Nanoparticles Based on Hydrophobic Polysaccharide Derivatives—Formation Principles, Characterization Techniques, and Biomedical Applications

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Polysaccharide (PS) nanoparticles (NP) are fascinating materials that combine huge application potential with the unique beneficial features of natural biopolymers. Different types of PS-NP can be distinguished depending on the basic preparation principles (top-down vs bottom-up vs coating of nanomaterials) and the material from which they are obtained (native PS vs chemically modified PS derivatives vs nanocomposites). This review provides a comprehensive overview of an approach towards PS-NP that has gained rapidly increasing interest within the last decade; the nanoself-assembling of hydrophobic PS derivatives. This facile process is easy to perform and offers a broad structural diversity in terms of the PS backbone and the additional functionalities that can be introduced. Fundamental principles of different NP preparation techniques along with useful characterization methods are presented in this work. A comprehensive summary of PS-NP prepared by different techniques and with various PS backbones and types/amounts of hydrophobic substituents is given. The intention is to demonstrate how different parameters determine the size, size distribution, and zeta-potential of the particles. Moreover, application trends in biomedical areas are highlighted in which tailored functional PS-NP are evaluated and constantly developed further.

1. Introduction

Polysaccharide (PS) nanoparticles (NP) are intensively studied in various branches of basic and applied sciences; from polymer chemistry over material research to advanced biomedical fields of application (Figure 1). Nature already uses PS to form highly complex hierarchical structures and composite materials such as wood and native hydrogels. By understanding the principles behind supramolecular self-assembling of PS it is possible to exploit PS for the fabrication of innovative bio-nanomaterials. PS are “sustainable” and “biobased” materials. In the context of current nanosafety debates, these traits are of increasing importance to the scientific community and general public alike. As opposed to many artificial nanomaterials, e.g., derived from synthetic polymers or inorganic materials, PS are inherently nontoxic, biocompatible, and frequently also possess beneficial bioactive effects. These features are highly desired for biomedical applications of NP, such as drug delivery and in vitro/in vivo sensing.

The NP covered in this review are best described by phenomenological definitions as materials with a dimension in the nm-scale (1–1000 nm) that possess unique physical, chemical, and/or biological properties that are different from both the individual molecules as well as the macroscopic bulk material of the same chemical composition. This could include among others thermodynamic (phase transitions, heat capacity), optical (surface plasmon absorption, fluorescence), magnetic (magnetism), or catalytic effects (electron affinity, ionization potential) that are altered as a result of the change in the density of electron states. NP in general and PS-NP in particular, are rather diverse classes of materials. One approach toward classification of NP is according to their chemical composition (Figure 2). Inorganic NP, which include metal-, metal oxide-, silica-, and carbon particles, are generally considered as “hard” nanomaterials. They are rigid (no deformation under shear), nonporous and have a well-defined surface (smooth, chemically homogeneous) that is characterized by a steep change in density when going from the NP surface to the surrounding medium. Organic NP are derived from synthetic organic polymers, copolymers, and oligomers, biopolymers (e.g., PS, proteins, virus particles, DNA/RNA aggregates), as well as semisynthetic compounds that are obtained by chemical modification of biopolymers to alter their properties. These organic nanomaterials are considered as “soft” because their surface is less defined and characterized by polymer brushes that extend from the particle surface into the surrounding medium. This results in a gradual density change across
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Reviews describing this type of materials can be found in the recent scientific literature.[11–14] PS-NP with the biopolymer as dominating component are fabricated either by II) top-down approaches in which PS-bulk materials are disassembled into nanoscaled objects or III) bottom-up approaches in which individual PS macromolecules self-assemble into larger scaled NP. “Cellulose nanocrystals” and “nanofibrillated celluloses” are typical examples for PS nanomaterials obtainable by top-down procedures. The materials have received increasing popularity in scientific and applied research in the last...
decades and have been covered in many recent comprehensive review articles already. \[15–19\] In bottom-up approaches, PS-NP are prepared starting from individual PS macromolecules using different processes: i) chemical crosslinking, ii) ionic crosslinking, and self-assemblying by iii) supramolecular interaction or iv) hydrophobic interaction.

i. Chitosan-based NP have been prepared by chemical crosslinking of the NH$_2$-group containing PS with epichlorohydrin or bifunctional epoxides, aldehydes, and carboxylic acids. \[20,21\] In order to obtain nanoscaled materials, emulsion-based techniques have to be employed. High reactivity of the PS and the crosslinker is required to achieve covalent linking of the polymer chains under aqueous conditions. This approach, however, raises safety issues (toxicity of residual crosslinker) and makes it more difficult to control the reaction in such a way that NP are obtained. Other methods for preparation of PS-NP are usually preferred.

ii. PS-NP can be obtained by crosslinking of ionic PS with an oppositely charged polyelectrolyte (biobased or synthetic ones) or multivalent molecules (e.g., tripolyphosphate) and metal ions (e.g., Ca$^{2+}$, Al$^{3+}$). This approach has been described frequently for native PS polyelectrolytes, most notably chitosan (cationic) or alginate (anionic). Prominent examples are the combination of chitosan and tripolyphosphate, alginate and Ca$^{2+}$, as well as chitosan and anionic PS (alginate, hyaluronic acid, carboxymethylated PS derivatives). \[22–24\] PS-NP prepared by ionic crosslinking have already been described comprehensively in the literature and are not part of this review. They are rather hydrophilic materials and consequently have different physical, chemical, and biological properties than the hydrophobic PS-NP described herein.

iii. Supramolecular self-assembling is an approach that is well known for preparing nanomaterials from synthetic polymers. \[25\] It relies on the directed association of macromolecules by weak, noncovalent interaction between two polymers carrying specific complementary functional groups. One common example for this “host-guest” principle is the interaction of β-cyclodextrins with molecules such as adamantanе that match the size of the hydrophobic cavity provided by the cyclic oligosaccharides. \[26\] Among other polymers, dextran and pullulan derivatives with β-cyclodextrin-/adamantane moieties were employed for supramolecular self-assembling into PS-NP. \[27,28\] The supramolecular approach involves considerable synthesis efforts to introduce the host-/guest substituents into the PS backbone and requires at least two components.

iv. The present review focuses on PS-NP that are obtained by hydrophobicity induced self-assembling of hydrophobically modified PS derivatives. This class of nanomaterials received a lot of attention in recent years due to the application potential in biomedicine and the facile preparation. PS can be chemically modified with various types of hydrophobic substituents to induce nano-self-assembling behavior. Contrary to the above described self-assembling by host-guest interaction, no secondary component is required because the process is induced by a solvent exchange. Henceforward, the term “PS-NP” refers to materials that meet this description (unless explicitly specified otherwise). This review will provide a comprehensive overview of basic preparation principles, analytical tools, representative examples, and selected biomedical applications for NP derived from hydrophobic PS derivatives.

2. Preparation Techniques for Polysaccharide Nanoparticles

Several processes can be exploited to shape hydrophobically modified PS derivatives from a dissolved state into NP (Figure 4). The following section will describe the main approaches and highlights their specific benefits and limitations in the light of physicochemical principles.

2.1. Solvent Displacement

In the “solvent-displacement” process (also known as “nanoprecipitation” or “solvent shifting”), a nonsolvent (usually water) is added to a polymer solution in a miscible organic solvent. Thus, solubility of the polymer is gradually decreased and it precipitates as NP. \[29\] This can be performed either by dialysis or by dropping technique. The dialysis method utilizes a membrane to separate polymer solution and water, which allows a slow mixing of both phases. The solvent is gradually removed by several changes of the surrounding dialysate and an aqueous suspension of particles is obtained. \[30\] In the dropping technique, water is added gradually to the polymer
solution under stirring or vice versa the polymer solution is dropped into water. Particles are formed by self-assembling.\cite{31,32} In contrast to the dialysis approach, the solvent has to be removed, e.g., by heating or dialysis, to yield the final aqueous suspension.

Both methods are easy to perform without any specific technical equipment. The dialysis approach is not laborious and applicable for many organic solvents, including non-volatile dipolar aprotic solvents that are known to be excellent solvents for many hydrophobically modified PS derivatives. However, the solvent exchange is intrinsically slow and takes about 1–2 d to yield aqueous particle dispersions that are free of organic solvent. The dropping approach is faster but is preferably employed for solvents with a low boiling point that can easily be removed from aqueous mixtures by evaporation (e.g., THF, acetone), which requires the PS to be soluble in these solvents.\cite{33} Compared to other procedures for PS-NP preparation, the solvent displacement in general is more dependent on the initial polymer concentration as only polymer concentrations below the critical overlap concentration are applicable (see also 4.1).\cite{34} The ionic strength of the aqueous non-solvent can be another limiting factor.\cite{35}

The principle of particle formation by solvent displacement is based on the fluctuations of polymer concentration in the boundary layer between solvent and precipitant during the solvent to nonsolvent exchange.\cite{34,36} The boundary layer is initially the site of the highest supersaturation where interfacial deposition of the polymer is induced.\cite{37,38} The ternary phase diagram of polymer, solvent, and precipitant includes the homogeneous (one phase) region at high content of solvent and the heterogeneous (two phases) region at low content of solvent (Figure 5). Both regions are separated by the binodal. The spinodal within the binodal comprises a region of spontaneous decomposition where only macroscopic aggregates are formed. Between binodal and spinodal there is the metastable region where the polymer forms nuclei upon concentration fluctuations in the supersaturated solution. The so called “Ouzo region” is a rather narrow regime of low polymer concentration within the metastable region where the polymer nuclei aggregate into NP without forming macroscopic precipitates. In the remaining metastable region, microparticles as well as NP are formed from the initial nuclei, because the concentration is high enough to allow the formation of large particles and the coagulation of these particles to even larger microparticles. The starting point in a solvent displacement process is usually a diluted polymer solution (red point in Figure 5). During the process, non-solvent (precipitant) is added to the one phase system consisting of polymer and solvent (red line in Figure 5). The system composition shifts, crosses the binodal at the side

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**Figure 4.** Schematic representation of fundamental approaches for the preparation of polysaccharide nanoparticles by self-assembling of hydrophobic PS derivatives.

**Figure 5.** Ternary phase diagram for a mixture of solvent, polymer, and precipitant. The red point schematically describes a diluted polymer solution as starting point for addition of precipitant. Upon addition, the composition of the system shifts along the red line and finally reaches the “Ouzo region.” Adapted with permission.\cite{38} Copyright 2013, Elsevier B.V.
of low polymer concentration, and stays in the “Ouzo region,” which results in NP formation.

NP preparation by solvent displacement requires two basic conditions. First, the polymer is dissolved in a molecular dispersed state to ensure that the individual polymer chains form spherical nuclei upon reducing solubility in the medium.\textsuperscript{[132]} Thus, a good polymer solvent is required and the initial polymer concentration should be below the critical overlap concentration. If the concentration is higher, polymer chains start to entangle in the solution, which results in irregular aggregation upon solvent exchange. Second, the regeneration of the dissolved polymer has to be slow enough so that the free energy of the system is minimized and equilibrium morphology is reached (“controlled precipitation”). The free interface energy that determines the spherical particle shape is a crucial thermodynamic parameter in this regard.\textsuperscript{[30]}

Two mechanisms influence the particle size and the size distribution, indicated by the polydispersity index (PDI), of the resulting NP in the suspension: i) nucleation-growth and ii) nucleation-aggregation.\textsuperscript{[31]} The initial nucleation of macromolecules is initiated by concentration fluctuations that are induced by mixing of polymer solution and nonsolvent. Growth of the nuclei into NP can occur by capturing macromolecules as the solubility is gradually decreased. The number of nuclei can also increase to an extent where they collide with each other and aggregation into larger particles takes place. Both mechanisms together determine the mean particle diameter and the particle size distribution. They are concentration dependent and generally favor the formation of larger particles when polymer concentration increases.

Solvent displacement approaches for NP formation are low-energy methods. This means that intrinsic physicochemical parameters, including solvent solvation power and the hydrophobic–hydrophilic balance along the polymer backbone, govern the resulting properties of the NP.\textsuperscript{[34,39]} These parameters strongly depend on the molecular structure of the polymers. Moreover, nanoprecipitation enables control of particle properties by varying several extrinsic factors, such as solvent/precipitant ratio, initial polymer concentration, dropping rate, and to a certain extent also physical shearing (i.e., stirring rate).\textsuperscript{[40,41]}

2.2. Nanoemulsions

Nanoemulsions are, just like macroemulsions, thermodynamically unstable yet kinetically stabilized systems.\textsuperscript{[42,43]} The size of the droplets in nanoemulsions, however, is much smaller (about 20–500 nm instead of 1–100 \textmu{}m) with a more narrow size distribution. The formation of nanoscaled emulsions usually requires a high energy input (e.g., ultrasonication, high mechanical shear forces) and surface-active stabilizers (e.g., polyvinyl alcohol, ionic/nonionic surfactants) are added to prevent coalescence of the droplets.

Polymer-based NP have been prepared taking advantage of nanoemulsions as well-defined templates.\textsuperscript{[19,44]} An approach that is solely restricted to synthetic polymer materials is in situ polymerization within the nanovesicles in which case one of the phases usually is the pure monomer. Another possibility is to start with preformed polymers dissolved in an organic solvent. Nanoemulsions are formed with a suitable nonmiscible nonsolvent and the polymer solvent is removed from the nanodroplets (preferably by evaporation) to yield the desired polymer NP dispersion. This approach is suitable for many different polymers, including PS, PS derivatives, and other biopolymers. However, the selection of polymers is dictated by their solubility. For most application purposes, water is the preferred dispersion medium. Thus, only water-immiscible, volatile solvents such as chlorinated hydrocarbons can be employed as polymer solvents, which means that the polymers themselves need to be rather hydrophobic to become soluble.

Nanoemulsion-based approaches offer some unique benefits and limitations for the preparation of PS-NP. Variation of external parameters, such as quantity of energy, sonication time, and type/amount of surfactant, enables a certain control over the size and size distribution of the nanodroplets and in consequence of the final NP because the process is not driven by thermodynamics.\textsuperscript{[39]} Thus, PS-NP with relatively small diameter and narrow distribution are obtainable.\textsuperscript{[34]} It is also possible to employ higher polymer concentrations above the critical overlap concentration. However, nanoemulsion approaches have a higher energy demand (ultrasonication, solvent evaporation) and can be more restrictive in terms of solubility of the PS derivatives. One important aspect of PS-NP prepared from nanoemulsions is that their surface is (at least partially) covered with a stabilizer, whereas PS-NP prepared by solvent-displacement are “naked.” The implications, e.g., for physicochemical properties and surface modification of the particles, have to be considered when comparing these two types of PS-NP.

