Journal of International Scientific Publication:
Materials, Methods & Technologies, Volume 3

INFRARED STUDY OF BIOCOMPATIBLE MAGNETIC NANOPARTICLES

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Abstract

Magnetic fluids mainly consist of nano sized iron oxide particles (Fe_3O_4) that are suspended in carrier liquid. In recent years, substantial progress has been made in developing technologies in the field of magnetic nanoparticles. Techniques based on using of these biocompatible magnetizable complex systems have found application in numerous biological fields. Generally, the magnetic component of the particle is coated by a biocompatible polymer such as PEG (polyethylene glycol), polysacharids as dextran, starch, have been developed. The coating acts to shield the magnetic particle from the surrouding environment. This review is focused on describing the infrared spectrosopy as method that allows checking starting materials and reactions while work is in progress. FTIR method is correlated with others spectral methods as XRD, TEM.

Key words*: Magnetic nanoparticles, infrared spectroscopy, biomedicine*

1. INTRODUCTION.

Nanotechnology is an area of science devoted to the manipulation of atoms and molecules leading to the construction of structures in the nanometer scale size range (often 100 nm or smaller), which retain unique properties. Colloidal gold, iron oxide crystals, and quantum dots (QDs) semiconductor nanocrystals are examples of nanoparticles, whose size is generally in the region of 1–20 nm, and have diagnostic applications in biology and medicine. QDs can label biological systems for detection by optical or electrical means in vitro and to some extent *in vivo* [1].

The study on magnetite nanoparticles (MNPs) have been intensively pursued in recent years for their widely potential applications in biomedical fields, such as magnetic resonance image (MRI), hyperthermia, drug delivery, cell and DNA labeling, etc. It is difficult to synthesize the nanocomposites with magnetite nanoparticles embedded in polymeric host for the immiscibility between organic polymer and inorganic magnetite nanoparticles. In-situ synthesis provided a good solution to synthesize silica-based magnetic nanocomposites for its facility and effectivity. In this paper, we present two synthesis methods for magnetite nanoparticles and their medical aplication.

Magnetic particles ranging from the nanometer to micrometer scale are being used in an increasing number of medical applications. The important properties of magnetic particles for medical applications are nontoxicity, biocompatibility, injectability, and high level accumulation in the target tissue or organ; the most important property among those mentioned above is nontoxicity. Magnetic nanoparticles offer attractive and versatile applications in the field of biotechnology, such as DNA and RNA separation, cell separation, drug delivery system (DDS), magnetic resonance imaging (MRI), and hyperthermia [1-6]. For these applications, magnetic iron oxides such as Fe_3O_4 or gamma-Fe_2O_3 are employed as a magnetic phase because they are stable and harmless to the living bodies. Recently, nano-scale materials have brought about many changes and new research opportunities in physics, materials science, biology, and so on. Iron oxide nanoparticles have potential applications in biomedicine. To make them bind to a biological entity, their surfaces are usually modified with an appropriate compound as a biocompatible polymer such as starch, dextran, chitosan or polyethylene

glycol (PEG). The coating creates strong hydrophilic and biodegradable behavior. Polymers like starch, dextran, and recently chitosan has been used as a stabilizer (coating agent) for iron oxide nanoparticles so as to improve the nanoparticle's biocompatibility and injectability. More or less to improve limitations stated above, several derivatives of chitosan have been proposed based on reactions with the free amino groups.

2. EXPERIMENTAL SECTION

2.1. Materials

Analytical grade ferric chloride hexa-hydrate ($FeCl_3 \cdot 6H_2O$), ferrous chloride tetra-hydrate ($FeCl_2 \cdot 4H_2O$), ammonium hydroxide (NH_4OH, 25% of ammonia) were purchased from Sigma Analytic. Dextran, and sodium citrate were purchased from Sigma Aldrich Co., Ltd.

2.2. Synthesis of superparamagnetic particles

The chemical reaction of Fe_3O_4 precipitation is expected to proceed as follows:

$$FeCl_2.4H_2O + 2FeCl_3.6H_2O + 8NaOH \text{ −! } Fe_3O_4 + 8NaCl + 20H_2O \qquad (1)$$

First 1.08 grams $FeCl_2.4H_2O$ and 3.0 grams $FeCl_3.6H_2O$ [Fe_2+: Fe_3+ = 1 : 2] were dissolved in about 50ml de-ionized water, and this solution was stirred under strong ultrasonic agitation while the solution was heat to 70°C. Next, this iron solution source was dropped slowly into 1M NaOH (300 ml) source under strong ultrasonic agitation for 30 minutes. Black Fe_3O_4 particles were decanted by permanent magnet and cleaned by de-ionized water.

