Studies of Complex Magnetic Nanoparticles with Anticancer Agents for Cancer Therapy

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***Abstract*- An impressive increase in the survival rates of rats infected with Guerin carcinoma has been demonstrated, following the injection of complex magnetic nanoparticles (abbreviated as MCN) to induce hyperthermia and targeted drug delivery. Here, we present the latest developments in our technology for the synthesis of MCN comprising magnetite nanoparticles Fe_3O_4 and molecules of the anticancer drug anthracycline antibiotic doxorubicin (DR) with KCl. MCN were synthesized in a mechanic reactor with magnetic elements, by magneto-mechano-chemical dry synthesis. DR and its nanocarrier Fe_3O_4 with KCl were subjected to mechanical activation. It is demonstrated that under the influence of mechano-chemical and magneto-mechano-chemical synthesis of the MCN, the concentration of paramagnetic centers increased. In pilot preclinical studies we showed that following the injection of the MCN synthesized by our methods, into rats with Guerin carcinoma subjected to external electromagnetic radiation, the survival rates increased by 25% as compared to the treatment with conventional DR alone.**

Keywords*- Magneto-Mechano-Chemical Synthesis; Reactor; Magnetic Nanoparticles; Magnetic Moment; Paramagnetic Centers; Free Radicals; Doxorubicin; Guerin Carcinoma

I. INTRODUCTION

There has been considerable interest in the use of magnetic nanoparticles in biomedicine, since they have unique characteristics. First, their sizes ranging from a few nanometers up to tens of nanometers, can be controlled, so that it matches those of biological entities of interest. The nanoparticles can be coated with biological moieties to enable interaction with numerous biological entities. Therefore, the nanoparticles can be employed to deliver anticancer drugs to a specific area of the body, hence, the interest in using them in the battle against cancer. Nanoparticles have also been used in inducing hyperthermia, i.e the particle heats up and delivers toxic amounts of thermal energy to tumors. They achieve that by resonantly responding to a time-varying electromagnetic field; thereby absorbing energy from the excitation field to the nanoparticles and subsequently to the target area. In combination with chemotherapy and radiotherapy, a moderate degree of tissue heating delivered by the nanoparticles can result in more effective treatment. All these potential applications can be utilized to advance biomedicine and health care as a result of the physico-chemical characteristics of these biomagnetic nanoelements [1–3].

Experimental and clinical studies conducted in the last 10 years testify to the potential use of complex magnetic nanoparticles (MCN) comprising iron oxide (magnetite) Fe_3O_4 and antitumor drugs adsorbed on their surface, such as anthracycline row antibiotic doxorubicin (DR), as a significant aid in the battle against cancer. Some of the known methods for synthesis of the MCN are oxidization, chemisorption on their surface, directed modification of surface, thermolysis, mechano-chemical activation. It is also known that modifying the nanoparticles surface layer, will change their magnetic properties with respect to their nucleus, and the interaction between them results in significant changes in the physical and chemical properties of the nanoparticles [4, 5].

Magneto-mechano-chemical technology is a new method for the synthesis of the MCN [6]; the method is based on the integration of two known methods: mechano-chemical synthesis [7, 8] and synthesis of enzymes in a micro-reactor with magnetic elements [9]. The principle of the mechano-chemical synthesis is based on the fragmentation and formation of an ensemble of paramagnetic centers, such as free radicals in MCN structure [10, 11]. The increase in the concentration of free radicals of the MCN is suggestive of the potential increase of the drug antitumor effect under the influence of external radio frequency energy [12].

Magnetite Fe_3O_4 is the material with high saturation magnetic moment m_s in a wide range of temperatures. When magnetite nanoparticles are used in the synthesis of MCN under the influence of external magnetic irradiation, strong local magnetic fields near the particle-components and other nanocomposites will appear. Effects such as, the magnetic (or nuclear-spin) isotope effect, the chemically induced magnetic polarization of electrons and nuclei, the radiofrequency chemical maser, the high-frequency magnetic-resonance modulation of the rates of physico-chemical processes involving paramagnetic particles and magnetic effects in molecular physics, are all consequences of spin selection rules and spin evolution [13].

In this study, we have examined some of the physical and chemical characteristics of the MCN synthesized by the method of magneto-mechano-chemical activation.

