Water-soluble nanoparticles as an universal imaging tool

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ABSTRACT

Here we present a continuous flow approach for the phase transfer of hydrophobic nanoparticles (NPs) into aqueuous media. This concept is build up of two steps. The first step is an exchange of the stabilizing agent on the particle surface against a multidental binding short chain polymer. In the second step the modified NPs are encapsulated in micellar nanocapsules and transferred into an aqueous solution. The encapsulation is performed by using microfluidic components in a continuous flow phase transfer approach (CFPTA) to ensure best reproducibility and up-scaling capacity. Finally we crosslink the micelles shell to ensure a good separation of NPs from the surrounding media. This enhances the long term stability as well as decreases the toxicity of the capsule.

The opportunity of using block-copolymers with different end-groups gives us the possibility to functionalize our micelles with affinity molecules like antibodies.

Keywords: nanoparticles, phase transfer, water solubility, microfluidic, CANdots®

1 INTRODUCTION

To improve the early stage diagnostic of widespread diseases like cancer or diabetes highly sensitive and specific imaging tools are needed. Preliminary concepts often used organic dies functionalized with bioactive affinity molecules. One mayor drawback of this approach is caused by the low stability of these dies [1]. In contrast semiconducting inorganic nanoparticles (NPs) exhibit a strong fluorescence with low photo bleaching effects. Superparamagnetic NPs are allready used as MRI contrast agent and offers an additional read-out [2].

To profit from these advantages water soluble NPs are needed. Therefore two different synthesis strategies are used: on the one hand it is possible to synthesize the NPs directly in water, respectively in an aqueous solution, which can be used without further phase transfer steps. But this approach leads to poor crystalline NPs with non-uniform shapes and broad size distributions. Because of that the performance of these NPs as imaging tools is dramatically decreased. On the other hand it is possible to achieve high crystalline NPs with homogeneous shape and narrow size distribution by using high temperature syntheses in organic solvents. A hindrance of this approach is that these NPs are not soluble in aqueous solutions. For biomedical applications a phase transfer process is necessary after the synthesis [3, 4].

The solubility of NPs is not only determined by the inorganic particle itself but mostly by the surrounding shell of organic molecules. An exchange of these ligands causes a change in the solubility. The adjustment of the solubility is not the only requirement concerning the ligands. They should also protect the particles of uncontrolled aggregation and chemical reaction with the surrounding media, e.g. oxidation. In addition a ligand modification can introduce further functionalitiy to the NPs.

The critical point during a phase transfer process is the prevention of any uncontrolled changes in the properties of the marker.

2 RESULTS

The schematic application flow of our continuous flow phase transfer approach (CFPTA) is shown in figure 1. In the first step the NPs, which are coated with the ligands used during the synthesis, get mixed with a short chain polyisoprene functionalized with a multi amino group to bind strongly on the particle surface. Afterwards the precoated NPs get encapsulated with an amphiphilic poly(isoprene-*b*-ethylene oxide) block copolymer (PI-PEO). During this step, AIBN is coencapsulated so that the PI part of the shell can get crosslinked to the PI ligands on the particle surface. The final product can get autoclaved and the functional groups at the outer shell of the capsule are suitable for further coupling to affinity molecules like antibodies or peptides [5].

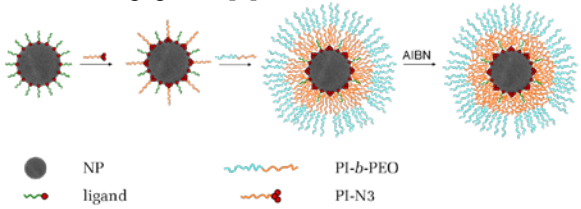

Figure 1: application flow of phase transfer

The encapsulation procedure can be carried out by injection of a solution containing the NPs and the polymer into water by hand or by rapid mixing of both solutions in a microfluidic mixing chip.

Figure 2 shows a SEM image of a nanocapsule loaded with a cluster of iron oxide nanoparticles manufactured using the CFPTA.

