*Romanian Biotechnological Letters* *Vol. 23, No. 5, 2018*

**ORIGINAL PAPER**

# *In vitro* evaluation of glycerol coated iron oxide nanoparticles in solution

DOI:10.26327/RBL2018.219

Received for publication, March, 5, 2018
Accepted, July, 8, 2018

**ALINA MIHAELA PRODAN[1,2], MIRCEA BEURAN[1,2], CLAUDIU STEFAN TURCULET[1,2], MARCELA POPA[3], ECATERINA ANDRONESCU[4,5], CORALIA BLEOTU[6], STEFANIA MARIANA RAITA[7], MARIAN SOARE[8], OLIVERA LUPESCU[1,2,*]**

[1]*Emergency Hospital Floreasca, 8 Calea Floresca, Bucharest, Romania,*
[2]*Carol Davila University of Medicine and Pharmacy, 8 Eroii Sanitari, Bucharest, Romania,*
[3]*Earth, Environmental and Life Sciences Section, Research Institute of the University of Bucharest, 1-3 Portocalelor Lane, 77206 Bucharest, Romania*
[4]*UPB Department of Science and Engineering of Oxide Materials and Nanomaterials, University Politehnica of Bucharest, Romania*
[5]*Academia Oamenilor de Știință din România, Romania*
[6]*Institute of Virology, Antiviral Therapy Department "Stefan S. Nicolau", 285 Mihai Bravu, 030304 Bucharest, Romania*
[7]*Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine, Bucharest, Romania.*
[8]*Nuclear NDT Research & Services SRL, 104 Berceni Street, Bucharest, Romania*
***Address for correspondence to:** oliveralupescu65@@gmail.com*

## Abstract

*Stable glycerol coated iron oxide nanoparticles in solution (G-IONPs) were prepared using an adapted coprecipitation method. The morphology of the G-IONPs was investigated by Scanning Electron Microscopy (SEM). The in vitro investigation regarding the toxicity of G-IONPs performed on HeLa cells suggested that they are not toxic even after 72 hours of exposure. Furthermore, the studies presented in this paper revealed that G-IONPs exhibited antimicrobial properties. On the other hand, the antibacterial effect of the sample based glycerol coated iron oxide were strongly active against P. aeruginosa 1397 and E. faecalis ATCC 29212. Moreover, a good antibacterial effect was also observed on C. krusei 963, C. albicans ATCC 10231 and K. pneumoniae 2968.*

**Keywords**: iron oxide, glycerol, *in vitro*, antimicrobial properties.

## 1. Introduction

In the early 20th century the leading cause of mortality across the globe were infectious diseases (HUH & KWON [1]; DE KRAKER et al. [2]). The introduction of antibiotics in the last centuries has significantly decrease the rate of mortality due to infectious diseases (COHEN [3]; GOLD & MOELLERING [4]). Nowadays, due to the emergence of antibiotic resistant microbial strains, the need of new antimicrobial agents has led to tremendous efforts in the development of new antimicrobial drugs (LIMBAN & CHIFIRIUC [5]; ICONARU et al. [6]; ICONARU et al. [7]). Nevertheless, there is no guarantee that the attempt of obtaining new reliable antimicrobial agents can match the rapidly development of resistance of the microbial pathogen. In the last years, the occurrence of resistant infections in hospitals caused by both Gram-positive and Gram-negative bacterial pathogens has spread, seriously threatening human health and patient recovery (GUILFOILE & ALCAMO [8]; PALANIAPPAN & HOLLEY [9]). Recently, the emergence of bacterial strains resistant to traditional antibiotics has been responsible for exploring the use of nanomaterials as

