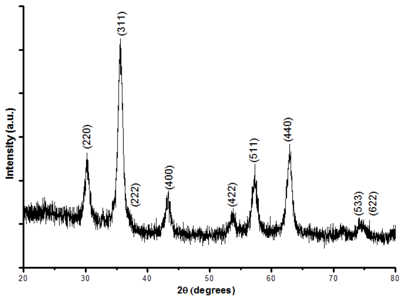

Fig. 2 – XRD analysis of Fe_3O_4

Functionalization (physical and/or chemical surface preparation) of magnetite with carboxymethylcellulose - CMC was performed by preparing a magnetic fluid ultrasounded for 15 minutes, after which a solution of carboxymethylcellulose was added. The use of carboxymethylcellulose is justified by recent studies dealing with its high biocompatibility-biodegradability and feasibility in manufacturing biocompatible nano-composites, coated particles or hybrid drugs, as in (Capanema *et al.*, 2018). The resulting mixture was ultrasounded again for 1 hour at 50°C. TEM analysis of the magnetite functionalized with carboxymethylcellulose reveals the presence of the cellulose derivative in the proximity of the inorganic component of the system, with a homogenous distribution, concluding that the functionalization with carboxymethylcellulose does not affect the size distribution of the magnetite particles or the value of the average size.

The development of gadolinium oxide composites was based on commercial gadolinium oxide that was functionalized with carboxymethyl-cellulose, by an adapted methodology as used for superparamagnetic iron oxide. Initially, the commercial gadolinium oxide was characterized by electron microscopy and it is observed that it consisted of irregular and highly agglomerated particles. The first attempt for reducing particles agglomeration was based on ultrasonic dispersion in a concentrated solution of acetic acid, but the results were modest. Finally, after the functionalization with carboxy-methylcellulose form the dispersion in acetic acid, the same TEM analysis, Fig. 4, demonstrated that the functionalization process allowed the inorganic particles to be fully disaggregated in the presence of the considered polymer. The functionalized gadolinium oxide particles had dimensions resulting in an average size of 10.78 nm, as in Ciobanu *et al.*, (2018).

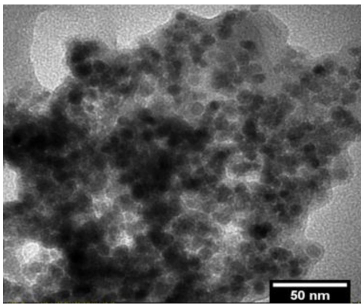

Fig. 3 – TEM analysis of CMC - Fe_3O_4.

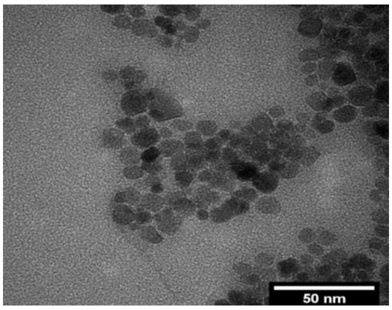

Fig. 4 – TEM analysis of CMC – Gd_2O_3.

3. Functional Characterization of Gadolinium Oxide and Superparamagnetic Iron Oxide Composites

The dielectric properties were tested with a broadband impedance dielectric spectroscope. The extended study included pure and activated Fe_3O_4 and Gd_2O_3 particles, and also the activation precursor of Gd_2O_3 particles, *i.e.* Gd_2O_3 in acetic acid (Gd_2O_3 AC). The results indicate that the functional activation of the Fe_3O_4 and Gd_2O_3 particles had a small influence on the dielectric permittivity, Fig. 5, which mainly depends on the base particles, thus presenting higher values for Fe_3O_4 and an expected decrease with frequency for all compounds. Relevant, however, was the variation of the characteristics vs. frequency of dielectric losses, which indicates that the activation of Fe_3O_4 and Gd_2O_3 nano-particles with carboxymethylcellulose conferred significant dielectric losses at higher frequencies, Figure 6, i.e the composites presented a greater optical-electromagnetic activity. It is expected that the affinity of these particles to cancerous tissue to lead to increased sensitivity and contrast in THz and MRI imaging.

The optical properties of the activated particles of Fe_3O_4 and Gd_2O_3 were analyzed using the UV-Vis spectrophotometer. The results confirmed the observations for dielectric losses, i.e. the absorbance is higher in the particles activated with carboxymethylcellulose, with approx. 30% higher than for inactivated particles, especially in the field of interest for medical imaging, respectively in the wavelength range of 250 nm, and more, there is an extension of the domain for which a high absorbance is obtained and towards the domain of 300 nm wavelength, Figs. 7 and 8.

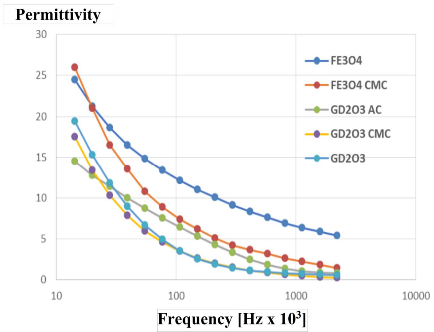

Fig. 5 – Permittivity *vs*. Frequency.

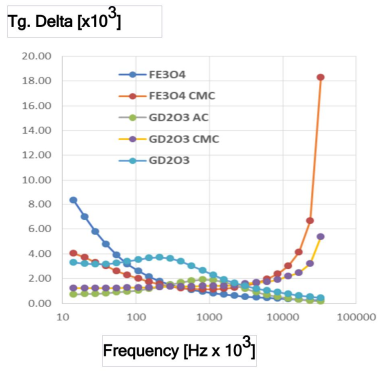

Fig. 6 – Tg Delta *vs*. Frequency.