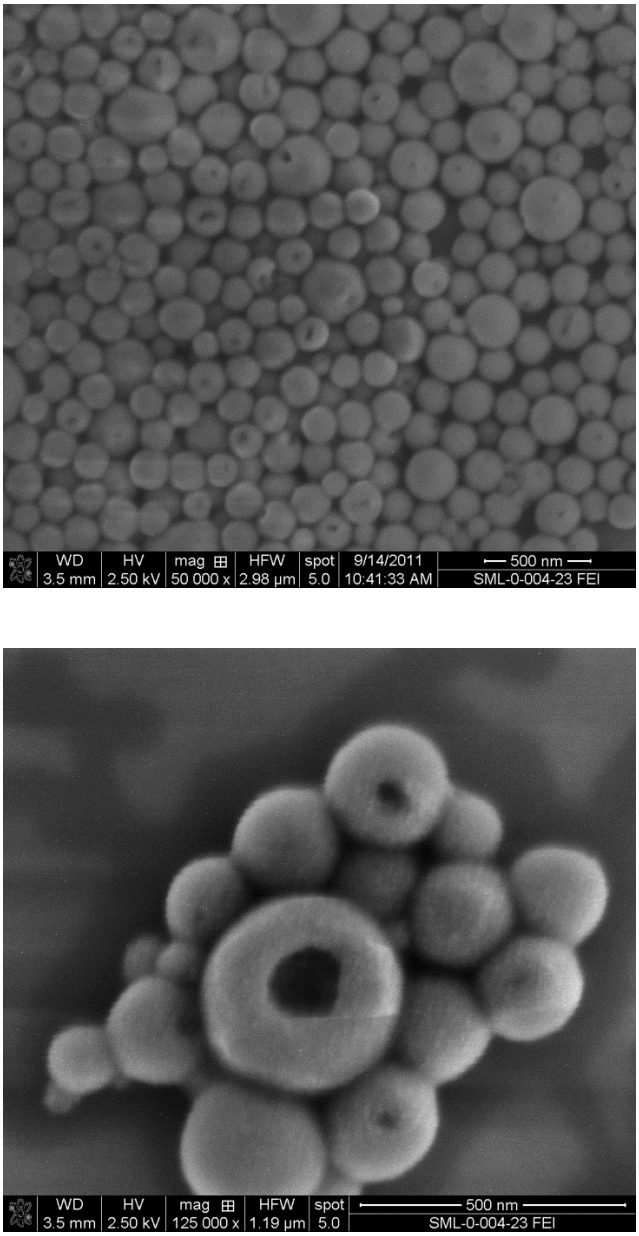

Figure 2: SEM images of PI-PEO nanocapsules filled with clusters of iron oxide nanoparticles. The clustering can be tuned using different settings during the CFPTA

2.1 Precoating

The precoating of the particles is needed to enhance their stability during the encapsulation procedure. Especially for fluorescent quantum dots the increased stability is needed to maintain their quantum yield. The ligand for the precoating is done by adding a short chain polyisoprene polymer to a diethylenetriamine. The resulting polymer (PI-N3) is mixed with the NP dispersion in an unpolar solvent like hexane. After the ligand exchange the particles get purified via centrifugation and dissolved in THF. The sucsessful ligand exchange can be seen in an increase of the hydrodynamic diameter from 8 to 20 nm measured with DLS.

2.2 Encapsulation by hand

To the solution of precoated particles AIBN and PI-PEO polymer are added. Using the standard encapsulation technique this solution is injected fast into an excess of water via a syringe. Because the THF mixes well with water, the sudden increase of polarity in the solution leeds to the formation of micelles with the PEO part facing towards the solution and the PI chain to the hydrophobic core. The nanoparticles get incorporated inside this core due to their hydrophobic PI ligands.

As the outcome of this encapsulation step is highly sensitive to local concentration inhomogeneieties of the polymer and the particles as well as the injection speed neither the particle size nor the properties like quantum yield or relaxivity are well reproducible. In addition up-scaling is not possible over a certain extend as the mixing behavior changes rapidly with increasing injection volume. This results in a inhomogeneiety of the properties because some capsules are filled with more than one particle as other remain empty.

