Chapter 1

Silica Nanoparticles for Biomedical Applications

Abstract. Inorganic nanoparticles are attractive materials due to their unique properties and prominent role in the fields of material science, nanotechnology and nanomedicine. Modern therapies aim to deliver drugs specifically to defective cells and mesoporous silica nanoparticles (MSNs) are considered to be promising candidates for this goal. In the first part of the introduction the synthesis, characterization and bio-applications of silica nanoparticles will be discussed. In the second part of this introduction the potential use of silica nanoparticles as emulsion stabilizers will be reviewed. This type of emulsification leads the formation of sub-millimeter particles which might be used as implantable drug delivery systems. Finally the outline of this thesis is given.

In 1992 scientists at the Mobil Corporation synthesized ordered mesoporous silica nanomaterials, and this discovery was recognized as an important breakthrough in material science that could lead to a variety of applications from food manufacturing to pharmaceutical technology.[1, 2] Mesoporous silica nanoparticles (MSNs) have attracted attention in recent years due to their intrinsic properties such as uniform inner mesoporosity, ease of chemical modification and biocompatibility.[3, 4] Great strides have been made in the field of nanomedicine during the last decade due to the rapid development of nanomaterials.[5] Colloidal particles, with a size range typically from 30 nanometers to 600 nanometers, were explored as potential drug delivery systems. Moreover, surface modifications resulted in modified physical properties such as water dispersibility or control over the release of guest molecules responding to exogenous stimuli.[6] The creation of a multifunctional platform based on nanoparticles for theranostic purposes that afford both therapeutic and diagnostic capabilities is much desired.[7-9] The topology of these nanomaterials can be dissected in three distinct domains which can be functionalized independently: the silica framework, the nanopores inner area and the nanoparticle's outermost surface.[10] The combined unique properties of MSNs enables the creation of site specific drug delivery systems which can transport guest molecules, fluorescent or contrast agents for bioimaging as well as pharmacological entities for therapy, to a targeted site in therapeutic significant concentrations. Moreover, the prevention of uncontrolled content leakage lowers the side effects associated with these molecules.[11-13]

The first part of this introduction gives an overview of general methods of MSN synthesis and biological applications with some relevant examples demonstrating the current state of the art of nanomaterials as drug delivery systems. In the second part of this introduction, the use of nanoparticles as a stabilizer for Pickering emulsions will be described and the creation of micro-capsules as implantable carriers for the delivery of therapeutic agents *in vivo* is discussed.

1.1 General methods of MSNs synthesis.

For the application of MSNs in the field of biomedicine some parameters have to be taken in to account such as: well-controlled nucleation and growth rate of the nanoparticles in order to create nanomaterials with a uniform size distribution. Several strategies were used to synthesize nanosized particles, including the use of templating agents, base or acid catalyzed processes and co-solvents. Silica nanoparticles were first synthesized by Stöber and coworkers who studied the formation of monodisperse nanoparticles by means of

hydrolysis and polycondensation of silicates in alcoholic solutions.[14] Subsequently Kresge *et al.* modified this process and synthesized ordered mesoporous molecular sieves using a quaternary ammonium surfactant as a template.[1] Mobil Composition of Matter No 41 (MCM-41) was the initial name of these mesoporous materials used as molecular sieves. The most striking feature of MCM-41 is the long range ordered framework with uniform mesopores, while the walls are composed of amorphous silica. Using non-ionic surfactants lead to materials with a different mesoporosity. SBA-15 (Santa Barbara matter No 15) is another mesoporous silica material synthesized using the non-ionic template Pluronic-123 (block co-polymer EO_{20}-PO_{70}-EO_{20} in which EO is ethylene oxide and PO propylene oxide) under acid catalyzed conditions.[15] TUD-1 (Technische Universiteit Delft matter No 1) is an example of mesoporous material achieved with a based catalyzed surfactant free synthesis. In this material the mesoporous structure is randomly connected in the three dimensions.[16] In this thesis mesoporous silica nanoparticles with a MCM-41 porous morphology will be discussed.

The Stöber synthesis of silica spheres was modified by Grün through the adoption of a cationic surfactant to the reaction mixture, resulting in sub-micrometer sized MCM-41 spheres.[17] In contrast, lower concentrations of surfactants were found crucial by Cai *et al.* and Nooney *et al.* to obtain MCM-41 materials with a particles size of less than 100 nm.[18, 19] Recently Ying *et al.*[20] have synthesized particles in the size range of 100-800 nm particles with ordered mesoporosity using the fluorocarbon surfactant FC-4 ($C_3F_7O(CFCF_3CF_2O)_2CFCF_3CONH(CH_2)_3N^+(C_2H_5)_2$-$CH_3I^-$) in the procedure. These selected examples show that mesoporous silica particles with a size range of 50-1000 nm can be prepared by adjusting the reaction conditions and the proper choice of the surfactant.