For this reaction has been used the installation from Fig.1.

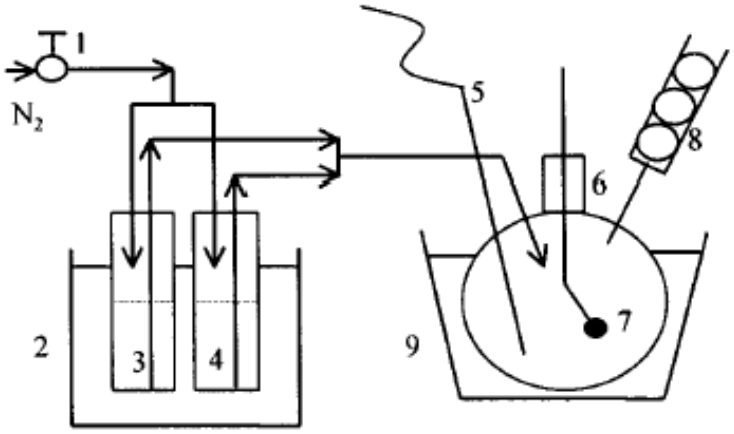


Figure 1. Reaction apparatus. 1: Valve, 2: Water-bath, 3: Ferrous and ferric solution, 4: NaOH solution, 5: Controlled thermometer, 6: Reactor, 7: Stirrer, 8: Reflux condenser, 9: Electrical heater.

2.3. Synthesis of colloidal suspension

2.3.1. For starch

a. Suspend 10 grams of soluble potato starch in 100 grams of boiling de-ionized water and mix.

b. Dissolve 3.0 grams of $FeCl_3.6H_2O$ and 1.08 grams of $FeCl_2.4H_2O$ (2:1 molar ratio Fe III to Fe II) in a total volume of 50 ml of de-ionized water.

c. Drop wise the iron solution into the starch solution, mix thoroughly and add 500 ml NaOH (13M) under strong ultrasonic agitation.

d. Heat the resulting solution so as to increase the temperature to 89°C over 4 hours, and maintain at 89°C for a further 50 minutes, deoxygenated by bubbling N_2 gas continuous.

e. After the 290 minute heating on the water bath, chill overnight at 10°C.

f. Washing by de-ionized water through settled gel suspension until pH is less than 8.5.

g. Excess material was removed by washing with de-ionized water and centrifuging the solution many times. The supernatant colloidal particles were separated from sedimented larger particles agglomerates.

2.3.2. For Dextran

a. Dissolve 4g grams dextran in 50ml de-ionized water and increase temperature of solution to around 90°C.

b. Prepare a mixture of iron salts as in 2.3.1.b and then pour this solution into the boiling solution in 2.3. 2. a.

c. Another solution of 60ml NaOH (1.5M) was placed in a three-necked fleck under strong ultrasonic agitation and N2. The solution was then heated to above 90°C and dropped slowly into the mixture of iron salts and Dextran in 3.2.b for 2 hours. The result was maintained at 89°C for 60 minutes and then cooled abruptly under 8°C and chilled overnight.

d. After 24 hours, the product was washed with cooled water several times until pH was neutral and then centrifuged at low temperature for 3 hours. The products were collected from sediment of larger particle agglomerates and continued keeping under 10°C.

2.3.3. For PEG

a. The coprecipitation method of ferric and ferrous salts in an alkali aqueous medium was used to prepare magnetite particles. Sodium oleate ($C_{17}H_{33}COONa$) as a first surfactant was used for the modification of prepared magnetic particles to prevent their agglomeration. To improve stability and in-crease the circulation half time of the particles, the polyethylene glycol (PEG) as a second surfactant was added to the system magnetite-oleate and stirred over 3 h. This magnetic fluid-PEG had a solid loading of 110.8 mg/ml. The solid loading in this case includes the weights of the iron oxide, sodium oleate and PEG. The composition was: 37 wt% magnetite, 58 wt % sodium oleate and 5 wt% PEG.

3. INSTRUMENTS

Fourier transform infrared spectroscopy (FT-IR) analysis was performed on a Perkin Elmer spectrophotometer employing a KBr pellet method. The dispersion property of Fe_3O_4 in organic solvent was performed on H-600 transmission electron microscope (TEM).

