PREPARATION OF FLUORESCENT, FUNCTIONALIZED, SILICA-COATED MAGNETIC NANOPARTICLES MARKED WITH MONOCLONAL ANTIBODIES

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Magnetic nanoparticles have been intensively studied for their potential applications in biomedicine. These magnetic nanoparticles are tested for in-vivo use, either in diagnosis as contrast agents for NMR imaging, or in therapy, for targeted drug delivery and for the treatment of cancer cells with magnetic hyperthermia. There are also many applications where these magnetic nanoparticles are used in-vitro, for example, in various types of bioseparations, cell sorting and purification, enzyme immobilization, and many others [1,2].
However, the properties of the magnetic nanoparticles need to be adapted for a specific application. The most important properties in the biomedical application of nanoparticles are their magnetic properties, size, biocompatibility and capacity for the selective binding of different molecules onto their surfaces [3]. For intravenous and intra-arterial applications, which are the most common for drug administration, magnetic nanoparticles of a suitable size are applied in the form of a stable aqueous suspension. For the preparation of a stable suspension, nanoparticles are expected to exhibit superparamagnetic properties in order to avoid inter-particle magnetic interactions that could cause agglomeration. Moreover, the nanoparticles should not be recognized by the immune system and eliminated from the blood. To ensure long circulation times, the nanoparticles should be coated with biocompatible molecules, such us polyethylene glycol. For the selective binding of different molecules (drugs, antibodies, etc.), the nanoparticles' surface should contain specific functional groups, i.e., they have to be functionalized. Usually, the nanoparticles for biomedical applications are composed of a magnetic core and an organic or inorganic shell, which provides biocompatibility and functionalization [4].

Superparamagnetic nanoparticles of maghemite (γ-Fe_2O_3) are the most frequently used core materials [5]. They are believed to be nontoxic and were approved for in-vivo applications by the Food and Drug Administration (FDA). The surface of the maghemite core is relatively inert and usually does not allow strong covalent bonds with biological molecules. The reactivity of the maghemite nanoparticles can be improved by coating a thin layer of silica onto their surfaces. This rigid layer of silica provides reactive –OH surface groups for strong covalent binding. The silica layer should be continuous and homogeneous, but as thin as possible so as not to impair the magnetic properties.

In this work, the coating of maghemite nanoparticles with a thin layer of silica was investigated. The magnetic nanoparticles were synthesized with co-precipitation from aqueous solutions of Fe^{2+} and Fe^{3+} ions with a concentrated ammonia solution. The synthesized nanoparticles were dispersed in an aqueous medium using citric acid as a surfactant. A thin layer of silica was coated onto the nanoparticles using hydrolysis and the condensation of tetraethyl orthosilicate (TEOS) and the nucleation of the formed silica on their surfaces. The coatings were characterized with transmission electron microscopy and magnetization measurements, and the homogeneity of the layer was evaluated using leaching tests. The coated nanoparticles were leached in HCl. The iron oxide is very soluble in acid, whereas the silica is practically insoluble. Thus, the nanoparticles coated with the more homogeneous silica layer were dissolved to a lesser extent.

First, the influence of various experimental parameters on the homogeneity of the coating was evaluated. The optimization of the coating procedure enabled a decrease in the thickness of the layer, even to a monolayer of -Si-OH, without significantly impairing its homogeneity.

The reactivity of the silica-coated nanoparticles was tested by grafting (3-aminopropyl) triethoxysilane (APS) onto their surfaces. The surface concentration of APS was determined using conductometric titrations. Even when the nanoparticles' surfaces were only covered by a monolayer of Si-OH, the surface concentration of bonded APS reached values suggesting full surface coverage (4.5 molecules of APS / nm^2), whereas with uncoated nanoparticles a much lower surface concentration of APS (< 1 molecule of APS / nm^2) can be grafted.

The suitability of the APS-functionalized, silica-coated nanoparticles was tested for the subsequent bonding of different molecules. To enable tracking of the nanoparticles using optical microscopy, fluorescein isothiocyanate (FITC) was attached to the nanoparticles' surface. FITC binds to the terminal amino group of the APS with its isothiocyanate functional group.

Finally, monoclonal antibody (mAb) [6] was covalently bound to the fluorescently labelled nanoparticles using a water-soluble crosslinking agent for covalent binding between the amino group of the APS and the amino group of the mAb. Recognition of the epitope on cancer cells by the mAb-marked fluorescent nanoparticles was investigated using MCF-7 cells. In a co-culture of breast tumor MCF-7 cells and pro-monocytic U937 cells, mAb-marked nanoparticles specifically recognized and internalized tumour cells.

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