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Influence of the separation procedure on the properties of magnetic nanoparticles: Gaining *in vitro* stability and T_1–T_2 magnetic resonance imaging performance

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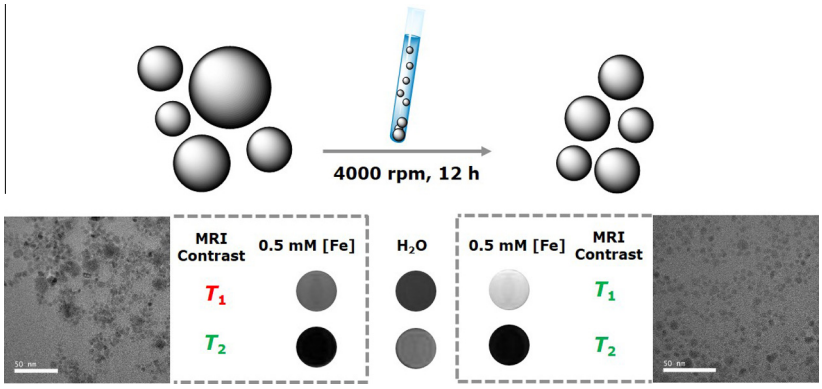
GRAPHICAL ABSTRACT



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ABSTRACT

Ultrasmall superparamagnetic iron oxide nanoparticles (USPIOs) coated with polyacrylic acid (PAA) were synthesized by a hydrothermal method in gram-scale quantity and extensively characterized. Only the nanoparticles subjected to an additional centrifugation step showed narrow size distribution, high polymeric coverage, and ideal superparamagnetism. In addition to improved physico-chemical properties, these nanoparticles feature high stability *in vitro* as well as dual T_1–T_2 performance as contrast agents

Abbreviations: aq., aqueous; BCA, bicinchoninic acid; BSA, bovine serum albumin; CA, contrast agent; Cy3-NH_2, cyanine3 amine; D_h, hydrodynamic diameter; D_{hkl}, crystallite size; DLS, dynamic light scattering; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; EDX, energy-dispersive X-ray; FOV, field of view; FSE, fast spin echo; FT-IR, Fourier transform infrared spectroscopy; h, hours; HAADF-STEM, high-angle annular dark-field scanning transmission electron microscopy; H_c, coercive force; ICP-OES, inductively coupled plasma optical emission spectroscopy; IMDM, Iscove's Modified Dulbecco's Medium; LDH, lactate dehydrogenase; M_s, saturation magnetization; MRI, magnetic resonance imaging; MW, molecular weight; **NP-ac**, nanoparticles after centrifugation; **NP-bc**, nanoparticles before centrifugation; NP, nanoparticle; PAA, polyacrylic acid; PB, phosphate buffer; PI, polydispersity index; PTFE, poly(tetrafluoroethylene); XRD, powder X-ray diffraction; *r*, relaxivity; rMSCs, rat mesenchymal stem cells; rpm, revolutions per minute; RSD, relative standard deviation; RT, room temperature; STEM, scanning transmission electron microscopy; *t*, time; TEM, transmission electron microscopy; TGA, thermogravimetric analysis; USPIOs, ultrasmall superparamagnetic iron oxide nanoparticles; VSM, vibrating sample magnetometry.

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(CAs) for magnetic resonance imaging (MRI), highlighting the importance of the additional separation step in obtaining material with the desired properties.

1. Introduction

USPIOs, with a mean hydrodynamic diameter below 50 nm, possess characteristics such as biocompatibility, long plasma half-life, and interesting magnetic properties, which make them suitable for a wide range of biomedical applications in both therapy and diagnosis [1]. MRI is one of the most used techniques in the medical field for the diagnosis of diverse diseases due to its high spatial resolution, rapid acquisition times, and the absence of exposure to ionizing radiation. However, CAs are frequently employed to distinguish between adjacent tissues, for example to enhance the visualization of tumor morphology or coronary angiography.

CAs affect the relaxation time of water protons' nuclei by two different processes: the longitudinal relaxation or T_1, where the contrast obtained is bright or positive, and transversal relaxation or T_2, with dark or negative contrast. Commonly, CAs are helpful for the reduction of either T_1 or T_2, e.g. gadolinium chelates function as T_1 and iron oxide nanoparticles (NPs) as T_2 CAs. Dual T_1–T_2 CAs would help to distinguish interferences, such as hemorrhagic regions, bond calcification, metal deposits, and susceptibility artifacts [2], leading to a more accurate and early diagnosis [3]. Additionally, dual behavior of a single CA platform within the same technique simplifies the acquisition due to identical penetration depths and time scale in both imaging modes. Consequently, dual T_1–T_2 systems have attracted attention in recent years, and complex nanostructures from coupled materials have emerged in order to fulfil both T_2 and T_1 requirements, for example iron oxide NPs attached to gadolinium compounds [4–8]. Despite the success of these structures in possessing attractive MRI properties, questions remain that need to be addressed before the clinical application of dual CAs can become a reality [9]. Some of such concerns are the requirement of facile synthesis procedures to obtain crystalline material on a sufficient scale with the desired magnetic properties. Additionally, interactions between NPs and biomolecules should be addressed to assess the suitability of the nanomaterial for biomedical applications [10,11]. The chemical stability and the behavior of NPs in a biological environment are key factors in biodistribution, toxicity, and their eventual efficiency as imaging probes [12].