The X-ray diffraction (XRD) scans were recorded at room temperature on anX-ray diffractometer (XRD, SHLMADZU- 6000) with CuK radiation at a scan rate of 0.0167°/s.

4. RESULTS AND DISCUSSION

XRD patterns for sample powder (Fig. 1) demonstrate that the product is the magnetite and Fe_3O_4 is the dominant phase in all samples. Using XRD data, the average size of magnetite particles were calculated according to Scherrer equation. Calculations shown that the average size of Fe_3O_4 particles is between 8 and 10 nm. Figure 2 shows the X-ray diffraction (XRD) pattern of dextran and starch matched with the magnetite (Fe_3O_4) phase as compared to standard XRD patterns. The sharp peaks which appeared approximately $2\theta = 30°, 35°, 43°, 53°, 57°$ and 62° were due to Fe_3O_4 [1].

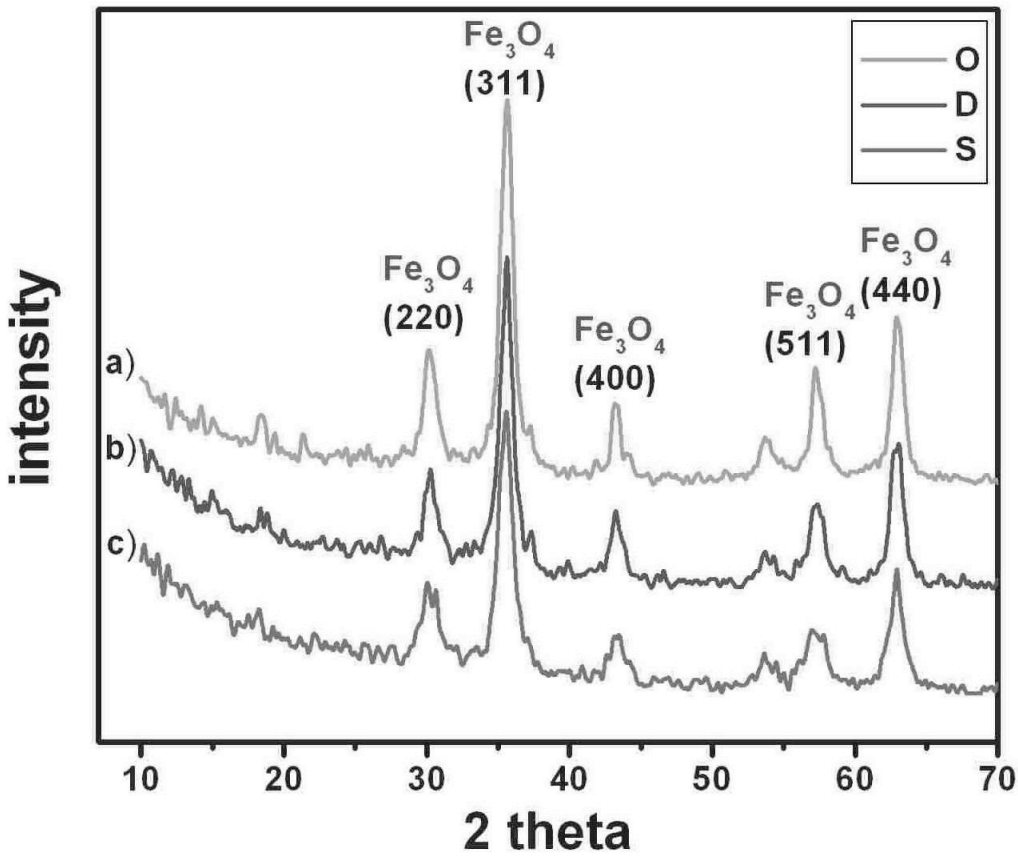


Fig. 2. X-ray powder diffraction patterns of a) uncoated particle; b) Dextran coated particle; c) starch coated particle

The FTIR spectra of samples are shown in Fig. 3. The 430-582 cm−1 peak belongs to the stretch vibration mode and torsional vibration mode of Fe-O bonds at tetrahedral sites and in the octahedral sites. The band near 1500 cm^{-1} relates to C-C and C-O modes of polysaccharide backbone. It indicates that the surfactant has coated the particle surface and formed a chemical bnd with the magnetic particles, and the spectra of magnetite nanoparticles coated with the starch shows little difference from

pure starch, which indicated that the interactions between the starch and magnetite nanoparticles are intermolecular interactions.

