"Alexandru Ioan Cuza" University Iași

Faculty of Physics

SUMMARY OF PhD THESIS

ADVANCED MATERIALS WITH BIOMEDICAL APPLICATIONS

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Introduction

The purpose of this thesis is the preparing and the study of the properties of nanoparticles of Ni, Co, Mg ferrite, hematite and magnetite, non isolated and superficially isolated with biocompatible polymers which can disperse and stabilize in various biocompatible liquids such as saline solution. We chose these types of particles as according to the latest studies, they are less toxic for the human biological environment. The research described here wishes to be an important contribution to the national and international research in the field of the biomedical applications of the magnetic nanoparticles.

The thesis is structured in four chapters, preceded by an introduction and followed by conclusions. In the first two chapters we present some theoretical aspects concerning the magnetic particles, the synthesis methods and the latest techniques used for the structural and functional investigation. The last two chapters state the personal contributions to this field.

Chapter I
Current research in the field of advanced materials with biomedical applications

In order to analyse the magnetic properties of the nanoparticles in a satisfactory manner it is necessary to have data concerning the geometry (size, shape, composition, crystalline structure) and their behaviour in a magnetic field (temperature dependency of the magnetization, saturation magnetization, remanent magnetization, coercive field, blocking temperature).

The magnetic particles used for biomedical applications are those of iron oxides such as magnetite (Fe_3O_4) and two oxide products such as tetragonal maghemite (γ-Fe2O3) and hexagonal hematite (α-Fe2O3).

It has been shown that the size of ferrite nanoparticles, the methods and conditions of synthesis can lead to various degrees of inversion of the spinel structure, non stoichiometry of the chemical composition, changes in the parameter of the elementary cell, apparition of secondary phases [1-5].

The protection of nanoparticles includes their covering with organic species (surfactants, polymers) or with an inorganic layer (silica, carbon).

The modification in the surface chemistry has an effect on the hydrodynamic dimension of the particle, changing its transport and biodistribution properties.

The covering increases the colloidal stability of the particles to aggregation and deposition and reduces the dipole-dipole interaction forces.

Chapter II
Methods of synthesis and structural and functional characterization of advanced materials with biomedical applications

In this chapter we describe the synthesis methods (polyol method, chemical co-precipitation, auto combustion sol-gel) and covering of the magnetic nanoparticles, the polymers used for their covering, as well as the analysis techniques of the structural properties (X-ray diffraction, FTIR spectroscopy), morphological (Scanning Electron Microscopy, Transmission Electron Microscopy), composition (X-ray Photoelectron Spectroscopy), magnetic (Vibrating Sample Magnetometry, Spin Electron Spectroscopy) and dielectric (Impedance spectroscopy).

Chapter III
Synthesis and characterization of nanoparticles with biomedical applications

3.1. Synthesis of magnetic nanoparticles for biomedical applications

3.1.1 Synthesis of ferrite and hematite nanopowders by the auto-combustion sol-gel method

We synthesized nickel ferrite, cobalt ferrite, magnesium ferrite, hematite and magnesium ferrite doped with copper nanopowders using the auto-combustion sol-gel method. We used glycine, $C_2H_5NO_2$, as chelating/combustion agent (Tab. 3.1) [6-9].

Table 3.1. The synthesis condition and thermal treatment of magnetic nanoparticles.