antimicrobial agents. Some recent studies, revealed that a couple of nanomaterials are known to possess antimicrobial activities, rendering them able to be utilized in controlling infectious diseases (LEE & al. [10]; MORONES & al. [11]; STOIMENOV & al. [12]). In the last decades, due to their unique properties, nanometer scale particles have attracted researchers from various fields such as physics, medicine, biology and material science. Moreover, nanomaterials with antimicrobial properties have been intensively researched because compared to traditional antibiotics they bring many advantages like low cost, good biocompatibility, small size, etc (CIOBANU & al. [13]; COSTESCU & al. [14]; CIOBANU & al. [15]). The most studied materials for their exquisite properties are the ones exhibiting magnetic properties (CIOBANU & al. [15]; WAN & al. [16]; PREDOI [17]; PREDOI & VALSANGIACOM [18]). Among these, iron oxide nanoparticles, mostly magnetite ($Fe_3O_4$) and maghemite ($\Upsilon$-$Fe_2O_3$) have been intensively studied due to their current and future promising applications in magnetic resonance imaging (MRI) (KIM & al. [19]), *in vivo* magnetically guided drug delivery (ROULLIN & al. [20]), tumor hyperthermia (JORDAN & al. [21]), cell and DNA separation (BUCAK & al. [22]), immunoassays (MURA & al. [23]), magnetic storage media (CHAKRABORTY [24]), etc. Usually, iron oxide nanoparticles tend to aggregate due to the strong magnetic dipole-dipole attractions and large surface energy, therefore, surfactants or polymeric compounds with specific functional groups are used to prevent particle aggregation ( WAN & al. [16]; PREDOI & VATASESCU-BALCAN [25]; PRODAN & al. [26]; WOODING & al. [27]). Several studies reported the increase of magnetic nanoparticles by using organic surfactants as coatings. However, these types of magnetic fluids were still not stable enough for specific applications such as separation and purification of biomolecules (WAN & al. [28]; SHEN & al. [29]; WAN & al. [30]). Shen et al. reported the preparation of magnetic fluids with improved stability by using the c-ray-induced polymerization of an olefin-terminated surfactant bilayer coating on the magnetic particles (SHEN & al. [29]). Recent studies have evidenced the use of polymers for obtaining stable iron oxide magnetic particles in solution (PREDOI [31]; GUPTA & al. [32]; LAURENT & al. [33]). Polymers such as dextran, dextrin, sucrose, chitosan, glycerol, etc., have been investigated as stabilizers for iron oxide nanoparticles (ICONARU & al. [34]; GUPTA & al. [35]). Recently due to its use in the medical and pharmaceutical industry, glycerol has attracted much attention and has been studied as a possible enhancer of the antimicrobial properties of iron oxide nanoparticles. Glycerol is already used in pharmaceutical products such as cough syrups, elixirs, toothpaste, mouthwashes, but also in skin care products like shaving cream, hair care products, soaps, hand creams, etc (RALF & al. [36]). In some extreme conditions, glycerol is also used intravenously to reduce pressure inside the brain for patients who suffers from encephalitis, Reye's syndrome, central nervous system trauma, strokes, meningitis, etc (MACDONALD & al. [37]). This study focuses on the investigation of the biocompatibility properties of glycerol coated iron oxide nanoparticles in solution prepared by an adapted co-precipitation method and on the antimicrobial behavior of these materials. Scanning electron microscopy studies have been conducted to obtain information about the morphology of the obtained G-IONPs. The biocompatibility studies were conducted at different time intervals using HeLa cells and the antimicrobial activity of the G-IONPs was assessed using the most common Gram positive (*S. aureus 0364*, *B. subtilis*, *E. faecalis ATCC 29212*), Gram negative (*P. aeruginosa 1397*, *E. coli* ATCC 25922, *E. coli* 714, *K. pneumoniae* 2968, *E. cloacae* 61R) and fungal (*C. krusei* 963, *C. albicans* ATCC 10231) microbial strains.

## 2. Materials and Methods

### *2.1. Materials*

Ferrous chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$), ferric chloride hexahidrate ($FeCl_3 \cdot 6H_2O$), natrium hydroxide (NaOH), and chlorhidric acid (HCl), were purchased from Merck. Glycerol (99.5%) was purchased from Sigma. Deionized water was used in the synthesis of nanoparticles.

### *2.2. Synthesis of glycerol coated iron oxide ferrofluid*

Glycerol coated iron oxide nanoparticles in solution used in this study were synthesized using the co-precipitation method as described in previously reported studies ( ICONARU & al. [38]). The final iron concentration in the solution of glycerol coated iron oxide was 0.35 $mol \cdot L^{-1}$.

### *2.3. Morphological characterization*

The morphology of the (G-IONPs) was investigated by scanning electron microscopy using a Quanta Inspect F scanning electron microscope (SEM), operating at 25 kV in vacuum.

