Synthesis of Novel Silica Nanomaterials for Biomedical and Metrological Applications

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1. Indroduction

Silicon dioxide, also known as silica, comprises a large class of products with the chemical formula SiO_2. Its application ranges from structural materials to water filtration through components used in the food and cosmetic industries.

Over the last two decades with the emergence of nanotechnology silica has received significant attention. Nanotechnology is the still evolving diverse and interdisciplinary branch of science presenting original solutions to current requirements. It is dealing with nanosized or nanometrically organized materials owning attractive properties compared to conventional ones. The new requirements resulted in countless varieties of silica nanomaterials, by using "bottom up" approaches, where molecular building blocks connecting in a stepwise manner.

The new applications such as drug delivery however are bringing forth very strict requirements of porosity, specific surface area, colloidal stability and dispersity, thus the optimization of such synthesis approaches is still a challenging issue requiring careful consideration of reaction parameters.

The objective of my work was to synthesize and characterize novel silica based nanomaterials for biomedical and metrological applications. For the synthesis I used wet-chemical approaches, in which the preparation of the nanomaterials took place in solution under mild conditions. During the preparation and surface modification much efforts were devoted to maintain the colloidal stability and the high monodispersity of the silica particles.

2. Experimental part

2.1 Amino-functionalization of silica NPs

20 nm sized silica nanoparticles (NPs) were prepared by the Stöber method. The native silica was amino-functionalized with 3-aminopropyl(diethoxy)methylsilane (APDEMS). The effect of acetic acid on amino-functionalized NPs' size distribution was investigated. The comparison of the different purification techniques for the removal of unreacted aminosilane molecules from the surface-modified silica sol was performed. The NPs were characterized comprehensively.

2.2 Manganese(II) chelating microporous silica NPs

Microporous silica NPs (MSNPs) with 84 nm mean particle diameter were synthesized by a modified surfactant assisted aqueous method. In order to remove the unreacted silica precursor the particles were dialyzed against 2M acetic acid-ethanol solution.

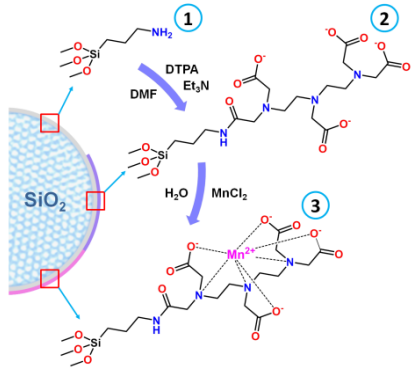

Figure 1. Schematic illustration of surface functionalization steps.

The particles were amino-functionalized, reacted with diethylenetriaminepentaacetic (DTPA) dianhydride and finally deposited with Mn^{2+} (**Figure 1**). Once characterized, the MRI properties of functionalized MSNPs were investigated both *in vitro* and *in vivo*.

2.3 Si-29 silica NPs

The preparation of ^{29}Si-TEOS ($^{29}Si(OEt)_4$) was carried out in a two-step synthesis as a modified combination of conventional methods described earlier in literature for isotopically non-enriched TEOS. First, the elementary silicon-29 was converted to its tetrachloride upon heating in chlorine gas, and then treated with abs. ethanol to give the silane ester. Three modifications over the traditional synthetic methods of $SiCl_4$ and TEOS was performed. One of them included the dilution of the volatile ($SiCl_4$; b.p. 57.65 °C) and moisture-sensitive $^{29}SiCl_4$ in an inert medium thus increasing the sample volume and decreasing the product loss due to evaporation. The second modification was the use of a multistep (+12 °C, -12 °C, -18 °C) cooling system in order to inhibit the volatilization of $^{29}SiCl4/CCl_4$ solution caused by gas stream and, simultaneously, prevent too much chlorine gas to get absorbed in the mixture (**Figure 2**). The third change was the use of triethylamine as a proton scavenger to neutralize residual hydrogen chloride dissolved in the mixture after ethanolysis. For the NP preparation, a basic amino acid catalysis route was used to get particles with narrow size distribution. After comprehensive characterization, preliminary experiments were performed by FFF/MALS/ICP-MS (field-flow fractionation coupled with multi-angle light scattering and inductively coupled plasma mass spectrometry) to investigate the applicability of the ^{29}Si-silica NPs as spikes for IDMS (isotope dilution mass spectrometry).