2.3. Other Processes

A recently emerged process for preparing polymer NP is the so called flash nanoprecipitation.\textsuperscript{[45–52]} A microfluidic device with different inlets (multi-inlet vortex mixer/MIVM) is used that allows simultaneous rapid mixing of several volumetric streams containing, e.g., polymer solution, precipitant, surfactant, and even additional substances (drug, dye, etc.) for encapsulation within the NP.\textsuperscript{[45]} The process enables control over the size and size distribution by adjusting the flow rates of each stream and the turbulence within the device that is expressed by the Reynolds number.\textsuperscript{[46,47]} Flash nanoprecipitation is still rather unexplored in the field of PS-NP but the method has a potential in particular for encapsulation of substances within the particles.

Aerosol flow reactors can be used to prepare polymer NP in a continuous process.\textsuperscript{[45,51]} Therein, a polymer solution is atomized into nanodroplets that are transferred through a heated reactor tube with aid of a carrier gas. The solvent is evaporated and dry NP are collected with an electrostatic precipitator. Stabilizers are not required in this process. The size of the final NP is governed by the initial droplet size (adjustable by surface tension and viscosity of the solution), drying rate of the particles, and the polymer concentration. This technique can be interesting for preparation of dry PS-NP in a continuous way. However, no examples were found where
it was explicitly employed for hydrophobically modified PS derivatives.

3. Characterization of Polysaccharide Nanoparticles

Comprehensive characterization of PS NP is an integral part of the development of new preparation procedures and for the continuous validation of established methods. Information on chemical composition and molecular structure are relevant for confirming purity of the products and to check if chemical conversions occur during formation or storage of the PS-NP. It can be assessed by various spectroscopic methods (e.g., IR-, Raman-, NMR-, UV/Vis-, fluorescence spectroscopy) either on native particles in aqueous dispersion, after drying, or after dissolution of dried particles in an appropriate solvent. Parameters that are particularly relevant for NP are the particle diameter, the size distribution that is measured as PDI, and the zeta potential. Among others, they determine crucial properties for the desired application like cellular uptake, interaction with cellular membranes or DNA molecules, and the half-life time during blood circulation. Information on particle sizes is also crucial for any attempt to quantitatively evaluate the various literature reports on PS-NP in a comprehensive review like the present one, most notably because values obtained by research groups using different methods are frequently not directly comparable. The following chapter will provide an overview of methods that can be used to gain information on the particle size of PS-NP. It is highly recommended to not focus on a single method but to employ complementary techniques in general for a comprehensive characterization.

3.1. Dynamic Light Scattering

One of the most applied characterization methods for PS-NP is dynamic light scattering (DLS). The measurement principle is based on the size-dependent scattering of laser light by a NP dispersion within a measuring cuvette (Figure 6). The scattered light can be detected at a certain angle; usually 13° (forward scattering), 90°, or 173° (back scattering).[54,55] Depending on the size of any scattering object in correlation to the wavelength of the laser light, scattering is described by Rayleigh or Mie theory.[56] The intensity of the scattered light that is detected is subjected to certain fluctuations caused by Brownian motion of the scattering NP during the measurement. These fluctuations are expressed by a correlation function $G(\tau)$ in the form of an integral of products of intensities $I$ at time $t$ and delayed time $(t + \tau)$[54]

$$ G(\tau) = \langle I(t)I(t+\tau) \rangle $$

or in its normalized form

$$ g(\tau) = \frac{\langle I(t)I(t+\tau) \rangle}{\langle I(t) \rangle^2} $$

The normalized correlation function $g(\tau)$ is linked with the electric field by the Siegert relation. Assuming monodisperse

Figure 6. Schematic principle of the determination of particle sizes by dynamic light scattering.
NP, the correlation factor of the electric field decays exponentially, which yield the following equation after substitution of the related decay constant\[54\]

$$g(\tau) = 1 + \beta e^{-2Dq^2/\tau}$$ (3)

where $\beta$ is the coherence factor, $D$ is the diffusion coefficient, and $q$ is the Bragg wave vector. Thus, the measured intensity fluctuation correlates with the diffusion of NP that directly depends on the hydrodynamic particle radius $R$ according to the Stokes-Einstein equation

$$D = \frac{k_BT}{6\pi\eta R}$$ (4)

where $k_B$ is the Boltzmann constant, $T$ is the temperature, and $\eta$ is the viscosity of the medium. A cumulant analysis is performed to calculate the particle sizes and PDI from the measured DLS data.\[54,55,57\] Alternatively, an inverse Laplace transform (CONTIN analysis) can be employed to gain the particle sizes.\[55\] For assessment of DLS results, it should be noted that the values that are generated represent the size of a hypothetical perfectly spherical object that diffuses in the same way as the examined particles. The method provides no information about the actual particle shape unless angular-dependent measurements are carried out. Moreover, the hydrodynamic diameter is measured, i.e., the diameter of a solvated particle including any shell of solvent molecules. DLS is of limited accuracy when investigating polydisperse samples (PDI $> 0.2$) and CONTIN analysis has to be employed in these cases.\[55,56\] Moreover, multimodal particle distributions with mean diameters close to each other ($\lesssim 30$ nm) cannot be distinguished. The sample must not absorb at the wavelength of laser light.\[58\]

### 3.2. Electron Microscopy

While DLS is useful for particle size measurements of NP in aqueous suspension, size and shape of dried NP can be characterized by electron microscopic experiments (Figure 7). In electron microscopy, a sample is scanned in high vacuum by an electron beam. Interaction of the scanned object with electrons is used for image generation. Usually, secondary electrons are detected with this method (scanning electron microscopy/SEM) but it is also possible to detect transmitted electrons after their transition behind the sample (transmission electron microscopy/TEM).\[59\] Electron microscopy provides information on size, size distribution (by software assisted image analysis), as well as shape of NP in their dried state, i.e., without solvent layer. TEM additionally allows insight into the inner structure of particles (e.g., core–shell morphologies).\[60\]

For sample preparation, diluted NP dispersions (about 0.01–0.1 mg mL\(^{-1}\)) are dried on a planar carrier material (usually mica surface). Thus, this method does not display particles in their previous state in suspension. Interaction of organic samples with the electron beam is rather weak because light atoms such as hydrogen, carbon, and nitrogen exhibit only a low electron density. For this reason, PS-NP have to be covered with a thin metal layer (e.g., platinum, gold) by sputtering.\[58\] Cryo-TEM enables characterization of NP in suspension close to their native state. The sample is plunge-frozen in liquid gases (e.g., nitrogen, ethane) upon which the liquid phase solidifies in an amorphous state. Subsequently, the frozen samples can be studied by electron microscopy (transmission mode). This method avoids structural changes of particles caused by drying or sputtering. In general, electron microscopy requires a high experimental effort and is not the method of choice for routine characterization of larger sample batches. DLS is more suited in this regard. When both methods are compared for the very same NP sample, DLS measurements should provide higher values because they assess solvated (and potentially swollen) particles whereas SEM images are taken for dried (and potentially shrunken) particles.

### 3.3. Atomic Force Microscopy

In atomic force microscopy (AFM), a very fine tip (ideally only one atom) is moved in close proximity to the surface along a fixed $x$–$y$-direction-raster of a sample. The potential energy between tip and sample results in a force in $z$ direction that is measured to get a topographic image of the sample surface. Different components can contribute to this force, including short-range...
chemical forces (fractions of nm) and long-range forces (up to 100 nm) such as van der Waals-, electrostatic-, and magnetic interaction. The force is sensed by measuring the deformation of a piezoelectric cantilever with a well-defined spring constant in z direction. Alternatively, the distance is kept constant and the force is measured as a function of the modulated piezoelectric current. Images can be recorded in contact mode (close proximity, primarily repulsive forces) or non-contact mode (higher distance to surface, primarily attractive forces). Moreover, AFM can be operated in a static mode or with an oscillating cantilever (amplitude or frequency modulation). [59]

AFM has a very high resolution, even down to atomic levels, and is a well-suited method for characterization of nanoassemblies (Figure 7c). Unlike 2D electron microscopy, it also provides a 3D topography with parameters like particle volume, aspect ratio, and height. [33,62,63] Moreover, mechanical properties like stiffness or elastic modulus can be probed. [33,63,64] However, sample shape perturbations may occur by tip-broadening effects leading to artifacts. [63] Recorded shapes may differ from the cross section measured by TEM. Thus, combination of AFM experiments with complementary DLS and electron microscopy methods is recommended.

3.4. Field-Flow Fractionation

Field-flow fractionation (FFF) is a group of hydrodynamic separation methods used for polymers, biomacromolecules, and NP. [65–67] The samples are injected into a capillary and separation methods used for polymers, biomacromolecules, and NP. [65–67] The samples are injected into a capillary and separation is induced by a “separation field” perpendicular to the laminar flow of the mobile phase. Different subtypes of FFF have been described based on the physical nature of the separation field including sedimentation-, gravitational-, thermal-, electrical-, and the most widely used technique flow-FFF (referred to as 4F). The separation principle of 4F is based on a second flow of mobile phase that is applied perpendicular to the longitudinal, laminar flow. This second flow (cross-flow) drives the particles to one side of the channel capillary that is equipped with a semipermeable membrane (accumulation wall). Due to Brownian motion, particles will constantly detach and move further along the capillary towards the outlet. Under “normal elution” conditions (Brownian elution mode), smaller particles, which have a higher diffusion coefficient, will move further toward the center where the flow rate is higher, i.e., they will elute first. However, this can shift depending on the channel geometry if a certain size (≥1 μm) is exceeded (steric elution mode). Different channel designs have been developed that also differ in how the sample is injected in the system. Asymmetric 4F is the most popular method (Figure 8). For more details, the reader is forwarded to the literature in which the theoretical backgrounds and experimental details are described. [65–67] FFF is rarely employed for comprehensive characterization of PS-NP although it has great potential in this regard. [68] It is an automated high throughput method and can be coupled with many different detector systems that can provide additional information on molecular structure and morphology, including DLS, multangle laser light scattering, small-angle X-ray scattering, spectroscopy (e.g., NMR, fluorescence), mass spectrometry, and electron/optical microscopy.

3.5. Zeta Potential

PS-NP dispersions need to be stable against aggregation and sedimentation. The energetic interaction within these colloid systems can be described by the DLVO theory (named after Derjaguin, Landau, Verwey, and Overbeek; Figure 9). [69] Two approaching NP exhibit attractive (van der Waals interaction) and repulsive forces (electrostatic interaction) that increase at different scales with decreasing particle distance. This results in a maximum of potential energy at short particle distance responsible for colloidal stability. An additional steric stabilization effect might be induced by more or less flexible macromolecules at the particle surface. [70]

In liquid phase, particles with a net surface charge form an electric double layer together with ions of the surrounding medium that consist of i) an inner layer (Stern layer) in which counter ions are tightly adsorbed on the surface and ii) an outer layer (the diffuse layer) in which the ions are only loosely bound (Figure 9). [71] When brought into an electric field, colloid particles will move directionally and parts of the diffuse layer are sheared off. The electric potential at this hydrodynamic shear plane is defined as the zeta potential. The zeta potential ζ of NP dispersions is determined by measuring the velocity v of the particles in an external electric field E using laser Doppler anemometry. [56,72] Based on some model assumptions (negligible surface conduction and
polarization of the electric double layer, large diameter and thin ion shell), the following equation can be employed for calculating the zeta potential

\[ \zeta = \frac{v \cdot \eta}{E \cdot \varepsilon_0 \varepsilon} \]  

(5)

where, \( v \) is the velocity of particles, \( \eta \) is the viscosity of the medium, \( E \) is the electric field, and \( \varepsilon_0 \varepsilon \) is the permittivity.\[73\]

Several factors can contribute to the net charge at a PS-NP surface, which is the basis for developing a pronounced zeta potential. Dissociation of ionogenic surface groups or adsorbed molecules results in a charged particle surface.\[74\] For non-ionogenic polymers, preferential adsorption of certain ions on the particle surface is a major contribution.\[75\] If other electrolytes are absent in the aqueous medium, also hydroxide ions can adsorb, which results in a negative surface charge.

The zeta potential is affected by the pH value and ionic strength of the medium as well as by the particle size and particle concentration.\[56,69\] At acidic pH values, adsorption of protons is favored due to their increasing concentration (zeta potential becomes more positive). The reversed effect occurs at basic pH values due to higher concentration of hydroxide ions. At a certain pH value (isoelectric point), the zeta potential might become zero, which results in unstable colloids and particle aggregation. An increasing electrolyte concentration in the medium generally leads to shielding of electric charges. This results in compression of the electric double-layer until ultimately the electrostatic repulsion becomes too small and the colloid coagulates.\[76\] Polymeric NP are “soft materials” and their surface is usually not completely smooth and homogeneous. Individual “polymer hairs” might extend into the surrounding medium and these polymer chains might collapse with increasing electrolyte concentration.\[70,77\] As a consequence, the shear plane shifts closer to the particle surface and the zeta potential increases to a certain extent.

Colloid particles with an absolute value for the zeta potential larger than about 30 mV (i.e., \( \zeta > +30 \text{ mV} \) or \( \zeta < -30 \text{ mV} \)) are usually considered as being stable against aggregation and sedimentation.\[56\] It should be noted, though, that the zeta potential is not the only factor that defines colloidal stability. Stable PS-NP with zeta potentials around 0 mV are possible with a sufficient steric stabilization or by adsorption of proteins on the particle surface.\[33,78,79\]

In the following chapters and the comprehensive Tables 1 and 2, which summarize examples for PS-NP from the literature, zeta potential values will be included. However, many studies in this area seem to underestimate the added value of zeta potential measurements. They can provide valuable information on the surface properties of PS-NP. The zeta potential can also affect the interaction of PS-NP with proteins and cells, which is of great relevance in many biomedical applications.