2.3 Encapsulation by CFPTA

To overcome these problems we have developed a continuous flow phase transfer approach. Figure 3 shows a schematic line up of our assembly.

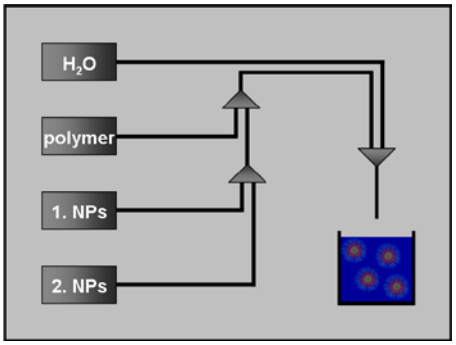

Figure 3: line up of the CFPTA

A dispersion of the precoated NPs get mixed with a solution of the amphiphilic polymer in the first mixing chamber. A second solution of different nanoparticles can be used for co-encapsulation. The formation of the nanocapsules takes place in the second mixing chamber. Here the organic solution get mixed with an excess of pure water which leads to a sudden raise of polarity in the solution. The formation of the micelles is finished instantaneously after the mixing, so no further ripening step is needed. Figure 4 shows DLS measurements of 6 different lots of encapsulated quantum dots produced all with the same settings.

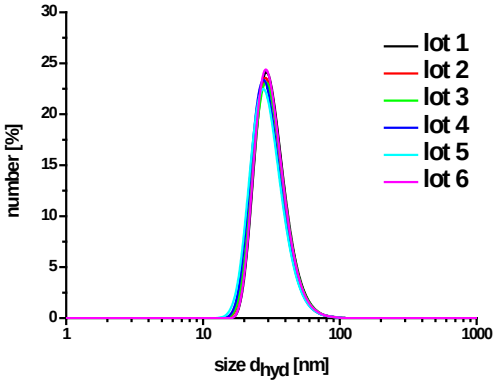

Figure 4: DLS measurements of 6 different lots of encapsulated quantum dots in water after CFPTA

It can be seen that the reproducibility of the size and size distribution exceeds the capability of traditional batch methods. This makes the study of the influence factors possible and is essential for the use of the particles in further processes like coupling to affinity molecules for the production of marker materials. Figure 5 and 6 compare the absorption and emission spectra of CANdot Series A quantum dots after the synthesis in toluene and after the CFPTA in water.

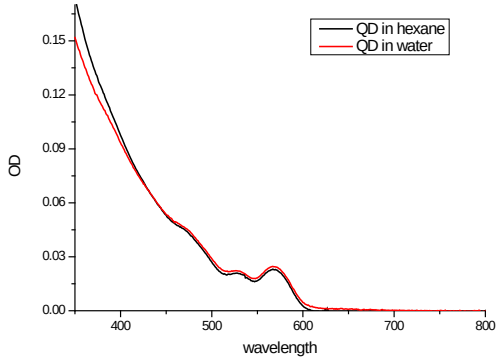

Figure 5: Absorption measurements of quantum dots (QD) before and after CFPTA

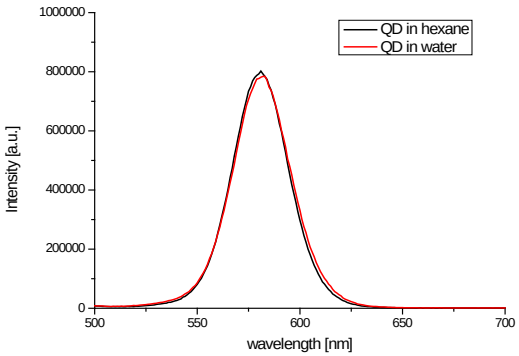

Figure 6: Emission measurements of quantum dots (QD) before and after CFPTA

As the absorption and emission spectra of the quantum dots remain identically, no agglomeration or quechning accures during the phase transfer.

Due to the fact that the micelles get crosslinked after the formation, the resulting capsule is highly stable and prevent the particles from agglomeration. Figure 7 shows DLS measurements of nanocapsules filled with magnetig iron oxide particles.