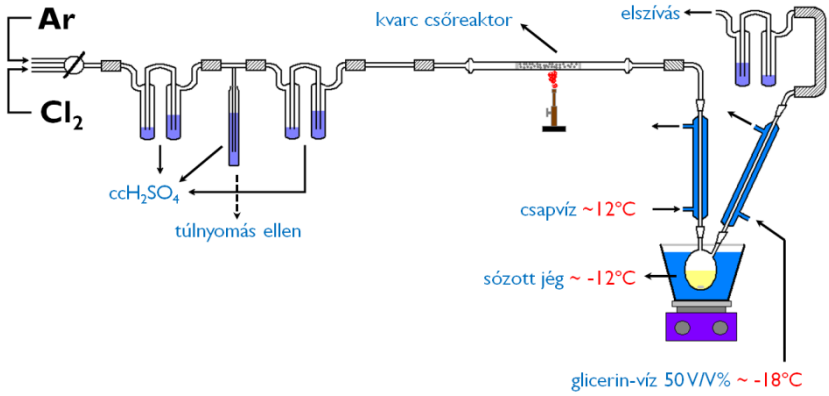

Figure 2. Apparatus scheme for the preparation of $^{29}SiCl_4$.

2.4 Organosilica hollow spheres

Organosilica hollow spheres (OSHSs) were prepared by the combination of a basic amino acid catalysis route with a hard template approach. In the aqueous/organic (water/cyclohexane) biphase procedure 1,2-bis(triethoxysilyl)ethane (BTEE) was used as organosilica precursor, L-arginine as base catalyst and different sized (nominal 200 nm, nominal 400 nm) silica nanospheres as template. The template silica was etched by using NaOH solution. Finally the OSHSs were characterized comprehensively.

3. Results

3.1 Amino-functionalization of silica NPs

I developed a new synthesis method for the preparation of 20 nm sized amino-functionalized silica NPs destined for biomedicinal use. TEM and SAXS measurements proved that polycondensation of the silane coupling agent and irreversible aggregation of the particles was successfully avoided at the chosen reaction parameters (**Figure 3**). The covalent binding of the aminopropyl groups was proved by FTIR and solid-state NMR investigations. It is an important fact that the silylation reaction making use of a divalent silane coupling agent has become controllable by the addition of small amount of acetic acid after appropriate reaction time (**Figure 4**). I demonstrated that centrifugation is not a convenient separation technique for such small NPs, while either ultrafiltration or dialysis preserve the dispersity of the sol sample even during solvent exchange to water (**Figure 4**).

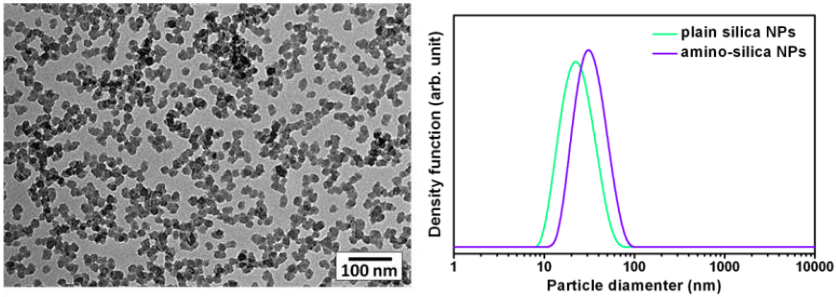

Figure 3. TEM analysis of APDEMS-functionalized silica NPs stabilized with acetic acid (left). DLS analysis of plain and amio-functionalized silica NPs in ethanol.

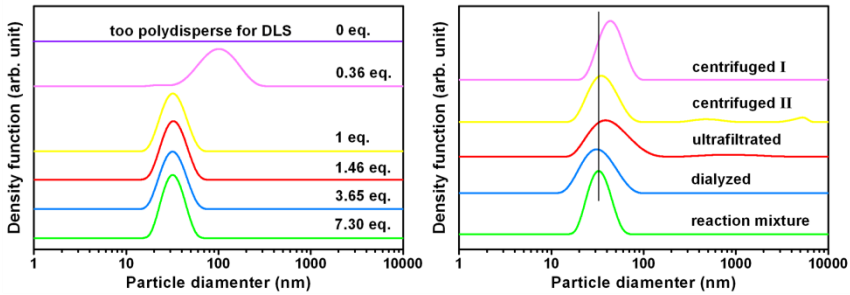

Figure 4. DLS size distribution function of the reaction mixture after addition of 0-7.3 molar equivalent of acetic acid (related to APDEMS) (left). Comparison of size distribution functions after various purification processes.