Herein, we report on the straightforward gram-scale synthesis of water-dispersed USPIOs. A separation procedure was found to have a great influence on size distribution, polymeric coverage rate, and coercive force of the NPs. Only the NPs with smaller hydrodynamic diameter, high organic loading and ideal superparamagnetism displayed high *in vitro* stability and excellent performance as a dual T_1–T_2 CA for MRI, all important features to enable this class of CAs to approach the clinic in the future.

2. Experimental

2.1. Chemicals

Agar, $FeCl_3{\cdot}6H_2O$, $ZnCl_2$, $MnCl_2{\cdot}4H_2O$, NH_4OH, polyacrylic acid sodium salt (MW 5100 Da), and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) were purchased from Sigma-Aldrich and used without further purification. Potassium bromide (99% for spectroscopy, IR grade) was purchased from Acros Organics. Cyanine3 amine (Cy3-NH_2) was purchased from Lumiprobe. Ultrapure water produced by Milli-Q Advantage A10 system (Millipore). Bicinchoninic acid (BCA) protein assay was purchased from Life Technologies. Rat mesenchymal stem cells (rMSCs) (Cultrex, Trevigen, Gaithersburg, MD, USA) were cultured in IMDM (78%), fetal bovine serum (10%), horse serum (10%), penicillin-streptomycin (1%) (Gibco, Invitrogen, Paisley, UK), and amphotericin B (1%) (Sigma-Aldrich, St. Louis, MO, USA).

2.2. USPIOs synthesis procedure

NPs were synthesized using a modification of a previously reported hydrothermal method [13]. Briefly, 14 mmol of $FeCl_3{\cdot}6H_2O$, 5.6 mmol of $MnCl_2{\cdot}4H_2O$, and 1.4 mmol of $ZnCl_2$ were dissolved in 10 mL of water (deoxygenated via bubbling N_2 through the solution for 30 min prior to use) in a 40 mL poly(tetrafluoroethylene) (PTFE) vessel. Next, 15 mL of aq. 25–30% NH_4OH were added, and subsequently 0.4 mmol of poly(acrylic acid) sodium salt in 5 mL of water were rapidly added to the reaction mixture. The PTFE vessel with the resultant black suspension was capped and placed into a stainless steel autoclave. The autoclave was sealed and kept at 200 °C for 24 h under autogenous pressure. NPs were isolated using a magnet, the solution was removed, and NPs were redispersed in 200 mL of water and isolated magnetically again. Redispersion was repeated twice in total. The resultant NPs were centrifuged at 3000 rpm for 10 min, the supernatant was kept and stored to yield sample **NP-bc** (1.8 g). **NP-bc** was subjected to an additional centrifugation of 4000 rpm for 12 h and the supernatant was kept to yield sample **NP-ac** (1.0 g).

2.3. Characterization

Transmission electron microscopy (TEM), high-angle annular dark-field scanning transmission electron microscopy (HAADF-STEM) and energy-dispersive X-ray scanning transmission electron microscopy (STEM-EDX) studies were performed using Titan ChemiSTEM (FEI, 0.08 nm STEM resolution) electron microscope, operated at 200 kV and equipped with a Super-X detector. The samples were prepared by dropping 10 μL of a diluted dispersion of the nanoparticles onto a Cu-grid coated by ultrathin carbon film on lacey carbon support film followed by evaporation of the solvent in vacuum at room temperature.

Dynamic light scattering (DLS) experiments were carried out in a SZ-100 nanoparticle analyzer (Horiba) at 173° detection angle. Results were represented in the histograms as volume scattered light. Hydrodynamic diameter and standard deviation of both samples were calculated as an average of 5 successive measurements.

Thermogravimetric analysis (TGA) was performed in a TGA/DSC 1 STARe system, Mettler-Toledo, fitted with OmniStar GSD320 gas analysis system (Pfeiffer Vacuum), with 10 K/min gradient from 30 to 900 °C under 30 mL/min of Ar flow.