### *2.4. Cell viability assay*

The toxicity of glycerol coated iron oxide nanoparticles in solution was investigated using HeLa cells as described in our studies previously reported (PRODAN & al. [39]; POPA & al. [40]; PRODAN & al. [41]). HeLa cells were treated with a solution of glycerol coated iron oxide nanoparticles (200 µl) diluted 100 times and 50 times respectively. The effects on the cell viability were evaluated after 24 h, 48 h and 72 h. The fluorescence studies were carried out using an Observer D1 Carl Zeiss microscope.

### *2.5. Antimicrobial assay*

The *in vitro* qualitative evaluation of the antimicrobial activity of G-IONPs was carried out using and adapted agar diffusion technique previously reported (CHIFIRIUC & al. [42]; CHIFIRIUC & al. [43]). The antimicrobial properties of G-IONPs were tested against Gram positive (*S. aureus* 0364, *B. subtilis*, *E. faecalis ATCC* 29212), Gram negative (*P. aeruginosa* 1397, *E. coli* ATCC 25922, *E. coli* 714, *K. pneumoniae* 2968, *E. cloacae* 61R) and fungal (*C. krusei* 963, *C. albicans* ATCC 10231) microbial strains. Antimicrobial activity was assessed by measuring the growth inhibition zones diameters expressed in mm.

## 3. Results and discussion

The SEM studies presented in Figure 1 revealed that the G-IONPs have spherical shape and present a uniform morphology. Furthermore, the SEM image confirm the nanometric size of the obtained particles.

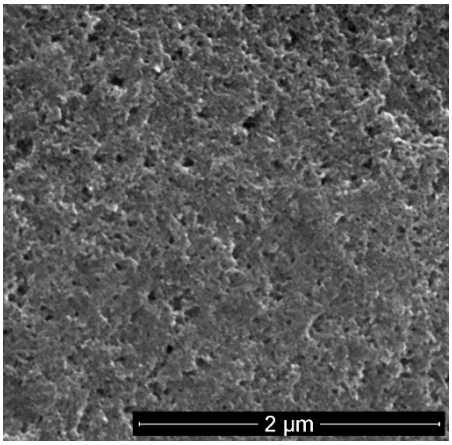


**Figure 1.** SEM image of G-IONPs.

In this paper, the toxicity of the glycerol coated iron oxide nanoparticles on HeLa cells was also evaluated. The cell viability investigations were performed by exposing HeLa cells to a 200 µL solution of glycerol coated iron oxide nanoparticles diluted 50 times and 100 times. To have a better understanding of the cells interaction with the G-IONPs solution, the cell viability was investigated at 24 h, 48 h and 72 h. The cytotoxicity assay of the G-IONPs solution on HeLa cells is presented in Figures 2-4. After 24 h (Figure 2), the cell viability investigations performed using HeLa cells and a G-IONPs solution (200 µL) diluted 100 times (Figure 2B) and 50 times (Figure 2C) have shown that the G-IONPs do not have a toxic effect on the cells. The absence of dead cells proves the nontoxic effect of G-IONPs on the HeLa cells. Red cells (dead cells), due to the propidium iodide staining were not observed (Figure 2 B-C). More than that, in the fluorescence microscopy image it can be observed that the cells exhibit normal features and there is no cell degradation compared to the control cell culture (Figure 2A) used in the experiments.