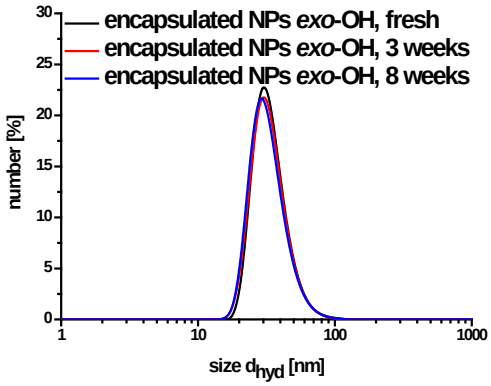

Figure 7: DLS measurements of encapsuled iron oxide particles in water, freshly prepaired, 3 weeks after and 8 weeks after the CFPT

The sample shown in figure 7 was not filtrated or anyhow separated after the phase transfer or between the measurements. The hydrodynamic diameters stays at 30 nm with no agglomerates above 100 nm which makes them suitable for biomedical applications.

2.4 Cross-linking and purification

After the CFPTA the resulting solution is heated up to 75 °C for 2h to evaporate the THF and to crosslink the PI shell by radiacal polymerisation startet by the decomposition of the AIBN.

2.5 Coupling to affinity molecules

For the attachment of affinity molecules to the nanocapsule functional groups on the micelle surface are needed. This can be achieved by using PI-PEO polymers with different groups at the PEO end. Usually we use -OH, -COOH or -NH_2 functionalities, but further groups like -CHO, -CH_1CH_2 or halogenids are possible, too. This offers us the possibility to use a broad range of different coupling strategies.

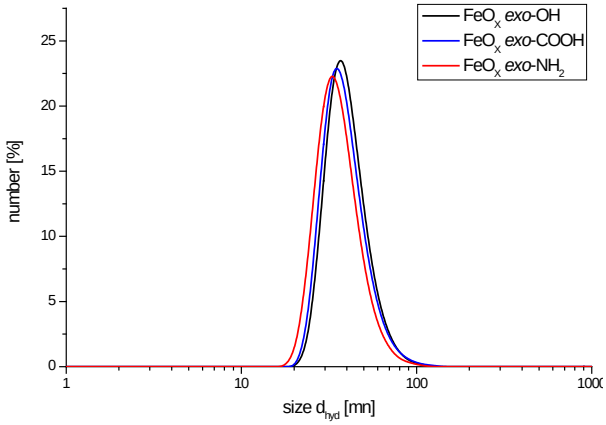

Figure 8: DLS measurements of encapsuled iron oxide particles in water, exhibiting different functional groups at the outer surface: -OH, -COOH, -NH_2

In generall two different methods are possible for the coupling of affinity molecules: to the polymer before the CFPTA (pre assembly) or after the CFPTA (post assembly).

For pre assembly chemical coupling reactions using the -COOH or -NH_2 groups are possible. The mayor drawback of this approach is the fact that the affinity molecule has to survive the encapsulation and clean up procedure which incluedes organic solvents and temperatures up to 75 °C. This is usually only working with small molecules like sugars as large peptides or antibodies would loose their specifity.

For sensible affinity molecules post assembly prooved to be more suitable. Here a general approach like the coupling via biotin/neutravidin in can be used to attach different affinity molecules. This works well for peptides, antibodies and small molecules, but can lead to a large increase in hydrodynamic diameter of the final product. In this case a special developed coupling reaction should be preferred.

3 CONCLUSION

Using the continuous flow phase transfer approach it is possible to encapsulate hydrophobic nanoparticles like quantum dots or iron oxide in a polymer micelle. This procedure is optimized in a way that the particle properties like quantum yield remain unchanged straight after the transfer. High stability against agglomeration is ensured by crosslinking of the nanocapsule after their formation. As this procedure is based on microfluidic components and can get fully automated a high degree of reproducibility and productivity is obtained. A further functionalization of the particles is possible by using pre or post assembly approaches with affinity molecules using functional groups on the surface of the capsules.

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