In the synthetic process there are several steps which lead to the mesoporous particle formation: nucleation, growth and aggregation as shown in **Figure 1**. Ideally, a short initial burst of nucleation is followed by a uniform growth resulting in the synthesis of homogenous particles. Hence, alkaline and highly diluted conditions are typically used to synthesize negatively charged particles avoiding inter-particle aggregation. For this reason the most commonly used surfactant is the cationic molecule hexadecyltrimethylammonium bromide (CTAB) which is used as the templating agent in the base catalyzed synthesis of uniformly sized MCM-41 materials. Three approaches are used for the formation of mesoporous silica nanoparticles, and are described in the following paragraphs.

1.1.A) Growth-quench approach

In this method the size of the particles is controlled by changing the pH, while quenching the silica condensation reaction, and increasing the solvent volume in order to restrain the polycondensation reaction. By using a different time-delay between dilution of the reaction mixture and pH neutralization, the particles size can be varied from 20 nm to 100 nm.[21] The rate of the reaction can be lowered by the addition of triethanolamine[22] due to the ability of to deprotonate all silanols groups still available for the condensation reaction. The process can also be queched by using alcohol as co-solvents[23] under basic conditions in which the deprotonated state of the silanols are favored and the alcohol acts as a proton donator to the electron rich silanol.

However, poor condensation of the silica precursor is a drawback of this method resulting in nanomaterials which are often less ordered and less stable in solution. This method is also difficult to scale up due to high amount of solvents used for dilution. When high volumes are required, triethanolamine is substituted by NaOH due to a minor impact on the environment.

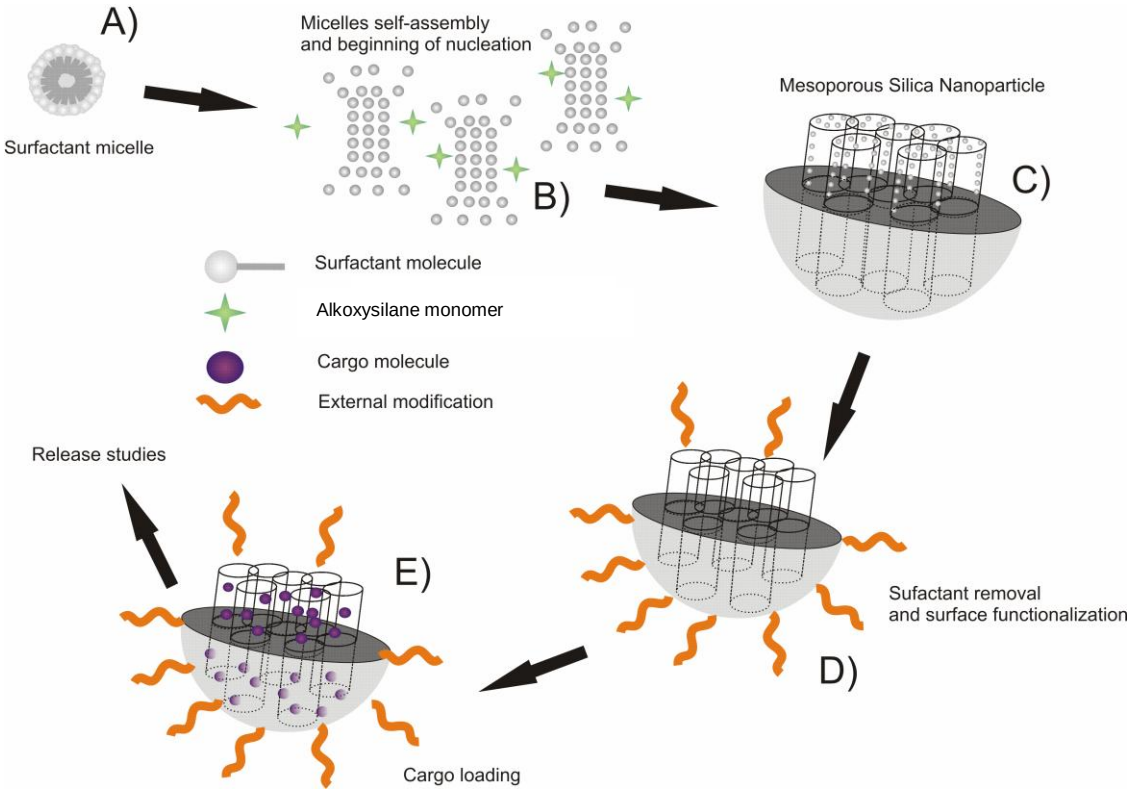

Figure 1. General scheme of surface modified mesoporous silica nanoparticles. A and B) In an emulsion the parallel arrangement of surfactant micelles occurs. C) After the polycondensation of alkoxysilanes the formation of mesoporous silica nanoparticles is observed. D) To achieve mesoporosity the surfactant template is removed. Moreover, the surface is chemically modified to control physical properties. E) Guest molecules are loaded in the mesoporous structure. Next, the surface modification is completed in order to finalize the nanovalve system for drug release.

