Magnetic nanoparticle effects on the red blood cells

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Abstract. In vitro tests on magnetite colloidal nanoparticles effects upon animal red blood cells were carried out. Magnetite cores were stabilized with citric acid in the form of biocompatible magnetic fluid administrated in different dilutions in the whole blood samples. The hemolysis extent was found increased up to 2.75 in horse blood and respectively up to 2.81 in the dog blood. The electronic transitions assigned to the heme group were found shifted with about 500 cm⁻¹ or, respectively, affected by supplementary vibronic structures. The Raman vibrations assigned to oxyhemoglobin were much diminished in intensity probably due to the bonding of OH group from citrate shell to the heme iron ion.

1. Introduction
The utilization of magnetite nanoparticles represents a subject of great interest in the field of multidisciplinary research since various applications in biology and medicine were carried out. The magnetite (ferric and ferrous ion oxides), in the form of functionalized and suspended nanoparticles, is used as contrast agent in Magnetic Resonance Imagery, in drug targeting techniques - by magnetic guiding of colloidal magnetite by means of adequately arrangements of magnetic fields - in cell tagging, in cancer therapy (hyperthermia), etc. The side effects induced by biological tissue contamination with magnetic compounds is also an important issue, since the magnetic field produced by them is of orders of magnitude greater than the geomagnetic field, so they can be a source of magnetic exposure, facilitating free radical formation around the magnetic particles. Until now, the consequences of nanoparticle use in medical purposes, is not fully understood.

Many reports have presented data regarding magnetic nanoparticle interaction with living tissues, especially with red blood cells. Since in most of these applications, the blood cells interaction with the magnetic nanoparticles is unavoidable, the response of erythrocytes membranes to low levels of magnetic nanoparticle concentrations remains an actual challenge. The presence of small quantities of ferromagnetic material within human body tissue was first detected while monitoring physiological magnetic fields from cardiac electric currents. Also, the liver and spleen are tissues known as able to uptake magnetic material from blood flow. Putative relaxation of magnetic nanoparticles concentrated within a tissue after intravenous injection was also reported in hamsters. The iron ions from the
hemoglobin heme core might be involved in the interaction with the local magnetic field produced by the magnetic nanoparticles attached to red blood cells during fulfilling their carrier function. Generally, literature reports present positive results in pointing up the magnetic fluid’s in vitro biocompatibility with the red blood cells, the cytotoxicity tests proving no hemolysis activity. There are a few reports mentioning the hemolytic effects of magnetic nanoparticles. For instance, the accelerated hemolysis was evidenced in the frame of an experimental study involving both electromagnetic exposure (visible radiation) and magnetic nanoparticle addition to in vitro red blood cells [1]. The tests described in literature as being applied to evaluate nanoparticle hemolytic effect were conducted using different methods, and even when similar approaches were used, factors such as plasma anti-coagulant, blood incubation times, centrifugal forces, assay detection wavelength varied from study to study [2-3]. Consecutively, it was really impossible to conclude what nanoparticle properties were responsible for the hemolysis – i.e. did it depend on size, charge, surface groups or was due to the particle absorbance.

Considering the large investigation possibilities offered by spectroscopy methods on hemoglobin behavior, the experimental investigation presented below was carried out. The present paper is focused on the magnetic nanoparticles interaction with the erythrocytes, since such situation could frequently occur during medical procedures (clinical diagnosis and therapy) or in biomedical research. The spectral behavior of animal blood in the presence of magnetic nanoparticles was investigated applying two spectral methods (electron absorption spectroscopy and vibrational Raman spectroscopy). The spectra were recorded for hemolysed blood before and after the incubation with magnetic nanoparticles, the modifications of the main spectral bands being discussed.