3.2 Manganese(II) chelating microporous silica NPs

I elaborated a new synthesis route for the preparation of manganese(II) chelating microporous silica NPs with the purpose of using them as liver-specific positive MRI contrast agent. The NPs were synthesized with enlarged porosity and specific surface area (**Figure 5**), and surface functionalized in three steps. The NPs exhibited excellent colloidal stability and preserved high monodispersity after each surface modification step (**Figure 5**). The surface modification was proved by FTIR and zeta potential measurements (**Figure 6**). The resulting MSNPs exhibited substantial MRI contrast enhancement both *in vitro* and *in vivo*, which suggests that this material is well suited for further MRI investigations (**Figure 6**).

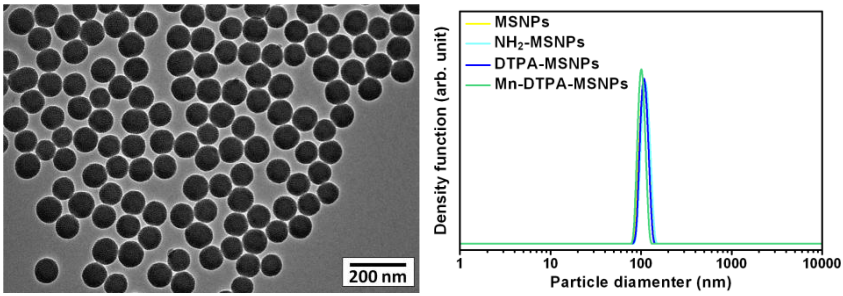

Figure 5. TEM (left) and DLS analysis of microporous silica NPs.

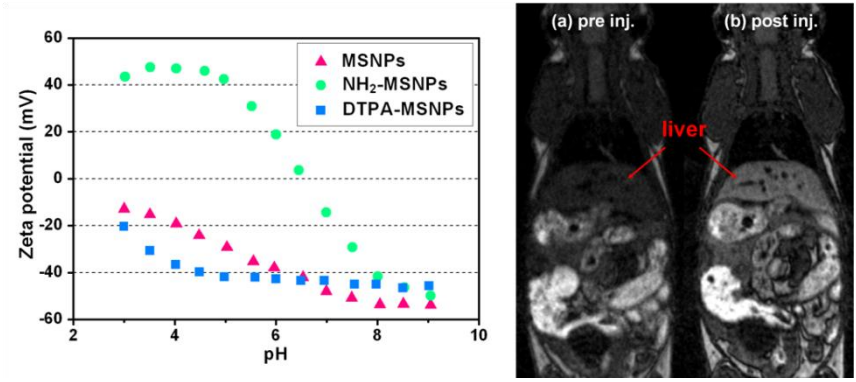

Figure 6. Zeta potential of plain, amino- and DTPA-functionalized MSNPs as a function of pH (left). Axial T1-weighted MR images of mouse before and 1 h after intravenous administration of Mn-DTPA-MSNPs.

3.3 Si-29 silica NPs

I developed a simple method for the synthesis of $^{29}SiCl_4$ and $^{29}Si(OEt)_4$ starting from elementary silicon-29. With the dilution of the volatile and moisture-sensitive $^{29}SiCl_4$ in an inert medium and by using a multistep cooling system the product loss due to evaporation was decreased (**Figure 2**). The synthesized $^{29}Si(OEt)_4$ was suitable for the preparation of silica NPs

by a basic amino acid catalysis route (**Figure 7**). The prepared NPs were highly monodisperse (**Figure 7**) and exhibited excellent colloidal stability. The preliminary FFF/MALS/ICP-MS experiments proved the applicability of the Si-29 silica NPs as spikes for IDMS (**Figure 8**).

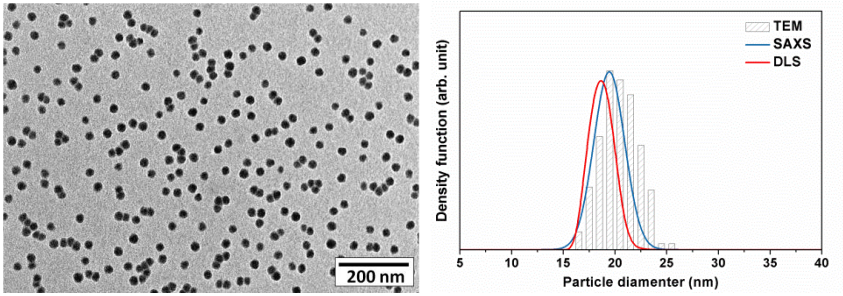

Figure 7. TEM analysis of ^{29}Si-silica NPs (left). Size distributions of ^{29}Si-silica NPs calculated from TEM, SAXS and DLS measurements.