Sample	The synthesis condition	Thermal treatment
NF-773K	$Ni(NO_3)_2 \cdot 6H_2O/Fe(NO_3)_3 \cdot 9H_2O/Fe(NO_3)_3 \cdot 9H_2O$ = 1:2:3 348K, magnetic stirring - xerogel, sand bath heating 373K - 623K 50K/h, autocombustion at 573K	773K, 2h
NF-973K		773K, 2h heating at 973K, cooling in the oven
NF-1173K		773K, 2h heating at 1173K, cooling in the oven
CF-773K	$Co(NO_3)_2 \cdot 6H_2O/Fe(NO_3)_3 \cdot 9H_2O/Fe(NO_3)_3 \cdot 9H_2O$=1:2:3 348K, magnetic stirring - xerogel, sand bath heating 373K - 623K 50K/h, autocombustion at 573K	773K, 2h
CF-973K		773K, 2h heating at 973K cooling in the oven
CF-1173K		773K, 2h heating at 1173K, cooling in the oven
MF-773K	$Mg(NO_3)_2 \cdot 6H_2O/Fe(NO_3)_3 \cdot 9H_2O/Fe(NO_3)_3 \cdot 9H_2O$=1:2:3 348K, magnetic stirring - xerogel, sand bath heating 373K - 623K 50K/h, autocombustion at 573K	773K, 2h
MF-973K		773K, 2h heating at 973K cooling in the oven
MF-1173K		773K, 2h heating at 1173K, cooling in the oven
$MFCu_x$-1173K	$((mMg(NO_3)_2 \cdot 6H_2O+ nCu(NO3)2•3H2O)/Fe(NO_3)_3 \cdot 9H_2O/C_2H_5NO_2$=1:2:3 348K, magnetic stirring - xerogel, sand bath heating 373K - 623K 50K/h, autocombustion at 573K m and n were chosen as Cu/(Mg+Cu)=x=0,17; 0,34; 0,50; 0,67	773K, 2h heating at 1173K, cooling in the oven
H-773K	$Fe(NO_3)_2 \cdot 6H_2O/Fe(NO_3)_3 \cdot 9H_2O/Fe(NO_3)_3 \cdot 9H_2O$=1:2:3 348K, magnetic stirring - xerogel, sand bath heating 373K - 623K 50K/h, autocombustion at 573K	773K, 2h
H-973K		773K, 2h heating at 973K cooling in the oven
H-1173K		773K, 2h heating at 1173K, cooling in the oven

3.1.2 Synthesis of the magnetite nanoparticles covered in polymers

Synthesis of magnetite nanoparticles

Magnetite nanoparticles (NP) were created by the co precipitation of the ferrous chloride ($FeCl_2.4H_2O$) and the ferric chloride ($FeCl_3.6H_2O$) with the help of an alkaline solution of sodium hydroxide (NaOH). The creation of NP was proved by the apparition of a black precipitate stabilized with tetramethylammonium hydroxide in order to stabilize the NP [10].

Covering the MNP in polymethacrylate (PMMA)

The covering in polymer was achieved by emulsion polymerization with a high surfactant/monomer ratio [10]. We studied the influence of the monomer/surfactant weight ratio by choosing a mixture of methyl methacrylate monomers (MMA) and acrylic acid (AAc) on a weight ratio of 90/10.

Table 3.2. Conditions of polymerization (v-Stirring speed).

Sample	Fe^{2+}/Fe^{3+}	SDS %w	MMA+AAc %w	T K	v rpm
NPP1	1:2	2	634	338K 24h	300
NPP2	1:2	4	634	338K 24h	300
NPP3	1:2	2	12,68	338K 24h	300

In the process of polymerization we used sodium dodecyl sulfate (SDS) as surfactant, which was added to the magnetite suspension earlyer synthesised (2% wt) (Tab. 3.2).

Synthesis of the magnetite nanoparticles covered in dextran, polyethylenglycol and polyvinylalcohol

In order to obtain magnetite nanoparticles covered in dextran (NP-D), polyethylenglycol (NP-PEG) and polyvinylacohol (NP-PVA) we proceeded as in the earlier stage: synthesis of the nanoparticles followed by their covering in polymers.