II. EXPERIMENTAL

A. Magneto-Mechano-Chemical Synthesis and Activation

The nanoparticles Fe_3O_4 with KCl (International Center for Electron Beam Technologies of EO Paton Electric Welding Institute, Ukraine) with diameters in the range 20–40 nm and DR (Pfizer, Italy) were processed in our high-precision magneto-mechanical-reactor (NCI, Ukraine). Mechano-chemical activation (MCA) or magneto-mechano-chemical activation (MMCA) of the individual nanoparticles (without DR) in the reactor without and with magnetic field respectively, was performed. Mechano-chemical synthesis (MCS) or magneto-mechano-chemical synthesis (MMCS) of the MCN with the drug DR was also performed without and with magnetic field respectively.

B. Energy-Dispersive Spectroscopy

The elemental composition (chemical composition) of the MCN was examined by a scanning electron microscope “JEOL JSM-6490LV” (Jeol, Japan) using an energy dispersive spectrometer “Inca Energy 450” manufactured by “Oxford Instruments Ltd.”

C. Electron Spin Resonance (ESR) Spectroscopy

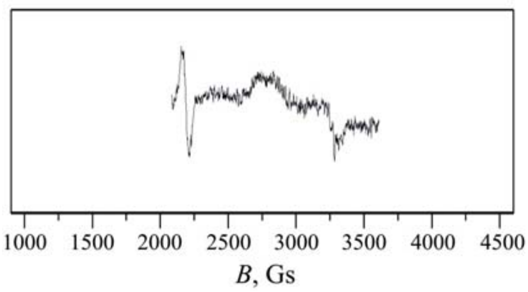
In order to measure the g-factor and the concentration of paramagnetic centers in the samples, Electron paramagnetic resonance spectra were recorded with the spectrometer RE1307 at liquid nitrogen temperatures (77 K) in a cylinder resonator with the mode H_{011}, with frequency 9.15 GHz. The power microwave radiation was 40 mW and the magnetic field modulation frequency 100 kHz. The samples were placed in a quartz Dewar with an inner diameter of 4.5 mm. g-factor calculated according to the formula of the resonance condition:

$$h\nu = g\beta B, \tag{1}$$

where $h\nu = 9.15$ GHz is microwave frequency, $\beta = 1.39968$ GHz/kGs is Bohr magneton, and B is magnetic induction.

III. RESULTS

The weight composition of the MCN elements is shown in Table 1. In Fe_3O_4 with KCl, iron was up to 70% of weight composition on average. In the MCN, that contained DR in addition to Fe_3O_4 and KCl, iron content decreased to 14.5% on average. Weight composition of oxygen varied from 24.05 to 76.65%.



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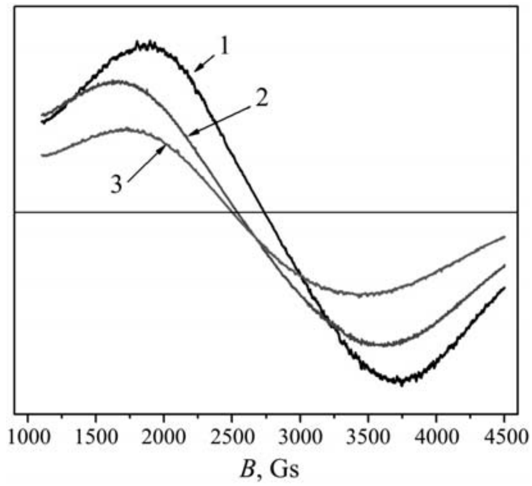

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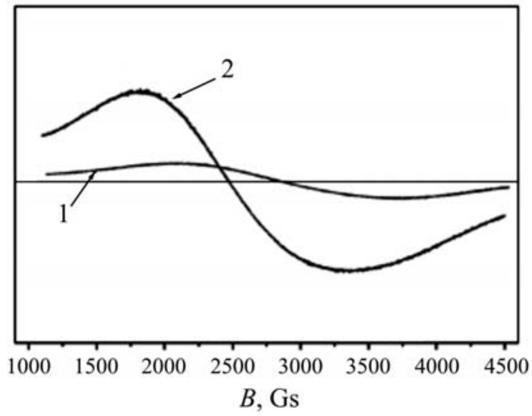

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Fig. 1 ESR spectra. B – magnetic induction. a – conventional DR; b – Fe_3O_4 + KCl: 1 – without activation, 2 – MCA, 3 – MMCA; c – Fe_3O_4 + KCl + DR: 1 – MCS, 2 – MMCS

The effect of MCA and MMCA on nanocomposites without DR resulted in decreased concentration of paramagnetic centers. MCS and MMCS of nanocomposites increases 10^2–10^3-fold compared with the usual state at a given temperature.