4. Polysaccharide-Based Nanoparticles

The basic procedures for particle preparation, which are described in chapter 2, have been employed on a vast variety of hydrophobic PS derivatives with various molecular structures in terms of PS backbone and hydrophobic substituents (Table 1, Figure 10). This chapter will provide a general, qualitative overview of how PS-based NP with tailored properties can be obtained depending on i) the chemical modification of the PS and ii) the methods employed for particle formation. Many studies deal with PS-NP from cellulose or dextran but also other PS are frequently employed. For a systematic overview, the following chapters were subdivided based on the primary polymer backbone of the hydrophobic derivatives used. Typical examples from literature are presented. In this context, it should be noted that direct quantitative comparison between different studies obtained from different research groups can be difficult. The particle properties subtly depend on many process parameters that are difficult to reproduce between different individual labs.
Table 1. Literature overview of nanoparticles of hydrophobically self-assembled polysaccharide derivatives.

| Method  | Polysaccharide backbone | Substituent | DS \(^{(1)}\) | Additive \(^{(2)}\) | Particle properties \(^{(4)}\) | Refs. |
|---------|-------------------------|-------------|----------------|-----------------|-----------------------------|-------|
|         |                         |             |                |                 | Size [nm] | PDI   | ζ [mV] |       |
| Dialysis| Cellulose Acetate       | 1.65 to 3.00| 180 to 240     | 0.08 to 0.23    | –            | [32] |
|         | Cellulose Acetate       | 2.30        | 280 to 390     | 0.11 to 0.14    | –32 to –28 | [96] |
|         | Cellulose Acetate       | 0.18        | 400            | 0.10            | –            | [32] |
|         | Cellulose Propionate    | 2.40        | 370            | 0.25            | –            | [32] |
|         | Cellulose Acetate       | 0.16        | 2.54           |                | –            |       |
|         | Cellulose Acetate       | 1.8         | 295–485        | 0.08–0.14       | –46 to –34  | [96] |
|         | Cellulose Stearate      | 2.99        | 250–530        | 0.02–0.05       | –45          | [40,186,187] |
|         | Dextran Cyoanoethyl     | 2.4         | 200            | –               | –            | [123] |
|         | Dextran Ethyl carbonate | 0.71–2.80   | 150–600        | –               | –            | [116] |
|         | Dextran Propyl carbonate| 1.07–2.77   | 150–600        | –               | –            | [116] |
|         | Dextran Butyl carbonate | 0.35–3.00   | 150–600        | –               | –            | [116] |
|         | Dextran Propionate      | –           | 60–670         | –               | –            | [188] |
|         | Dextran Tryptophanate   | –           |                |                | –            |       |
|         | Dextran Propionate      | 1.70        | 200–600        | –               | –            | [111] |
|         | Dextran Pyroglutamate   | 0.26        |                |                |              |       |
|         | Dextran Propionate      | 1.04        | 100            | –               | –            | [113] |
|         | Dextran Pyroglutamate   | 1.96        |                |                |              |       |
|         | Dextran Propionate      | 1.75        | 460            | –               | –            | [113] |
|         | Dextran Pyroglutamate   | 1.13        |                |                |              |       |
|         | Dextran Furoate         | 0.12        |                |                |              |       |
|         | Dextran Pyroglutamate   | 1.27        | 515            | –               | –            | [112] |
|         | Dextran Furoate         | 0.79        |                |                |              |       |
|         | Dextran Propionate      | 0.87–1.51   | 250–450        | –               | –            | [112] |
|         | Dextran Pyroglutamate   | 1.27–1.34   |                |                |              |       |
|         | Dextran Furoate         | 0.22–0.79   |                |                |              |       |
|         | Dextran Propionate      | 1.70–3.00   | 90–445         | –               | –            | [154] |
|         | Dextran Pyroglutamate   | 0.26–1.96   |                |                |              |       |
|         | Dextran Propionate      | 1.98        | 100            | –               | –            | [154] |
|         | Dextran Furoate         | 1.02        |                |                |              |       |
|         | Dextran Pyroglutamate   | 1.27        | 520            | –               | –            | [154] |
|         | Dextran Furoate         | 0.79        |                |                |              |       |
|         | Dextran Propionate      | 1.87–2.78   | 200–500        | –               | –            | [154] |
|         | Dextran Pyroglutamate   | 0.72–1.34   |                |                |              |       |
|         | Dextran Furoate         | 0.12–0.79   |                |                |              |       |
|         | Dextran Tosylate        | 1.02        | 420            | –               | –            | [162] |
|         | Dextran Tosylate        | 1.02        | 160            | –               | –            | [162] |
|         | Dextran Propionate      | 1.98        |                |                |              |       |
|         | Dextran Decanoate       | 0.23        | 280            | 0.10            | –            | [189] |
|         | Dextran GφP-PLG         | M5: 1.7–6.2 | 110–230        | –               | –            | [190] |
| Pullulan | Acetate                | 2.6         | 285            | –               | –18          | [132] |
| Pullulan | Acetate                | 1.85        | 60–125         | –               | –            | [35] |
Table 1. Continued.

| Method(1) | Polysaccharide | Additive(1) | Particle properties(1) | Refs. |
|-----------|----------------|-------------|------------------------|-------|
|           | Backbone | Substituent(2) | DS(1) | Size [nm] | PDI | \(\zeta\) [mV] | |
| Pullulan  | Acetate | 1.17 | 120 | – | – | [136] |
| Pullulan  | Acetate | 1.17 | 55–140 | – | – | [136] |
| Pullulan  | ODSM | 0.23–1.03 | – | – | – | – |
| Pullulan  | Acetate | 1.16 | 80–125 | – | – | [134] |
| Pullulan  | Biotin | 0.07–0.39 | – | – | – | – |
| Pullulan  | Acetate | 0.7–0.8 | 130–415 | – | – | [191] |
| Pullulan  | Acetate | – | \(~80\) | – | – | [192] |
| Pullulan  | Cholesterol | 0.05 | \(~60\) | – | – | [193] |
| Pullulan  | Cholesterol | 0.05 | 100–180 | 0.15–0.21 | –10 to –4 | [194] |
| Cellulose | Acetate | 2.46 | 90–240 | 0.04–0.27 | – | [32] |
| Cellulose | Acetate | 2.49 | 225–710 | 0.06–0.62 | – | [34] |
| Cellulose | Acetate | 2.50 | T-20, SDS, HTAB, PF-68 | 60–220 | 0.19–0.28 | –39 to –7 | [41] |
| Cellulose | Acetate | 2.50 | 120 | 0.21 | –37 | [84] |
| Cellulose | Acetate | – | T-20, T-80, M-52, SHS-15, KP, PF-68, PF-127 | 85–135 | 0.16–0.26 | –22 | [101] |
| Cellulose | Acetate | – | PVA + HCl | 265–370 | <0.12 | – | [100] |
| Cellulose | Acetate | 0.37 | 260 | – | – | [86] |
| Cellulose | Tosylate | 1.03 | – | – | – | – |
| Cellulose | Hexanoate | 0.26 | 220 | – | – | [86] |
| Cellulose | Tosylate | 1.04 | – | – | – | – |
| Cellulose | Stearate | 2.95–3.00 | 80–395 | 0.03–0.18 | –51 to –45 | [40,186,187] |
| Cellulose | Stearate | 0.3–3.0 | 85–200 | 0.04–0.13 | –48 to –27 | [85] |
| Cellulose | Laurate | 3.0 | 95 | 0.09 | –48 | [85] |
| Cellulose | Hexanoate | 3.0 | 80 | 0.17 | –54 | [85] |
| Cellulose | Ethoxy | 48 wt% | 40–135 | – | –59 to –50 | [87–91] |
| Cellulose | Acetate | – | T-20, T-80, M-52, SHS-15, KP, PF-68, PF-127 | 85–135 | 0.16–0.26 | –22 | [101] |

Phthalate

Dextran | PDP | 0.05–0.19 | 120–305 | – | – | [166] |
| Method| Polysaccharide | Additive\(^b\) | Particle properties\(^d\) | Refs. |
|-------|----------------|----------------|---------------------------|-------|
|       | Backbone       | Substituent\(^c\) | DS\(^i\) | Size [nm] | PDI | \(\zeta\) [mV] |       |
| Dextran| CAR            | 0.05            | –           | 90      | –   | –           | [166] |
| Dextran| Stearate       | 0.07            | –           | 25      | –   | –           | [166] |
| Dextran| Cholesterol    | 3 wt%           | –           | 125     | –   | –           | [121] |
| Dextran| Graft-PLA      | 4–8 wt%         | –           | 80–100  | –   | –           | [121] |
| Dextran| Graft-PLA      | 0.45            | –           | 140–225 | 0.05–0.13 | – | [122] |
| Dextran| Graft-PCL      | 0.52–0.76       | –           | 100–350 | –   | –           | [119] |
| Dextran| Benzoate       | 0.61–1.62       | –           | 50–150  | –   | =0          | [33]  |
| Dextran| Methacrylate   | 0.14–0.31       | –           | –       | –   | –           |       |
| Dextran| Hexanoate      | –               | –           | –       | –   | –           | [33]  |
| Dextran| Methacrylate   | –               | –           | –       | –   | –           | [33]  |
| Dextran| 2-Hydroxy-3-phenoxypropyl | 1.30–2.10  | None       | 60–170  | –   | –           | [124] |
| Dextran| 2-Hydroxyoctyl | 3.00            | Phenoxydextran | 75–215 | –   | –           | [124] |
| Dextran| 2-Hydroxydodecyl | 0.52–1.64 | Phenoxydextran | 180–300 | – | –           | [124] |
| Pullulan| Acetate       | 2.7–3.0         | PVA         | 190–425 | –   | –           | [130] |
| Pullulan| Acetate       | 2.7             | PVA         | 185     | –   | –           | [131] |
| Pullulan| Acetate       | 2.6             | =250        | =0.18   | =3  | –           | [133] |
| Pullulan| Acetate       | 0.8             | =80         | –       | –   | –           | [199] |
| Pullulan| Benzoate      | –               | –           | –       | –   | –           | [33]  |
| Pullulan| Methacrylate  | –               | –           | –       | –   | –           |       |
| Pullulan| Cholesterol   | 0.02            | =20–30      | –       | –   | –           | [139,140] |
| Pullulan| Cholesterol   | 0–0.1           | 135–235     | 0.11–0.20 | =4.5 | –           | [200] |
| Pullulan| Hydrophobic lysin dendrons | 0.2 | MS ≈0.1 | 55 | 0.13 | – | [201] |
| Starch | Benzoate      | –               | –           | –       | –   | –           | [33]  |
| Starch | Methacrylate  | –               | –           | –       | –   | –           |       |
| Starch | Graft-PEG     | 78–90 wt%       | 130–165     | –       | –   | –           | [150] |
| Starch | Acetate       | 2.62–2.97       | 80–720      | 0.01–0.28 | – | –           | [144] |
| Starch | Acetate       | 2.58–2.78       | 150–850     | 0.08–0.15 | – | –           | [202] |
| Starch | Palmitate     | 0.005–0.20      | –           | –       | –   | –           |       |
| Starch | Acetate       | 2.41            | 150–230     | –       | –   | –           | [185] |
| Starch | Graft-PNIPAAM | 26 wt%          | –           | –       | –   | –           |       |
| Starch | Acetate       | 2.3             | 270         | 0.13    | –   | –           | [147] |
| Starch | Allyl         | 0.48            | –           | –       | –   | –           |       |
| Starch | Acetate       | 2.34            | NaCl        | 360–2905 | 0.17–0.46 | =–22 to –9 | [203] |
| Starch | Phthalate     | 0.35            | –           | –       | –   | –           | [149] |
| Starch | Hydroxyethyl  | 0.5             | 20–350      | –       | –   | –           | [199] |
| Starch | Laurate       | MS = 0.1        | –           | –       | –   | –           |       |
| Emulsion| Cellulose     | Acetate         | PVA         | 200–255 | 0.02–0.10 | – | [34] |

\(^a\) Method and Polysaccharide structure. \(^b\) Additive and concentration. \(^c\) Backbone and Substituent. \(^d\) Particle properties: Size [nm], PDI, \(\zeta\) [mV]. \(^e\) Refs. [121, 122, 119, 124, 130, 131, 133, 199, 33, 139, 140, 200, 201, 150, 144, 202, 185, 34].
4.1. Cellulose-Based Nanoparticles

Cellulose is one of the most renowned PS and highly abundant in nature. \[^{80}\] So called “nanocelluloses” (cellulose nanocrystals, nanofibrillated cellulose) are intensively studied in various scientific areas with potential applications of industrial relevance. \[^{15-19,81}\] However, these materials are fundamentally different from the nanomaterials described in this review. They are prepared in a top-down approach by chemical or mechanical disintegration of cellulosic fibers as opposed to the bottom-up self-assembling described in this review. The nanocellulosic bulk material is mostly unmodified, which results in overall hydrophilic properties. Subtle chemical modification occurs at the fiber surface only.

The molecular structure of cellulose is very uniform and composed of $\beta$(1$\rightarrow$4) glucopyranosyl units that are accessible for chemical modification (Figure 10), most notably esterification, etherification, nucleophilic displacement reactions, and oxidation. \[^{82}\] Cellulose esters, particularly cellulose acetate (CA), are the most frequently employed derivatives for PS-NP formation.

### Table 1. Continued.

| Method (a) | Polysaccharide | Additive (b) | Particle properties (c) | Refs. |
|-----------|----------------|-------------|-------------------------|-------|
| Cellulose | Acetate        | 0.10        | PVA                     | 210   | 0.05 | – | [34] |
| Cellulose | Propionate     | –           | –                       | –     | –    | – | – |
| Cellulose | Acetate        | 0.15        | PVA                     | 215   | 0.06 | – | [34] |
| Cellulose | Butyrate       | 2.10        | –                       | –     | –    | – | – |
| Dextran   | Graft-PCL      | 0.10–0.23   | SC                      | 190–210 | <0.17 | –30 to –18 | [118] |
| Dextran   | Graft-PCL      | 9–94 wt%    | PVA                     | 140–190 | 1.34–1.93 | –29 to –13 | [120] |
| Dextran   | Graft-PCL      | 0.18        | SC                      | 70\(^{PDA}\) | –    | – | [117] |
| Dextran   | Acetel         | –           | PVA/PBS                 | 220–245 | –    | – | – |
| Dextran   | 2-Hydroxydodecyl | 1.64       | Phenoxypolymer          | 125   | –    | – | – |
| Dextran   | 2-Hydroxy-3-phenoxypolymer | 0.65–2.10... | – | – | – |
| Dextran   | 2-Hydroxyoctyl | 0.85–3.00   | None                    | 370–850 | –    | – | – |
| Dextran   | 2-Hydroxyoctyl | 0.85–3.00   | None                    | 370–850 | –    | – | – |
| Starch    | Phosphate      | –           | –                       | –     | –    | – | – |
| Starch    | Phosphyl       | 1.05–1.45   | PVA                     | 150–185 | 0.08–0.12 | –8 to –6 | [148] |
| Starch    | Deoxycholate   | 0.05–0.09   | PBS                     | 185–250 | –    | –23 to –16 | [206] |
| AeFR      | Cellulose      | Graft-PAAM  | 58.9 wt%                | 170   | 1.4\(^{GSD}\) | – | [53] |
| Cellulose | Graft-PDMAAM   | 45.0 wt%    | 120                     | 1.4\(^{GSD}\) | – | – | [53] |
| Cellulose | Graft-PDMAAM   | 81.0 wt%    | 150                     | 2.1\(^{GSD}\) | – | – | [53] |
| Micr      | Cellulose      | Butyrate    | 1.64                    | 200   | 0.19 | –41 | [46] |
| Micr      | Acetate        | 0.44        | –                       | –     | –    | – | – |
| Micr      | Carboxymethyl  | 0.33        | –                       | –     | –    | – | – |
| Cellulose | Acetate        | –           | 320                     | 0.37  | –30 | – | [46] |
| Cellulose | Propionate     | –           | –                       | –     | –    | – | – |
| Cellulose | Acetate        | –           | 370                     | 0.45  | –35 | – | [46] |
| Cellulose | Butyrate       | –           | –                       | –     | –    | – | – |
| Reac-dial | Cellulose      | Trimethylsilyl | 1.35–1.94          | 175–265 | 0.11–0.28 | – | [105] |
| Reac-drop | Cellulose      | Acetate     | 3.0                     | 40–110 | –    | – | – |
| 15-Pyrene | End group      | –           | –                       | –     | –    | – | – |