1.1.B) Confinement approach

A different method for the synthesis of size controlled nanomaterials is the use of confined media, where the growth of the nanoparticles is limited. Brinker and co-workers[24] have developed an aerosol-assisted self assembly method for the synthesis of mesoporous spherical nanomaterials. The process relies on the evaporation of aerosol solvent droplets where the polymerization reaction takes place. Although this method results in the synthesis of a high amount of mesoporous nanomaterials, it is not widely adopted due to need of special equipment for aerosol production.

Another confinement strategy is based on using oil-in-water microemulsions as the reaction medium, since these emulsions are in thermodynamic equilibrium their phase domain is rather uniform in size.[25, 26] Mou and collaborators have used this methodology to prepare MSNs with a uniform size either with a hollow or solid core.[25] Different reaction compositions were used and more recently hollow mesoporous nanomaterials were synthesized in water-heptane-CTAB nanoemulsions,[26] or using liposomes as a template.[27]

1.1.C) Separation of nucleation and growth

This method occurs principally in three steps: in the first step the total amount of surfactant mixed with a small amount of silane form a clear solution containing the nuclei of the particles. In the second step a larger amount of the same silane is added to start the growth process without further nucleation. Subsequently in the final stage, the original material gets exhausted and the condensation reaction is not able to proceed.[28] In this way the particle size is defined.

The nucleation process was studied in 1905 by Aelion and coworkers.[29] This study showed that the hydrolysis rate of tetraethyl-orthosilicate (TEOS) increases linearly with the concentration of OH^- in the basic medium. When the pH is in a range of 9-10 the hydrolysis rate of TEOS reaches its maximum. Thus, this process leads to the formation of more hydrolyzed silicates species in the solution. Next, they self-assembly with the surfactant to form micelles which condense in the nuclei bearing a 2D hexagonal structure. Interestingly, at higher values of pH (pH=11) the silica condensation rate decrease as the silicates are increasingly charged. Thus, there is a smaller amount of nuclei resulting in particles with bigger size.

1.2 Surface functionalization

Surface functionalization of MSNs with different chemical entities, results in the modification of the physical properties of these nanoparticles which are crucial for

potential biomedical applications. Three methods are typically used for the synthesis of surface modification of nanomaterials: grafting, co-condensation and the surfactant displacement strategy.

1.2.A) Grafting Process

In a mesoporous nanoparticle two different surfaces can be distinguished: an internal surface which covers the nanopore channels and an external surface at the periphery of the nanoparticle.[10] The internal surface in MSNs is accessible only after the surfactant template has been removed; thus surface modification after template removal affects both the external surface as well as the chemical groups located inside the pores. The grafting process is the most popular method to modify the surface of MSNs. The reaction takes place between the silanol groups on the surface and organoalkoxysilanes or organochlorosilanes. The main problem encountered using this method is the inhomogenity in surfaces[30] coverage because the silanols located on the exterior surface and at the opening of the mesopores are kinetically more accessible than silanols located in the interior pore walls. Thus, the majority of the grafted organic functionalities have been found to accumulate on the surface and to congregate at the mesoporous opening.[31]

1.2.B) Co-condensation Process

Another common method to functionalize the nanoparticle surface is the co-condensation of two or more alkoxysilanes. In this method, the organoalkoxysilane is introduced to a mixture of silanol precursor (i.e. TEOS, TMOS) and CTAB. In the case of addition of alkoxysilanes with polar substituents the polycondensation process leads to nanoparticles with spherical shape. However, using alkoxysilanes with aliphatic substituents, rod-shaped nanoparticles are favoured.[10] Thus, the formation of long individual cylindrical micelles are stabilized by intercalating the hydrophobic groups of the alkoxysilanes in the micelles. In this manner interactions with the hydrocarbon tails of the surfactant templates are established and thereby the charge density of the head groups is reduced. In contrast when the nature of the functional groups is more hydrophilic there is no further stabilization of micelles. Polar substituents do not favour by the interaction with the surfactants; therefore they show little “side-on” condensation with the consequent formation of spherical particles. (**Figure 2**)

1.2.C) Co-condensation and Grafting Combined Process

A combination of co-condensation followed by grafting methods has been used to create MSNs with a bifunctional surface. In this process the nanoparticles are first created with

the co-condensation process, and the free silanols groups are then functionalized using the grafting method in a supercritical fluid medium. In this synthesis template-based, the surfactant is then removed with an alcohol acidic extraction revealing the mesoporous structure.[32-34] In acidic conditions the interaction between the template and the silica backbone weakens, thus the surfactant is removed by proton exchange and solvent extraction. The methods results in an uniform monolayer coverage of organic functional groups on the inner and outer surface. This synthesis results in the formation of spherical nanoparticles and the addition of the alkoxysilanes does not perturb the final morphology.

