

Blood Clearance of Citric Acid-Coated Superparamagnetic Iron Oxide Nanoparticles in Rats - a Pilot Study

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ABSTRACT: Superparamagnetic iron oxide nanoparticles are primarily utilized for different biomedical applications such as magnetic resonance imaging (MRI), hyperthermia, cancer treatment, targeted delivery of drugs or genes and biosensors. Nanoparticles are interesting due to their unique proprieties together with minor side effects. It is essential to determine the blood clearance of superparamagnetic nanoparticles (SPIONs) for *in vivo* biomedical applications, to ensure their optimum clinical use. The purpose of this study was to evaluate the elimination kinetic of citric-acid iron oxide nanoparticles in blood via intravenous injection in rats. Animals were blood sampled at different time intervals, ranging from 30 minutes to 24 hours after injection. The decay of SPIONs in blood was analyzed using electron paramagnetic resonance (EPR) technique. The results suggest that the injected iron oxide nanoparticles are rapidly cleared from circulation, with half-life of elimination process from the bloodstream about 14.06 minutes.

KEYWORDS: SPIONs, citric acid, EPR, clearance, animal model

Introduction

Lately, the development of nanoparticles and investigation of their properties have attracted the interest of physicists, scientists, researchers and chemists. Enthusiasm towards nanoparticles emerges from the way that the mechanical, electrical, attractive and optical properties are rather different from their mass properties and depend on the particle size. Rise of nanoparticles in the medical field brought huge promises for early diagnosis and treatment of diseases such as cancer [1].

Superparamagnetic iron oxide nanoparticles (SPION), the only metal oxide nanoparticles approved for clinical use, hold tremendous potential in an ample range of biomedical applications, for example, magnetic resonance imaging (MRI), focused on conveyance of medications or genes, tissue building, eradication of tumor through hyperthermia, tissue engineering, magnetic transfections and iron detection [2-7].

SPION are divided into three main categories depending on their hydrodynamic diameter: oral SPION, diameter between 300 nm to 3.5 μm; standard SPION (SSPIO), diameter between 50-150 nm; ultra small SPION (USPIO) with diameter less than 50 nm [8]. Ideal SPION for intravenous administration have a diameter

between 10-100 nm, while particles larger than 200 nm and respectively below 10 nm are sequestered by the spleen or excreted by renal clearance [8]. For diagnostic imaging or drug therapy, a large range of functional groups like peptides, antibodies or small molecules to target tumors, can be connected to their surface [9,10].

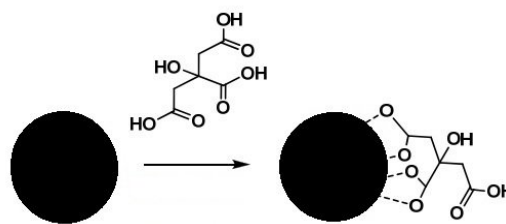


Fig.1. Surface modification of SPIONs with citric acid.

Among various small molecules, citric acid ($C_6H_8O_7$), a biocompatible short-chained tricarboxylic acid, has been extensively used for the preparation of aqueous stable iron oxide nanoparticles for biomedical applications [11-18]. Accordingly, citric acid can be adsorbed onto the surface of the iron oxide nanoparticles by coordinating via one or two of the carboxylate functionalities, leaving at least one carboxylic acid group exposed, as shown in Fig. 1, making the nanoparticle surface hydrophilic,

preventing particle agglomeration and providing functional groups to be used for further surface derivation [19] (Fig.1).

Citric acid has been employed commercially as coating surfactant of iron oxide nanoparticles, as the case of the MRI contrast agent VSOP C184 [12]. Citrate covered iron oxide nanoparticles have additionally been broadly concentrated on for their applications in medication delivery, focused on cell imaging, hyperthermia, and biodetection [11,13-18].

The iron from the nanoparticles enters the body's common iron metabolic pathway, is sequestered in the iron stockpiling protein ferritin and in the end is transformed in the hemoglobin in red blood cells [19].

The aim of the present work is to investigate the blood clearance of biocompatible Fe_3O_4 nanoparticles covered with citric acid, after intravenous administration to Sprague Dawley rats. In this study, electron paramagnetic resonance (EPR) technique is used to determine the iron content found in rat blood [20].

Materials and methods

Ferrofluid

The biocompatible ferrofluid consisted of a suspension of 14 mg/ml citric acid coated Fe_3O_4 superparamagnetic nanoparticles in water. Magnetite particles were obtained at the Institute

of Macromolecular Chemistry "Petru Poni" (Iasi, Romania) by co-precipitation reaction of iron salts ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$) in the presence of ammonia solution and were coated with citric acid. The hydrodynamic diameters are between 20–150 nm.

Experimental procedure using animals

Ten SD (Sprague Dawley) male rats with an average body weight of 400 g and ninety days old were used based on the written approval of the Ethics Committee of the University of Medicine and Pharmacy Craiova (UMFCV), Romania, and according to the European Legislation regarding animal rights, Directive 2010/63/EU. All animals were maintained in special locations at the animal facility of the UMFCV, with a controlled temperature of 20-22 degrees Celsius, in separate cages and subjected to fasting and liquids for 24 hours, respectively 6 hours, prior the procedure.