\(^{a}\)AeFR, aerosol flow reactor; Diss, spontaneous self-assembly after dissolution; Micr, microfluidic channel, Reac-Dial, reaction dialysis, Reac-Drop, reaction dropping technique; \(^{b}\)15-Pyrene, 1-deoxy-1-N(15-(1-pyrenebutylamino)-pentadecanoyl)amino, CAR, 2-(3-pentadec-7-enyl)phenoxoyacetate; graft-PAAM, graft-polyacrylamide; graft-PCL, graft-poly(ε-caprolactone); graft-PDMAAM, graft-poly(N,N-dimethylacrylamide); graft-PEG, graft-poly(ethylene glycol); graft-PCA, graft-poly(glutamic acid); graft-PLA, graft-poly(lactic acid); graft-PLG, graft-poly(ε-lactide-co-glycolide); graft-PNiPAM, graft-poly(N-isopropylacrylamide); OSDM, oligo(methacryloyl sulfadimethoxine); PDP, 2-(3-pentadecyloxyethyl)phenoxyacetate; \(^{c}\)DS, degree of substitution; weight percentage is provided of no DS values were available, –: no information available; \(^{d}\)HTAB, hexadecyltriethylammonium bromide; KP, Kolliphor TPGS; M-52, Myrj 52; PBS, phosphate buffered saline; PF-68, Pluronic F-68; PF-127, Pluronic F-127; Phenoxypolymer, 2-hydroxy-3-phenoxypolymer dextran; PVA, polyvinyl alcohol; S-80, Span 80, SC, sodium cholate; SDS, sodium dodecyl sulfate; SHS-15, Solutol HS 15; T-20, Tween 20; T-80, Tween 80; \(^{e}\)Particle size (determined by dynamic light scattering/DLS or electron microscopy), polydispersity index (PDI), and zeta-potential ($\zeta$), GSD, geometric standard deviation.
formation by hydrophobically induced self-assembling. Esterification allows for facile modification of hydroxyl groups, and thus enables adjustment of the hydrophobic–hydrophilic balance of PS derivatives. In addition, several hydrophobic cellulose ethers, mixed cellulose ether esters, and cellulose graft-copolymers have been employed for the preparation of cellulose-based PS-NP. A comprehensive compilation of literature examples is provided in Table 1. In the following chapter, selected examples will be presented that provide a broad overview of this topic.

Fundamental investigations of NP formation were performed using commercially available CA, cellulose acetate propionate (CAP), cellulose acetate butyrate (CAB), and cellulose acetate phthalate (CAPh) as well as CA that was prepared under lab conditions.[32] PS-NP in the range of 85 to 400 nm were obtained by dialysis and dropping technique. The overall degree of substitution (DS) but also the distribution of substituents showed a very pronounced effect on the self-assembling behavior. CA with DS of 2.05 and uniform substitution pattern formed well-defined PS-NP by dialysis (diameter ≈ 170 nm, polydispersity index/PDI ≈ 0.1), whereas only macroscopic aggregates were obtained in case of products with a predominant acetylation at C-2 and C-6. It can be speculated that the state of dissolution is different for nonregioselective and regioselective cellulose esters. Surprisingly, fully acetylated samples (DSacetate = 3.00) only showed aggregation, which indicates that a certain hydrophilic/hydrophobic balance is required for preparing PS-NP.[83]

Particle preparation by dropping technique was evaluated for CA and it was observed that dropping an acetone solution of the polymer into water generally yields smaller particles (Ø ≈ 85 to 140 nm) compared to the vice versa approach of dropping water into the polymer solution (Ø ≈ 160–240 nm).

In a detailed examination of nanoprecipitation techniques, the critical overlap concentration was highlighted as the limit above which irregular precipitation occurs (Figure 11).[34] It was also
demonstrated for a series of cellulose esters (acetates, propionates, butyrates) that the emulsion-evaporation approach is very useful for obtaining PS-NP with a very narrow size distribution (PDI ≈ 0.02–0.10). A microfluidic channel device has also been employed to prepare PS-NP from mixed esters CAP and CAB.[46] These materials had a rather large diameter (Ø ≈ 350 nm) and a wide particle distribution (PDI ≈ 0.5).

A modified dropping technique for preparing CA-based NP was proposed using a mixture of water/THF (nonsolvent/solvent) as dispersion medium (instead of water) in combination with ultrasonication and surfactants.[41] Relatively small particles (≤100 nm) could be obtained and the size was shown to depend on stirring velocity (250–500 rpm) and temperature (5–30 °C) in the process (Figure 12). In addition, this process was employed to obtain composite PS-NP by combining hydrophobic CA with various hydrophilic PS derivatives, including hydroxyethyl cellulose, carboxymethyl cellulose, chitosan, and amino cellulose.[84]

In addition to cellulose esters with rather short alky moieties (acetates, propionates, butyrates), fatty acid esters of cellulose (caproates, laurates, stearates) have been employed for preparing PS-NP preferably by the dropping technique.[85] Contrary to CA, also fully acylated derivatives (DS ≈ 3) could be employed and it was found that the particle diameter strongly increased from 60 nm (C6) to 95 nm (C10) and finally to 200 nm (C18) with increasing length of the alky chain. Moreover, decreasing the degree of polymerization of cellulose stearates (DS ≈ 3.0) from 212 via 157 to 36 resulted in a stepwise decrease in particles size from 200–185 nm and finally to 155 nm. In particular at high DS and length of the alkyl chain, PS fatty acid esters have rather low melting points (≤100 °C) when compared to short chain PS esters (≥200 °C). This thermal behavior can also affect the structure of PS-NP. It was reported that the diameter and spherical shape of PS-NP obtained from cellulose stearate (DS ≈ 3) was nearly independent from the temperature while the light reflectance of the particle dispersions dropped significantly upon heating from 25 °C to 75 °C. This was ascribed to a change from crystalline solid NP (25 °C) to amorphous nanodroplets (75 °C).[85]

Figure 11. (Top) Particle size (z-Average) and polydispersity index (PDI) of nanoparticles obtained by dialysis of solutions of cellulose acetates with a degree of acetylation of a) 2.95 or b) 2.49 in N,N-dimethylacetamide at different mass concentrations (cw). (Bottom) Reduced viscosity (η_red) and critical overlap concentration (c*) for the same solutions. Reproduced with permission.[34] Copyright 2013, Springer Science Business Media Dordrecht.

Figure 12. Dependency of particle diameter on temperature and stirring velocity of cellulose acetate nanoparticles prepared by dropping technique. Adapted with permission.[41] Copyright 2010, Elsevier B.V.
Most literature on cellulose-based PS-NP describes the use of carboxylic acid esters for the hydrophobic self-assembly process. Nevertheless, several other types of derivatives have also been employed such as cellulose-graft-copolymers with polyacrylamide and poly(N,N-dimethylacrylamide) side chains as well as mixed cellulose esters with a high content of aromatic tosylate groups (DS = 1.0) and a low content of aliphatic acetate or hexanoate moieties (DS = 0.3 to 0.4).[^53,^86] Cellulose ethers have also been evaluated for preparation of PS-NP because they are commercially available and well accepted in food and pharma applications.[^87] NP derived from ethyl cellulose were prepared by the dropping technique. Using different PS solvents, the particle size was varied from “medium” (100–200 nm from acetone) to “small” (20–50 nm from isopropanol).[^88–^91] A series of different drug-loaded ethyl cellulose-based particles with sizes ranging from 120 to 1055 nm were prepared using this approach (see chapter 5.2.).[^62,^92–^94] Hydroxyethyl cellulose as well as hydroxypropyl cellulose, which were additionally esterified with oloxoain (DS = 0.5 and 0.7), were used to prepare PS-NP with sizes around 100 to 250 nm by the dialysis approach.[^95]

PS-NP featuring reactive groups in addition to the hydrophobic substituents are of high interest for obtaining responsive and/or functional nanomaterials suited for advanced applications (see also chapter 5.1.). Hydrophobic cellulose derivatives with an additional carboxyl group, such as CAPh, can be further modified by covalent or electrostatic immobilization of additional functionalities.[^96–^98] One of the first reports on CAPh-based NP described particles with sizes in the range of 150 to 200 nm produced by an emulsion-evaporation process.[^99] PS-NP prepared by dialysis of CAPh solutions in DMA showed a similar size range (Ø ≈ 290–485 nm) but a more negative surface charge (ζ ≈ −40 mV) in comparison to CA-based PS-NP (Ø = 280–370 nm; ζ = −30 mV) prepared under the same conditions.[^96] Several publications described the use of CAPh as a matrix for the encapsulation of drugs (e.g., dexamethasone, efavirenz, dolutegravir, fluorouracil) or dyes (rhodamine 6G, 2-aminooantraquinone, sudan red, sudan black) within composite PS-NP.[^96,^97,^100–^102] The compounds were simply added to the cellulose ester solution and incorporated within the particles, which could result in drastic changes of the particle properties depending on the amount and compatibility of the additional compounds within the cellulose ester matrix. In addition to CAPh, CA-based mixed esters with aliphatic dicarboxylic acids (adipate, sebacate) have been reported for the preparation of PS-NP.[^51]

Carboxymethylated cellulose esters were studied for the preparation of PS-NP because they also provide a reactive ionic group that can be exploited for further functionalization. Particles with a size of about 200 nm were prepared from carboxymethyl CAB using a microfluidic device.[^46] Thereby, the particle size increased linearly with the Reynolds number (Figure 13). In a series of similar studies, acetylated carboxymethyl cellulose was further modified by grafting poly(ethylene glycol) (PEG) chains as well as various hydrophobic drugs (podophyllotoxin, docetaxel, or cabazitaxel) onto the carboxyl group of the repeating unit. The derivatives obtained were shaped into PS-NP with diameters from about 20–200 nm depending on the PEG- and drug content.[^49,^50,^52,^103] Either microfluidic devices or flash nanoprecipitation were employed. These two techniques were also used for physical incorporation of drugs into carboxymethyl CAB-based PS-NP with diameters of about 100–200 nm, which proved to be advantageous over dropping techniques for this kind of hydrophobic cellulose esters with an additional anionic ether group.[^46,^48,^51]

Carboxyl group containing PS-NP can be functionalized with amino group containing target molecules (e.g., dyes, proteins, antibodies), which results in the formation of chemically stable amide bonds. Therefore, multistep in situ activation procedures such as carbodiimide mediated coupling are required. In a similar fashion, PS-NP with amino groups on the surface can potentially be converted with carboxyl group containing molecules after in situ activation. Alternatively, highly reactive isocyanate and thiocyanate reagents can be used to functionalize PS-NP with amino groups. As an example, PS-NP derived from cellulose carbamates with additional 6-deoxy-6-(aminoalkyl)amino moieties were successfully converted with rhodamine B isothiocyanate without significantly affecting the size of the particles.[^104] However, only a very limited number of such reagents with specific target functionalities is readily available or can be prepared because the isocyanate/thiocyanate group is highly reactive and does not tolerate certain functionalities within the same molecule (e.g., amine, thiol, hydroxyl). Thus, more efficient and generally applicable procedures for a direct functionalization of PS-NP are desired (see also chapter 5.1.).

The cellulose-based PS-NP described thus far were prepared by self-assembling of hydrophobic derivatives and consequently possess a rather hydrophobic surface. A few examples are described in literature in which hydrophilic PS-NP are prepared by self-assembling of a hydrophobic cellulose derivative that is converted into cellulose by cleavage of the labile substituent during the particle formation procedure. Spherical shaped cellulose NP (Ø = 175–265 nm) were prepared by dialysis of trimethylsilyl cellulose (DS = 1.4 to 1.9), dissolved in DMA, against water.[^105] During the solvent exchange, the labile silyl ether groups were cleaved hydrolytically and particles were formed by a combined hydrophobic self-assembling and regeneration followed by aggregation of cellulose. PS-NP with a hydrophilic...
cellulose shell and a hydrophobic core were prepared from short chain CA oligomers (degree of polymerization ≈ 10 to 30) that were modified at the reducing end group with a pyrene linker of variable length.[106] Parallel to the self-assembling, which was induced by the aromatic pyrene system, saponification of the ester groups was initiated by addition of an organic base. The final size of the aggregates (Ø ≈ 40–110 nm) depended on the length of the cellulose chain and the end group linker.

4.2. Dextran-Based Nanoparticles

Dextran is a homo-PS predominately composed of α-(1→6) glucopyranosyl units (> 50%), however, with varying amounts of α-(1→3)- and occasionally also α-(1→4)- or α-(1→2)-linkages depending on its source.[107,108] It is produced biotechnologically on an industrial scale using Leuconostoc mesenteroides and several other bacteria strains. The biopolymer is widely accepted in biomedical areas because of its good biocompatibility and biodegradability.[109,110] Chemically modified hydrophobic dextran derivatives have been employed to obtain PS-NP in a similar fashion as described for cellulose derivatives (chapter 4.1.).

In some of the very first comprehensive studies on the preparation of PS-NP by hydrophobic self-assembling, a series of dextran mixed esters with a combination of pyroglutamate, propionate, and furoate moieties was employed to prepare particles (≈100–600 nm) by dialysis.[111–113] An overall DS ≥ 2.0 was found to be important in order to obtain PS-NP with a regular spherical shape. Following a similar approach, light-responsive PS-NP with diameters of 115 to 215 nm were prepared by dialysis of photoactive dextran ester with and without additional propionate groups (see also chapter 5.4.).[114]

The preparation of dextran-based PS-NP by a dropping-in approach was studied using mixed dextran benzoate methacrylate derivatives.[33] By variation of the total amounts of both substituents, the hydrophobicity of the compounds was adjusted gradually. However, the effect on the particles size was minor (Figure 14). On the contrary, it was found that the particle diameter increased significantly in a linear fashion with increasing polymer concentration in the organic phase. It was possible to stabilize these PS-NP by photoinduced crosslinking of the methacrylate moieties in such a way that these particles could be lyophilized and redispersed. The original size of the particles (≈180 nm) was only slightly increased by this procedure (≈ 205–260 nm).