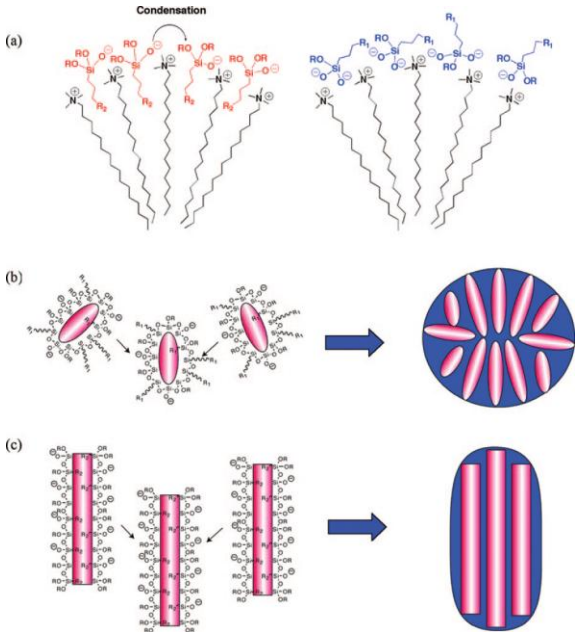

Figure 2. Correlation between nanoparticles morphology and the type of of alkoxysilanes. In the figure, R refers to methyl or ethyl groups, R_1 to hydrophilic functional groups and R_2 to hydrophobic functional groups. (a) Templated assisted condensation process of alkoxysilanes. (b) In case of nanoparticle synthesis with hydrophilic alkoxysilanes the formation of spherical particles is the main process. (c) However, the use of hydrophobic alkoxysilanes leads to the formation of rod-shaped nanoparticles.

1.3 Functionalization for Biomedical Applications

Surface functionalization controls the physical properties such as dispersibility in aqueous media. In this section two important strategies used to modified the nanoparticles surface will be discussed: charge control and functionalization for receptor targeting with biological ligands.

1.3.A) Functionalization for surface charge control

Internalization of nanocarriers in cells is strongly influenced by its physicochemical properties, and the surface charge is one of the most important parameters.[35] Due to the silanol groups located on the surface which can be easily modified, the surface charge can be controlled by the modification with different organic groups that bear a specific charge

and chemical structure. 3-aminopropyltrialkoxysilane (APTES) is one of the most used precursor that introduces amino groups at the surface resulting in a positive surface charge depending on the pH.[36] Positively charged nanocarriers have a stronger interaction with the plasma membrane due to its negative charge, thus the cellular up-take rates of MSNs are enhanced.[37] In addition other organic moieties are used to increase the cellular uptake such as (N-trimethoxysilyl)-propyl-N,N,N-trimethylammonium (TA).[38] In contrast unmodified mesoporous silica nanoparticles have a negative surface charge at physiological pH and this negatively influences the cellular uptake.[39, 40] Moreover, aggregation and clustering in aqueous media due to inter-particle interaction negatively affects the cellular uptake. For this reason Lu and collaborators developed a protocol where the dispersibility of nanoparticles in physiological media is enhanced due to the presence of phosphonate groups on the nanocarrier surface.[41, 42]

However, when new MSNs with a new surface modification, special attention has to be given to the colloidal stability. Certain types of chemical entities (i.e. hydrophobic substituents) may disturb the colloidal properties of these nanomaterials resulting in aggregation and clustering in aqueous media which results in a decreased uptake into cells.

1.3.B) Functionalization for ligand-receptor interaction

In order to target specific cells the external surface of MSNs is modified with chemical entities acting as ligands for cellular membrane receptors. This is important when cell recognition is desired, while the inner mesopores can be functionalized differently to ensure maximum guest molecule loading. Bifunctional nanoparticles were synthesized by combining the co-condensation and grafting method as reported by Bein and coworkers.[43] In this study bifunctional colloidal mesoporous silica nanoparticles were synthesized by the formation of nucleation seeds from TEOS followed by a co-condensation with trialkoxysilanes. In this manner the inner surface and the outer surface have different functionalizations which can be modified independently for the design of ligand targeted nanocarriers.

The identification of overexpressed folate receptors on the surface of cancer cells led to the design of nanocarriers modified with folic acid.[44] Zink and collaborators have developed MSNs systems bearing this functionality in order to deliver hydrophobic anticancer drugs in tumor cells.[45, 46] They also synthesized magnetic silica mesoporous nanoparticles for magnetic resonance analysis for *in vivo* investigation in tumor affected mice.[47] Fan and coworkers have developed a similar system in which folic acid is covalently bound to the surface of MSNs.[48] The nanoparticles were subsequently loaded

with doxorubicin, a potent anticancer drug, and the cellular uptake with other analysis were studied in order to establish the efficacy of drug delivery using MSNs.

