Experimental investigation on blood magnetic contamination in the presence of drug molecules

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Abstract. The purpose of the present project was to study the interference of magnetic nanoparticles with drug molecules – rifampicin, used in lung infectious disease and respectively, sodium diclofenac, an antiinflammatory steroid. The controlled magnetic contamination was accomplished using colloidal nanoparticles supplied from diluted magnetic fluids. Various concentrations of diluted aqueous magnetic fluids, based on magnetite cores coated with citric acid and respectively sodium oleate, were tested. The experiment was focused on the capacity of the magnetic nanoparticles to form reversible complexes with the drug molecules, as well as on the monitoring of the nanoparticle-drug complex dynamics, under the action of external magnetic field. The level of released rifampicin ranged between 4 mg/100 ml and 7 mg/100 ml for the magnetic exposure of 20 mT, while the sodium diclofenac decomplexation level was not higher than 2.5 mg/100 ml under magnetic exposure of 60 mT. The experimental arrangement was proved to be an adequate model for the dynamical study of magnetite reversible complexation with drug molecules, evidencing certain specific values of drug concentration and magnetic field induction that favour such interactions.

1. Introduction
The interaction magnetite-biological molecules could occur following accidental magnetic contamination of human body tissues or blood, since very fine magnetic nanoparticles seem to be present in most biotic and abiotic elements of the environment – the magnetic loading of water, air and soil increasing continuously in the modern industrial era. In the same time, environmental magnetic fields enhance the risk of chronic magnetic exposure. The present study was motivated by the issue of magnetic contamination of both the environment and living bodies, that shaped more and more during latest decades, based on the iron abundance – the fourth element on the Earth, on the detection of ferromagnetic particles in aerosols, foods and sterile laboratory liquids, as well as on the existence of magnetosomes. According to [1], ferromagnetic particles can be present in the body tissues mainly as contaminants of the lungs and other organs. The magnetic domains of these particles can be aligned by the application of an external magnetic field; the magnitude and time course of the resultant field depend on the quantity of magnetic material and the degree of particle motion. In this respect, other authors [2-6] formulated a possible explanation of the living body sensitivity to electromagnetic fields,
based on the hypothesis of magnetic particle contamination. According to this theory, small polluting magnetic particles from the environment are present not solely in the air dust, but they are also deposited on the surface of laboratory devices and penetrate into plastics and glass, into chemicals and purified water [7]. Their mean size is about $10^{-5}$ cm and they consist of ferro- and ferrimagnetic compounds (they possess spontaneous magnetization). The authors have shown that routine laboratory procedures like pouring or/and rinsing give rise to enrichment of the cell cultures by pollutants. The number of particles in cultures could be ten times more than that of cells. The energy of such a particle is approximately three orders of magnitude greater than kT. In the authors [8-9] opinion, the magnetic particle absorbed by a cell membrane can transfer its energy to the adjacent biophysical structures, for example, mechanically activated ion channels. On the other hand, internalized magnetic nanoparticles are shown to be an endogenous source of chronic magnetic exposure that increases the local concentration of free radicals. Experimentally, ultrafine particles (12–14 nm in size) were shown to be internalized by human monocyte cells and significantly increase, by 40–45%, the release of free radicals [10]. An enhanced level of leukaemia in early childhood is assumed to originate from magnetic nanoparticles located in hematopoietic stem cells [11], while progressive neurodegeneration is supposed to be initiated by metal crystalline compounds [12-14].

The main objective of the experiment presented below was to investigate the putative side effects of residual magnetic nanoparticles, supplied by environmental sources or following insufficiently controllable medical procedures (such as Magnetic Resonance Imagery or cancer therapy with magnetic fluids) in the case of patient consecutive treatment with usual pharmaceutics. Rifampicin antibiotic was chosen due to its ability to interact with iron ions, but sodium diclofenac was also tested in the frame of physical model proposed below.