Esterification of dextran is a convenient approach to obtain hydrophobic PS derivatives for nano-self-assembling. The derivatization reactions can be performed under homogeneous conditions to obtain well-defined products.[115] Nevertheless, other dextran derivatives have been studied for the preparation of PS-NP. Dextran alkyl carbonates with varied substituents (ethyl, propyl, and butyl) formed PS-NP in a range of 150–600 nm by dialysis.[116] Several dextran graft-copolymers were reported for the preparation of PS-NP, including graft-poly(ε-caprolactone), graft-poly(lactic acid), and graft-poly(DL-lactide-co-glycolide).[117–122] Controlling the type, amount, and length of the oligomer-/polymer side chains enables a certain control over the particle morphology (e.g., size distribution, core–shell structure, surface chemistry) as well as parameters such as swelling behavior and drug loading efficiency.
Hydrophobic dextran cyanoethyl ethers along with pullulan cyanoethyl ethers have been employed for preparing PS-NP with the intention to incorporate iron oxide NP.\textsuperscript{[123]} A series of dextran hydroxyalkyl ethers was evaluated for the preparation of PS-NP by an emulsion–evaporation process.\textsuperscript{[124]} The hydrophilicity of these derivatives was gradually decreased by increasing the DS and by changing the substituent from 2-hydroxy-3-phenoxypropyl to the more hydrophobic 2-hydroxyoctyl and 2-hydroxydodecyl groups. This alteration resulted in a significant increase of the particles sizes from about 370 to 850 nm and finally to µm-sized aggregates. It was speculated that the coverage with hydrophobic macromolecules at the water–oil interface is too low to provide sufficient colloidal stability. Much smaller particles (≈150–300 nm) could be obtained independent on the hydrophobicity of the derivatives when a water soluble 2-hydroxy-3-phenoxypropyl dextran ether with low DS was added as surfactant. The concentration of the hydrophobic dextran derivative in the organic phase had an influence on the particle size but changing the surfactant concentration had little effect.

A rather unique approach for obtaining hydrophobic dextran derivatives is the formation of acetalates by conversion of the PS with 2-methoxypropene under acid catalysis.\textsuperscript{[125]} These derivatives were stable at a pH value of 7.4 but at a pH value of 5.0 they completely dissolve in water due to acid catalyzed hydrolysis of the acetal groups. PS-NP with sizes of about 230 nm were prepared from acetalated dextran using an emulsion-evaporation process.\textsuperscript{[78,79,126–128]} Similar to CA (chapter 4.1.) and dextran acetates (chapter 4.2.), these hydrophobic pullulan derivatives have been employed for NP formation mostly using dialysis or dropping techniques.\textsuperscript{[15,130,131]} The particles sizes appear to be dependent on the degree of acetylation (Table 1). DS$_{ac}$ < 2 seems to favor the formation of PS-NP with diameters ≈50–150 nm. For pullulan acetates with high DS$_{ac}$ in the range from 2.7 to 3.0, it was demonstrated that particle size and polydispersity increase significantly with increasing hydrophobicity, i.e., the particles became less defined.\textsuperscript{[130]} This has been ascribed to a higher resistance of the mass transfer during the diffusion of polymer solvent into the aqueous nonsolvent phase (dialysis process). A similar finding has been reported for PS-NP derived from highly functionalized cellulose esters.\textsuperscript{[12]} The ionic strength in the aqueous nonsolvent is another parameter that has an impact on the particle size.\textsuperscript{[35]} Increasing the salt concentration results in decreasing critical aggregation concentration for dissolved pullulan acetate and yields larger PS-NP.

NP derived from pullulan acetate are biocompatible and showed no cytotoxicity, neither in cell viability assays nor in animal tests.\textsuperscript{[132,133]} Thus, they received interest as carrier for drug delivery purposes (see chapter 5.3.). Pullulan acetates have been further modified prior to the particle formation by derivatization with additional substituents to enhance drug encapsulation and/or release efficiency as well as to tailor the interaction with cells. In order to achieve tumor selective drug delivery, pullulan acetate derivatives have been modified with biotin or folic acid and PS-NP have been prepared therefrom by hydrophobically induced self-assembling.\textsuperscript{[11,114,115]} Tumor cells are known to overexpress receptors for binding these two vital vitamins. Pullulan acetate with additional oligo(methacryloyl)sulfamidethione) substituents (OSDM) has been employed to obtain pH-sensitive PS-NP.\textsuperscript{[136]} The OSDM group is responsive

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4.3. Pullulan-Based Nanoparticles

Pullulan is a nonionic exo-PS that is produced commercially by yeast-like fungi strains such as Aureobasidium pullulans.\textsuperscript{[129]} It is composed of repeating units with three α-(1→4)-linked glucopyranosyl units that are α-(1→6)-linked to form a linear glucan polymer chain (Figure 10). Different hydrophobic pullulan derivatives have been described for the preparation of PS-NP by self-assembling.

Pullulan acetate with varying DS values can be prepared by homogeneous esterification of pullulan, e.g., in formamide.\textsuperscript{[130]} Similar to CA (chapter 4.1.) and dextran acetates (chapter 4.2.), these hydrophobic pullulan derivatives have been employed for NP formation mostly using dialysis or dropping techniques.\textsuperscript{[15,130,131]} The particles sizes appear to be dependent on the degree of acetylation (Table 1). DS$_{ac}$ < 2 seems to favor the formation of PS-NP with diameters ≈50–150 nm. For pullulan acetates with high DS$_{ac}$ in the range from 2.7 to 3.0, it was demonstrated that particle size and polydispersity increase significantly with increasing hydrophobicity, i.e., the particles became less defined.\textsuperscript{[130]} This has been ascribed to a higher resistance of the mass transfer during the diffusion of polymer solvent into the aqueous nonsolvent phase (dialysis process). A similar finding has been reported for PS-NP derived from highly functionalized cellulose esters.\textsuperscript{[12]} The ionic strength in the aqueous nonsolvent is another parameter that has an impact on the particle size.\textsuperscript{[35]} Increasing the salt concentration results in decreasing critical aggregation concentration for dissolved pullulan acetate and yields larger PS-NP.

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Figure 15. a) Reaction scheme for the synthesis and hydrolysis of acetalated dextran (AcDex). b) SEM images of nanoparticles (NP) of AcDex (scale bar: 2 µm). c) Dextran release and images d) of AcDex-NP, stored in buffered solution at 37 °C. Adapted with permission. Copyright 2008, American Chemical Society.
to small pH changes under physiological conditions, which results in a significant decrease of particles size from about 230–100 nm upon increasing pH from 6.8 to 7.4 (Figure 16). This has a direct influence on drug release properties. For a similar purpose, PS-NP with pH-sensitive swelling behavior have been prepared from pullulan derivatives with protonatable urocanate groups.\(^{[137,138]}\)

“Hydrophobized pullulan” derivatives bearing cholesterol substituents are frequently described in literature in the context of nanomaterials for drug delivery.\(^{[139,140]}\) The derivatives and NP derived thereof are somewhat different from the previously described hydrophobic PS derivatives. The DS\(_{\text{cholesterol}}\) values are significantly lower (usually < 0.1), which means that the polymer chains still possess a strong hydrophilic character. When the derivatives are dispersed in water by sonication with ultrasound or by diluting a DMSO solution with water, nanoaggregates with particles sizes \(\approx 30\) nm are formed. Instead of a solvent exchange induced self-assembling, the mechanism for particle formation follows a spontaneous aggregation of the cholesterol-modified pullulan derivatives in water when a certain concentration is exceeded. The nanoaggregates formed can be regarded as nanogels in which cholesterol groups act as physical crosslinking points.\(^{[141]}\)

### 4.4. Starch-Based Nanoparticles

Starch is highly abundant in nature and produced by many green plants in the form of granules (0.1–200 µm) for the purpose of long-term energy storage.\(^{[142]}\) Native starch is a mixture of two homo-PS components; amylose (15%–35% depending on the source) and amylpectin. Amylose is primarily composed of linear, unbranched \(\alpha-(1\rightarrow4)\)-linked glucopyranosyl units. Amylopectin is a highly branched PS in which about 5% of the repeating units contain additional \(\alpha-(1\rightarrow6)\)-branches. Starch is of major importance in food and paper applications, for creating bioplastics, and as a renewable feedstock for biofuel production. It is also of interest for developing materials for biomedical applications because it is biocompatible and, unlike cellulose and several other PS, fully degradable in the human body. Procedures have been developed for preparation of starch-based NP either by regeneration of unmodified starch from solution (bottom-up) or chemical and/or mechanical treatment of macroscopic starch granules (top-down).\(^{[143]}\) However, the methods employed as well as the materials obtained are different from the ones presented in this review. The following chapter will present examples for the preparation of starch NP by self-assembling of hydrophobic starch derivatives.

Starch acetates (DS\(_{\text{acetate}}\) 2.62 to 2.97) have been studied in the context of NP formation by hydrophobic self-assembling.\(^{[144]}\) Dropping an acetone solution of starch acetate into water yielded rather small PS-NP (80–130 nm) with a broad size distribution (PDI from 0.18 to 0.28). The reversed process (dropping water into the acetone solution) resulted in narrower size distributions (PDI from 0.01 to 0.04), however, in larger particle sizes (250 to 720 nm). Thereby, the particle size increased with increasing concentration of starch acetate (1–20 mg mL\(^{-1}\)). The slow increase of nonsolvent content, which is achieved when dropping water into the PS solution...
enables a uniform self-assembling of NP under thermodynamic control. Increasing the volume ratio of water to acetone resulted in a reduction of the particle size (595–460 nm), which was explained by reinforced interfacial interaction of acetone and water followed by compression of the particles. Also the chain length of the PS macromolecules had an influence on the NP formation; increasing the molecular weight from 21,500 to 345,000 g mol\(^{-1}\) resulted in a reduction of the particle size (595–460 nm), which was explained by the decreased interfacial interaction of acetone and water.

Dialysis yielded smaller particles. Presumably, the high water content decreased the dialysis rate, which led to more compact NP. The particle size decreased by increasing the DS and/or decreasing the polymer concentration. Moreover, these parameters affected the encapsulation efficiencies. Functional PS-NP could also be obtained using starch acetates. A mixed starch acetate derivative with additional allyl moieties (DS\(_{\text{acetate}}\) 2.3; DS\(_{\text{allyl}}\) 0.5) was used to prepare crosslinkable PS-NP by the dropping technique. TEM experiments with phosphotungstic acid (adsorbs onto the acetate ester bonds) and osmium tetroxide (reacts with the allylic double bond) as contrast agents revealed a core–shell-like structure with acetate groups predominately located in the interior bulk and allyl groups concentrated on the surface (Figure 17). The particles were employed as crosslinkers to fabricate acrylamide-based hydrogels.

In addition to starch acetates, several other starch esters, ethers, and mixed derivatives have been evaluated for the preparation of PS-NP. Starch 3-carboxy-undec-5-enoate was employed to prepare PS-NP with diameters of 65 to 130 nm by dialysis. The particle size decreased by increasing the DS and/or decreasing the polymer concentration. Moreover, adding small amounts of water to the polymer solution prior to the dialysis yielded smaller particles. Presumably, the high water content decreased the dialysis rate, which led to more compact NP. PS-NP with diameters around 150–180 nm and zeta potentials of about −6 to −8 mV were found for propyl starch. The DS\(_{\text{propyl}}\) (1.0 to 1.5) had little effect on the particle properties. The propyl starch-based NP were employed for physical encapsulation of drugs (flufenamic acid, testosterone, and caffeine) with loading capacities of ≈45 wt%. Hydroxyethyl starch was converted with different fatty acids (lauric, palmitic, and stearic acid) to obtain hydrophobized mixed ether ester derivatives. Interestingly, only hydroxyethyl starch laurate with low MS formed NP (250–350 nm) with a vesicle-like structure as demonstrated by freeze fracture TEM experiments. The other mixed derivatives formed only macroscopic aggregates. A graft-PEG starch derivative, synthesized by esterification of the polysaccharide with carbomyl-terminated PEG, was used to prepare PS-NP (130–160 nm) by dropping a fivefold excess of water into a DMSO solution of the copolymer. The particles sizes were independent of the grafting ratio. However, size and critical micelle concentration both decreased with decreasing length of the PEG block from 5000 to 2000 g mol\(^{-1}\). In addition, PS-NP derived from PEGylated starch with crosslinkable aldehyde groups were obtained and employed for encapsulation of doxorubicin.

4.5. Xylan-Based Nanoparticles

Xylan is the major hemicellulose constituent in lignocellulosic biomass from hardwood species and grasses. Xylan is primarily composed of a linear chain of 4-(1→4)-linked xylopyranosyl units. Depending on the plant source and extraction method, the main chain can be modified in positions 2 and 3, including acetyl groups, glucuronic acid (unmodified or as 4-O-methyl ether), L-arabinose, and oligosaccharides (composed of D-xylose, L-arabinose, D- or L-galactose and D-glucose). The molecular structure of xylan differs from the previously discussed PS (cellulose, dextran, pullulan, starch). It is composed of pentose repeating units rather than hexoses and lacks a primary hydroxyl group, which makes the polymer chain more hydrophobic and alters its ability to form 3D networks through hydrogen bonding. Moreover, only two instead of three hydroxyl groups per repeating unit are available for chemical modification, which means that at a given DS value the ratio of hydrophilic and hydrophobic groups is different. These parameters have to be considered when comparing xylan-based PS-NP with those derived from other PS. However, a comparative study on this issue is currently missing.

The particle formation of mixed xylan esters with furoate and pyrogulamate substituents was studied using the dialysis approach. The PS-NP were comparably small (60–85 nm) and formed only at a sufficiently high overall DS > 1.3. PS-NP obtained from a comparable dextran furoate pyrogulamate derivative with an overall DS of 2.06 showed significantly higher particles sizes of about 520 nm. A strong influence of the PS backbone on the nano-selfassembling has also been observed for PS phenyl carbonates. Xylan phenyl carbonates formed well defined PS-NP with a size of about 160 nm by dialysis whereas the corresponding cellulose derivatives only formed macroscopic aggregates.

Several xylan-based nanoprodrugs prepared by dialysis have been reported in which the hydrophobic substituent was a drug molecule covalently attached to the PS backbone. Xylan was esterified with curcumin monosuccinate and the corresponding product formed PS-NP of about 205 nm. Conjugates of xylan and ibuprofen (DS from 0.36 to 1.24) were reported to form PS-NP with sizes of about 340 to 475 nm. Interestingly, introducing a small amount of sulfate groups (DS from 0.02 to 0.6) into the polysaccharide chain improved the particle formation. The particle size decreased with increasing size with increasing polymer concentration and decreasing water to acetone ratio. Moreover, these parameters affected the encapsulation efficiencies. Functional PS-NP could also be obtained using starch acetates. A mixed starch acetate derivative with additional allyl moieties (DS\(_{\text{acetate}}\) 2.3; DS\(_{\text{allyl}}\) 0.5) was used to prepare crosslinkable PS-NP by the dropping technique. TEM experiments with phosphotungstic acid (adsorbs onto the acetate ester bonds) and osmium tetroxide (reacts with the allylic double bond) as contrast agents revealed a core–shell-like structure with acetate groups predominately located in the interior bulk and allyl groups concentrated on the surface (Figure 17). The particles were employed as crosslinkers to fabricate acrylamide-based hydrogels.

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**Figure 17.** Transmission electron microscopy images of allyl starch acetate nanoparticles: a) untreated, b) after staining with phosphotungstic acid, and c) after staining with osmium tetroxide. Reproduced with permission.[147] Copyright 2009, WILEY-VCH.
0.25) reduced the particle size by about 90 nm and significantly improved the drug release from the particles by hydrolysis (see also chapter 5.2.).