1.4 Cellular uptake

One of the most important barriers a nanocarrier has to overcome in order to deliver the payload in specific cells, is the cellular membrane. Thus, understanding the mechanisms of cellular uptake is of utmost importance for the development of efficient nanocarriers for a potential application in nanomedicine. There is a wide variety of cellular pathways for particle uptake, and new variants are still discovered each year. However all these mechanisms can be categorized in two main classes: endocytosis and pinocytosis.[49, 50] Endocytosis is a multistep complex process starting with the interaction between the cell membrane and the nanocarrier, invagination and pinching off, followed by tethering of the newly formed vesicles in the intra-cellular trafficking process.[49, 50, 51] Unfortunately no "rule of thumb" is available to the predict which mechanism will be involved in the cellular uptake of a particular nanoparticle; therefore understanding the internalization is important to optimize the efficacy of a potential delivery system based on MSNs.

To understand the endocytosis of MSNs several blocking agents are used to elucidate the mechanism. Huang *et al.* showed that clathrin-mediated endocytosis plays an important role in the uptake of nanocarriers in human mesenchymal stem cells (hMSC). MSNs internalization was shown to be highly dependent on the sucrose concentration in the medium; thus changing the media conditions may modify the nanoparticles uptake by hMSC.[52, 53] Huang and coworkers studied the cellular uptake of MSNs in 3T3-L1 fibroblast cell line confirming the previous hypothesis of clathrin dependent uptake mechanism.[38] Recently Lu *et al.* studied the energy-dependent internalization pathway by incubating cells (PANC-1 pancreatic cancer cells) with MSNs at different temperatures. This showed an energy-dependent internalization as the most efficient MSNs uptake was achieved at 37° C while at 4 °C the endocytotic pathway was not very active.[54]

Several factors which have to be taken into account for nanoparticles cellular uptake such as: the cell line type,[52, 53] the surface charge[4] and chemistry as previously discussed,[48] the particle size[55-60] and the shape[61] showing the complexity of MSNs cell uptake. Several studies investigated the influence of particles size on cellular uptake;[35, 62-64] and the most important findings were: I) the particles size limit for internalization depends on the cell type, II) particles smaller than 500 nm typically are taken up *via* endocytosis pathways, III)

above this limit nanocarriers are hardly engulfed within the cell. Micrometer sized nanocarrier are normally internalized by macrophages *via* a phagocytosis pathway.[65]

The developments in the field of nanomaterials led to the discovery of different shaped nanoparticles for drug delivery such as nanorods. However rod-shaped nanocarriers are internalized less efficiently in cells. Gao [66] developed the "wrapping time" concept, which describes how cell membranes interact with particles having different shapes. When nanospheres interact with the cellular membrane the internalization is more efficient, as engulfing a sphere is faster than wrapping an entire rod which has a larger surface area. Lin and coworkers[67] have studied the cellular uptake kinetics for nanorods (600 nm x 100 nm) and nanospheres (80-100 nm in diameter) in hamster ovarian cells (CHO) and fibroblasts. Faster internalization of both spheres and rods was observed in CHO cells; however nanospheres were quantitatively taken up within 3 hours, while rod-shape particles reached the same level of uptake only in 6 hours, which is in agreement with the "wrapping time" theory.

1.5 MSNs as a drug delivery system, controlled cargo release

Vallet-Regi[68] published the first report on applying MSNs as a drug delivery tool. Efficient drug delivery is crucial for the development of novel therapies, in particular in the case of therapeutic agents which cause severe side effects if administered systematically. Ideally the use of MSNs as a drug delivery system results in the protection of the payload from systemic metabolisms, and at the same time the guest molecules are delivered in a specific area of the body. To release therapeutic molecules MSNs capped with cadmium,[69] gold[70] and iron oxide[71], and soft nanoparticles such as dendrimers,[72] proteins[73] and polymers[74-76] towards drug delivery systems have been developed. Here the payload is entrapped in the pores while the entrance of the nanochannels is blocked with bulky particles; hence the name "gatekeeper" was coined due to its original function. This surface modification is one of the most popular strategies to encapsulate anticancer drugs, while preventing premature release and allowing their "smart" release due to an external stimulus (i.e. pH reducing environment).

Stimuli responsive drug delivery systems (DDS) can release their cargo upon specific activation. These triggers may be classified in two main categories: internal stimuli such as pH, reducing environment and enzymatic activity, and external stimuli such as light radiation, ultrasound or electromagnetic fields which can be applied.[77, 78]

Lai and co-workers[69] demonstrated that CdS-modified MSNs delivers the cargo with "zero" premature release before reaching the targeted site. Polymers were also used to entrap guest molecules in MSNs, Radu *et al.* used dendrimers (G2-PAMAM) molecules at the surface of the nanocarriers. [74] The positive charges of the dendrimers strongly interact with the cellular membrane. The positive surface charge can also be used to electrostaticly bind nucleic acids and as a result the nanosystem acts as a gene delivery system.[79]

Photoswitchable molecules were used as "nanoimpellers". When the system is irradiated with a specific wavelength both isomers have the same extinction coefficient, thus the molecules undergo to continuous dynamic wagging, this imparts motion to the molecules entrapped in the nanopores and forcing them out of the MSN. One of these molecules is amino benzene (AB) which was used as nanoimpellers by Lu *et al.*[80] and used for the controlled release of a drug from MSNs. Although these systems have fascinating controlled drug release properties, the potential application as delivery systems in living cells is still a pristine area of research.