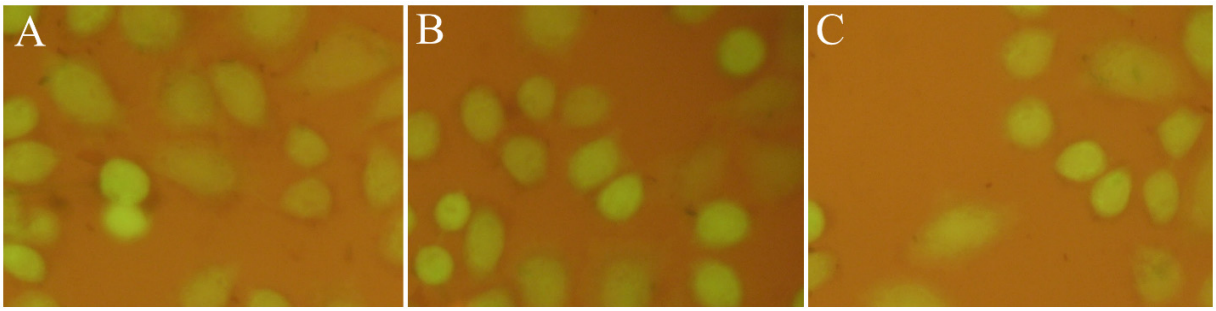


**Figure 2.** Fluorescence microscopy image of HeLa cells after 24 h exposure to a solution of G-IONPs (200 µL) at various concentration: (B) - diluted 100 times; (C) - diluted 50 times. Control HeLa cells cultured in free medium (A) (×200).

In spite of the fact that, after 24 h of incubation with G-IONPs solution the cells were viable for both nanoparticles concentrations, the fluorescence microscopy image shows a slight decrease of the number of cells when the G-IONPs solution was diluted 50 times. Figure 3 illustrates the fluorescence microscopy image of HeLa cells after 48 h exposure to a G-IONPs solution (200 µL) diluted 100 times (Figure 3 B) and 50 times (Figure 3 C) and also of the control HeLa cells cultured in free medium (Figure 3 A). The results reveal that after 48 h, the G-IONPs solution has a toxic effect on the cells depending on the concentration used. 48 h after incubation (compared to the control), a slightly toxic effect on HeLa cells has been observed at higher concentrations of the G-IONPs solution (200 µL of the G-IONs solution diluted 50 times). Dead cells (red cells stained with propidium iodide) were observed (Figure 3 C).

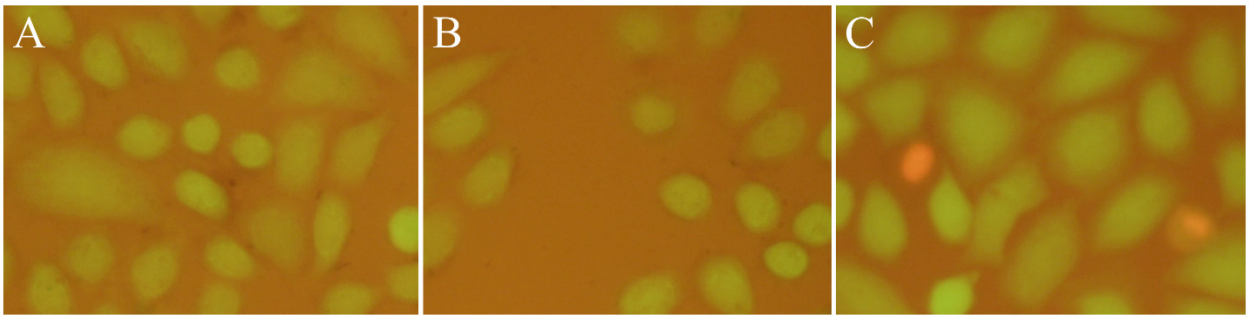


**Figure 3.** Fluorescence microscopy image of HeLa cells after 48 h exposure to a solution of G-IONPs (200 µL) at various concentration: (B) - diluted 100 times; (C) - diluted 50 times. Control HeLa cells cultured in free medium (A) (×200).

The fluorescence microscopy images of HeLa cells after 72 h exposure to a solution of G-IONPs (200 µL) diluted 100 times (Figure 4 B), 50 times (Figure 4 C) and the control HeLa cells cultured in free medium (Figure 4 A) are presented in Figure 4.