5. Biomedical Application of Polysaccharide-Based Nanoparticles

PS-NP have been evaluated for various kinds of applications, mostly in the biomedical field. Typical examples will be discussed in the following chapters. A key issue is “functionalization” of the particles, i.e., the introduction of functional groups or molecules that support a given task. An overview of various types of functional PS-NP that have been reported in the literature is provided in Table 2.

Two approaches can be pursued to functionalize PS-NP. i) Active compounds such as drugs and dyes can be incorporated physically within the particles during the nano-self-assembling process. Thereby it needs to be studied how the particle formation is affected by the incorporation of an additional compound. ii) Specific functional groups as well as more complex molecules can be fixed covalently to PS-NP. In many cases, this has been achieved by chemical modification of the PS chains with functional substituents prior to the actual particle formation. A key question that needs to be studied is how derivatization with a desired functionality influences the nano-self-assembling of polymer chains. No additional hydrophobic substituents might be needed to induce self-assembly if the functional substituent itself is sufficiently hydrophobic and the overall degree of substitution (DS) is high enough. PS-NP can also be functionalized after their formation. This approach requires efficient coupling reactions to achieve high conversion rates as well as to avoid crosslinking and aggregation of the NP.

5.1. Reactive Polysaccharide Nanoparticles

PS-NP can be chemically modified at the surface, but it has to be considered that the particles are dispersed in an aqueous medium and that the functionalization must not induce aggregation, e.g., by chemical crosslinking or a loss of repulsive forces between the individual particles. Different functional moieties can be located on the particle surface depending on the material composition and the preparation techniques employed. Efficient chemical modification of PS-NP at the hydrophobic alkyl/aryl groups or the residual hydroxyl groups from the PS backbone is not feasible under mild aqueous reaction conditions. Thus, reactive groups are required that can be modified in the presence of water with reasonable reaction efficiency. It is also desired to minimize the number of reaction and processing steps (e.g., purification, centrifugation, solvent exchange) to avoid negative impacts on the particle properties (e.g., aggregation, sedimentation).

Cellulose phenyl carbamate-based PS-NP with various types of linear and branched substituents carrying terminal amino groups have been prepared.[104] Despite having a considerable amount of primary amino groups, the particles showed no aggregation or disintegration over a broad range of pH values from 2 to 12. Moreover, it was possible to chemically modify the particle surface with a dye using rhodamine B isothiocyanate. The reagent is rather reactive toward amines and can be employed in aqueous media to form stable thiourea bonds. The dye-functionalized PS-NP showed pronounced fluorescence, which could be exploited to localize the particles after incubation within living cells by confocal laser scanning microscopy (see also chapter 5.2.). PS-NP with carboxyl groups can be functionalized with amines, including biomolecules such as proteins and antibodies, forming stable amide bonds. However, activation of the carboxyl group is required in order to facilitate coupling with high reaction efficiency under aqueous reaction conditions.[158] PS-NP derived from cellulose acetate phthalate (CAPh) was successfully coupled with antibodies by in situ activation with 1-ethyl-3-[3(dimethylaminopropyl)carbodiimide (EDC)/sulfo-N-hydroxysuccinimide (NHS), which converted the carboxyl group at the particle surface into an activated ester that readily reacted with amino groups.[96] The antibody-functionalized PS-NP were employed as nanolabels in immunoassays (see chapter 5.3.). Following a similar synthesis approach, CAPh-NP have been modified with amino-functionalized dextran to increase the drug loading capacity of these particles.[97]

Although PS-NP with amino- or carboxyl groups can be functionalized under aqueous conditions, their reactivity is comparably low, which requires multistep activation procedures and/or highly reactive reagents (e.g., isocyanates, isothiocyanates) that are not readily available for most desired functionalities. For broad applicability, reactive PS-NP should feature groups that can be functionalized i) in water, ii) under mild conditions, iii) with high reaction efficiency, and iv) with high chemoselectivity. Advancing the concept of carboxyl group containing PS-NP that require in situ activation, activated NHS ester derivatives were prepared by homogeneous conversion of CAPh.[98] The NHS-CAPh derivatives obtained were stable under aqueous conditions (i.e., no hydrolysis of the activated ester occurred) and formed spherical PS-NP using the dialysis approach with particle diameters in the range from 200 to 385 nm depending on the initial polymer concentration (Figure 18). Contrary to the original CAPh-NP, NHS-CAPh-NP directly reacted with amines (e.g., amino group containing dyes) without the need for a previous activation. High coupling efficiencies of up to 90% could be achieved for a functionalization of PS-NP under mild aqueous conditions (25 °C, pH value of 8.9).

“Click chemistry” approaches could become very valuable for the functionalization of PS-NP because they proceed with high selectivity and reaction efficiency.[159] The synthesis and post-modification of several PS derivatives with “click chemistry” substituents has been reported in the literature.[160,161] However, only few reports have been published in this context for PS-NP. Reactive PS-NP with a size of 360 nm derived from deoxy-azido dextran tosylate have been described that could potentially be converted with alkynes in a chemoselective 1,3-dipolar cycloaddition.[162] PS-NP derived from acetylated dextran tosylate have been described that could potentially be converted with alkynes in a chemoselective 1,3-dipolar cycloaddition. PS-NP that require in situ activation, activated NHS ester derivatives were prepared by homogeneous conversion of CAPh.[98] The NHS-CAPh derivatives obtained were stable under aqueous conditions (i.e., no hydrolysis of the activated ester occurred) and formed spherical PS-NP using the dialysis approach with particle diameters in the range from 200 to 385 nm depending on the initial polymer concentration (Figure 18). Contrary to the original CAPh-NP, NHS-CAPh-NP directly reacted with amines (e.g., amino group containing dyes) without the need for a previous activation. High coupling efficiencies of up to 90% could be achieved for a functionalization of PS-NP under mild aqueous conditions (25 °C, pH value of 8.9).

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Table 2. Literature overview of functionalized and/or loaded polysaccharide nanoparticles (PS-NP).

| Method | Polysaccharide | Substituent | DS | Entrapment | Substance | Additive | Particle properties | Refs. |
|--------|----------------|-------------|----|------------|-----------|---------|---------------------|-------|
| Dialysis | Cellulose Acetate | 2.30 | None | 2-aminoanthraquinone | 33–50 wt% | 270–395 | 0.18–0.24 | – | 32 to –29 | [96] |
|         | Sudan red IV | 20–50 wt% | 330–760 | 0.14–0.37 | – | 31 to –28 | [96] |
|         | Sudan black B | 20–50 wt% | 585–910 | 0.21–0.36 | – | 30 to –26 | [96] |
|         | Cellulose Acetate | 1.19 | None | 2-aminoanthraquinone | 33–50 wt% | 295–485 | 0.08–0.14 | – | 46 to –34 | [96] |
|         | Sudan red IV | 20–50 wt% | 680–910 | 0.21–0.28 | – | 37 to –27 | [96] |
|         | Sudan black B | 20–50 wt% | 235–445 | 0.09–0.26 | – | 39 to –33 | [96] |
|         | Cellulose N-phenyl carbamate | 1.41 | Tosylate | AEA | 0.46 | (*Functional substituent) | 215 | 0.12 | 44 | [104] |
|         | Cellulose N-phenyl carbamate | 1.41 | Tosylate | AEA | 0.46 | (*Functional substituent) | 175 | 0.09 | 50 | [104] |
|         | Cellulose N-phenyl carbamate | 1.41 | Tosylate | RBITC | 0.7 | (*Functional substituent) | 175 | 0.09 | 50 | [104] |
|         | Cellulose N-phenyl carbamate | 1.19 | Tosylate | TAEA | 0.49 | (*Functional substituent) | 105 | 0.11 | 62 | [104] |
|         | Cellulose N-phenyl carbamate | 1.02 | Tosylate | BAEA | 0.57 | (*Functional substituent) | 175 | 0.06 | 65 | [104] |
|         | Cellulose Tosylate | 0.26–0.98 | ABZ | AEA | 0.12–0.29 | (*Functional substituent) | 80–390 | 0.13–0.54 | 44–61 | [180] |
|         | Cellulose Tosylate | 0.26–0.98 | FTC | AEA | 0.12–0.29 | (*Functional substituent) | 80–390 | 0.13–0.54 | 44–61 | [180] |
|         | Cellulose Tosylate | 0.26–0.98 | ABZ | FTC | – | (*Functional substituent) | 80–390 | 0.13–0.54 | 44–61 | [180] |
|         | Cellulose Hydroxyethyl | 1.5–3.0; MS: 2.5–3.5 | Ofloxacin | U-MPA | 3.0 | (*Functional substituent) | 150–320 | 0.08–0.15 | – | 48 to –44 | [184] |
|         | Dextran MCA | 0.33–0.72 | Dextran | OF10 | 0.47–0.71 | (*Functional substituent) | 115–130 | 0.15–0.21 | – | [114] |
|         | Dextran AZO | 0.81–1.89 | Dextran | AZO | 0.81–1.40 | (*Functional substituent) | 160–170 | 0.06–0.11 | – | [114] |
|         | Dextran Propionate | 1.60–2.19 | Dextran | COU | 0.44–2.32 | (*Functional substituent) | 130–315 | 0.08–0.18 | – | 38 to –25 | [114,181] |
|         | Dextran | 1.60–2.19 | Dextran | COU | 0.44–2.32 | (*Functional substituent) | 130–315 | 0.08–0.18 | – | 38 to –25 | [114,181] |
|         | Dextran Propionate | 1.01–2.29 | Dextran | TPY | 0.23 | (Functional substituent) | 960 | 0.55 | – | [207] |
|         | Dextran Propionate | 1.01–2.29 | Dextran | TPY | 0.23 | (Functional substituent) | 960 | 0.55 | – | [207] |
|         | Dextran | 1.7–6.2 | Dextran | Doxorubicin | 5.7–7.5 wt% | 140–280 | – | – | [190] |
|         | Dextran | 0.50–2.08 | Dextran | Doxorubicin | 5.7–7.5 wt% | 140–280 | – | – | [190] |
|         | Dextran | 0.41–1.62 | Dextran | Naproxen | 0.41–1.62 | (Functional substituent) | 180–390 | 0.06–0.21 | – | [167] |
|         | Dextran | 1.81 | Dextran | Propionate | 1.81 | 375 | 0.13 | – | [165] |

continued
Table 2. Continued.

| Method(a) | Polysaccharide | Entrapment Additive(e) | Particle properties(f) | Refs. |
|-----------|----------------|------------------------|------------------------|-------|
| Dextran   | FTC” *         | –                      | (‘Functional substituent) | –     |
| Dextran   | Propionate     | –                      | –                      | 540   | 0.23 | –     | [165] |
| Dextran   | SRB *          | –                      | (‘Functional substituent) | 500   | –    | –     |
| Dextran   | Tosylate 0.74  | –                      | (‘Functional substituent) FTC-dextran propionate 12.5 wt% | 360   | –    | –     | [162] |
| Dextran   | Deoxy-azido*   | 0.55                   | (‘Functional substituent) Horseradish peroxidase 1.5 wt% | 305   | 0.11 | –     | [189] |
| Dextran   | Decanoate 0.23 | –                      | (‘Functional substituent) Iron oxide nanoparticles 0.3–6.7 mmolFe /gPS | 250–445 | –    | –     |
| Dextran   | Cyanoethyl” 2.40 | –                      | (‘Functional substituent) – | 240   | –    | –     | [208] |
| Dextran   | Aminopropyl” 0.43 | –                      | (‘Functional substituent) – | –     | –    | –     | [208] |
| Dextran   | Cyanoethyl” 0.85–2.31 | –                      | (‘Functional substituent) – | –     | –    | –     | [208] |
| Dextran   | Aminopropyl” 0.44–0.63 | –                      | (‘Functional substituent) VAN” 0.19–0.48 | –     | –    | –     | [162] |
| Dextran   | Aminopropyl” 1.31 | –                      | (‘Functional substituent) BHT” 0.15 to 0.70 | –     | –    | –     | [208] |
| Pullulan  | Acetate 0.8    | Clonazepam 10 wt%      | 200                    | 175–225 | 0.15–0.24 | – to –12 | [135] |
| Pullulan  | Acetate 0.8    | Clonazepam 10 wt%      | 180                    | 0.15–0.24 | –     | –     | [209] |
| Pullulan  | Acetate 0.1    | Clonazepam 10 wt%      | 200                    | 175–225 | 0.15–0.24 | – to –12 | [135] |
| Pullulan  | Acetate 0.05   | Clonazepam 10 wt%      | 180                    | 0.15–0.24 | –     | –     | [209] |
| Pullulan  | Acetate        | None                   | =80                    | 60–90 | 0.13–0.19 | –     | [211] |
| Pullulan  | Cholesterol    | Clonazepam 10 mol%     | 80–100                 | 50–60 | –     | –     | [192] |
| Pullulan  | Cholesterol    | Clonazepam 10 wt%      | 60–90                  | 50–60 | –     | –     | [190] |
| Pullulan  | Biotin 0.2–0.4 | –                      | –                      | 240   | –    | –     | [192] |
| Pullulan  | Cyanoethyl 2.42 | –                      | Iron oxide nanoparticles 0.3–6.7 mmolFe /gPS | 400–600 | –    | –     |
| Pullulan  | Urocanate 0.08 | Doxorubicin 9–20 wt%   | 200–440                | 175–225 | 0.15–0.24 | – to –12 | [137] |
| Starch    | Graft-PGA” 0.04–0.23 | –                      | (‘Functional substituent) – | –     | –    | –     | [196] |
| Starch    | 3-Carboxy-undec-5-enoate” 0.67–0.74 | –                      | (‘Functional substituent) – | –     | –    | –     | [197] |
| Xylan     | Stearate 0.83  | None                   | 195                    | 215   | 0.10 | –     | [198] |
| Xylan     | Stearate 0.83  | None                   | 215                    | 0.10 | –    | –     | [198] |
| Xylan     | Ibuprofen” 0.36–1.24 | –                      | (‘Functional substituent) – | –     | –    | –     | [157] |
| Xylan     | Ibuprofen” 0.36–1.24 | –                      | (‘Functional substituent) – | –     | –    | –     | [157] |
| Xylan     | Sulfate 0.02–0.25 | –                      | (‘Functional substituent) – | –     | –    | –     | [157] |
| Xylan     | Curcumin monosuccinate” 24 wt% | –                      | (‘Functional substituent) – | –     | –    | –     | [156] |
| Dropping  | Cellulose 2.50 | Acetate 82 wt%         | 90                     | 205   | –    | –19   | [156] |
|          | Cellulose 2.50 | Acetate 82 wt%         | –                      | 205   | –    | –     | [156] |
|          | Cellulose 2.50 | Acetate 82 wt%         | –                      | 205   | –    | –     | [156] |

continued
Table 2. Continued.