Animals were weighted, and were anesthetized with an intraperitoneal injection of Ketamine 100 mg/kg body (MSD Animal Health, Germany) and Xylazine 10 mg/kg body (Bioveta A.S., Czech Republic). After 15 min, they had their cervical region tricotomized, and through a little cut in the skin, the subcutaneous plane was exposed and debrided until the jugular vein was visible.

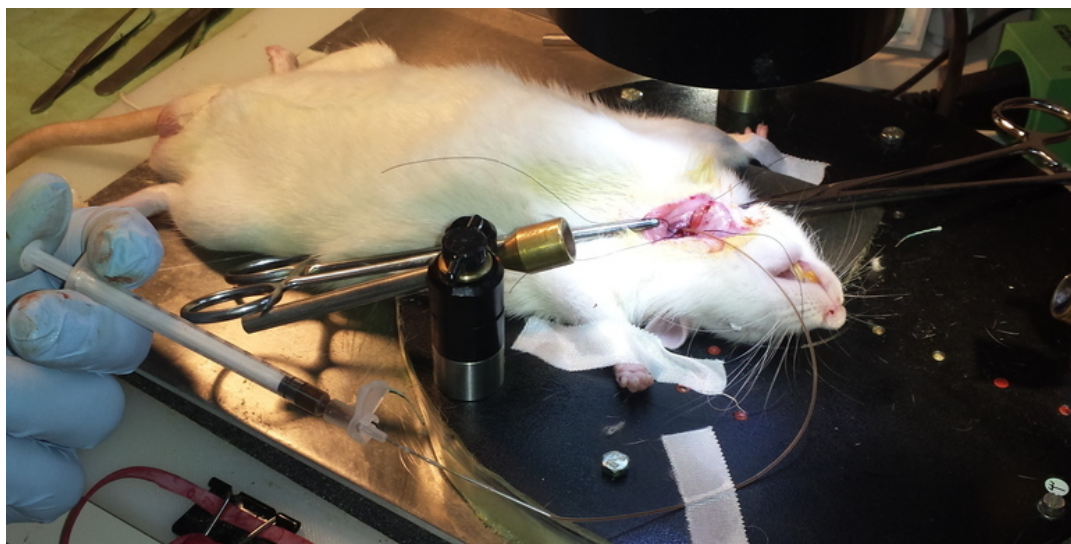


Fig.2. SPIONs being injected via a microcatheter inserted into the jugular vein of the rat

Next, they were injected in the jugular vein with a volume $V(\mu\text{L})$ equivalent to concentration of 15 μmol of Fe/kg of biocompatible ferrofluid, composed of citric acid coated Fe_3O_4 superparamagnetic

nanoparticles, diluted in an isotonic glycolized solution, to a final volume of 0,5 mL (Fig. 2). Previously, ferrofluid was dispersed with an ultrasonic bath, to prevent aggregation.

$$V(\mu L) = \frac{\text{rat body weight(grams)}}{2}$$

Following each period of 0, 30, 90, 150, and 240 minutes respectively one day, blood samples were collected. The control samples were collected from two rats who did not receive ferrofluid.

The blood was lyophilized in order to be homogenized for the EPR analysis. To obtain a material without humidity the lyophilization process spanned a period of 24 hours.

Results

Ferrofluid blood elimination kinetics was determined using electron paramagnetic resonance. The EPR line intensity at $g=2.1$ was found to be proportional to the concentration of SPIONs and optimum temperature for spectra acquisition is the room temperature ($T=298$ K) [20]. EPR spectra were recorded within the time set in the above protocol and are described in Fig. 3.