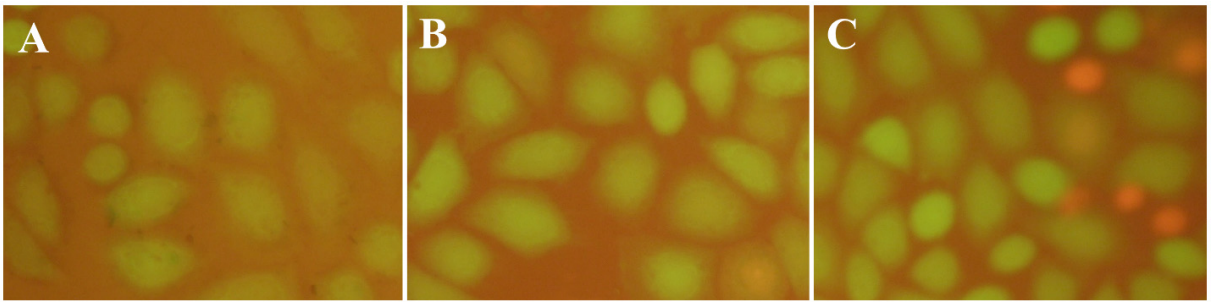


**Figure 4.** Fluorescence microscopy image of HeLa cells after 72 h exposure to a solution of G-IONPs (200 µL) at various concentration: (B) - diluted 100 times; (C) - diluted 50 times. Control HeLa cells cultured in free medium (A) (×200).

The results confirm that the concentration of G-IONPs solution plays a major role in cell damage. In the fluorescence images, both G-IONPs solution concentration and exposure time have a major role in HeLa cell destruction. In addition, a slight change in the morphology of HeLa cells was observed after a 72 h exposure to a high concentration of the G-IONPs solution. Morphological changes observed after 72 h exposure to a G-IONPs solution (200 µL) diluted 50 times marks the beginning of cells degradation (Figure 4C). The results obtained in the case of HeLa cells exposed 48 h and 72 h respectively to the G-IONPs solution revealed that the number of dead cells increased with the increase of the exposure time and the concentration. The antimicrobial properties of the G-IONPs solution were investigated against Gram positive (*S. aureus* 0364, *B. subtilis*, *E. faecalis* ATCC 29212), Gram negative (*P. aeruginosa* 1397, *E. coli* ATCC 25922, *E. coli* 714, *K. pneumoniae* 2968, *E. cloacae* 61R) and fungal (*C. krusei 963*, *C. albicans ATCC* 10231) microbial strains. The qualitative results of the antimicrobial assay are presented in Table 1. The results have evidenced that G-IONPs proved to have antimicrobial properties against some of the tested strains.

**Table 1.** Qualitative antimicrobial assay of G-IONPs.

| **Microbial strain** | G-IONPs antimicrobial activity |
|---|---|
| *P. aeruginosa* 1397 | + |
| *C. krusei* 963 | ± |
| *C. albicans* ATCC 10231 | ± |
| *E. coli* ATCC 25922 | - |
| *E. faecalis* ATCC 29212 | + |
| *B. subtilis* | - |
| *K. pneumoniae* 2968 | ± |
| *E. cloacae* 61R | - |
| *E.coli* 714 | - |
| *S. aureus* 0364 | ± |

Inorganic nanoparticles are largely known as large spectrum antimicrobial agents (PURDOIU et al [44], BUTEICA et al [45], KAYGUSUZ et al.[46]). According to the results of the antimicrobial studies, G-IONPs exhibited antimicrobial activity against Gram negative *P.*

*aeruginosa* 1397 bacterial strain and also against Gram positive *E. faecalis* ATCC 29212 bacterial strain. These results are in good agreement with previous studies (ICONARU & al. [41]). Furthermore, the studies presented here revealed that the G-IONPs exhibited antimicrobial properties against fungal strains *C. krusei* 963 and C. *albicans* ATCC 10231. A small antibacterial activity was also observed in the case of Gram negative *K. pneumoniae* 2968 and Gram positive *S. aureus* 0364 bacterial strains.

## 4. Conclusion

An adapted coprecipitation method was successfully used to obtain highly stable glycerol iron oxide nanoparticles in solution. The round shape morphology of the particles has been evidenced by SEM investigations. More than that, the toxicity of the glycerol coated iron oxide nanoparticles on HeLa cells was also evaluated in this paper. The results revealed that glycerol iron oxide nanoparticles in solution were nontoxic to HeLa cells, proving that the G-IONPs maintain their biocompatibility even after 72 h. The antimicrobial assay of the G-IONPs emphasized that these particles present antimicrobial properties. The results presented in this paper demonstrated that glycerol iron oxide nanoparticles in solution possess a good biocompatibility and exhibit antimicrobial properties making them ideal candidates to be further investigated for possible medical applications.

**Acknowledgements**
This research was financially supported by the Ministry of Education of Romania through Project PN II Contract Nr. 131/2014.