| Method | Polysaccharide | Entrapment | Additive | Particle properties | Refs. |
|--------|----------------|------------|----------|---------------------|-------|
|        | Backbone | Substituent | DS | Substance | Amount | Size [nm] | PDI | ζ [mV] |
| Method a) | Polysaccharide | Entrapment Additive e) | Particle properties f) | Refs. |
| Cellulose | Acetate | – | None | PVA + HCl | 265–370 | <0.12 | – | [100] |
| Cellulose | Acetate | – | None | T-20, T-80, M-52, SHS-15, KP, PF-68, PF-127 | 85–135 | 0.16–0.26 | – | [101] |
| Cellulose | Acetate | – | None | PF-127 | 95 | 0.2 | – | [97] |
| Cellulose | Acetate | – | None | Amino dextran | 160–315 | <0.12 | – | [100] |
| Cellulose | Acetate | – | None | Saline, PBS, sucrose | 120–135 | 0.08–0.10 | – | [103] |
| Cellulose | Acetate | – | None | Phenoxy dextran | 145–215 | 0.06–0.13 | – | [122] |
| Cellulose | Acetate | – | None | Piroxicam | 165–475 | 0.10–0.74 | – | [93] |
| Cellulose | Acetate | – | None | Rhodamine B | 180–360 | 0.06–0.20 | – | [182] |
| Cellulose | Acetate | – | None | Camptothecin | 170–360 | 0.27–1.00 | – | [92] |
| Cellulose | Acetate | – | None | PNA, T-80, PF-68, CA-25 | 145–215 | 0.06–0.13 | – | [122] |
| Cellulose | Acetate | – | None | Dextran PDP | 0.05 | 1.8 wt% | 170 | – | – | [166] |
| Cellulose | Acetate | – | None | Ibuprofen | 0.74–2.08 | 70–280 | 0.08–0.18 | – | [167] |
| Cellulose | Acetate | – | None | Graft-PLA | 0.45 | 20–50 wt% | 145–215 | 0.06–0.13 | – | [122] |
| Cellulose | Acetate | – | None | 2-Hydroxydodecyl | 1.64 | 10–20 wt% | 170–200 | – | – | [204] |
## Table 2. Continued.

| Method^a) | Polysaccharide | Backbone Substituent^b) | DS^c) | Entrapment Additive^d) | Substance^e) | Particle properties^f) | Refs. |
|-----------|----------------|-------------------------|-------|------------------------|--------------|------------------------|-------|
| Dextran—β-acetated dextran (block copolymer) | None | None | TEA | 105 | 0.11 | 3 | [78] |
| Dextran | Benzoate | 0.61–1.62 (Functional substituent) | Curcumin | 3.0 wt% | TEA | 100 | 0.10 | −6 | [33] |
| | Methacrylate* | 0.14–0.51 | | | | | | |
| Dextran | Benzoate | 1.12 | BODIPY | 0.01–0.05 wt% | 145–170 | – | – | [33] |
| | Methacrylate* | 0.22 (Functional substituent) | FTC* | – | – | – | – | |
| Pullulan | Acetate | 2.7–3.0 | None | PVA | 190–425 | – | – | [130] |
| | | | | | | | | |
| Pullulan | Acetate | 2.7 | None | PVA | =5 wt% | 355–540 | 0.16–0.32 | – | [131] |
| | | | | | | | | |
| Pullulan | Acetate | 2.7 | None | PVA | 185 | – | – | [131] |
| | | | | | | | | |
| Pullulan | Acetate | 2.7 | Epirubicin | 0–6 wt% | PVA | 200–350 | – | – | [131] |
| | | | | | | | | |
| Pullulan | Acetate | 2.7 | Epirubicin | 2–7 wt% | PVA | 260–390 | – | – | [131] |
| | | | | | | | | |
| Pullulan | Acetate | 0.8 | None | PVA | 250 | 0.18 | ≈3 | [133] |
| | | | | | | | | |
| Pullulan | Acetate | 0.8 | None | PVA | 345 | 0.20 | −2 | [199] |
| | | | | | | | | |
| Pullulan | Acetate | 0.1 | Epirubicin | 10 wt% | PVA | 5–10 wt% | 270–315 | 0.25–0.27 | −2 to −7 | [135] |
| | Biotin | ≈0.05 | | | | | | |
| Pullulan | Acetate | – | Curcumin | 5–10 wt% | PVA | =125 | – | – | [213] |
| | | | | | | | | |
| Pullulan | Cholesterol | 0.02 | None | PVA | 220–30 | – | – | [214, 215] |
| | | | | | | | | |
| Pullulan | Cholesterol | 0–0.1 | None | PVA | 355–540 | 0.09–0.51 | −32 to −5 | [145] |
| | Urocanate | – | Doxorubicin | 5–20 wt% | PVA | 190–315 | 0.10–0.11 | – | [138] |
| | | | | | | | | |
| Pullulan | Urocanate* | 0.06 (Functional substituent) | Ciprofloxacin | 6–21 wt% | PBS, DTT | 210–245 | 0.11–0.16 | −5 | [138] |
| | Methotrexate* | 0.18 (Functional substituent) | Combretastatin A4 | 9–16 wt% | | | | |
| Pullulan | Hydrophobic lysin dendrons | 0.2 | None | PVA | 55 | 0.13 | – | [201] |
| | Lactobionate | 0.3 | Doxorubicin | 17 wt% | 70 | 0.14 | – | |
| Starch | Grafi-PEG | 77.8–89.5 wt% | None | PBS | 120–180 | – | – | [150] |
| | | | | | | | | |
| Starch | Grafi-PEG | 77.8–89.5 wt% | None | PBS, DTT | 80–115 | – | – | [150] |
| | Lipoate* | – | | | | | | |
| Starch | Acetate | 0.33–2.66 | Ciprofloxacin | 6–21 wt% | TEA | – | – | [145] |
| | Acetate | 1.82–2.85 | Ibuprofen | 15–25 wt% | TEA | – | – | [146] |
| | Acetate | 2.14 | | | | | | |
| | FTC* | 0.002 (Functional substituent) | | | | | | |

*Continued*
Table 2. Continued.

| Method | Polysaccharide | Backbone | Substituent(s) | DS | Substance | Amount | Size [nm] | PDI | ζ [mV] | Refs. |
|--------|----------------|----------|----------------|----|-----------|--------|----------|-----|--------|-------|
| Starch | Acetate        | 2.58–2.78| None           |    | Rhodamine | 0.4 wt%| 150–850  | 0.08–0.15 | –   | [202] |
| Starch | Maleate monoester* | 1.64 | (‘Functional substituent) | 0.06 | FTC* | | | | | [216] |
| Emulsion | Cellulose | Acetate | – | Dolutegravir | 7 wt% | PF-127 + PVA | 215 | 0.19 | –25 | [102] |
| | Phthalate | – | Rhodamine 6G | 5 wt% | PF-127 + PVA | 165 | 0.17 | –27 | |
| Cellulose | Ethyl | – | Dexamethasone | 1–3 wt% | PVA | 120–175 | 0.06–0.15 | –36–18 | [94] |
| | | | co-PMMA-PMA | 49 wt% | PVA | 100 | 0.11 | 34 | |
| Dextran | Graft-PCL | 0.18 | BSA | 1.6–15.8 wt% | SC | 190–210 | 0.09–0.17 | –5 to –1 | [118] |
| | | | BmoLL | 1.2–2.7 wt% | | 100–105 | – | – | |
| | | | LCL | 3.0–4.0 wt% | | 115–130 | – | – | |
| Dextran | Graft-PLA | 0.36 | PLA | 50–71 wt% | Dex-g-PLA | 200–260 | – | – | [217] |
| Dextran | Acetal | – | PVA in PBS | | | 220–260 | 0.07 | – | [126] |
| Dextran | AEEEC* | 0.4 | (‘Functional substituent) | | | | | | |
| Dextran | Acetal | 1.73 | PVA in PBS | | | 230 | – | –6 | [127] |
| Dextran | Spermine* | 0.07 | (‘Functional substituent) | | | | | | |
| Dextran | 2-Hydroxydodecyl | 1.64 | Monomyristin | 5–50 wt% | Phenoxy-dextran | 115–600 | – | – | [204] |
| Dextran | (Boronic pinacol ester) benzyl carbonate* | – | None | | PVA in PBS | 100 | – | – | [183] |
| Dextran | Acetal | – | Ovalbumin | 1.6 wt% | PVA in PBS | – | – | – | |
| Dextran | | | None | | PVA in PBS | 245 | – | – | [79] |
| Dextran | | | AF-350 alkoxyamine | 1.2 wt% | PVA in PBS | 310 | – | – | |
| Dextran | | | Unstained CPP | 0.12 wt% | Tris/EDTA, PVA in PBS | 285 | – | –1 | |
| Dextran | | | Stained CPP | 0.12 wt% | Tris/EDTA, PVA in PBS | 280 | – | 1 | |
| Dextran | | | FTC-BSA | 5 wt% | PVA in PBS | 260 | – | – | |
| Dextran | Acetal | – | Iniquimod | 4.1 wt% | PVA in PBS | – | – | – | |
| Dextran | Acetal | 2.19 | None | | PVA in PBS | – | – | – | [125] |
| Dextran | | | Ovalbumin | 3.7 wt% | PVA in PBS | 230 | – | – | |
| Dextran | | | Pyrene | 3.6 wt% | PVA in PBS | 260 | – | – | |
| Dextran | | | FTC-dextran | 5 wt% | PVA in PBS | – | – | – | |
| Pullulan | Acetate | – | Ursodeoxycholic acid | ≈10 wt% | PVA | 100 | – | –28 | [218] |
| Starch | Propyl | 1.05–1.45 | None | | PVA | 150–185 | 0.08–0.12 | –8 to –6 | [148] |
| | | | Caffeine | ≈50 wt% | PVA | 160–185 | 0.11 | 10–16 | |
| | | | Flufenamic acid | ≈50 wt% | PVA | 160–185 | 0.06–0.14 | 12–16 | |
5.2. Drug Delivery

Polymeric NP are intensively studied in the context of targeted drug release.[163,164] PS-based materials are well suited in this regard due to their natural biocompatibility and biodegradability. Cell viability tests with PS-NP derived by nano-self-assembling of various hydrophobic PS derivatives demonstrated that this type of nanomaterials is nontoxic.[178,92,94,100,126–128,148,156] Moreover, these particles are taken up by cells (Figure 19), which opens the possibility for target specific in vivo drug delivery.[101–104,120,150,165,166] Two principles for drug delivery with PS-NP can be distinguished: i) covalent binding of the drug to the backbone of the NP forming PS derivative and ii) physical entrapment within the PS matrix of the NP. Both routes have
been employed for various kinds of drugs and various types of PS-NP (Table 3). Thus, selected examples will be discussed in the following chapter.

Covalent fixation of drugs to the backbone of a PS or PS derivative can be achieved by advanced synthesis approaches depending on the molecular structures of both components. The products obtained might be able to form PS-NP by self-assembling if the drug substituent is hydrophobic and the DS is reasonably high. Otherwise, an additional hydrophobic substituent might be required. In addition to the synthesis and characterization of the derivatives itself, it needs to be studied how type and amount of the drug substituent affect the NP formation. Moreover, the final drug release is a crucial issue because it usually requires a cleavage of the active drug from the prodrug conjugate under specific physiological conditions (e.g., pH value above or below 7.4, presence of specific enzymes) that are relevant for the desired in vivo target (e.g., tumor, skin, intestine, blood stream, specific cells or cellular compartments).

Conjugates of xylan and dextran with ibuprofen or naproxen have been prepared by facile one-step synthesis.\cite{157,167} Both drugs contain carboxyl groups that are accessible for in situ-activated esterification with the hydroxyl groups within the PS backbone, e.g., using N,N’-carbonyldiimidazole. Without the need of additional hydrophobic substituents, these drug-modified derivatives formed PS-NP in the range of about 100 to 500 nm using either the dialysis or the dropping approach. At reasonable DS values in the range of 0.4 to 2.0, rather high drug loading (30–70 wt%) could be achieved by covalent linking. Ester linkages can be hydrolyzed under basic conditions. Ibuprofen was slowly released from the PS-NP within 72 h at elevated pH values >10 (Figure 20). The hydrophobicity of the particles, which increased with increase DS\textsubscript{ibu-profen}, was found to be an important parameter that decreased the rate of hydrolysis. By incorporation of ionic sulfate groups into the PS backbone it was possible to achieve faster hydrolysis at lower pH values. Under physiological conditions (pH 7.4), NP of sulfated xylan ibuprofen-based NP exhibited significant long-term degradation over 4 weeks in contrast to stable non-sulfated NP.

Hydroxyethyl and hydroxypropyl cellulose were esterified with the antibiotic drug ofloxacin and the products obtained formed PS-NP using a dialysis approach.\cite{95} Both types of drug-conjugated particles were examined in rabbit models and showed sustained drug release in plasma and an increased drug half-life time of 18 to 20 h compared to 3 to 5 h for the controls (unmodified drug, physical mixtures of drug and cellulose ethers). Conjugation of the antibiotic to the polymer backbone of a PS-NP contributed significantly to the drug bioavailability.

If the desired drugs are not available with a carboxyl group, alternative coupling techniques have to be employed. Drugs containing hydroxyl groups can be converted with dicarboxylic acid derivatives (e.g., succinic anhydride) to introduce carboxyl groups, thus enabling esterification with the PS backbone. Xylan-curcumin conjugates that formed PS-NP of about...
200 nm size were prepared by this approach. The drug-functionalized PS-NP were found to be non-cytotoxic, blood compatible, and gradually released curcumin under physiological (pH 7.4, slow) or acidic conditions (pH 5.0, increased release rate). PS derivatives bearing carboxyl groups, e.g., carboxymethyl derivatives or derivatives converted with a dicarboxylic acid, can be reacted with drugs containing hydroxyl- (esterification) or amino groups (amide formation). Activation of the carboxyl group is required and the majority of the hydroxyl groups of the PS backbone should be functionalized with hydrophobic substituents in order to prevent crosslinking of the polymer chains. Drug-conjugated PS-NP that showed slow drug release/little cytotoxicity at physiological pH value (7.4, healthy tissue) and increased drug release/moderate to high toxicity at low pH value (6.8, tumor tissue) were obtained starting from pullulan acetate. The PS derivative was converted with succinic anhydride to introduce carboxyl groups and subsequently reacted with sulfadimethoxine, which is a drug with antitumor activity that possesses a primary amino group for the formation of stable amide bounds.

Acetylated carboxymethyl cellulose was converted with PEG chains and a series of antitumor drugs (docetaxel, podophyllotoxin, cabazitaxel). PS-NP with sizes ranging from about 20 to 200 nm were prepared from these derivatives using either flash nanoprecipitation or dialysis. It was found that increasing the drug content yielded larger particles, presumably because the increased hydrophobicity resulted in the formation of a larger hydrophobic core. As a result, larger particles showed a higher hydrolytic stability and consequently a slower drug release. The drug-functionalized PS-NP prepared in this manner showed preferential accumulation in tumor tissue, a slow and controlled drug release, and improved plasma stability, thus, increasing bioavailability and cytotoxicity against tumor cell lines in comparison to the nonconjugated drug.