In contrast with the nanoimpellers which can be positioned on the surface of the nanochannels, so-called nanovalves are located at the pore openings. Nanovalves can be both supramolecular and mechanically interlocked entities (i.e. rotaxanes), in which a cyclic component can move with respect to a stationary stalk counterpart. In this specific system the movement generates two states which correspond to an open state and to a close one. The groups of Stoddart and Zink have developed these systems and successfully applied these modified MSNs as a drug delivery system in cancer cells.[81] Bistable [2]rotaxane acted as nanopore gates. The tether in this rotaxane was composed of two electron-rich moieties and encircled by a electron-deficient cyclophane. Upon oxidation the cyclophane moves from one station to the other resulting in the open and the closed position of the system, thus the nanocarrier releases the payload only when the electronpoor ring is located in the opening position. However, the position of the two electron-rich moieties is critical in order to prevent "zero" premature release.[82] When the rotaxane were attached inside the nanochannels no leakage was observed, however the cargo release was considerably slow when the valve was opened showing that there is still room for improvement.

The nanovalve approach was also applied using pseudorotaxanes: in this case the ring can dissociate reversibly with the stalk determining the opening of the system.[83] In another example the system was modified in order to be activated with light.[84] Also pH controlled nanovalves were reported. MSNs were functionalized with dibenzylammonium acting as a

stalk on which dibenzo-24-crown-8 was encircled.[85] A strong hydrogen bond between the ring and the stalk allows the system to stay in the closing position, while the opening was determined by basic conditions. It is interesting to notice that many of these nanosystems can operate in water, a crucial prerequisite whenever the system is envisioned to be used in living cells.

Cyclodextrins can be tethered on different stalks such as polyethyleneimine[86] or ferrocene carboxylate and are therefore used as pseudorotaxanes.[87] Interestingly the ferrocene system can be activated upon electrochemical oxidation when the cyclodextrin was tethered on the chain. In other cases the nanovalves was a combination of aminobenzene nanoimpellers located in the channels and pH-switchable *pseudo*rotaxanes located at the orifices.[88] The azabenzene stalk changes its conformation at basic pH allowing the release of the cyclodextrin and the cargo molecules are released only upon light activation.

1.6 *In vitro* experiments

One of the main challenges in modern nanomedicine is a combination of a drug delivery system able to target a specific cell type, and MSNs are promising candidates to accomplish this challenge. Achieving this goal would advance current cancer treatment therapies, as current drugs are administered systematically resulting in severe side effects for the patient. Therefore efforts are made to create MSNs able to target specific tissues without damaging surrounding healthy cells. Several strategies are studied to direct nanoparticles towards specific cells such as targeting folate receptors which are over-expressed on the surface of cancer cells. Lin and coworkers[36] have developed MSNs bearing folic acid moieties at its surface, and demonstrated a receptor-mediated endocytosis pathway since the internalization is inhibited by high levels of folic acid in medium. Linden *et al.*[89] used poly(ethyleneimine) coupled with folic acid groups on the surface of the nanoparticles. The authors showed that endothelial cancer cells (HeLa) internalized actively this nanosystem while embryonic kidney cells did not. Tamanoi and coworkers[45] have shown targeted release in pancreatic cancer cells (PANC-1) of camptothecin/paclitaxel using folate modified MSNs. Moreover, Mou *et al.*[90] used monoclonal antibodies for selective targeting in breast cancer cells. In addition other successful applications have been reported for gene delivery and photo-induced therapy.[86, 91]

1.7 *In vivo* experiments

The first *in vivo* experiment was recently reported by Wu *et al.*[92] who inoculated magnetic MSNs in mice at a concentration of 2 mg/kg. In this study the mice remained in good condition at the end of the treatment and no damage to the vital organs was observed. In contrast Hudson and collaborators[3] found that injected mesoporous silica nanomaterials were toxic for mice. At the end of the experiment, organs showed abnormalities due to accumulation of nanoparticles. However, these effects are most likely due to the very high dose of MSNs used in this treatment (1.2 g/kg).

Mice injected with 0.5 mg of phosphonated MSNs were studied by Lu *et al.*[47] and after 14 days and 2 months blood samples were taken and organ histology was performed. After 2 weeks the majority of the animals were found to be healthy, while only a minority showed liver toxicity. After 2 months of treatment with phosphonate MSNs the mice appeared healthy and neither histological lesion in tissues nor pathological abnormalities were detected. Tamanoi and coworkers[42] have suggested a dose for pharmacological applications of 50 mg MSNs per kg.