TABLE I WEIGHT COMPOSITION (%) FE, O, K AND CL IN COMPLEX MAGNETIC NANOPARTICLES (MCN)

N	Samples	Elements			
		Fe	**O**	**K**	**Cl**
1	Conventional DR without activation	–	33.63	–	0.83
2	Fe_3O_4 + KCl without activiation	70.47	27.15	1.27	1.12
3	Fe_3O_4 + KCl mechano-chemically activated (MCA)	65.92	31.78	1.12	1.18
4	Fe_3O_4 + KCl magneto-mechano-chemically activated (MMCA)	73.61	24.05	1.23	1.13
5	Fe_3O_4 + KCl + DR mechano-chemically synthesized (MCS)	13.83	76.65	1.00	7.29
6	Fe_3O_4 + KCl + DR magneto-mechano-chemically synthesized (MMCS)	15.12	32.24	0.38	2.15

ESR spectra of nanocomposites are shown in Figure 1 and Table 2. The effect of MCA and MMCA on nanocomposites without DR resulted in decreased concentration of paramagnetic centers, while the synthesis of nanocomposites in the conditions of MCS and MMCS resulted in increased concentration of paramagnetic centers. During MMCS the g-factor increases up to 2.64 for nanocomposite Fe_3O_4 + KCl + DR, implying substantial changes in its electronic structure given that ESR signals with g-factors 2.0839 and 2.18838 are typical for Fe_3O_4 nanoparticles [14].

TABLE II THE CHANGES IN PARAMAGNETIC CENTERS CONCENTRATION (C, MG^{-1})

N	Samples	g-factor	Paramagnetic centers concentration
1	Conventional DR (without activation)	1.97	$5\cdot10^{17}$
		2.003	$8\cdot10^{17}$
		2.005	$4\cdot10^{17}$
2	Fe_3O_4 + KCl (without activation)	2.38	$4\cdot10^{19}$
3	Fe_3O_4 + KCl (MCA)	2.57	$3\cdot10^{19}$
4	Fe_3O_4 + KCl (MMCA)	2.62	$2\cdot10^{19}$
5	Fe_3O_4 + KCl + DR (MCS)	2.31	$3\cdot10^{20}$
6	Fe_3O_4 + KCl + DR (MMCS)	2.64	10^{21}

IV. DISCUSSION

A theoretical analysis of the experimental data follows next. We begin with the elementary act of collision of the balls with the walls of the chamber, inside which the nanoparticles of magnetite and DR are synthesised. It will be shown that the collision energy is sufficient to break down the weak bonds and to eject an electron from MCN molecule. The average movement speed of the chamber is determined by the formula:

$$v = Af, \tag{2}$$

where A is an amplitude of the vibration of the chamber and f is the vibration frequency.

The balls inside the chamber are moving chaotically due to the continuous mechanical collisions with the chamber walls and between themselves. The approximate average speed of the balls in the chamber is v. During a collision of the ball with the chamber wall the ball deformed and elastic waves are propagated in MCN. Part of the kinetic energy of the ball motion is irreversibly transferred to the internal energy of the chamber wall and ball, caused by presence of internal friction in solids [15]. The energy conservation coefficient k, which is defined as the ratio of kinetic energy following the collision, to the kinetic energy before the collision during the collision of the ball against a fixed wall, is characteristic of the material of the chamber walls and balls.

On average, in a collision of a ball with the chamber wall energy ΔU is absorbed

$$\Delta U = (1-k)\frac{m_b v^2}{2}, \tag{3}$$

where m_b is the ball mass.

If a nanoparticle of DR or magnetite is situated on the point of contact of the ball and chamber wall, this energy is partially transferred to the nanoparticle. The area of the collision of the ball against the wall at the place of contact can be estimated from the deformation of the ball by Hook's law.

$$\frac{F}{S} = E\frac{\Delta d_b}{d_b} \tag{4}$$

where F is the collision force; S is the collision area; d_b is the ball diameter; $\Delta d_b/d_b$ is the ball deformation and E is Young's modulus.