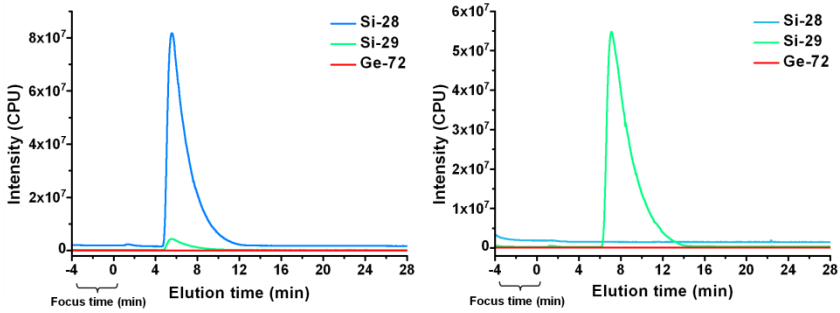

Figure 8. FFF/MALS/-ICP-MS analysis of natural (left) and ^{29}Si-silica NPs.

3.4 Organosilica hollow spheres

In summary, an optimized synthesis route was developed for the preparation of organosilica hollow spheres (OSHSs) (**Figure 9**). The use of cyclohexane ensured a very slow increase of the solution supersaturation, thus the uniformity of thickness of the organosilica layer improved

significantly. The OSHSs had narrow size distribution and exhibited excellent colloidal stability. The performed flow cytometry results demonstrated the applicability of OSHSs as reference beads for the size characterization of extracellular vesicles (**Figure 10**).

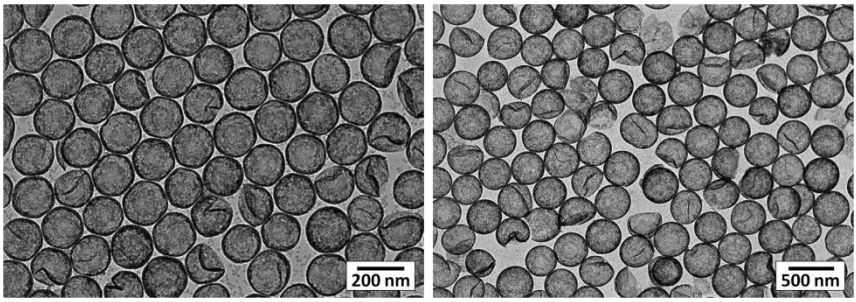

Figure 9. TEM analysis of OSHSs prepared by using nominal 200 (left) and 400 nm sized silica templates.

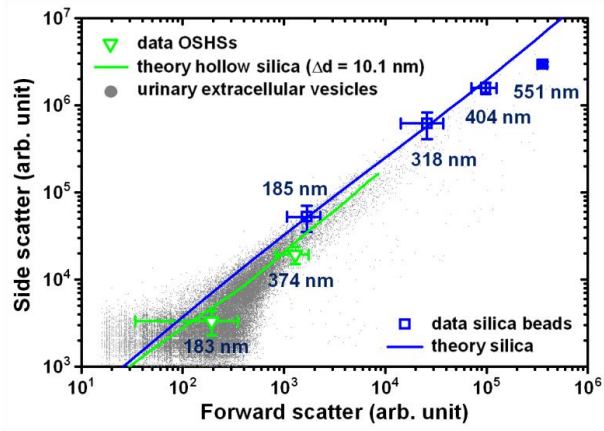

Figure 10. Side scatter versus forward scatter for silica beads (185, 318, 404 and 551 nm), nominal 200 and 400 nm sized OSHSs and vesicles from human urine.

Theses

1. I developed a new synthesis method for the preparation of amino-functionalized silica NPs. It was shown that the silylation reaction has become controllable by the addition of small amount of acetic acid after appropriate reaction time. I compared the different purification techniques for the removal of unreacted aminosilane molecules and demonstrated that dialysis is the most appropriate method. (Pálmai et al., 2013)

2. I elaborated a new two-step synthesis route for the preparation of manganese(II) chelating microporous silica NPs. The plain NPs were amino-functionalized and reacted with diethylenetriaminepentaacetic (DTPA) dianhydride. I performed the comprehensive characterization of the NPs. (Pálmai et al., 2017)

3. I developed a simple method for the synthesis of ^{29}Si-TEOS, which was suitable for the preparation of 20 nm sized Si-29 isotopically enriched silica NPs. After comprehensive characterization, I performed FFF/MALS/ICP-MS experiments and I proved the applicability of the Si-29 silica NPs as spikes for IDMS. (Pálmai et al., 2015)

4. I developed a new method for the preparation of organosilica hollow spheres by using BTEE as silica precursor, L-arginine as base catalyst and cyclohexane as co-solvent. After comprehensive characterization I proved the applicability of organosilica hollow spheres as reference beads for the size characterization of extracellular vesicles in flow cytometry measurements.

Publications

Papers

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