Hollow silica mesoporous nanoparticles[7] were also studied *in vivo* and the injected animals were found to be healthy and no abnormalities were detected in the liver, spleen and kidney. The excretion time of such nanoparticles was estimated to be more than 4 weeks.

Mesoporous silica nanoparticles have shown to be potential candidate for the establishment of innovative therapies for the treatment of different diseases, as it has been revealed by the animal studies discussed. However, several parameters have still to be studied in depth, as organ clearance and biodegradability of nanoparticles in long time experiments, leaving room for further research.

1.8 Silica Nanoparticles as stabilizers of Pickering emulsions

In this second part of the introduction the application of silica nanoparticles as stabilizers for Pickering emulsions and their potential use as drug delivery systems is briefly reviewed. A macroemulsion is a mixture of two immiscible liquids in which one phase is dispersed as micron sized droplets within the other continuous phase. Due to the large surface area between the two phases, macroemulsions are thermodynamically unstable. However, the addition of solid colloidal particles can be used to impart kinetic stability to this kind of emulsion.[93] The resulting system is remarkably stable in the time, and the credit of this

finding was given to Pickering, after which these particular emulsions were named Pickering emulsions.[94]

In traditional emulsions the mixed phase is stabilized by surfactant molecules and the most important parameter which determines whether the surfactants reside in either the water or the oil phase is the hydrophilic-lipophilic balance (HLB). Aveyard[95] recognized that the packing parameter of the surfactant determines the tendency of these molecules to bend towards the oil or the water phase, but also to remain in a planar displacement between the two phases. This tendency is directed by the intrinsic geometry of the surfactant molecules. When hydrophilic surfactants have a bulky polar head group attached to a lipophilic chain the monolayer formed curves towards the oil phase resulting in an oil-in-water emulsion. On the other hand surfactant molecules with a bulky lipophilic chain and smaller hydrophilic head stabilize water-in-oil emulsions. For hydrophilic surfactants the volume of the chain exceeds that of the head group, thus the molecules have a tendency to curve towards the water phase allowing a stabilization of water-in-oil emulsions. However, in cases where the volume of the head is comparable with the steric hindrance of the lypophilic chain, zero curvature is observed resulting in bilamellar phases and bicontinuous emulsions.[96]

In the case of spherical particles which adsorb to the water-oil interfaces the most important parameter to consider is the contact angle θ which the particle makes with the interface (see **Figure 3**). In the case of hydrophilic particles, i.e. metal oxides, θ is smaller than 90°, meaning that a larger portion of the particle resides in the water phase then in the non-polar phase. In contrast for hydrophobic particles, i.e. surface modified silica nanoparticles, the contact angle is generally larger than 90° thus the particle resides better in the hydrophobic phase than in the polar phase.[97] These hypotheses were confirmed by Schulman and Leja[98] who used barium sulfate crystals/powders as a surfactant and measured the contact angle of the particle with the phases and determining the type of emulsion.

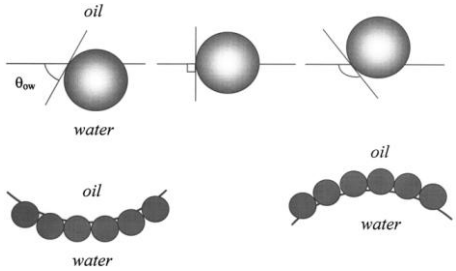

Figure 3. (upper) Position of a small spherical particle at a planar oil–water interface for a contact angle (measured through the aqueous phase) less than 90° (left), equal to 90° (centre) and

greater than 90° (right). (lower) Corresponding probable positioning of particles at a curved interface.

Silica colloids are able to stabilize different types of emulsion depending on the degree of hydrophobicity of the particle. This factor is revealed to the amount of total silanol groups (SiOH) located on the surface of the nanoparticles, such that if the silica surface is completely covered with silanols it is considered hydrophilic and hydrophobic when the percentage of SiOH groups is low.[97] Thus hydrophilic silica particles disperse in water, whereas more hydrophobic silica disperse in the oil phase.[99] Therefore, silica particles are able to stabilize Pickering emulsions. Depending on the oil to water ratio, emulsions of both types can be prepared.[100] This behavior is correlated with the energies of attachment of particles at the interface, although it remains unclear why both types of emulsions can be stabilized by the same kind of particle. Lumsdon *et al.*[101] have studied the effect of particle hydrophobicity on stability of toluene-containing emulsions in detail. They showed that considerations of the attachment energy of a single particle to the interface, gives an approximate prediction for the stability of the system. Moreover, they showed that emulsions can be stabilized by very hydrophilic or very hydrophobic particles resulting in droplets with an average diameter of 100 µm, albeit very unstable due to coalescence and inter-drop interactions. In contrast, emulsions stabilized with an intermediate degree of hydropobicity and hydrophilicity result in sub-micron sized droplet with higher stability. An important advantage of Pickering emulsions is their "emulsifier-free" character, making these systems attractive for biomedical applications in which surfactants may have a detrimental effect on living organisms.[102, 103, 104]