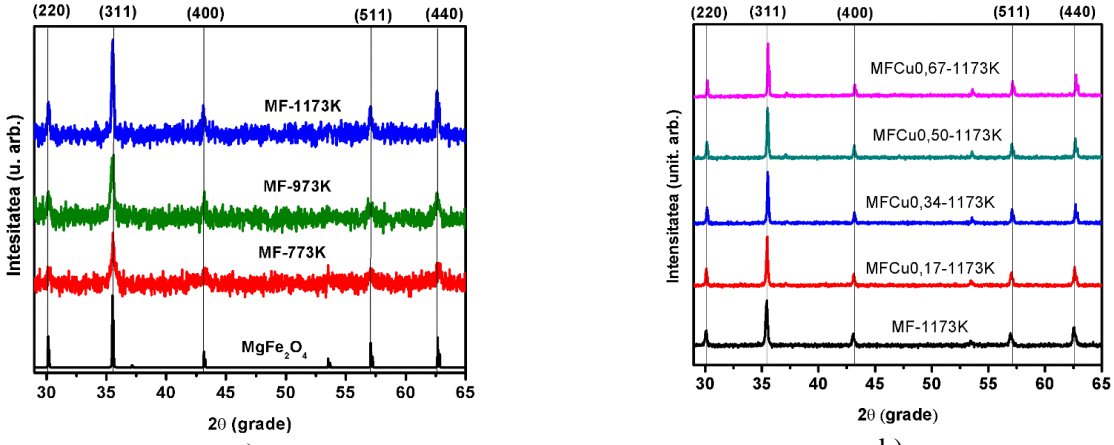
In order to obtain dextran covered NP, we mixed the ferrous chloride and ferric chloride solutions and set the under magnetic shaking (500 rpm) at 60°C. The dextrans solution was mixed with the NaOH solution and heated at 55°C, being added drop by drop in the first mixture. Thus we obtained a dark precipitate which was submitted to ultrasounds for 15 minutes. After having sedimented the nanoparticles by means of a magnet, they were washed in bidistilled water to a pH ≈ 7. The resulting sample was marked NP-D.

We procedeed in a similar manner for the nanoparticles covered in polyethylenglycol an polyvinylalcohol, marked NP-PEG an NP-PVA, respectively.

3.2 The study of the structure, morphology and chemical composition of the magnetic nanoparticles

3.2.1. Characterization of ferrite nanoparticles

From the X-ray diffractograms we could notice the diffraction peaks specific for the spinel structure for all the samples under thermal treatment, highlighting the nanostructured aspect of the samples. By comparing the three samples covered in polymers, we notice that the highs of the NPM non covered sample are well kept, without noticeable differences between them, except for the intensity of the peaks (Fig.3.1).

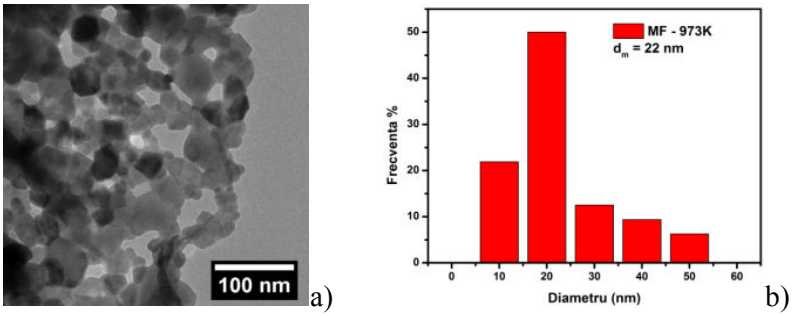


a) b)

Fig.3.1. MF (magnesium ferrite) and MF doped with Cu nanopowders diffraction patterns treated at different temperatures [6, 7].

The structural investigation by Infrared Spectometry (FTIR) proved the formation of the spinel type phase. We also noticed the disappearance of the organic and inorganic residues after each thermal treatment.

By the TEM analysis of the nanopowders we noted that the particles have a nanometric size (Fig.3.2).



a) b)

Fig. 3.2. a) TEM micrograph of MF (magnesium ferrite) nanopowders of thermally treated at 973K; b) distribution of MF nanoparticles.

By analysing the SEM images we note that the fluffy aspect of the powder diminishes with the growth of the temperature, as a result of the increase in size of the ferrite nanoparticles. The EDAX spectrum highlights the presence of iron, nickel and oxygen atoms. From the EDAX spectrum we determined the effective atomic concentrations and noticed the homogeneity of the distribution of the various elements of the system Mg – Fe – O (Tab. 3.3) [6].

