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A method for synthesis and functionalization of ultrasmall superparamagnetic covalent carriers based on maghemite and dextran

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Abstract

A new generation of susceptibility contrast agents for MRI and based on maghemite cores covalently bonded to dextran stabilizing macromolecules was investigated. The multistep preparation of these versatile ultrasmall superparamagnetic iron oxides (VUSPIO) consisted of colloidal maghemite synthesis, surface modification by aminopropylsilane groups, and coupling of partially oxidized dextran via Schiff's bases and secondary amine bonds. The dextran corona might be easily derivatized, e.g. by PEGylation.

Keywords: Ferrofluid; Infrared spectroscopy; MRI; Contrast agent; PEGylation; Silanation; Transmission electron microscopy; Zeta potential; Photon correlation spectroscopy; USPIO; VUSPIO; 3-Aminopropyltrimethoxysilane; α-Amino poly(ethylene glycol); α,ω-Diamino telechelic poly(ethylene glycol); Dextran; Maghemite

1. Introduction

Magnetic iron oxide nanoparticles have attracted attention in particular because of their usefulness as contrast agents for magnetic resonance imaging (MRI) [1,2]. The superparamagnetic behaviour of these subdomain magnetic cores (3–10 nm) is similar to that of paramagnetic substances, in that they lose their magnetization when the magnetic field is removed, but differs by the value of the magnetic moment which is markedly higher. Therefore their relaxivities are much higher than those of the widely-used Gd-chelates. In most situations, they are used for their significant capacity to produce predominantly T_2 relaxation effects, which result in signal reduction on T_2-weighted images. They are also called susceptibility agents or (U)SPIO for (Ultrasmall) SuperParamagnetic Iron Oxide.

For intravenous administration, they are generally synthesized in a one-step process by alkaline coprecipitation of iron (II) and iron (III)

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precursors in aqueous solutions of hydrophilic macromolecules, such as dextran [3], followed by chromatographic separation for narrowing the size polydispersity. The dextran macromolecules serve to limit the magnetic core growth during the synthesis and to stabilize via sterical repulsions the nanoparticle dispersion in water (and later in physiological medium). These colloidal contrast agents would be more realistically described as several magnetic cores, more or less aggregated, embedded in the dextran corona, which are sometimes cross-linked in a second step for enhancing the mechanical entrapment of the inorganic cores [4].

Two different classes of iron oxides are currently clinically approved or in phase-III trials. Because of their large overall hydrodynamic volume (over 40 nm in diameter), SPIO agents are efficiently accumulated in the organs of the Mononuclear Phagocyte System (MPS): ca. 80–90% of the injected dose in liver and 5–8% in the spleen with plasma half-life less than 10 min. Therefore, SPIO decrease liver and spleen signals and allow diagnosing malignant tumours or metastases in these organs. Thanks to their smaller size and the hydrophilicity of their dextran corona, USPIO act as stealth particles. Their plasma half-life is more than 2 h [5] and therefore they remain in the blood long enough to act as blood-pool agents (MR angiography) [6] or to be accumulated in the lymph nodes (MR lymphography) [7]. This size-dependent distribution in tissues (passive targeting) is a current limitation for the diagnosis of pathologies in any other organ. For instance, imaging specific tissues would need contrast agents as stealth as USPIO with an extra requirement, as their surface labelling with ligands that specifically bind to surface epitopes or receptors on the target sites (active targeting).

Only very few attempts for conjugating biomolecules to the dextran coating were reported using electrostatic or chelating interactions [8] or reduction of Schiff's bases formed from dextran activated by oxidation [9,10]. Indeed, the interactions between magnetic cores and dextran macromolecules, e.g. Van der Waals and hydrogen interactions [11], are too weak and generally prevent any efficient derivatization of dextran corona without macromolecule depletion [12]. Recently, for a similar purpose, attempts were made for preparing USPIO from dextran whose terminal sugar had been previously reduced [13].

The aim of this work is to investigate a new generation of USPIO, based on maghemite cores covalently bonded to their dextran corona through the use of silane coupling agents. Because of their potential ability to be tailor-derivatized, they are called VUSPIO for Versatile USPIO [14]. Their multistep synthesis and their characterization by IR spectroscopy, transmission electron microscopy (TEM), zeta potential measurement and photon correlation spectroscopy (PCS) are described and discussed in this article.

2. Experimental section

2.1. Materials and purification methods

Iron (III) chloride hexahydrate (98% +), iron (II) chloride tetrahydrate (99% +), iron (III) nitrate nonahydrate (99% +), sodium borohydride (99%) sodium metaperiodate (99%) and 3-aminopropyltrimethoxysilane APS (97%) were purchased from Aldrich. Dextran 70 kD (from leuconostoc mesenteroides B512) was obtained from Sigma. Monoamino- and diaminotelechelic poly(ethylene glycol), $M_W \sim 2000$ g/mol, were purchased from Huntsman, Salt Lake City. All other reagents were of analytical grade. In all experiments, water was previously deionized ($R < 10\,M\Omega$) by reverse osmosis (PM600, USF, France). Tangential ultrafiltration system consists of a perilstatic pump (Millipore N80EL005), silicon tubing and a poly(ethersulfone) membrane (Prep/Scale^{TM}, cut-off 100,000 g/mol). Dialysis tubing (cellulose, cut off 12,400 g/mol) was obtained from Sigma.

2.2. Maghemite ferrofluid preparation

Maghemite-based cationic ferrofluids were prepared according to a method previously described [15]. Briefly, the cores were synthesized by alkaline coprecipitation of iron (II) and iron (III) precursors in aqueous solution with an excess of