1.9 Pickering Emulsions as drug delivery systems

Emulsions have been used as a drug carrier, in particular to transport and release an effective dose of therapeutic agents while protecting the drug from undesired metabolism.[105] Oza and collaborators[106, 107] developed the first pharmaceutical emulsion-based vehicle in 1989. The authors described the release of lidocaine from water/oil/water emulsions. However this system contained a mixture of microcrystalline cellulose and conventional surfactants to stabilize the emulsions. Garti *et al.*[108-110] used nanoparticles as emulsions stabilizer because they could better protect the payload and a slow controlled release of a model drug was reported. Prestidge and collaborators[111] reported a synthesis of Pickering emulsions stabilized with fumed silica. Polydimethylsiloxane droplets in water were stabilized with Aerosil™. The release of guest molecules like dibutylphtalate from these nanocapsules was studied *in vitro*.

The controlled and the targeted delivery of poorly soluble drugs is a challenge in medicine. The use of combinatorial chemistry approaches led to the development of small therapeutic molecules that are often hydrophobic in nature. This results in a low solubility in water and biological fluids lowering its efficacy *in vivo*. Hence, the creation of a delivery system able to transport hydrophobic molecules to a specific site without disturbing the homeostasis of living organisms is desiderable. Simovic and coworkers[112] developed microcapsules stabilized with silica nanoparticles with an internal porous matrix structure to encapsulate poor water soluble drugs. *In vivo* oral administration of these microcapsules using indomethacin as a model compound was studied, showing higher bioavailability of the drug compared to oil-in-water emulsions and aqueous drug suspension.

1.10 Aim and outline of the thesis

The first part of the thesis describes the synthesis of functionalized mesoporous silica nanoparticles for site specific and controlled drug delivery. In **Chapter 2** Peptide-Modified Mesoporous Silica Nanoparticles are studied. A straightforward synthetic approach to this system is given and the particles are characterized with microscopy techniques such as scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The particle mesoporosity was analyzed with X-Ray diffraction (XRD) showing an homogenous size of the mesochannels in the particles. The nanoparticles were modified with nanovalves in which the peptide acts as a valve keeping the rotaxane ring tethered on the aliphatic stalk in place which opens in a reducing environment. The system results in a cell membrane targeted nanocarrier with controlled release of guest molecules. An ideal drug delivery system should not release any of its cargo before reaching the targeted site, thus the presence of the rotaxane valve is crucial to prevent any leakage from the system. The dispersibility in aqueous media is another important parameter when designing a drug delivery system. Aggregation and clustering may result in diminished uptake into cells. Therefore the inter-particles interactions were studied as a function of the spacer used in the rotaxane and the presence or absence of the entire rotaxane at the surface of the MSNs. Cellular uptake studies confirmed the membrane affinity due to the peptide chain. The system presents a high affinity towards cancer cells, however lacks selectivity. Since cancer cells have a high replication rate the uptake of folic acid from the environment is crucial for their survival. In order to enhance folic acid uptake these cells have an abundance of folic acid receptors on the membrane surface. Therefore a new nanovalve system was designed in which folic acid is an intricate part of the valve. **Chapter 3**

describes the synthesis and characterization of this system. The valves are activated by esterases that catalyze the hydrolysis of an ester bond present in the valve resulting in the release of the guest molecules. Laser confocal scanning microscopy analysis revealed the cellular localization of the MSNs, and interestingly the nanoparticles were also located in the nucleus. Thus this opens the possibility to a nuclear targeted delivery system for gene therapy. In **Chapter 4** mesoporous silica nanoparticles were studied *in vivo*, as a carrier for retinoic acid. The biocompatibility of the PEGylated MSNs was studied and the release of retinoic acid was analyzed *in vitro*, and in *Xenopus laevis* using morphological studies and *in situ* hybridization techniques. The nanoparticles were also investigated in zebrafish embryos as described in **Chapter 5**. The biocompatibility of the system and the effective release of the drug were studied. Mesoporous silica nanoparticles were found very attractive as drug delivery in animal embryos for drug screening as minimal amount of active compound can be delivered in the bioactive form and pharmacological effects are observed. In **Chapter 6** a micron sized delivery system was designed using silica nanoparticles as stabilizers for Pickering emulsions. The microcapsules were composed of a polymerized core in order to confer strength to the particle allowing the manipulation of a single microcapsule with tweezers to enable surgery. The release of the model drug retinoic acid was tested in adult zebrafish analyzing the regeneration delay of amputated tails.

Finally in **Summary and discussion** the results are summarized and discussed, and possibilities for future research are presented.

1.11 References and Notes

*The images of this chapter have been taken from the following references: **Figure 2**, Huh S. *et al.*, *Chemical Material* **2003**, 15, 4247-4756; **Figure 3**, Aveyard R. *et al.*, *Advances in Colloid and Interface Science* **2003**, 9, (1), 53-57.

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