Tab. 3.3. The elemental chemical composition of magnesium ferrite thermally treated at 1173K.

Element	*Wt%*	*At%*
CK	10,74	18,91
OK	42,12	55,68
MgK	15,38	13,38
FeK	31,75	12,02

Chapter IV
Investigating the magnetic and dielectric properties of the magnetic nanopowders

In this chapter we present the experimental results and discussions on the magnetic and dielectric properties in the context of using the synthesized nanopowders in biomedical

applications. At the end we study the application of magnetic nanopowders in the magnetic fluid hyperthermia used in the tumours treatment.

We noticed that the values of the saturation magnetization (M_S) of the compounds submitted to thermal treatments are significantly lower than the bulk state M_S [11, 12], they vary with the temperature of the thermal treatment [6-8]. The M_S variations are due to the increase in the surface effect (spin effect, canting), or to the spin disorder [13] with the decrease in the particle size (Fig.4.1) [14].

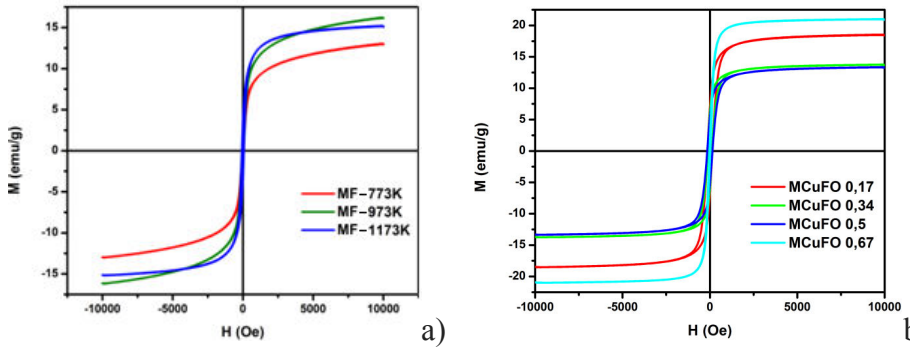


Fig. 4.1. Hysteresis curves of ferrite nanopowders a)Mg, b)Mg doped with Cu [6, 7].

The values of the coercive field change significantly following the treatment, the differences between the coercivities of the particles can be attributed mainly to the different morphology of the particles, to the presence of the shape anisotropy which can significantly intensify the magnetic properties [15, 16], as well as to the decrease in the content of Fe^{2+} ions on the tetrahedral positions and to the migration of Ni^{2+} ions on these positions [17].

From the analysis of the hysteresis for the magnetite nanoparticles biocompatibilized with dextran, PEG and PVA we notice their superparamagnetic behavior (Fig. 4.2).

