SCIENTIFIC REPORT

regarding the implementation of the project between May 2013 - December 2014

Title of the project: "DETECTION AND IDENTIFICATION OF BIOMOLECULES OF MEDICAL INTEREST BY USING MAGNETIC AND OPTIC PROCESSES"

Phase I. Synthesis of magnetic and non-magnetic *micro* and *nano-particles*, as markers and supports for magnetically controlled transport of the receptors (probes) and target biomolecules that are involved in the detection process.

Activity I.1. Synthesis of magnetic nanoparticles (for ex., magnetite by using chemical/physical methods).

To prepare magnetic nanoparticles, a physico-chemical method was used. As precursors of the magnetic reaction products, ferrous chloride ($FeCl_2 \cdot 4H_2O$), ferric chloride ($FeCl_3 \cdot 6H_2O$) and sodium hydroxide (NaOH) were used in a molar ratio of 1:2:23. The reagents, bought from Alfa Aesar company and used in their solid state, were mechanically mixed several minutes for homogenization. Then, NaOH was added, as precipitation agent, while continuously mixing. The reaction was powerful exothermic, the temperature, measured with a chromel-alumel thermocouple, getting to 140 ^oC.

After cooling at room temperature, the sample was washed with double distilled water until the pH of the suspension got to 6.5-7. The magnetic separation of the particles was performed by using a permanent magnet, NdFeB.

For characterization, the particles were used as powders or in colloidal form. For characterization in powder form, the magnetic particles were dried 3 hours at 85 ^oC, while for characterization in dispersed state, the suspension was sonicated in distilled water by using an ultrasonic homogenizer (Hielscher UP50H, Germany) at 90 % amplitude.

The size of nanoparticles were controlled by the pH and the solubility of the precipitate (Mohapatra et al. 2007; Tartaj et al. 2006). On the other hand, a general rule regarding the crystals precipitated from oversaturated solutions assumes that the mean size of the crystal, measured at the end of the crystallization process, decreases depending as the value of the oversaturation increases (Von Weimarn 1925; Barlow and Baird 2004). Therefore, an increase amount of NaOH was poured over solid solution of iron salts for inducing a quick precipitation of the reaction products, to keep them with low size, and obtain a very high pH. Also, due to the specific reaction conditions, the product between Fe^{2+}, Fe^{3+} si OH^- becomes much higher than the solubility product, favoring therefore the synthesis of the magnetic product.

Activity I.2. Synthesis of non-magnetic nanoparticles, i.e., Au nanoparticles by using chemical routes.

To prepare gold nanoparticles, a gold salt (chloroauric acid) and solution of trisodic citrate were used.
The synthesis process followed the following steps:

1. The glass vessels used to prepare the nanoparticles were treated one minute with 10 % hydrofluoric acid to remove any possible contamination of the inner surface of the glass vessels because the metal or salt tracks lead to quick agglomeration of the nanoparticles. Thereafter, the vessels were cleaned with ultrapure water several times and dried at 70 ^oC.
2. The magnetic stirrer was cleaned with aqua regia (1:3 v/v HNO_3: HCl)
3. 300 ml solution of $HAuCl_4$ 0.5 mM was heated to 100 ^oC.
4. Over the solution mentioned above, 30 ml of sodium citrate solution were quickly added under strong stirring.
5. Several seconds after, the solution became slightly blue, turning finally to dark red, indicating the presence of the gold nanoparticles.
6. The solution was maintained under stirring, at room temperature, another 30 minutes and then slowly cooled at room temperature.

The final concentration of the particles in the reaction medium was 10.8×10^{11} nanoparticule/ml and was calculated taken into account the weight of gold in the chloroauric acid, the mean size of the nanoparticles and the gold density.

Activity I.3. Synthesis of non-magnetic microparticles (as alternative – acquisition from a commercial firm).

To prepare non-magnetic particles, there was used an emulsion polymerization method by using styrene as monomer to prepare polystyrene microparticles.

The emulsion polymerization consists in polymerization of a free radical in a heterogeneous reaction. In the beginning of the reaction, the monomer is dispersed as small drops in the aqueous phase. The reaction should take place under strong stirring to maintain the dispersion of the polymer. If there is an insufficient polymerization, there will appear a limitation of the mass transfer of the monomer from its phase to that in which polymer particles forms.