2. Materials and methods
The biologic material used for the experiment consists in whole animal blood (horse and dog) freshly withdrawn in the presence of heparin. The nanoparticle source was consistent with a biocompatible magnetic fluid (magnetite cores coated with citric acid), two concentrations of magnetic fluid being tested: C1 = 0.5 % and C2 = 3.0 % (v/v). The volume fraction of ferrophase was of 4.5 %, saturation magnetization of 23 kA/m, while average physical diameter was of 11.44 nm (as shown by transmission electron microscopy – Figure 1) [4]. Aliquots of aqueous magnetic fluid were brought in direct contact with the blood samples, the concentration of magnetite nanoparticles being of $10^{-12}$-$10^{-13}$/ml. The blood samples were incubated at 37.5°C ± 0.1°C within a water bath, for 30 minutes, and then centrifuged for 5 minutes at 3 000 cycles/min, the supernatant being further investigated. The electronic absorption spectra of hemolysed blood were recorded using a Shimadzu UV 1700 spectrophotometer, the main differences, observed at the level of the two major bands in the visible domain, being discussed.

![Figure 1. Left: TEM image of magnetite nanoparticles. Right: Physical diameter distribution (N: number of particles).](image-url)
Hemolysis extent was assessed using the formula:

\[ H.E. = \frac{A_S - A_C}{A_C} \]  \hspace{1cm} (1)

where \( A_S \) is the absorbance of the magnetite loaded sample at 555 nm (the most intense of the two peaks observed in the animal blood at long wavelength) and \( A_C \) the absorbance of the control sample at the same wavelength.

The absorbance ratio for the peaks having maxima at the wavelengths of 555 nm and 588 nm (18 000 cm\(^{-1}\) and respectively 16 955 cm\(^{-1}\)) was also calculated, aiming to get semiquantitative expression of the changes induced in the blood samples compared to the control:

\[ R_A = \frac{A_{555}}{A_{588}} \]  \hspace{1cm} (2)

where \( A_{555} \) and \( A_{588} \) are the absorbance values at 555 nm and respectively 588 nm.

Raman vibration spectra were recorded at room temperature (200 Fourier transformation scans) using adequate installation provided with 400 mW laser device (Jobin Yvon T-64000 spectrometer).

Statistic significance of the investigation was ensured by working on three replies of every blood magnetite sample and controls and applying statistic t-test.

3. Results and discussion

Typical hemoglobin spectra were recorded for blood samples that were characterized by two electronic transitions in the visible range (Figure 2): relative high intensity band at 23 750 cm\(^{-1}\) (421 nm) and lower intensity and bifurcated band at 18 000-16 955 cm\(^{-1}\) (555 nm and respectively 588 nm). Significant changes were evidenced in the electronic transitions of horse blood hemolysates of magnetite loaded samples:

- the transition at 23 750 cm\(^{-1}\) was shifted toward higher wavenumber values with about 500 cm\(^{-1}\);
- the double vibronic transition at 18 000-16 955 cm\(^{-1}\) was accompanied by several new shoulders at 15 500 cm\(^{-1}\), 19 500 cm\(^{-1}\) and 21 000 cm\(^{-1}\).

One could say that following the colloidal magnetite interaction with the heme group the energies of the electronic levels are changed as well as the heme stretchings, so that complex interactions are supposed to occur within the hemoglobin molecules.

In the dog blood spectra the hemoglobin bands were found at slightly shifted wavenumbers - with no more than 85 cm\(^{-1}\) toward the blue edge of the visible spectral domain. The qualitative changes, following magnetite loading were the same as for the horse blood. In all magnetite loaded samples, the hemoglobin level was higher than in the control sample, revealing the intensification of hemolysis.
phenomenon (Table 1) following the addition of magnetic fluid dilutions (linear approach of hemolysis extent dependence on the magnetic fluid concentration). The hemolysis extent (measured at 555 nm) significantly increased for higher concentration of magnetic fluid, following theoretical lines, with correlation coefficients higher than 0.97. Statistic significance (p<0.05) was revealed by t-test.