## References

1. A.J. HUH, Y. J. KWON. "Nanoantibiotics": A new paradigm for treating infectious diseases using nanomaterials in the antibiotics resistant era. *J. Control. Release*, 156, 128-145 (2011).
2. M.E.A. DE KRAKER, P.G. DAVEY, H. GRUNDMANN. On behalf of the BURDEN study group. Mortality and hospital stay associated with resistant *Staphylococcus aureus* and *Escherichia coli* bacteremia: estimating the burden of antibiotic resistance in Europe. *PLoS Med* 2011, 8:e1001104.
3. M.L. COHEN. Changing patterns of infectious disease. *Nature*, 406, 762-767 (2000).
4. H.S. GOLD, R.C. MOELLERING. Antimicrobial-drug resistance. *N. Engl. J. Med.,* 335, 1445-1453 (1996).
5. C. LIMBAN, M.C. CHIFIRIUC. Antibacterial activity of new dibenzoxepinone oximes with fluorine and trifluoromethyl group substituents. *Int. J. Mol. Sci.,* 12(10), 6432-6444 (2011).
6. S.L. ICONARU, P. CHAPON, P. LE COUSTUMER, D. PREDOI. Antimicrobial Activity of Thin Solid Films of Silver Doped Hydroxyapatite Prepared by Sol-Gel Method. *Sci. World J.,* http://dx.doi.org/10.1155/2014/165351, (2014).
7. S.L. ICONARU, M. MOTELICA-HEINO, D. PREDOI. Study on Europium-Doped Hydroxyapatite Nanoparticles by Fourier Transform Infrared Spectroscopy and Their Antimicrobial Properties. *J. Spectrosc.*, http://dx.doi.org/10.1155/2013/284285, (2013).
8. P. GUILFOILE, I. E. ALCAMO. Antibiotic-Resistant Bacteria, , Chelsea House Publishers, 2007.
9. K. PALANIAPPAN, R.A. HOLLEY. Use of natural antimicrobials to increase antibiotic susceptibility of drug resistant bacteria. *Int. J. Food. Microbiol.,* 140(2-3), 164-168 (2010).
10. C. LEE, J.Y. KIM, W.I. LEE, K.L. NELSON, J. YOON, D.L. SEDLAK. Bactericidal effect of zerovalent iron nanoparticles on *Escherichia coli*. *Environ. Sci. Technol*., 42, 4927-4933 (2008).
11. J.R. MORONES, J.L. ELECHIGUERRA, A. CAMACHO, K. HOLT, J.B. KOURI, J.T. RAMIREZ, M.J. YACAMAN, The bactericidal effect of silver nanoparticles. *Nanotechnology*., 16, 2346-2353 (2005).
12. P.K. STOIMENOV, R.L. KLINGER, G.L. MARCHIN, K.J. KLABUNDE. Metal oxide nanoparticles as bactericidal agents. *Langmuir,* 18, 6679-6686 (2002).