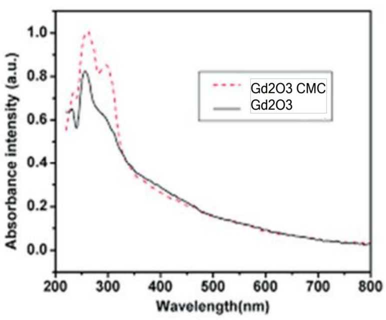

Fig. 7 – UV-Vi of Gd_2O_3/Gd_2O_3 CMC.

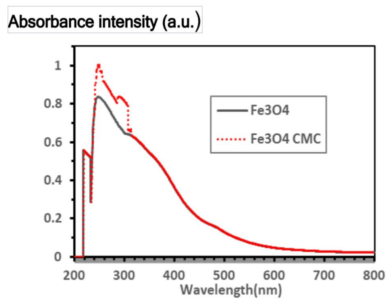

Fig. 8 – UV-Vis of Fe_3O_4/Fe_3O_4 CMC.

Finally, the basic bio-compatibility of the contrast agents was tested, in terms of experimental cell lines viability and survival. The cells were seeded into well-plates (10,000 cells/well) and after the incubation period, the cells were supplemented with the tested nanoparticles (Fe_3O_4 and Gd_2O_3 with carboxymethylcellulose) in consecutive dilutions during 24 hours. The cell viability was assessed by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium) assay. It was generally noticed that Fe_3O_4 nano-particles with carboxymethylcellulose induced a reduced cytotoxic effect in dilutions of between 1:100 – 1:10, followed by a moderate decrease in cell viability at dilutions up to 1:1. The maximum cytotoxic value was only 30%. In the case of Gd_2O_3 nano-particles with carboxymethylcellulose, the impact on cell viability is moderate in the dilution range 1:100 – 1:1. The maximum cytotoxic value was 50% in the case of maximum dilution (1:1).

Cellular tolerability provided to be similar for the two types of nanoparticles functionalized with carboxymethylcellulose, but slightly wider in terms of the dilution range for Gd_2O_3 nanoparticles. The existence of an acceptable tolerability of functionalized nanoparticles with a degree of over 70% at dilutions between the limits of 1:100 – 1:5, may suggest a similar behavior, *i.e.* non-toxic, in the case of administration to experienced animals.

3. Conclusions

The paper describes the laboratory development of new gadolinium oxide and superparamagnetic iron oxide composites, based on carboxymethylcellulose, with larger molecular weight, that can serve as contrast agents for tumor diagnosis, using both MRI and THz imaging. The functionalization process allowed the inorganic particles to be fully disaggregated in the presence of the considered polymeric medium, aspect extremely important when intending the dispersion of the respective contrast agents upon the tissues to be visualized. The functionalized metallic oxide particles had dimensions resulting in an average size of 9,…,11 nm, enough small to allow a good contact and a relevant resolution of image at cellular level.

The composites presented a greater optical-electromagnetic activity, with approx. 30% higher than for inactivated particles, especially in the field of interest for medical imaging, respectively in the wavelength range of 250 nm.

Preliminary tests for bio-compatibility demonstrated an acceptable tolerability of functionalized nanoparticles with a degree of over 70% at dilutions between the limits of 1:100,…,1:5, which may suggest a similar behavior, *i.e.* non-toxic, in the case of administration to experienced animals, so a good perspective for in vivo experiments.

Acknowledgments. Project PN-III-P2-2.1-PED-2016-1598 - NanoTeraPlasia and Project PN-III-P1-1.2-PCCDI2017-021 - 3PCCDI - Microfluic platform for detection of circulating tumor cells concentrated by dielectrophorosis-magnetophorosis and analyzed by dielectric and electrochemical impedance spectroscopy.

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DEZVOLTAREA ŞI CARACTERIZAREA DE NOI NANO-PARTICULE DE CONTRAST PENTRU IMAGISTICA MEDICALĂ

(Rezumat)

Lucrarea descrie dezvoltarea în laborator de noi tipuri de agenți de contrast atât pentru imagistica MRI, cât şi pentru imagistica în domeniul THz, cu greutate moleculară mai mare şi caracteristici îmbunătățite, pornind de la oxidul de gadoliniu şi de la oxidul de fier superparamagnetic, bazate pe compozitarea cu carboximetilceluloză. Analiza TEM a oxizilor metalici funcționalizați a evidențiat prezența carboxi-metilcelulozei în vecinătatea componentei anorganice a sistemului, cu o distribuție omogenă. În plus, procesul de funcționalizare a permis particulelor anorganice să fie complet dezagregate în prezența polimerului considerat. Particulele de oxid metalic funcționalizate au avut dimensiuni medii de 9,...,11 nm, ideale pentru depunerea pe țesuturi. Nano-compozitele au prezentat o activitate optică-electromagnetică superioară, în special în domeniul lungimii de undă de 250 nm. Testele preliminare pentru bio-compatibilitate au demonstrat o tolerabilitate acceptabilă a nanoparticulelor funcționalizate şi un grad de peste 70% de viabilitate, ceea ce poate asigura o perspectivă bună pentru experimentele ulterioare în vivo.