Table 1. Magnetite loading effect on hemoglobin electronic absorption spectra.

| magnetite level / horse blood | hemolysis extent | absorbance ratio | magnetite level / dog blood | hemolysis extent | absorbance ratio |
|------------------------------|------------------|------------------|----------------------------|------------------|------------------|
| Control                      | 0.00             | 1.045            | Control                    | 0.05             | 1.045            |
| 0.5 %                        | 0.75             | 1.055            | 0.5 %                      | 0.84             | 1.052            |
| 3.0 %                        | 2.75             | 1.065            | 3.0 %                      | 2.81             | 1.077            |

Thus, the red blood cells release significantly higher concentration of hemoglobin following the interaction with the magnetic nanoparticles, suggesting possible undesired side effects of the traces of magnetic fluid remaining for some time in the patient’s blood, when used in medical applications. The iron ions of the ferrophase from the magnetic fluid could affect the relative intensity of the two electronic absorption bands studied in here, probably due to the fact that these transitions are located at the hem level - the iron ions being also involved. The possible magnetic influence of iron ions from the ferrofluid nanoparticles upon the hem structure seems to be able to modify the transition probabilities, corresponding to the main two absorption bands of hemoglobin from the visible range (the effect being also suggested by the changes induced in Raman vibration spectra).

Figure 3. Raman vibrational spectra: 1) pure water; 2) horse blood; 3) horse blood incubated with magnetic nanoparticles.

In the Raman spectra of horse blood loaded with magnetic nanoparticles, several characteristic peaks at 1,563 cm\(^{-1}\); 1,450 cm\(^{-1}\); 1,337 cm\(^{-1}\); 1,002 cm\(^{-1}\) and 783 cm\(^{-1}\) (Figure 3) – that are missing in the water spectrum taken as reference – are shown being diminished significantly or they disappeared after magnetite loading. Another peak, that was present at 2,950 cm\(^{-1}\) in the horse blood hemolysate, disappeared after magnetite loading. Similarly Raman peaks, corresponding to the dog blood samples at 1,543 cm\(^{-1}\), 1,457 cm\(^{-1}\) and 1,232 cm\(^{-1}\) (Figure 4) – with lower intensity than in the horse blood – as well as that at 2,960 cm\(^{-1}\) diminished or vanished after magnetite loading; an additional peak appeared at 1,369 cm\(^{-1}\) following the interaction heme-nanoparticles. The Raman spectra allowed taking knowledge about the spectral shifts of some stretching bands corresponding to the vibration ranges, characteristic to the pyrrole ring from the heme bonded to different globins – in horse and respectively
in dog blood. The behavior of the most sensitive bands - assigned to the oxyhemoglobin or to the deoxyhemoglobin - to the addition of the iron oxide particles, was interpreted taking into account the physical nature of core shell assembly, suggesting the coupling of colloidal magnetite particles to heme iron, by means of OH hydrogen bonds (OH groups being provided by the molecules of citric acid used to functionalize the magnetite particles surface within the ferrofluid composition). These results are concordant with the report of Santana [5] and Soler [6] that monitored murine hemoglobin structural changes using Raman spectroscopy, by carrying out in vitro biological tests using a biocompatible magnetic fluid, based on carboxymethyl dextran-coated magnetite nanoparticles, emphasizing iron interaction with hemoglobin molecules. So one could say that red blood cells can be hemolysed following the magnetite loading and the released hemoglobin could interact with magnetite shell at the level of pyrrole group. It is also possible that some nanoparticles get attached to the red blood cells and exert magnetic irradiation on them, so that the electrons belonging to the heme group change their energetic levels – which can be seen in the electronic spectra behavior.

Figure 4. Raman vibrational spectra: 1) pure water; 2) dog blood; 3) dog blood incubated with magnetic nanoparticles.

4. Conclusion
The in vitro interaction of hemoglobin from animal blood with biocompatible magnetite nanoparticles was investigated in the frame of this study. Hemolysis extent was found significantly increased, so the hemolytic effect of magnetite nanoparticles seems to be evident. There are two possible ways that the magnetite nanoparticles influence the heme energetic levels – either electronic or vibrational ones: by interacting with heme from released hemoglobin molecules or by exerting chronic magnetic exposure on the heme iron following the addition to the red blood cell membranes. Further in vitro and in vivo investigations are planned to get detailed and deeper insight on the nature of the interactions between colloidal magnetite and heme structures.

References
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