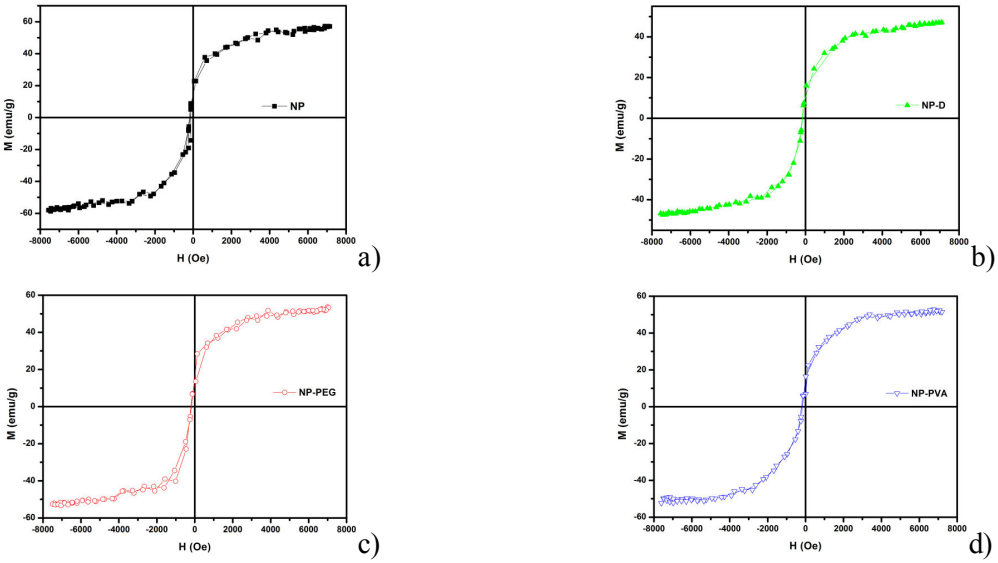


Fig. 4.2. Hysteresis curves of magnetite nanoparticles: a) NP; b) NP-D; c) NP-PEG și d) NP-PVA.

From the analysis of the spin electron resonance spectrum we notice the fact that the RES line is asymmetric for all the samples, being the result of the superposition of at least two RES signals. We notice that the width of the line is depending on the temperature (Fig. 4.3). This dependence can be attributed to a large distribution of the shape and size of the particles and to the increase in effective anisotropy of the system and to the presence of the particle interactions.

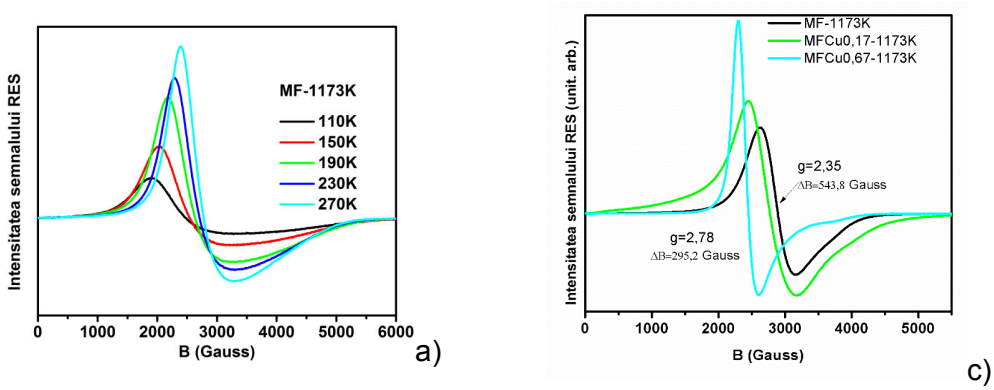


Fig. 4.3. ESR spectra of MF and MF doped with Cu nanopowders depending on the temperature thermally treated at different temperatures [6, 7].

The width of the RES signal depending on the temperature ΔB increases with the decrease of the temperature for the thermally treated samples. This linear increase is due to the de dependence between temperature and magnetization parallel to the increase in magnetic momentum inside the particles. The observed deviations can be produced because of the bipolar interactions between magnesium ferrite particles, a decrease in the heterogeneity of the bipolar fields occurring because of the disposition and order of the particles in the system (Fig. 4.4) [6].

The values of resonance field varying with the temperature of the sample thermally treated at 1173K are the lowest, noticing that the values of the resonance field are decreasing with the increase in the temperature of the thermal treatment. The resonance field is decreasing slowly with the temperature up to 120K where we see a deviation of the inclination of the graphic probably because of the change in magnetic anisotropy for temperatures under 140K, phenomenon encountered in other studies in the [18]. This change in the resonance field is a clue of the presence of the induced field which is the main cause for the spin disorder of the magnetic system consisting of magnesium ferrite magnetic particles (Fig. 4.5).

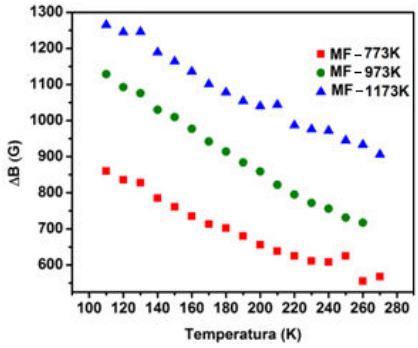


Fig. 4.4 . The temperature dependence of the ESR signal linewidth for MF nanoparticles annealed at different temperatures.