Figure 2 shows a representation of the ball deformation due to a collision with a chamber wall. The area of contact S can be estimated by the formula:

$$S = 4\pi a^2 = 4\pi\left((d_b/2)^2 - (d_b/2 - \Delta d_b/2)^2\right) \approx 2\pi d_b \Delta d_b \tag{5}$$

where a is the radius of contact spot.

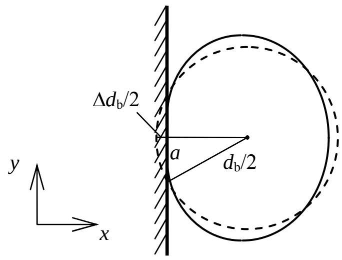

Fig. 2 Deformation of metallic ball as it collides with the walls of the reactor

Dotted line represents unstrained ball, solid line represents deformed ball

Substituting the expression for Δd_b from Formula (5) to Equation (4) we obtain:

$$F = \frac{ES^2}{2\pi d_b^2} \tag{6}$$

From where,

$$S = \sqrt{\frac{2\pi F}{E}}d_b \tag{7}$$

The impact force F can be estimated according to Newton's second law

$$F \approx \frac{\Delta p_x}{\Delta t} \tag{8}$$

where $\Delta p_x \approx m_b v$ is the change of momentum of the system "ball – chamber wall" in the direction of axis x, which is perpendicular to the chamber wall.

The time of impact can be estimated from the formula

$$\Delta t \approx \frac{2d_b}{v_s} \tag{9}$$

where v_s is the speed of sound in steel. Substituting the Expression (9) to (8), we obtain:

$$F \approx \frac{m_b v v_s}{2d_b} \tag{10}$$

Taking into account the last formula, Equation (7) for the contact area at impact of the ball with the chamber wall becomes:

$$S = \sqrt{\frac{\pi d_b m_b v v_s}{E}} \tag{11}$$

The area S_p of the nanoparticle is expressed by the formula:

$$S_p = \frac{1}{4}\pi d_p^2 \tag{12}$$

where d_p is the diameter of the nanoparticle. The internal energy of the nanoparticle increases by ΔU_p, that is the part of energy ΔU absorbed at impact, which is proportional to the ratio of particle area to the contact area at the impact of ball and wall:

$$\Delta U_p = \frac{S_p}{S}\Delta U \tag{13}$$

Substituting values for S_p, S, ΔU to (13) and taking (2) into account, we obtain a formula that links the increase of internal energy ΔU_p of one nanoparticle, situated at the contact area, with the parameters of mechano-magneto-chemical synthesis:

$$\Delta U_p = \frac{\sqrt{\pi}}{8}(1-k)d_p^2 Af\sqrt{\frac{EAfm_b}{d_b v_s}} \tag{14}$$

The energy absorbed at impact by the nanoparticle is transferred to the excitation of elastic vibrations in its crystal lattice and excitation of electrons. As a result of continuous collisions working balls with the chamber walls in the MCN, situated at the contact area, free radicals are initiated in great numbers. Under the influence of electromagnetic field free radicals are redistributed on the surface DR and magnetite nanoparticles.

According to the results of our experimental studies during MMCS of the MCN, the concentration of paramagnetic centers increases 10^2–10^3-fold compared with the usual state at a given temperature. This increase of the concentration of paramagnetic centers suggests a corresponding increase of the concentration of free radicals.

For nanoparticles of DR, MCS and MMCS results in the initiation of additional charge carriers and sharp growth of the concentration of paramagnetic centers.

Electron transfer processes take place in the MCN subjected to electromagnetic irradiation. A possible mechanism of electron transfer, the so-called spintronic mechanism, is related to the phenomena of giant magnetoresistance in thin layers of magnetite and DR. The giant magnetoresistance can mediate the electron transfer in DR benzene rings depending on whether the magnetization of adjacent magnetite layers are in a parallel or an antiparallel alignment. The overall resistance is relatively low for parallel alignment and relatively high for antiparallel alignment [16].

In addition to electron transfer, there are magnetoelastic waves in magnetite nanoparticles. These are ions oscillations of the magnetite crystal lattice accompanied by spins oscillations [17]. They take place due to Villari or magnetoelastic effect, which consists of the change of magnetic magnetization under the action of mechanical deformations. Magnetoelastic waves spread further to DR particles, changing the spin states of the paramagnetic centers and altering the magnetoresistance.