2. Materials and methods

Magnetic nanoparticles sources were consistent with magnetite stabilized with citric acid (CA) and respectively sodium oleate (SO), that were supplied as adequate volumes from various dilutions of the corresponding aqueous magnetic fluids (CA-MF and SO-MF) [15-16]. The dilutions (in distilled water), that favored the magnetite reversible complexation with the drug molecules, were: C1 = 1 ml CA-MF + 9 ml water; C2 = 2 ml CA-MF + 8 ml water; C3 = 4 ml CA-MF + 6 ml water for magnetite-citric acid complexation with rifampicin; C4 = 5.0 ml SO-MF + 5.0 ml water; C5 = 2.5 ml SO-MF + 7.5 ml water; C6 = 1.25 ml SO-MF + 8.75 ml water for magnetite-sodium oleate complexation with sodium diclofenac. The stock solutions of the tested drugs were prepared from pharmaceutical powders in the concentrations of 30 mg/100 ml rifampicin and 40 mg/100 ml diclofenac. Magnetic field (rare earth permanent magnets) induction was of 20 mT for rifampicin and 60 mT for diclofenac as measured with a Hall probe teslameter. Spectral device (Shimadzu UV-VIS 1700 spectrophotometer) was utilized for quantitative monitoring of drug molecules decomposition in aqueous medium.

The level of released sodium diclofenac was assessed in the ultraviolet range (UV) at 275 nm, while the absorption bands of rifampicin were recorded in the visible range (VIS) at 474 nm and ultraviolet domain at 334 nm. For each magnetic fluid concentration, the same percentage of drug solution volume was added: 50 % (v/v) - by careful stirring, followed by 24 hours incubation at the temperature of 35.0°C in the INCUCELL thermostatic room. Magnetic exposure was applied by placing all samples onto permanent magnets (Figure 1); distilled water columns of 10 ml were added very slowly onto the mixture of fluids - simplified physical model of blood serum, suitable for spectral assay based on light absorption. Aliquots of 0.5 ml were withdrawn to measure light extinction at t = 0, t = 0.5 h, t = 1.0 h, t = 1.5 h, t = 2.0 h, t = 2.5 h and t = 24.0 h – temperature monitoring being carried out continuously.

Three replicates of every experimental variant were considered in the experimental project in order to get the statistical insurance. Average values and standard deviations were taken into account for discussion; statistical t-test was applied to compare the rifampicin levels measured by using two absorption bands, as well as to discuss diclofenac behavior for the three tested concentrations.
3. Results and discussion

The dynamics of reversible interaction CA-MF - rifampicin was monitored by spectral assay, the rifampicin concentration being provided by light extinction measurement and calibration curve – in mg/100 ml or mg%. The data obtained for the three combinations mentioned above (noted with C1, C2 and C3: C1<C2<C3) are presented in Figures 2a, 2b and 2c. The concentration of rifampicin reached over 4 mg % in the case of C1 – as shown by graphical plots of both UV and VIS data (Figure 2a). In the case of C2, over 6 mg % were detected by spectral investigation (Figure 2b), while in the case of C3 (Figure 2c) the highest level of rifampicin decomplexation was evidenced – about 7 mg %. The data provided by the measurements carried out in the maximum of the rifampicin VIS absorption band are similar to those corresponding to the measurements in the UV band maximum, the standard deviation being of about 5 %. The differences between the two data series obtained for each spectral band and for each of the concentrations C1, C2, C3 are negligible - according to t-test - except for two time values, i.e. 25.5 and 26.0 h in the case of C1 (Figure 2a) and respectively, for 24.0 and 24.5 h in the case of C2 (Figure 2b) (p<0.05). The statistic comparison of any pair of data series (each pair of Ci, Cj concentrations, i,j=1,2,3) corresponding to each spectral band, resulted in statistically significant differences (p<0.05).

The interpretation of the rifampicin dynamics during its decomplexation is related to the specific conditions characteristic for the experimental project described above. So, the experiment was designed to model the situation when the magnetic contamination occurs during a patient treatment with rifampicin – it could be the case of magnetite nanoparticles up-taken by lung tissue simultaneously with antibiotic administration against infectious lung disease. This could happen actually in the case of professional exposure of mine workers; but in this case, we have to do with natural stores of iron and iron-oxidization compounds – namely magnetite – where magnetic field exposure is also unavoidable since local magnetic field is considerable increased around iron mines. Consequently, the rifampicin molecules could be temporarily retained by the magnetite nanoparticles present in the lung tissues, under the supplementary influence of the relative intense magnetic field. Then gradually – as it was shown by the data presented above – the water from the interstitial liquid as
well as that from the blood serum can extract rifampicin molecules from the complexes formed with magnetite nanoparticles. In nature, as well in the artificially arranged spaces, free magnetic nanoparticles seem to be dispersed in significant levels, but when they are internalized within the human body fluids, colloidal forms could develop as result of their interaction with various biomolecules.