13. C.S. CIOBANU, S.L. ICONARU, C.L. POPA, M. MOTELICA-HEINO, D. PREDOI. Evaluation of Samarium Doped Hydroxyapatite, Ceramics for Medical Application: Antimicrobial Activity. *J. Nanomater.* http://dx.doi.org/10.1155/2015/849216, (2015).
14. A. COSTESCU, C.S. CIOBANU, S.L. ICONARU, R.V. GHITA, C.M. CHIFIRIUC, L.G. MARUTESCU, D. PREDOI. Fabrication, Characterization, and Antimicrobial Activity, Evaluation of Low Silver Concentrations in Silver-Doped Hydroxyapatite Nanoparticles, *J. Nanomater.* http://dx.doi.org/10.1155/2013/194854, (2013).
15. C.S. CIOBANU, S.L. ICONARU, C.M. CHIFIRIUC, A. COSTESCU, P. LE COUSTUMER, D. PREDOI, Synthesis and Antimicrobial Activity of Silver-Doped Hydroxyapatite Nanoparticles. BioMed Res. Int., http://dx.doi.org/10.1155/2013/916218, (2013).
16. S. WAN, J. HUANG, H. YAN, K. LIU. Size-controlled preparation of magnetite nanoparticles in the presence of graft copolymers, *J. Mater. Chem.,* 16, 298-303 (2006).
17. D. PREDOI. A study on iron oxide nanoparticles coated with dextrin obtained by coprecipitation, Dig. *J. Nanomater. Biostruct.,* 2(1), 169-173 (2007).
18. D. PREDOI, C.M. VALSANGIACOM. Thermal studies of magnetic spinel iron oxide in solution. *J. Optoelectron. Adv. M.*, 9(6), 1797-1799 (2007).
19. D.K. KIM, Y. ZHANG, J. KEHR, T. KLASON, B. BJELKE, M. MUHAMMED. Characterization and MRI study of surfactant-coated superparamagnetic nanoparticles administered into the rat brain. *J. Magn. Magn. Mater.*, 255(1), 256-261 (2001).
20. V.G. ROULLIN, J.R. DEVERRE, L. LEMAIRE, F. HINDRE´, M.C.V. JULIENNE, R. VIENET, J.P. BENOIT. Anti-cancer drug diffusion within living rat brain tissue: an experimental study using [3H](6)-5-fluorouracil-loaded PLGA microspheres. *Eur. J. Pharm. Biopharm.*, 53(3), 293-299 (2002).
21. A. JORDAN, R. SCHOLZ, P. WUST, H. SCHBIRRA, T. SCHIESTEL, H. SCHMIDT, R. FELIX, Endocytosis of dextran and silan-coated magnetite nanoparticles and the effect of intracellular hyperthermia on human mammary carcinoma cells *in vitro*. *J. Magn. Magn. Mater.*, 194(1-3), 185-196 (1999).
22. S. BUCAK, D.A. JONES, P.E. LAIBINIS, T.A. HATTON. Protein separations using colloidal magnetic nanoparticles. *Biotechnol. Prog.,* 19(2), 477-484 (2003).
23. C.V. MURA, M.I. BECKER, A. ORELLANA AND D. WOLFF. Immunopurification of Golgi vesicles by magnetic sorting. *J. Immunol. Methods.*, 260-263 (2002).
24. A. CHAKRABORTY. Kinetics of the reduction of hematite to magnetite near its Curie transition. *J. Magn. Magn. Mater.,*204:57(1999).
25. D. PREDOI, R.A. VATASESCU-BALCAN. Osteoblast interaction with iron oxide nanoparticles coated with dextrin in cell culture. *J. Optoelectron. Adv. M.,* 10(1), 152, 157 (2008).
26. A.M. PRODAN, S.L. ICONARU, C.M. CHIFIRIUC, C. BLEOTU, C.S. CIOBANU, M. MOTELICA-HEINO, S. SIZARET, D. PREDOI. Magnetic Properties and Biological Activity Evaluation of Iron Oxide Nanoparticles. *J. Nanomater.,* 2013, http://dx.doi.org/10.1155/2013/893970, (2013).
27. A. WOODING, M. KILNER AND D.B. LAMBRICK. Studies of the double surfactant layer stabilization of water-based magnetic fluids. *J. Colloid Interface Sci*., 1991, 144, 236.
28. S. WAN, Y. ZHENG, Y. LIU, H. YAN, K. LIU, $Fe_3O_4$ Nanoparticles coated with homopolymers of glycerol mono(meth)acrylate and their block copolymers. | *J. Mater. Chem.,* 15, 3424, 3430 (2005).
29. L. SHEN, A. STACHOWIAK, A. HATTON, P.E. LAIBINIS, Polymerization of Olefin-Terminated Surfactant Bilayers on Magnetic Fluid Nanoparticles. *Langmuir,* 16, 9907, 9911 (2000).
30. S. WAN, Y. ZHENG, Y. LIU, H. YAN, K. LIU. $Fe_3O_4$ Nanoparticles coated with homopolymers of glycerol mono(meth)acrylate and their block copolymers. *J. Mater. Chem.,* 15, 3424, 3430 (2005).
31. D. PREDOI. Physico-chemical studies of sucrose thin films. *Dig. J. Nanomater. Biostruct.*, 5(2), 373, 377 (2010).
32. A.K. GUPTA, M. GUPTA. Synthesis and surface engineering of iron oxide nanoparticles for biomedical applications. *Biomaterials*, 26(18), 3995, 4021 (2005).
33. S. LAURENT, D. FORGE, M. PORT, A. ROCH, C. ROBIC, L. VANDER ELST, R.N. MULLER. Magnetic Iron Oxide Nanoparticles: Synthesis, Stabilization, Vectorization, Physicochemical Characterizations, and Biological Applications. *Chem. Rev.*, 108 (6), 2064, 2110, (2008), DOI: 10.1021/cr068445e.
34. S.L. ICONARU, A.M. PRODAN, M. MOTELICA-HEINO, S. SIZARET, D. PREDOI. Synthesis and characterization of polysaccharide-maghemite composite nanoparticles and their antibacterial properties. *Nanoscale Res. Lett.*, DOI: 10.1186/1556-276X-7-576, (2012).
35. A.K. GUPTA, R.R. NAREGALKAR, V.D. VAIDYA, M. GUPTA. Recent advances on surface engineering of magnetic iron oxide nanoparticles and their biomedical applications. *Nanomedicine*, 2(1), 23-39, DOI doi:10.2217/17435889.2.1.23, (2007).