Our plan is to develop the technology for tumor treatment based on the utilisation of magneto-mechano-chemically synthesized nanocomposites of the MCN comprising DR and iron oxides. This is based on well-known experimental and theoretical work about the role of free radical processes in the transformation of the normal cells to malignant and subsequent growth of neoplasm and their mutual relationship with other organs in the organism.

In our previous experimental studies, we demonstrated that the increase of paramagnetic centers (free radicals) concentration in MCN, increases cytotoxic action of the drug in a tumor under external inhomogeneous electromagnetic fields [6]. In the study, 56 male rats weighing 100 ± 15 g were bred in the vivarium of National Cancer Institute. The transplantation of Guerin carcinoma was performed according to the established procedure. All animal procedures were carried out according to the rules of the regional ethic committee. Inhomogeneous electromagnetic irradiation was applied with our prototype apparatus "Magtherm" (Cavendish NanoTherapeutics, Radmir).

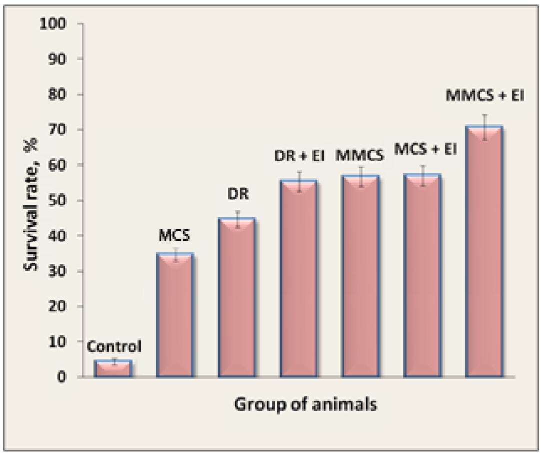

Fig. 3 Survival rates in rats with Guerin carcinoma after treatment: MCS– mechano-chemically synthesized nanoparticles with (doxorubicin) DR; DR – conventional DR; DR + EI as conventional DR with electromagnetic irradiation; MMCS – magneto-mechano-chemically synthesized nanoparticles with DR; MCS + EI – mechano-chemically synthesized nanoparticles with DR and electromagnetic irradiation; MMCS + EI – magneto-mechano-chemically synthesized nanoparticles with DR and electromagnetic irradiation

The most significant survival of animals with Guerin carcinoma (in comparison with all other experiments, $p < 0.05$) was observed after intravenous injections of MMCS $Fe_3O_4 + KCl$ nanoparticles with DR and electromagnetic irradiation.

The survival rate of animals 90 days after transplantation of Guerin carcinoma was 25% higher compared to the treatment with conventional DR. Figure 3 shows data from trials on animals injected with magnetic nanoparticles synthesised under the various conditions described above. The highest survival rate is shown for the animals which were injected with the MMCS nanoparticles and subjected to external inhomogeneous electromagnetic irradiation.

This is an experimental demonstration of the above-mentioned studies on the antioxidant properties of the MCN. We are planning to undertake future comparison studies of the antitumor, antimetastatic and toxicity effects of conventional DR and magneto-mechano-chemically synthesized MCN formed by Fe_3O_4 particles and DR for a wide range of experimental models of malignant tumors.

V. CONCLUSIONS

We have developed and presented our technology and a magnetic-mechano reactor for the magneto-mechano-chemical dry synthesis of the MCN comprising nanoparticles and the anticancer drug DR. Under the influence of MCS and MMCS, the MCN with DR shows a hysteresis curve which is typical for soft ferromagnetic materials. Very significant effects on the survival rates in animal experiments have been shown. We anticipate that this method of nanoparticles synthesis will lead to improvements in the use of hyperthermia and targeted drug delivery to aid the treatment of cancer patients.

ACKNOWLEDGMENTS

We would like to thank Dr. B.A. Movchan and Dr. Yu.A. Kurapov of the International Center for Electron Beam Technologies of E.O. Paton and the Electric Welding Institute of the National Academy of Sciences of Ukraine for providing the nanoparticles Fe_3O_4, as well as Dr. Yu.G. Mel'nik of the National Cancer Institute for technical assistance.

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