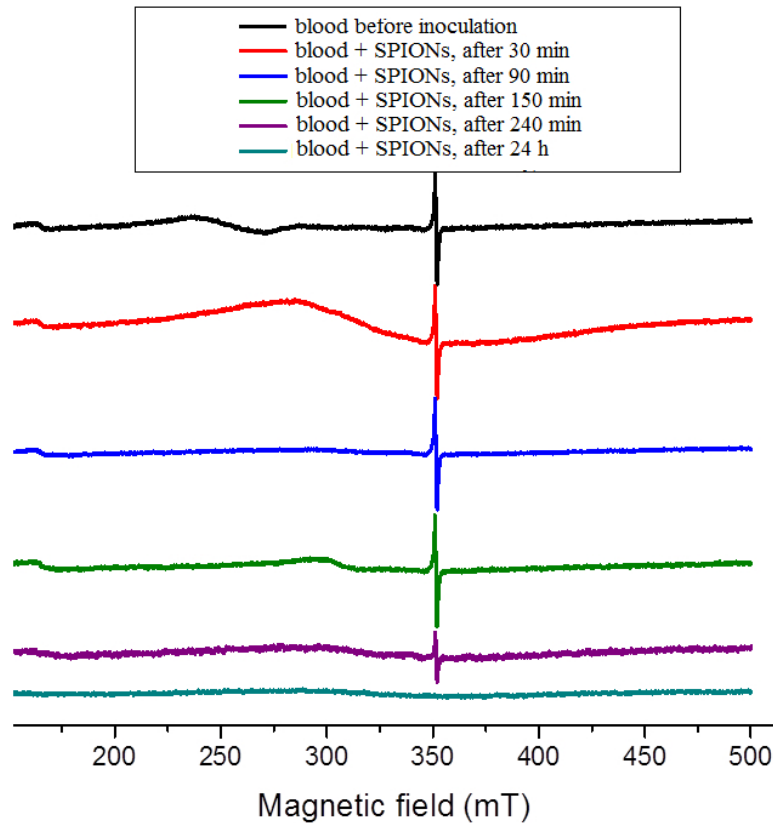


Fig.3. EPR spectra taken from the blood samples collected from the animals after administration of the ferrofluid

The time decay of the concentration of the coated iron oxide nanoparticles in blood it is shown in Fig. 4. It can be noticed that the injected ferrofluid is cleared rapidly from the bloodstream. The dashed line represents the fit of the data using a first order exponential decay.

The time dependence of nanoparticle concentration $C(t)$ was determined using the next equation [21-23]:

$$C(t) = C_0^B e^{-\frac{t}{\tau_B}} \quad (1)$$

- where C_0^B is the concentration of nanoparticles at time $t=0$ and τ_B is the rate constant of the iron oxide nanoparticles elimination from blood.

The best fitting of the blood data using Eq. (1) led to the parameter values $C_0^B = 5,909 \times 10^{12}$ particles/mm³ and $\tau_B = 20,29$ min with a correction factor equal to 0,177. The half-life associated with the nanoparticles elimination process in the blood circulation is given by $t_{1/2} = \tau_B \ln 2 = 14,06$ min.

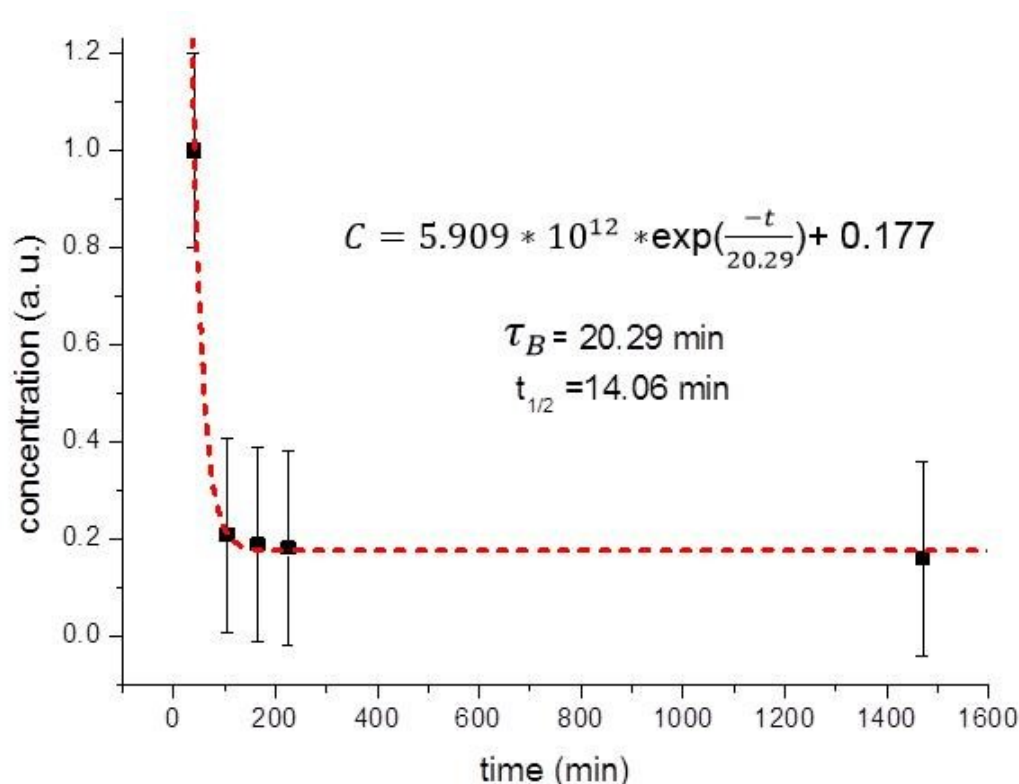


Fig.4. Blood concentration– time curve following intravenous administration of the ferrofluid. Dashed line represents the best fit of the experimental data with OriginPro v.8.6

Conclusions and future perspectives

After the EPR analysis it was noticed a decay kinetic of first order in the superparamagnetic nanoparticles concentration in the blood flow, with half-time of 14,06 min and the rate constant of the iron oxide nanoparticles elimination from blood of 20,29 min. The reduced circulation time in the bloodstream is due to nanoparticles uptake by macrophages [24]. A future objective of this research is to study the biodistribution of SPIONs in rat organs in order to observe the optimum moment when the maximum concentration is reached and henceforth to enhance the efficiency of our SPIONs for targeted delivery of drugs, imaging and cancer treatment.

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