**Figure 2a.** The dynamics of rifampicin - magnetite reversible complexation as resulted from measurements on the UV-VIS spectrum – relative low concentration (C1) - the time axis was scaled from 0 to 48 h by including the 24 hours incubation.

**Figure 2b.** The dynamics of rifampicin-magnetite reversible interaction as resulted from UV-VIS spectral assay – medium concentration (C2) - the time axis was scaled from 0 to 48 h by including the 24 hours incubation.

In the frame of the experiment discussed inhere, the utilization of magnetite powder was found to be inadequate since it does not disperse spontaneously in aqueous media, except when colloidal suspensions are used – this is why the supply of magnetite nanoparticles was carried out by means of diluted magnetic fluids. In this situation, the involving of the coating molecules could play a role in the interaction between the iron oxides and the drug molecules.

**Figure 2c.** The dynamics of rifampicin-magnetite reversible interaction as resulted from UV-VIS spectral assay – relative high concentration (C3) - the time axis was scaled from 0 to 48h by including the 24 hours incubation.

**Figure 3.** The dynamics of sodium diclofenac decomplexation for the concentrations C4, C5, C6 - the time axis was scaled from 0 to 48h by including the 24 hours incubation.

The rifampicin is a large molecule that was shown to be able to interact with iron ions like those composing magnetite, but when citrate ions are coupled to the magnetite particles, the complexation phenomenon could be more complicated. Though the measurements performed in the frame of this research could not provide details regarding the intimate molecular arrangements into the studied
magnetic systems, however it was evident that the level of rifampicin released following decomplexation - favored in aqueous environment - is increasing with the concentration of magnetite (Figures 2a, 2b, 2c). We need to mention that for higher magnetic induction (60 mT) also tested within this work, the decomplexation was rather slightly visible; on the other side, the mixture between rifampicin and SO-MF was not homogeneous enough to enable optimal quality spectral readings.

In Figure 3 the data obtained for the interaction SO-MF-diclofenac are shown. Since diclofenac absorbs only ultraviolet radiation, the measurements were carried out on a single spectral band. The dynamics of diclofenac decomplexation is described by similar curves as in the case of rifampicin, except the diminution tendency revealed for longer observation times. One could say that the precipitation of recently released drug molecules could occur in time, so that light extinction gradually diminishes. The maximum levels of drug molecules in the supernatant ranged between 1.6 and 2.5 mg/100 ml for the three mixtures of diclofenac solution and magnetic fluid (C4>C5>C6). It seems that for similar concentrations of the two drugs used in the present experiment, the decomplexation of diclofenac is several times weaker than that of rifampicin, which could be possibly related to the particular chemical features of diclofenac (sodium salt) and the magnetite shell (sodium oleate). The two sodium ion sources could generate competitive processes which could underlie the non-monotone variation of released diclofenac level to the monotone variation of magnetite concentration. Indeed, the highest diclofenac level was obtained for the medium SO-MF concentration – C5, the differences recorded between all three data series (considered at the same time moments) being statistically significant (p<0.05) for the time moments when the maxima of the three curves were reached: 24.5, 25.0 and 25.5 h. Neither the mixing of sodium diclofenac with CA-MF nor the application of the relatively low magnetic field (20 mT) did not lead to convenient homogeneous systems.

4. Conclusion

The magnetite nanoparticles in colloidal form were let to interact with two drug molecules in the presence of static magnetic field aiming to provide an experimental model of blood magnetic contamination and its putative side effects. The reversibility of magnetite complexation with rifampicin and sodium diclofenac was studied. Optimal experimental conditions for the observation of the decomplexation phenomenon were found testing two magnetic fluids – as magnetite sources – and two magnetic field inductions. The increased decomplexation of rifampicin was evidenced to the increase of magnetite ratio within the studied magnetic mixtures, the optimal experimental conditions involving a 20 mT magnetic field. Comparatively, the diclofenac reversible interaction with magnetite was found to release lower drug concentration in the surrounding aqueous medium while higher magnetic field was also needed. The study of these interactions could be of interest for the situations of people magnetic contamination – as in professional exposure - during simultaneous treatment with pharmaceutical products.

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