36. C. RALF, S.BERND, S. UDO, D. WOLFGANG, K. REETTA. "Glycerol". Ullmann's Encyclopedia of Industrial Chemistry. *Ullmann's Encyclopedia of Industrial Chemistry*. doi:10.1002/14356007.a12_477, (2006).
37. J.T. MACDONALD, D.L. UDEN. Intravenous glycerol and mannitol therapy in children with intracranial hypertension. *Neurology*., 32(4), 437,440 (1982).
38. S.L. ICONARU, A.M. PRODAN, P. LE COUSTUMER, D. PREDOI. Synthesis and Antibacterial and Antibiofilm Activity of Iron Oxide Glycerol Nanoparticles Obtained by Coprecipitation Method. *J. Chem,* 2013, 1-6, (2013).
39. A.M. PRODAN, C.S. CIOBANU, C.L. POPA, S.L. ICONARU, D. PREDOI, Toxicity Evaluation following Intratracheal Instillation of Iron Oxide in a Silica Matrix in Rats. *BioMed Res. Int.*, 2014 , 1-13(2014).
40. C.L. POPA, A.M. PRODAN, C.S. CIOBANU, D. PREDOI. The tolerability of dextran-coated iron oxide nanoparticles during in vivo observation of the rats. *Gen. Physiol. Biophys.,* 35(3), 299-310 (2016).
41. A.M. PRODAN, S.L. ICONARU, C.S. CIOBANU, M.C. CHIFIRIUC, M. STOICEA, D. PREDOI, Iron Oxide Magnetic Nanoparticles: Characterization and Toxicity Evaluation by In Vitro and In Vivo Assays *J. Nanomater*, 2013, 1-10 (2013).
42. M.C. CHIFIRIUC, V. GRUMEZESCU, A.M. GRUMEZESCU, C. SAVIUC, V. LAZĂR, E. ANDRONESCU. Hybrid magnetite nanoparticles/*Rosmarinus officinalis* essential oil nanobiosystem with antibiofilm activity. *Nanoscale Res. Lett*. 7, (2012).
43. M.C. CHIFIRIUC, C. STECOZA, L. VERONICA, O. DRACEA, C. LARION, A.M. ISRAIL. Antimicrobial activity of some new O-acyloximino-dibenzo[b,e]thiepins and O-acyloximino-dibenzo[b,e]thiepin-5,5-dioxides against planktonic cells, *Rom. Biotech. Lett.*, 15(2), 5134-5139, (2010).
44. PURDOIU L, IORDACHE PZ, CAPLAN DM , CÎMPEANU C , DINU PÎRVU C ,PURDOIU S , IVANA S. Comparative Analyses of Interactions Established between Nanoparticles and Emergent Bacteria. *Rom. Biotech. Lett.*, 21(4), 11479-84, (2016).
45. BUTEICĂ SA , MIHĂIESCU DE , ROGOVEANU I , MĂRGĂRITESCU DN , MÎNDRILĂ I. Chick Chorioallantoic Membrane Model as a Preclinical Tool for Nanoparticles. *Rom. Biotech. Lett.*, 21(4), 11684-90, (2016).
46. KAYGUSUZ K, LKHAGVAJAV N , YAŞA I , ÇELIK E. Antimicrobial Nano-Ag-TiO2 Coating for Lining Leather. *Rom. Biotech. Lett.*, 21(5), 11866-74, (2016).