The spectrum is consistent with magnetic (Fe_3O_4) and the signals associated to the magnetite appear as broad features at 408.9, 571.5 and 584.5 cm^{-1}. The IR spectra of iron oxide is highly consistent with magnetite (Fe_3O_4) (bands at 408.9, 560.0 and 585 cm-1). The spectra of PEG coated nanoparticles shows the small shift in the positions of the main peaks as compared to uncoated particles. This is due to the change in environment of the particles after PEG coating.

In order to confirm the coating of PEG onto the surface of Fe_3O_4 nanoparticles, FTIR spectra were performed and the result was shown in Figure 4. The bands of 1726 cm^{-1} and 1113 cm^{-1} were respectively assigned to the C=O and C-O stretching vibrations for carbonyl group of carboxylic ester, the wide band around 3423 cm^{-1} to the O-H stretching vibration, the bands of 2871 cm^{-1} and 1470~1380 cm^{-1} to the stretching and bending vibrations of methylene (-CH_2-), and the band of 1640 cm^{-1} to the characteristic vibrations of -CONH- group. Furthermore, the peak of 584 cm^{-1} in curves a and b was the characteristic band for Fe_3O_4 nanoparticles.

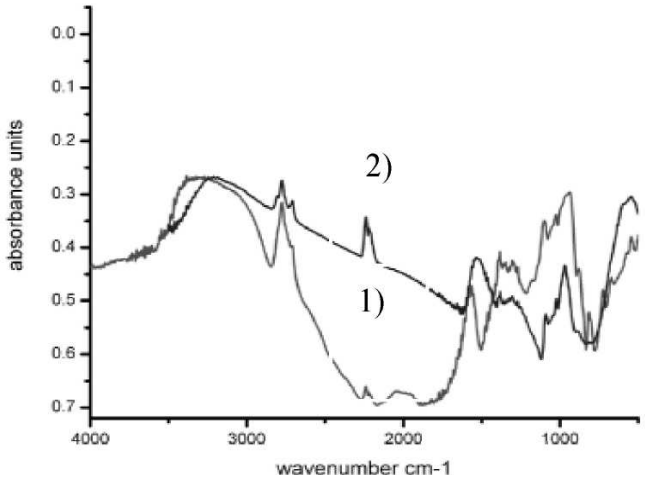


Fig. 3. The FTIR spectra of samples: 1) pure starch; 2) magnetite nanoparticles coated with starch

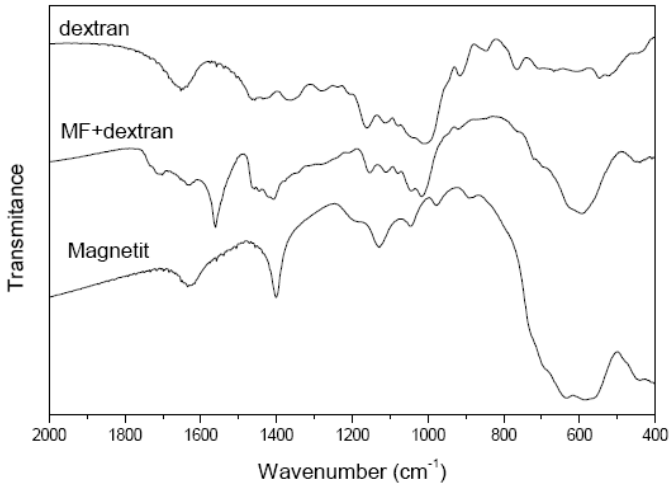


Fig. 4. The FTIR spectra of samples: 1) pure dextran; 2) magnetite nanoparticles coated with dextran