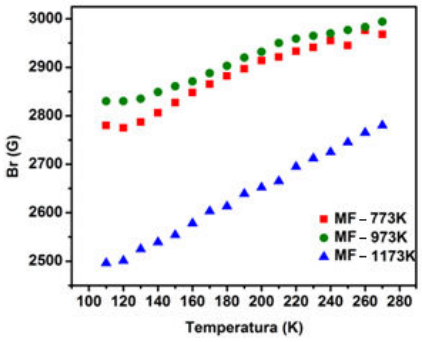


Fig. 4.5. The temperature dependence of the resonance field for MF nanoparticles annealed at different temperatures.

The dielectric measurements for the magnetic nanopowders were made in the frequency interval of 40 Hz and 110 MHz. The real part and the imaginary part of the permittivity, as well as the dielectric losses angle tangent were measured in comparison with the frequency.

The tendency for all the samples that were investigated is that the values of electric permittivity, the imaginary and the real parts, as well as the loss angle tangent decrease with the increase in frequency [6-8].

The real part of the permittivity in the frequency interval 40Hz – 0,5MHz, decreases suddenly with the increase in frequency, but for the interval 0,5MHz – 10,5MHz the electric

permittivity of the samples decreases almost linearly with the increase in frequency after having performed the thermal treatments on various temperatures (Fig. 4.6).

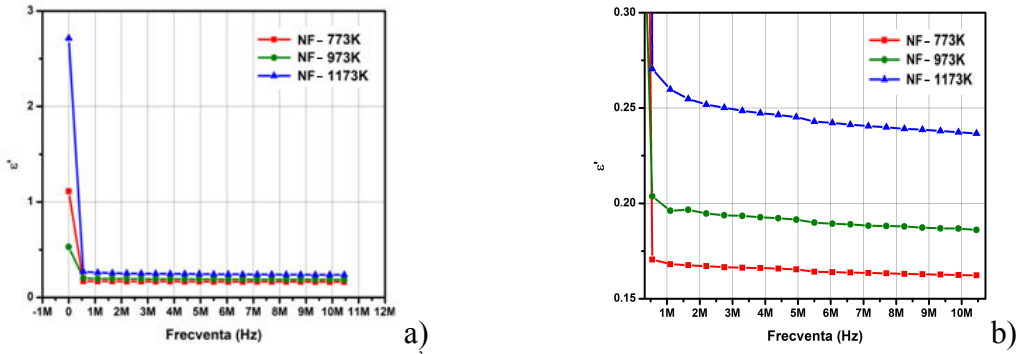


Fig. 4.6. The real part of permittivity (ε') as a function of frequency for thermally treated NF nanoparticles: a) 40Hz – 10,5MHz; b) 0,5MHz – 10,5MHz.

The behaviour of the imaginary part of electric permittivity and that of the loss angle tangent is similar to that of the real part of the permissiveness for the two frequency intervals mentioned above.

The structure of the sample, its homogeneity and porosity significantly influence the values of the dielectric constants and of the loss angle tangent.

We were able to see the sudden variation of the conductivity with the frequency of the sample treated thermally at 773K. The conductivity shows a gradual increase at low frequencies, and for high frequencies it increases exponentially [6].

4.3 Studies of heating the magnetic nanoparticles dispersed for applications in magnetic hyperthermia

For the test heating of the magnetic nanoparticles in view of their use in magnetic fluids hyperthermia we used two magnetic colloids. The first colloid, magnetite in PEG solution, and the second, magnetite in MEG solution (monoethylenglycol). The samples were heated by induction [19].

Were made graphics of the temperature variations over time of the two magnetic colloid concentrations [19]. The temperature interval between 309K and 319K was preferred in order to include all the transformations of the ferrofluid under the action of the alternative magnetic field in the organism [19, 20].

