



Fluorescence labeling of colloidal core–shell particles with defined isoelectric points for in vitro studies

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ABSTRACT

In the light of in vitro nanotoxicological studies fluorescence labeling has become standard for particle localization within the cell environment. However, fluorescent labeling is also known to significantly alter the particle surface chemistry and therefore potentially affect the outcome of cell studies. Hence, fluorescent labeling is ideally carried out without changing, for example, the isoelectric point. A simple and straightforward method for obtaining fluorescently labeled spherical metal oxide particles with well-defined isoelectric points and a narrow size distribution is presented in this study. Spherical amorphous silica (SiO₂, 161 nm diameter) particles were used as the substrate material and were coated with silica, alumina (Al₂O₃), titania (TiO₂), or zirconia (ZrO₂) using sol–gel chemistry. Fluorescent labeling was achieved by directly embedding rhodamine 6G dye in the coating matrix without affecting the isoelectric point of the metal oxide coatings. The coating quality was confirmed by high resolution transmission electron microscopy, energy filtered transmission electron microscopy and electrochemical characterization. The coatings were proven to be stable for at least 240 h under different pH conditions. The well-defined fluorescent particles can be directly used for biomedical investigations, e.g. elucidation of particle–cell interactions in vitro.

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1. Introduction

There is growing use of engineered nanoparticles in a wide-spread portfolio of products and processes [1,2], raising questions of risks such as toxicity and their general behavior in biological systems [3–10].

Ceramic nanoparticles of SiO₂, Al₂O₃, TiO₂, or ZrO₂ are used in products such as filling materials, coating materials, paints, biological sensors, chromatographic systems, filtration membranes, cosmetics, drug carriers, catalysts, photonic crystals, and toothpaste [8,11]. Some of these applications include the risk of nanoparticle release into the biological environment. Specific example of release is abrasive wear of orthopedic implants, e.g. artificial hip joints, in which Al₂O₃ and ZrO₂ are typically used [12–14]. The effects of these oxide nanoparticles on biological systems are still unclear and have not yet been elucidated in depth [9].

The general behavior of the particles in biological systems has been suggested to be influenced by particle size and type of material [6,15–17] and, therefore, an independent investigation of these two parameters is considered to be of use. While keeping their size and shape, the different biological impact of these nanoparticles can clearly be ascribed to the material and its surface properties.

Nanoparticle localization within biological systems is also of crucial importance. Fluorescent labeling of nanoparticles has been used to visualize them, or at least their agglomerates, within a cell environment and in vivo [18–21]. Adhesion to or chemical binding of a fluorescent dye to the nanoparticle surface is a suitable method to image them, but can alter the nanoparticles surface chemistry. This can be avoided by incorporating the fluorescent dye into the nanoparticle, preserving the surface material properties with no influence of the dye, as shown for silica [22,23]. However, fluorescent labeling of other oxide surfaces with controlled surface chemistries is lacking and has not yet been discussed in the literature.

In our approach we used amorphous silica nanoparticles as the substrate material and introduced different oxide coatings to the surface by sol–gel processing, illustrated in Fig. 1. The coatings were silica, alumina, titania and zirconia. During the coating process rhodamine 6G (R6G), which has been reported to be stabilized by inclusion in an oxide matrix [24], was used as the fluorescent dye.

2. Experimental section

2.1. Materials

Silica particles with a narrow size distribution (Angström-sphere™, Fibre Optics Center Inc., New Bedford, MA, purity