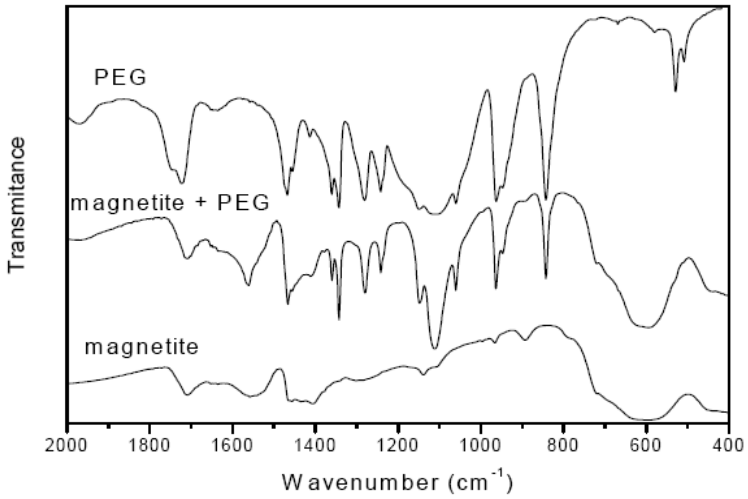
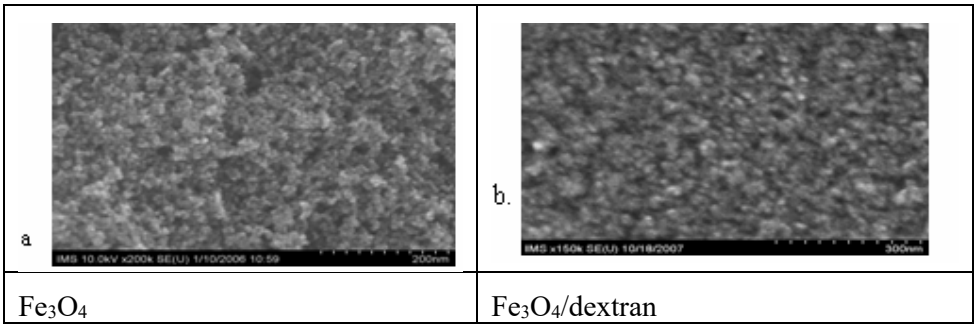
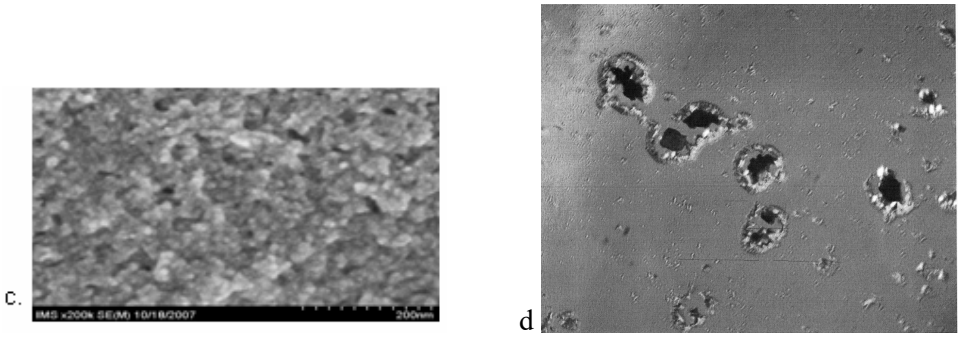


Fig. 5. The FTIR spectra of samples: 1) PEG; 2) magnetite nanoparticles coated with PEG

TEM and optical microscopy have been used to evaluate the shape and size of particles, as it is shown in Figure 6.



Fe_3O_4 | Fe_3O_4/dextran



Fe_3O_4/*starch* | Fe_3O_4/dextran optical microscopy

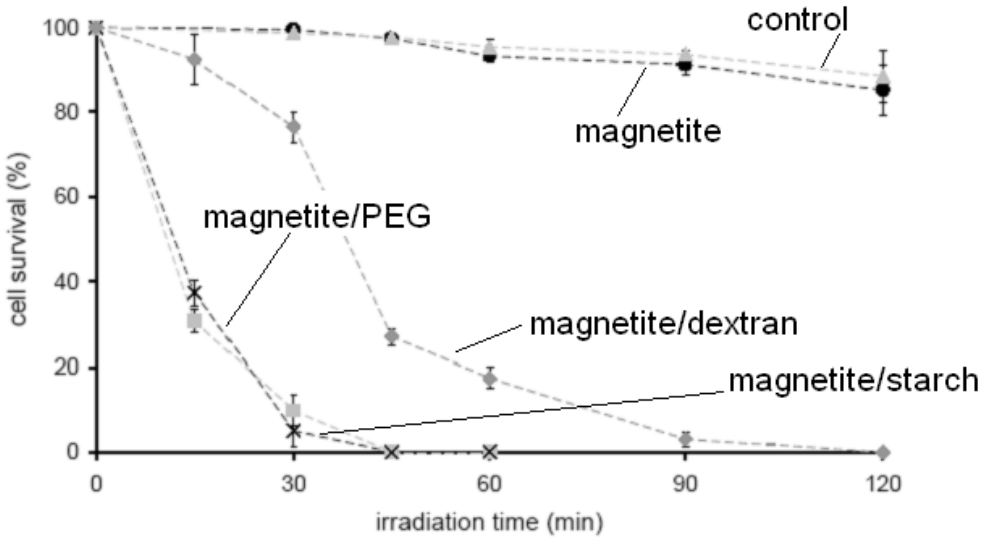
Figure 6.