As regarding the stabilizers, some of the polyvinyl alcohols and other types of water soluble polymers can initiate the polymerization process, though they do not form micelles and do not act as surfactant. It is assumed that all these polymers graft on the polymeric particles and stabilize them.

As surfactants, the most used are: fatty acids, sodium dodecyl sulphate and alpha olefin sulfonate. As polymerization initiators, the most used are represented by the persulfate salts; the persulfate ions brake into sulfate radical ions at temperatures higher than 50 °C, starting the polymerization process.

The preparation method

An aqueous phase composed by 200 ml distilled water, 0.1 sodium dodecyl sulphate (as surfactant), 0.1 g polyvinylpyrrolidone (stabilizer), 20 g glucose (stabilizer and reducing agent, hindering a possible quick and uncontrolled polymerization) was magnetically stirred 1 hour at 36 ^oC.

Over the aqueous phase, there were added 10 ml styrene, under stirring at 1000 rpm, the temperature being increased at 70 ^oC. After 3 hours, 10 ml solution of potassium persulfate (polymerization initiator, 5 g %) were added. After 24 hours there were added another 10 ml solution of potassium persulfate and temperature was increased at la 85 ^oC.

After 3 hours, the temperature was gradually decreased down to the room temperature, keeping a constant mixing. After stopping the mixing, the polystyrene microparticles were centrifugated and washed several times by using 0,1 g % sodium dodecyl sulphate solution.

Activity I.4. Evaluation of the structure, sizes, shapes and magnetic characteristics of the obtained particles.

Magnetic nanoparticles

In the case of magnetic nanoparticles, the structure was investigated by X-ray diffractometry (XRD); the shape was evaluated through scanning electron microscopy (SEM); the size was measured through a method based on dynamic light scattering (DLS); the magnetic properties were determined by using vibrating sample magnetometry (VSM).

The magnetization of the nanoparticles (fig. 1) was measured using a vibrating sample magnetometer Lake Shore 7410. The specific saturation magnetization (46 emu/g) was calculated by extrapolating the tangent to the curve obtained from the dependence of the magnetization, M, on the inverse of the applied field, 1/H, in the region where the M(1/H) dependence is a straight line.

The coercive field of the magnetic particles was 20 G, while the remanent magnetization was 1.5 emu/g, the nanoparticles being close to superparamagnetic behavior.

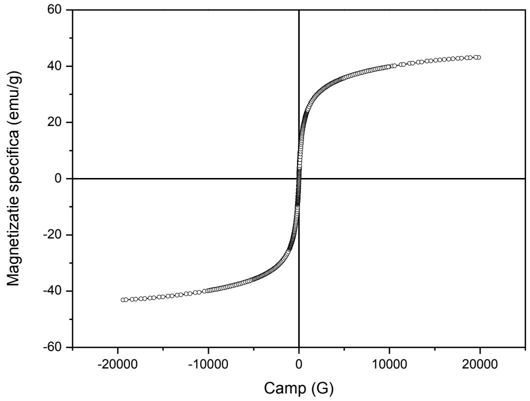

Fig. 1. The hysterezis curve of the magnetic samples.

The evaluation of the size was carried out by using a particle analyzer, Microtrac/Nanotrac 252. The size of magnetic nanoparticles ranged between 10 nm and 55 nm, with the mean diameter of 24 nm (fig.2)

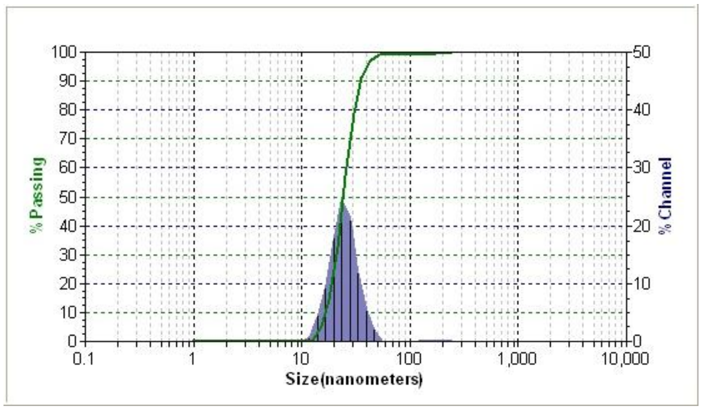

Fig. 2. Dimensional distribution of the magnetic nanoaprticles

The geometric shape of the particles was evaluated by using a FE-SEM / FIB, CrossBeam System Carl Zeiss NEON40EsB microscope. The SEM images (fig. 3) showed that the majority of the nanoparticles presents a cubic/octahedral profiles.