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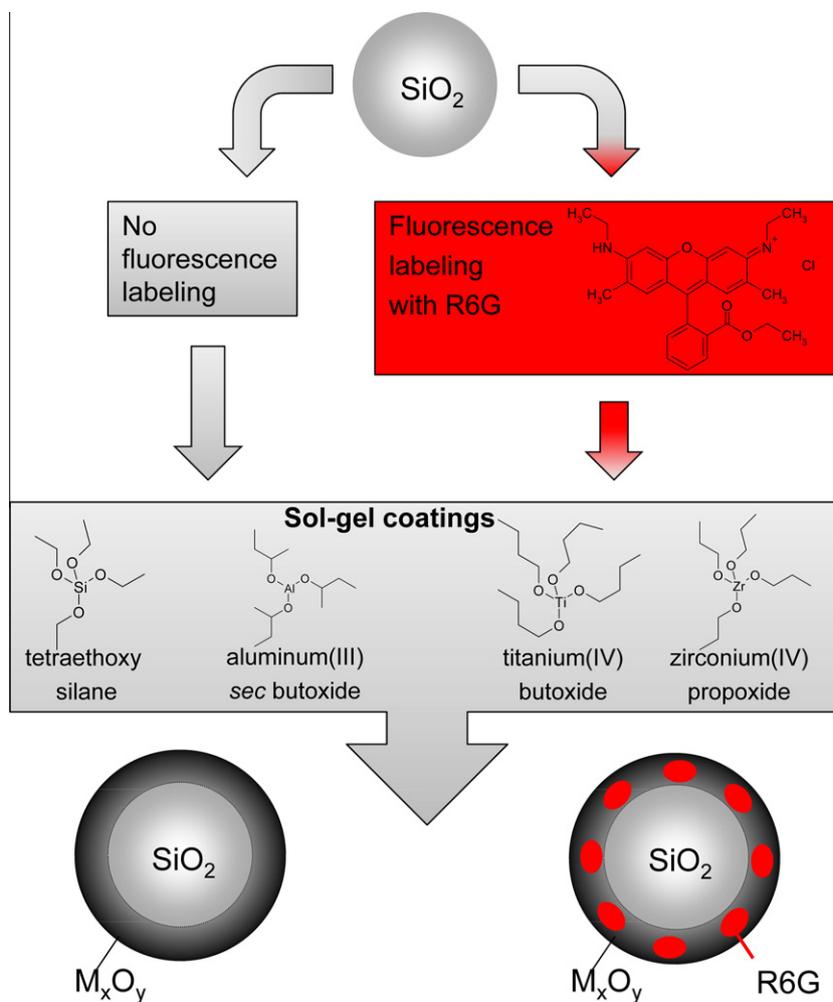


Fig. 1. Schematic of the sol-gel coating process (M_xO_y : SiO_2 , Al_2O_3 , TiO_2 , or ZrO_2).

>99.9%, lot No. 090320-01), 161 nm in diameter were used as the nanoparticle substrate. The applied precursors for the metal oxide coatings were aluminum(III) sec butoxide (purity 97%, batch No. MKAA3853, Sigma Aldrich, Germany), titanium(IV) butoxide (purity 97%, lot No. S43274-398, Sigma Aldrich), zirconium(IV) propoxide (70% solution in propan-1-ol, lot No. S60691-408, Sigma Aldrich), and tetraethoxysilane (TEOS) (purity 98%, lot No. STBB6034, Sigma Aldrich), listed in Table 1. 0.1–2 M hydrochloric acid, 1 M potassium hydroxide solution, 25% aqueous ammonia solution, and rhodamine 6G (R6G) (dye content ~95%, lot No. 32909175) were also purchased from Sigma Aldrich (Germany).

High purity water (conductivity $<0.4 \mu S cm^{-1}$) supplied by a Synergy[®] Ultrapure Water System (Millipore Corp., Billerica, MA) was used for all studies. Ethanol (purity 96%, Sigma Aldrich, Germany) was dried using a 0.4 nm molecular sieve from Carl Roth (Karlsruhe, Germany).

To prepare well-dispersed suspensions of silica nanoparticles the powder was added to the respective solvent (water or ethanol), stirred and sonicated using an ultrasound horn (Sonifier 450, Branson, Germany) at an ultrasound frequency of 20 kHz, with two ultrasound pulses of $150 W s^{-1}$ for 10 min.

2.2. Fluorescent labeling

The fluorescent labeling of nanoparticles was performed prior to coating. The substrate silica nanoparticles were suspended in

Table 1

Overview of the nanoparticles and their short notation used in this paper and the sol-gel precursor applied during coating.

Particle type	Short notation (core//shell)	Sol-gel-precursor (formula and name)
Silica nanoparticle	$SiO_2//$	–
Silica nanoparticle, silica coated	$SiO_2//SiO_2$	$Si(OCH_2CH_3)_4$ tetraethoxysilane
Silica nanoparticle, alumina coated	$SiO_2//Al_2O_3$	$Al(OCH_2CH_2CH_3)_3$ aluminum(III) sec butoxide
Silica nanoparticle, titania coated	$SiO_2//TiO_2$	$Ti(OCH_2CH_2CH_2CH_3)_4$ titanium(IV) butoxide
Silica nanoparticle, zirconia coated	$SiO_2//ZrO_2$	$Zr(OCH_2CH_2CH_2CH_3)_4$ zirconium(IV) propoxide

water or ethanol, depending on the coating. 15 mg of rhodamine 6G were added to the suspension and stirred for 24 h. The sol-gel process was started by adding the appropriate precursor

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