The first colloid had an almost linear behaviour when heated over time, with a rapid increase in the interval 309 - 3019K for 90 s. The second colloid had a much slower evolution.

We noticed that the heating of the magnetite particles depends on the frequency of the applied field, the exposure time, as well as on the size of the particles and the suspension environment (Fig. 4.7 and 4.8).

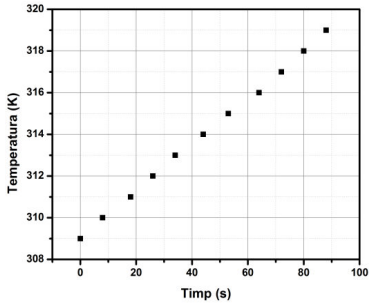


Fig. 4.7. Temperature evolution as a function of time in colloid I (magnetite with PEG).

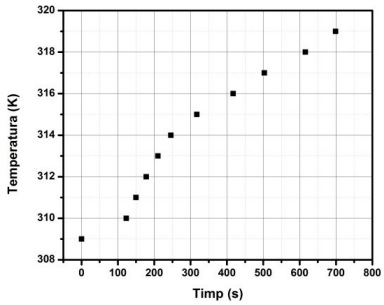


Fig. 4.8. Temperature evolution as a function of time in colloid II (magnetite with MEG).

CONCLUSIONS

1. We have run systematic tests on ferrite nanopowders in which the Fe2+ ion has been replaced with two other bivalent ions: Ni, Co, Mg, and of the hematite, magnesium ferrite doped with copper and magnetite nanoparticles.
2. We have used different experimental and result interpretation techniques (X-ray diffraction, scanning electron microscopy, magnetization, XPS, FTIR spectroscopy, RES spectroscopy, impedance).
3. We have obtained a number of experimental data which highlight original aspects of the structure, microstructure changes, of the magnetic and dielectric properties in the studied magnetic nanopowders. The investigation methods have proved to be accurate for the target, the characterization of the synthesized compounds being rigorous, at the level of the international requirements for the field of the nanoparticles.
4. The ferrite and hematite nanopowders were prepared by the auto-combustion sol-gel method and the chemical co-precipitation method. We proceeded afterwards to thermal treatments in order to completely eliminate the organic residues and to improve sample homogeneity.
5. The FTIR spectrum has proved the formation of the spine type phase characteristic for the ferrites and the disappearance of organic and inorganic residues.
6. The nickel ferrite structure and the characteristic parameters were determined with the help of the X-ray diffractometry which confirmed the formation of the spinel type structure after thermal treatment. We have determined the size of the crystallites and we have established that the network parameter value is within the values from the literature data with a size of 8,3-8,4 Å.
7. The TEM SEM images have confirmed the creation of nanometric particles of uniform distribution. The EDAX spectrum has offered data on the percentage of the weight and the atomic percentage of the elements, as well as information on the homogeneity of the samples.
8. The values of the saturation magnetization for all the samples are significantly lower than the value of the bulk state magnetization, specific for the nanometric size of the obtained powders.
9. From the RES spectra of the superficially non isolated nanopowders as well as of the nanopowders covered in polymers we noticed that the graphics of the width of the ΔB signal line, the values of the resonance field Br and the *g* factor describe normal trajectories for this type of ferrites.
10. The dielectric measurements have shown that the values of the real part, the imaginary part and the loss angle tangent decrease with the increase in frequency, becoming constant at high frequencies. This behaviour is due to the migration of electrons between Fe^{2+} and Fe^{3+}.
11. We have proved that the electric conductivity has a normal behaviour for ferrites, increasing with the decrease in frequency, especially after applying a thermal treatment at 773K.
12. The values of the saturation magnetization, as well as the values of coercivity show the fact that the magnetic nanopowders obtained can be used in medicine as carriers for drugs with targeted release, in hyperthermia as well as detection elements in biosensors.