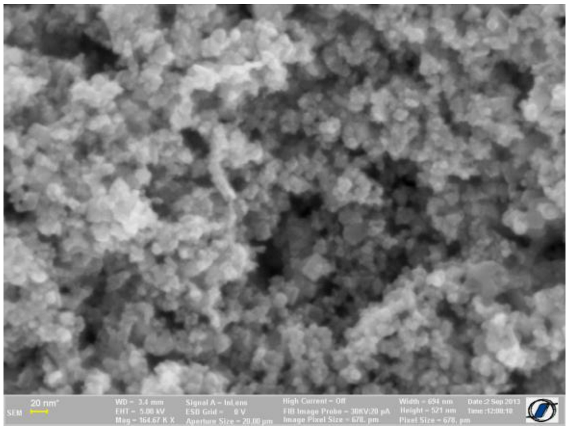

Fig. 3. SEM image of the magnetic nanoparticles.

The XRD spectra (fig. 4), obtained by using a Brucker AxS D8-Advance diffractometer indicates the presence of both magnetite and maghemite. The diffractogram of the magnetic sample presents diffraction maxima specific to the two compounds and corresponding to the diffraction planes (220), (222), (311), (400), (440), (422), (511), (533).

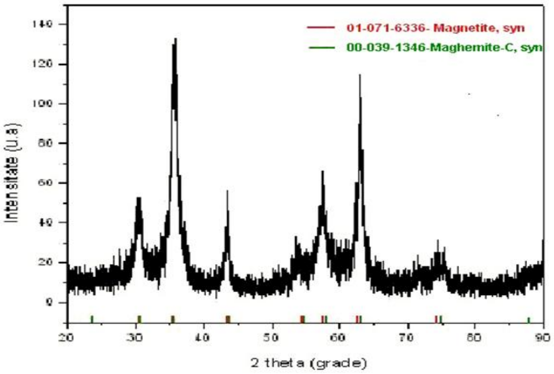

Fig. 4. The XRD spectrum of the magnetic samples.

The XRD analysis shows that the sample is composed by crystaline nanoaprticles, with a mean size of 8.26 nm. The size were calculated by using Sherrer's formula.

Given the fact that magnetite and maghemite are structurally very related , it is difficult to specify the purity of the XRD-analyzed sample.

Gold nanoparticles

The DLS measurements showed a gaussian distribution of the sizes of the nanoparticles, ranging from 10 nm to 60 nm, the mean size placing around 20 nm (fig. 5a); the sizes were confirmed also by the SEM images (fig. 5b).

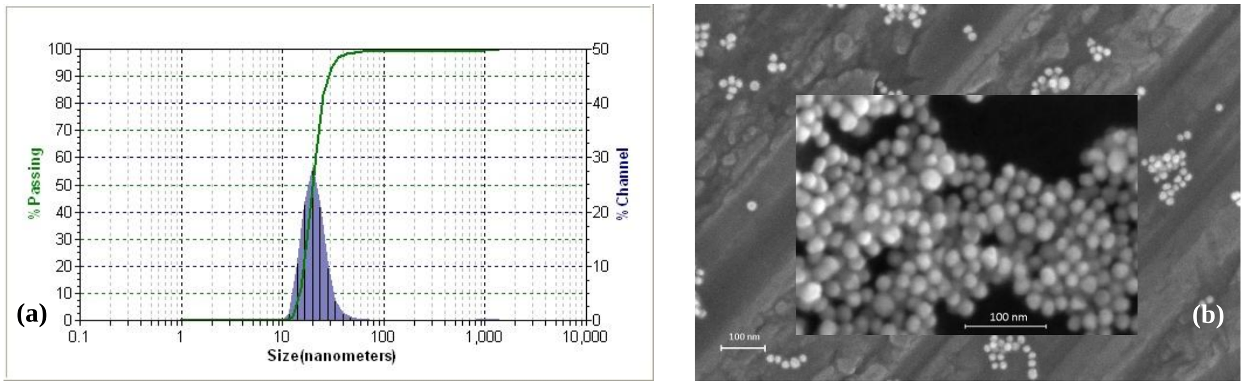

Fig. 5. Size distribution of the gold nanoparticles (a); SEM images of the Au nanoparticles (b).