5. EVALUATION OF CYTOTOXICITY

Evaluation of the cytotoxicity was performed by the MTT assay in K562 cells line. Briefly, K562 cells suspensions containing 1×10^4 cell/well in DMEM containing 10% FBS were distributed in a 96-well plates, and incubated in a humidified atmosphere containing 5% CO_2 at 37°C for 24 h. The cytotoxicity of magnetite coated with dextran, starch, PEG was evaluated in comparison with control cells. Cells were incubated for additional 24 h after the addition of defined concentration of magnetite. The mixture was replaced with fresh medium containing 10% FBS. Then, 20 µl of MTT solution (5 mg/ml in 1 × PBS) were added to each well. The plate was incubated for an additional 4 h at 37°C. Next, MTT-containing medium was aspirated off and 150 µl of DMSO were added to dissolve the crystals formed by living cells. Absorbance was measured at 490 nm, using a microplate reader (ELX 800; BIO-TEK Instruments, Inc.). The cell viability (%) was calculated according to the following equation:

Cell viability (%) = [OD 490(sample)/OD 490(control)] × 100

Figure 7. The viability of magnetite in different synthesis conditions



6. CONCLUSIONS

Researches in this topic have shown that development of magnetite is a current strategy designated to increase their hydrosolubility, biocompatibility, and their *in vivo* metabolic stability. Some functionalized magnetite by coating with starch, dextram or PEG, could contribute to antineoplastic chemotherapy efficacy displaying a low toxicity upon administration. The particular properties of such compounds could open new opportunities in tumor treatment with promising application in photodynamic therapy of cancer. From this point of view, the purpose of our study is the *in vitro* analysis of the novel compounds upon K562 leukemia cell line, in order to determine the toxicological outline of this modified magnetite and to evaluate its candidature for a potential photosensitizer agent.

REFERENCES

1. R.M. Ion, N*anocrystalline materials, FMR Ed., Bucharest, 2003;*
2. RM Ion, *Acta Bio-optica et Informatica Medica, 1, 2006, pp.37-49;*
3. Cheng, FY; Su, CH; Yang, YS; Yeh, CS; Tsai, CY; Wu, CL; Wu, MT; Shieh, DB. *Biomaterials.* 2005;26:729–738.
4. Pankhurst, QA; Connolly, J; Jones, SK; Dobson, J. *J Phys D-Appl Phys.* 2003;36:167–181.
5. Khor, E; Lim, LY. *Biomaterials.* 2003;24:2339–2349.
6. Woo, K; Hong, J; Choi, S; Lee, HW; Ahn, JP; Kim, CS; Lee, SW. *Chem Mater.* 2004;16:2814–2818.
7. Stoykova, E. , Sabotinov, O., Ion, R.-M., Alexandrova, R, *Biotechnology and Biotechnological Equipment* Volume 22, Issue 2, May 2008, Pages 754-758

8. A.Filip, S.Clichici, A.Muresan, C.Gherman, D.Daicoviciu, RM Ion, S.Dreve, N.Decea, R.Moldovan, *Cytokine*, 43(3), 260(2008);
9. C.Constantin, M.Neagu, G.Manda, I.Neagoe, M.Gherghiceanu, C.Stravaru, RM Ion, *J.Porph.Phthal*., 2008, 12(3/6) 651;
10. R.M.Ion, *Physiology*, 18(2), 58(2008), pp.37-38;
11. S.Clichici, M.Perde-Schrepler, A.Filip, D.Daicoviciu, P.Virag, E.Fischer-Fodor, I.Brie, RM Ion, C.Login, A.Muresaan, *Physiology,* 18(2), 58(2008), pp.17-18;
12. R.Cosgarea, S.Senila, I.Baldea, M.Susan, S.Dreve, RM Ion, *Physiology,* 18(2), 58(2008), pp. 19;
13. C.Constantin, M.Neagu, G.Manda, RM Ion, D.Iordachescu, R*omanian Archives of Microbiology and Immunology, 2005;*