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Scientific activity

Published or in press ISI articles

1. **G.G. Nedelcu**, A. Druc, M. Iacob, A. Popa, P. Postolache, M. Cazacu, L. Leontie, F. Iacomi, Structural, magnetic and dielectric properties of sol-gel auto-combustion synthesized magnesium ferrite nanoparticles, Beilstein Journal of Nanotechnology, trimisă spre publicare 2014.(Impact factor = 2,326; AI score = 0,2249)
2. **G.G. Nedelcu**, A. Nastro, L. Filippelli, M. Cazacu, C. Oliviero Rossi, A. Popa, D. Toloman, F. Iacomi, Synthesis and structural characterization of copolymer embedded magnetite particles, Applied Surface Science, trimisă spre publicare 2014. (Impact factor = 2,538; AI score = 0,5503).
3. A.C. Druc, A.I. Borhan, **G.G. Nedelcu**, L. Leontie, A.R. Iordan, M.N. Palamaru, Structure-dielectric properties relationships in copper-substituted magnesium ferrites, Materials Research Bulletin, 48 (2013) 4647-4654.(Impact factor = 1,968; AI score = 0,5473)
(http://www.sciencedirect.com/science/article/pii/S0025540813006673)

4. A.C. Druc, A.I. Borhan, A. Diaconu, A.R. Iordan, **G.G. Nedelcu**, L. Leontie, M.N. Palamaru, How cobalt ions substitution changes the structure and dielectric properties of magnesium ferrite?, Ceramics International, 40 (2014) 13573–13578.(Impact factor =2,086;AIscore=0,5346)(http://www.sciencedirect.com/science/article/pii/S0272884214007949)
5. S. Feraru, A. Borhan, P. Samoila, **G. Nedelcu**, A. Iordan, M. Palamaru, Influence of A-site cation on structure and dielectric properties in A2DyBiO6 (A=Mg, Ca, Sr, Ba) double perovskite, Australian Journal of Chemistry, 67 (2014) 250-255. (Impact factor = 1,644; AI score = 0,5728)
(http://www.publish.csiro.au/paper/CH13300.htm)
6. **G. Nedelcu**, The heating study of two types of colloids with magnetite nanoparticles for tumours therapy, Digest Journal of Nanomaterials and Biostructures, 3 (2008) 99 – 102.(Impact factor = 1,123; AI score = 0,2004)(http://www.chalcogen.ro/Nedelcu01.pdf)
7. **G. Nedelcu**, Magnetic nanoparticles impact on tumoral cells in magnetic fluid hyperthermia Treatment, Digest Journal of Nanomaterials and Biostructures, 3 (2008) 103-107.(ImpactFactor=1,123;AIscore=0,2004) (http://www.chalcogen.ro/Nedelcu12.pdf)

Papers presented at international conferences

1. **G.G. Nedelcu**, A. Nastro, L. Filippelli, M. Cazacu, C. Oliviero Rossi, A. Popa, D. Toloman, F. Iacomi, Synthesis and structural characterization of copolymer embedded magnetite particles, International Conference on Physics of Advanced Materials, ICPAM-10, Iaşi – Romania, 22 – 28 September, 2014.
2. **G.G. Nedelcu**, M.L. Craus, F. Iacomi, Structural and magnetic properties of superparamagnetic magnetite nanoparticles superficially isolated with biocompatible polymers, 1st Autumn School on Physics of Advanced Materials, PAMS-1, Iași – Romania, 22 – 28 September, 2014.
3. **Gigel Nedelcu**, Alfonso Nastro, Luigi Filippelli, Cesare Oliviero Rossi, Adriana Popa, Dana Toloman, Felicia Iacomi, Synthesis and characterization of polymer-coated magnetic nanoparticles for biomedical applications, TIM-13 Physics Conference, 21 – 24 November, Timișoara, 2013.
4. Alfonso Nastro, **Gigel Nedelcu**, Luigi Filippelli, Cesare Oliviero Rossi, Felicia Iacomi, Synthesis And Characterization Of Polymer-Coated Magnetic Nanoparticles For Biomedical Applications, prezentată poster la 9th International Conference on Physics of Advanced Materials – ICPAM 9, 20-23 september 2012, Iași, Romania.