The absorption spectra of the nanoparticles in suspension was carried out through UV-VIS spectroscopy. The maximum absorbance, which depends on the particles' concentration, presented a maximum for a wavelenght of 520 nm (fig. 6), specific for gold nanoparticles.

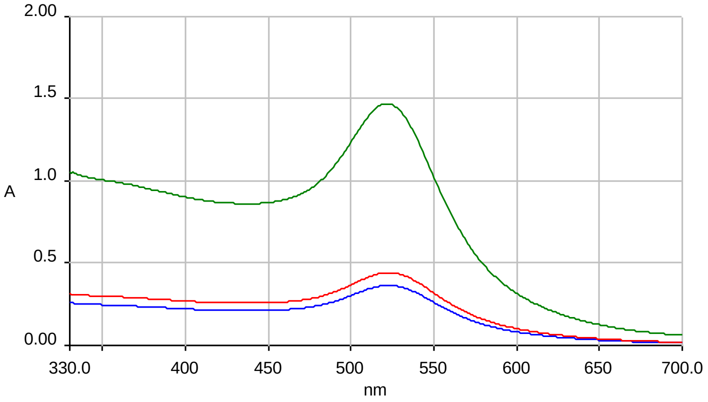

Fig. 6. UV-VIS absorption spectrum of the gold nanoparticles

Polystyrene microparticles

According to the dimensional analysis, performed by using the DLS, the size distribution of the microparticles ranged between 2 micrometers and 6 micrometeres (fig. 7a), showing a gaussian profile. The mean size was 3.35 micrometeres.

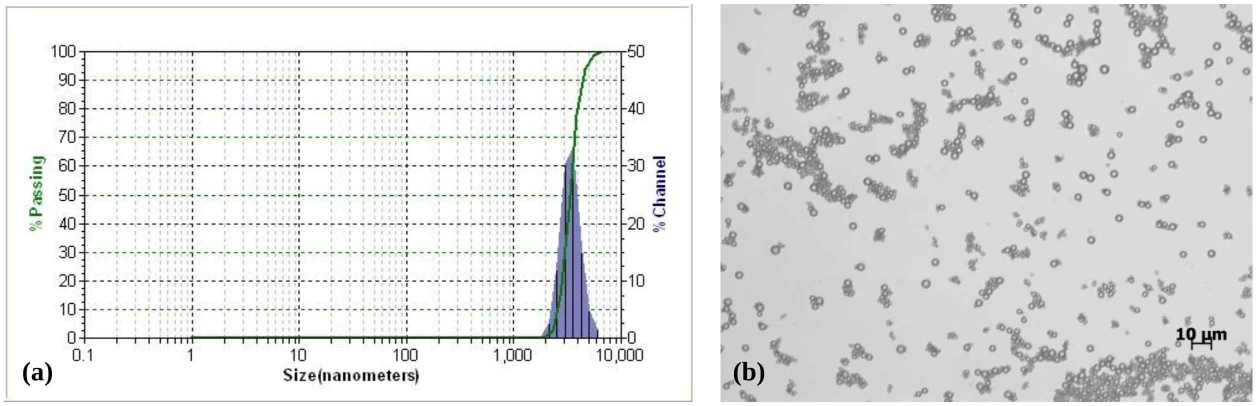

Fig. 7. Size distribution of the polystyrene microparticles (a); optical image of the polystyrene microparticles.

Optical images showed spherical shapes of the polystyrene microparticles (fig. 7b), and confirmed their micrometer dimension.

In conclusion, all the activities and objectives proposed for this phase of the project were succesfully carried out.

References

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Project Director,
Dr. Dumitru-Daniel Herea