Physical entrapment of drugs within PS-NP is a viable approach. PS and PS derivatives such as cellulose, starch, as...
Table 3. Literature of about polysaccharide (PS) nanoparticles employed in drug delivery studies.

| Immobilization | Drug          | PS backbone       | Additional substituents                                      | Refs. |
|----------------|---------------|-------------------|-------------------------------------------------------------|-------|
| Covalent       | Cabazitaxel   | Cellulose         | Acetate, carboxymethyl, graft-poly(ethylene glycol)         | [52]  |
|                | Curcumin      | Xylan             | –                                                           | [156] |
|                | Docetaxel     | Dextran           | Acetate, carboxymethyl, graft-poly(ethylene glycol)         | [103] |
|                | Ibuprofen     | Dextran           | –                                                           | [167] |
|                | Ibuprofen     | Xylan             | –                                                           | [157] |
|                | Methotrexate  | Pullulan          | –                                                           | [138] |
|                | Naproxen      | Dextran           | –                                                           | [167] |
|                | Olfoxacin     | Cellulose         | Hydroxyethyl                                                | [95]  |
|                | Podophyllotoxin| Cellulose         | Acetate, carboxymethyl, graft-poly(ethylene glycol)         | [49,50]|
|                | Sulfadimethoxine| Pullulan        | –                                                           | [168,210]|
| Incorporation   | Acyclovir     | Cellulose         | Butyrate, acetate, carboxymethyl                            | [47]  |
|                | Caffeine      | Starch            | Propyl                                                      | [150] |
|                | Camptothecin  | Dextran           | 2-(3-Pentadecylphenoxy)acetate                              | [166] |
|                | Ciprofloxacin | Starch            | Acetate                                                     | [145] |
|                | Clonazepam    | Pullulan          | Acetate, graft-poly(ethylene glycol)                        | [209] |
|                | Combrestatin A4| Pullulan        | Methotrexate                                                | [138] |
|                | Curcumin      | Cellulose         | Butyrate, acetate, carboxymethyl                            | [46,48]|
|                |               | Dextran           | Graft-poly(caprolactone)                                    | [120] |
|                |               | Dextran-b-acetalated dextran (block copolymer)               | –     | [78]  |
|                |               | Pullulan          | Acetate                                                     | [213] |
|                | Dexamethasone | Cellulose         | Acetate, phthalate                                          | [100] |
|                |               | Cellulose         | Ethyl                                                       | [94]  |
|                |               | Cellulose         | Hydroxypropyl, methyl, phthalate                            | [100] |
|                | Dolutegravir  | Cellulose         | Acetate, phthalate                                          | [102] |
|                | Doxorubicin   | Dextran           | Graft-poly(DL-lactide-co-glycolide)                         | [190] |
|                |               | Pullulan           | Cholesterol                                                | [211] |
|                |               | Pullulan           | Cholesterol, urocanate                                      | [200] |
|                |               | Pullulan           | Hydrophobic lysin dendrons, lactobionate                    | [201] |
|                |               | Pullulan           | Sulfadimethoxine                                           | [168,210]|
|                |               | Pullulan           | Hydroxyte                                                  | [137] |
|                |               | Starch             | Graft-poly(ethylene glycol), lipote                         | [150] |
|                | Efavirenz     | Cellulose         | Acetate, phthalate                                          | [101] |
|                |               | Cellulose         | Acetate, sebacate                                           | [51]  |
|                |               | Cellulose         | Butyrate, acetate, carboxymethyl                            | [46]  |
|                |               | Cellulose         | Propionate, acetate, adipate                                | [51]  |
|                | Epirubicin    | Pullulan           | Acetate                                                     | [135] |
|                |               | Pullulan           | Acetate                                                     | [130] |
|                |               | Pullulan           | Acetate                                                     | [133] |
|                |               | Pullulan           | Acetate, biotin                                             | [135] |
|                |               | Pullulan           | Acetate, folate                                             | [131] |
|                | Flufenamic acid| Starch             | Propyl                                                      | [148] |
|                | 5-Fluorouracil| Cellulose         | Acetate, phthalate                                          | [97]  |
|                | Ibuprofen     | Starch             | Acetate                                                     | [146] |
|                | Imiquimod     | Dextran           | Acetate                                                     | [128] |

*continued*
well as a series of their corresponding ethers, esters, and mixed ether ester derivatives are important excipients in many commercial drug formulations, in particular for oral delivery.[169,170] The PS matrices can control the overall drug release kinetics under specific physiological conditions (e.g., pH value, presence of hydrolytic enzymes) and increase solubility and, thus, bioavailability of poorly water-soluble drugs by preventing their crystallization. Due to their small size and the possibility to tailor the surface chemistry, nanoscaled drug carriers can facilitate selective uptake in specific tissue, cell types, or cellular compartments.[171] The release of the drugs could be gradual, i.e., by time-controlled dissolution of the drug from the nanocarrier, or burst-like, i.e., by disintegration of the particles under specific conditions.

Compared to a covalent immobilization of drugs onto PS-NP, physical entrapment is a less restrictive approach in terms of the molecular structures of both components. Thus, far more examples can be found in the literature for PS-NP in this regard and only selected examples can be discussed here (see Table 3 for a comprehensive overview). Physical entrapment within PS-NP is mostly suited for rather hydrophobic drugs. They need to be soluble in the organic media used to dissolve the hydrophobic PS derivatives prior to the self-assembling step. Moreover, the water solubility of the drug should be sufficiently low to prevent substantial leaking within the time scale of preparation of the aqueous particle dispersions and storage until therapeutic use. Key questions in most studies are the influence of the drugs onto the self-assembling process (i.e., size, zeta potential, and shape of the NP), the encapsulation efficiency and maximum loading capacity, as well as the drug release kinetics. These issues strongly depend on many factors including the PS matrix (e.g., type and amount of hydrophobic substituents), the drug (e.g., type, drug to matrix ratio), the technical parameters of the particle preparation step (see chapter 2), and the experimental conditions of the drug release study (e.g., composition of the aqueous medium, type of cells/tissue).[146] Thus, no general rules can be advanced.

PS-NP were evaluated as matrices for poorly soluble drugs. These compounds possess poor bioavailability because they readily crystallize, which prevents their dissolution under physiological aqueous conditions. It has been demonstrated that the incorporation of highly crystalline antiviral drugs (ritonavir,

Table 3. Continued.

| Immobilization Drug | PS backbone | Additional substituents | Refs. |
|---------------------|-------------|-------------------------|-------|
| Insulin             | Pullulan    | Cholesterol             | [214,215] |
| Ketoprofen          | Xylan       | Stearate                | [198] |
| Lemongrass oil      | Cellulose   | Acetate                 | [212] |
| Luciferase-encoding | Dextran     | Acetel, cell penetrating peptide | [79] |
| DNA                 |             |                         |       |
| Mitoxantrone        | Pullulan    | Cholesterol             | [194] |
| Monomorystin        | Dextran     | 2-Hydroxydodecyl        | [204] |
| Nimesulide          | Cellulose   | Ethyl                   | [62]  |
| Paclitaxel          | Pullulan    | Acetate                 | [199] |
| Piroxicam           | Cellulose   | Ethyl                   | [93]  |
| Ritonavir           | Cellulose   | Acetate, sebacate       | [31]  |
|                     | Cellulose   | Butyrate, acetate, carboxymethyl | [46] |
|                     | Cellulose   | Propionate, acetate, adipate | [51] |
| siRNA               | Dextran     | Acetel                  | [127] |
| Testosterone        | Starch      | Propyl                  | [148] |
| Vitamin C           | Cellulose   | Ethyl                   | [92]  |

Figure 20. Start of hydrolysis induced drug release from nanoparticles obtained from xylan esters with different degrees of substitution (DS) of ibuprofen and sulfate groups; a) pH 10, b) pH 11, c) pH 12, d) pH 13. Open bars indicate that no hydrolysis occurred within 72 h. Adapted with permission.[157] Copyright 2010, WILEY-VCH.
efavirenz) within PS-NP derived from cellulose acetate (CA) kept the drugs in an amorphous state. Similar results have been reported for incorporation within PS-NP derived from carboxymethylated cellulose acetate butyrate (CAB) or ethyl cellulose. The water solubility of the CAB-NP-stabilized drugs was significantly higher compared to the crystallized drugs. Moreover, the PS-NP showed a smoother drug release profile and higher initial drug release compared to micrometer-sized particles of the same material due to their higher specific surface area.

Oral application is the preferred route for most drug formulations. Ethyl cellulose-based PS-NP loaded with piroxicam, a nonsteroidal anti-inflammatory drug, were evaluated in vitro and in vivo in this context. The particles were prepared by an emulsion-evaporation process and depending on the type and amount of stabilizer that was used, drug encapsulation efficiencies between 70% and 85% were achieved at an initial ratio of 0.5 mg drug per mg PS derivative. Compared to a commercial drug formulation, the PS-NP showed a slower burst release of the drug in the in vitro experiments and significantly reduced gastric irritation in the in vivo studies on rats. PS-NP loaded with nimesulide, another nonsteroidal anti-inflammatory drug, that were prepared from ethyl cellulose by emulsion-evaporation process were also demonstrated to be hemocompatible.

Depending on their size, shape, and material, NP are able to penetrate through the different skin layers and/or enter the skin at the hair follicle sites. This approach is noninvasive, can provide a long-term sustained release, and avoids the hepatic first pass metabolism. Several studies described the use of PS-NP for the application on skin and for transdermal drug delivery. Moreover, incorporation of PS-NP into thermo-sensitive gels for vaginal application has been studied.

PS-NP prepared from two different propyl starches (DS = 1.05 and 1.45) were compared regarding the transdermal release of various entrapped drugs. In all cases, a linear permeation profile was observed in the experiments using a Franz diffusion cell. While no difference between free and encapsulated drugs was observed for caffeine and testosterone a 10-fold increase of drug release through the human epidermis was observed for flufenamic acid. Thus, it was suggested that the propyl starch-based NP itself might not enter the epidermis. Similar results were reported for dexamethasone-loaded ethyl cellulose-based PS-NP that showed lower overall drug release than a marketed dexamethasone cream. The slow drug penetration was attributed to small amounts of drugs being release from PS-NP that adhere to the skin layer due to opposite surface charges. However, follicular penetration, which was not addressed in the ex vivo experiments, is assumed to be the major skin penetration pathway for NP, i.e., in vivo experiments on real skin should provide different results. It was also proposed that PS-NP derived from CAPh or hydroxypropyl methyl cellulose phthalate should be more suited for transdermal drug delivery formulations than synthetic acrylate-based NP. They are more sensitive to the pH value and should dissolve faster in the hair follicle environment.

Due to their biocompatibility and low cytotoxicity, PS-NP have been considered as carrier for vaccines, siRNA therapeutics, and plasmids. These compounds are rather large molecule assemblies that usually cannot cross the cellular membranes and that are rapidly degraded in vivo if not properly protected by a matrix. It has been demonstrated for several types of PS-NP prepared from hydrophobic PS derivatives that these particles can be taken up by cells.
5.3. Sensing

Nanomaterials have a huge potential as sensors in medicine and biotechnology for the detection of analytes such as bacteria, viruses, antigens, and marker molecules. They have a high specific surface area for immobilization of receptors (e.g., antibodies, (bio)affinity ligands, metal chelators) and reporters (e.g., dyes). Due to their small size, NP can enter living cells acting as in vivo sensor, e.g., for monitoring pH values. PS-based nanomaterials possess great advantages over synthetic ones due to their inherent biocompatibility (see also chapter 5.2.). It has been demonstrated for several examples that PS-NP obtained by hydrophobic self-assembling are well suited for in vivo applications because they are noncytotoxic and taken up by cells.

For optical sensing applications, functionalization of PS-NP with a dye is required. Several examples can be found in the literature in which hydrophobic PS derivatives that are capable of forming NP were converted with readily available reactive dye reagents such as fluorescein isothiocyanate or sulforhodamine B acid chloride (see Table 2). Colored PS-NP dispersions were obtained from these derivatives using either the dialysis or the dropping approach.

It was demonstrated for PS-NP obtained from fluorescein-labeled starch acetate that their fluorescence intensity is less affected by the presence of a fluorescence quencher compared to the free dye molecule in solution. This was explained by a predominant localization of dye moieties in the interior of the particles, which keeps them protected. In addition, the fluorescence showed a strong dependence on the pH value in the physiologically relevant range from 5 to 8. Thus, it has been suggested to employ these dye-labeled PS-NP for pH value sensing. This approach is only viable if the same amount of particles is measured in each experiment. The particle concentration can be difficult or even impossible to control, especially for in vivo applications in which PS-NP are taken up and measured inside of living cells. The sensing systems become more accurate and independent on external parameters if a second dye is incorporated as an internal, pH independent reference standard. Following that concept, dextran propionates were functionalized with either sulforhodamine B or fluorescein to obtain derivatives that were capable of forming fluorescent PS-NP with diameters of about 525 and 375 nm, respectively. Moreover, mixed PS-NP containing both derivatives were prepared (Figure 22). Confocal laser scanning microscopy experiments showed an even distribution of both dyes along the particle cross section. Both dyes exhibited different emission maxima (sulforhodamine B: 581 nm; fluorescein: 516 nm) when excited at specific wave lengths (488 or 543 nm). Whereas the fluorescence intensity of fluorescein increased with increasing pH value from 5 to 8, the fluorescence intensity of sulforhodamine B remained constant. Using the latter as internal reference, it was possible to calibrate the system for different pH values without the need for accurate particle concentrations. Moreover, the PS-NP were stable during sterilization and found suitable for in vivo pH sensing in biological samples.

Immunoassays are a promising application field in which PS-NP can be employed as nanolabels for the quantitative detection of proteins and other biomolecules. The analytical method is based on the exceptionally strong avidity and specificity of antigen-antibody interactions. The nanolabels need to be functionalized with i) a dye to facilitate optical detection and ii) an antibody that captures the target analyte. PS-NP should provide a stronger color and maximize the number of successful binding events in order to improve both sensitivity and specificity of gold- and polystyrene-based NP that are frequently employed in immunoassays. Up to three different functional groups need to be introduced into the PS backbone for this purpose. A hydrophobic substituent is required to induce nano-self-assembling. UV/Vis- or fluorescent dyes need to be attached to enable an optical detection. Reactive moieties are required on the particle surface in order to facilitate an efficient immobilization of the respective capture antibodies. Functionalization of the PS derivatives with antibodies prior to the self-assembling is not straightforward because these biomolecules are very sensitive, expensive, and only active if located on the surface.

Dye-functionalized PS-NP with reactive amino groups that can potentially be used to immobilize antibodies have been reported. The corresponding mixed PS derivatives from which the PS-NP could be obtained were prepared by conversion of tosylated celluloses with a mixture of a hydrophobic benzyl amine (to induce self-assembling in conjunction with excess tosylate groups) and ethylene diamine (to introduce reactive amino groups). In addition, the NP forming derivatives were reacted with a sufficient amount of fluorescein isothiocyanate with the intention to use the final PS-NP in immunoassay applications. An alternative approach has been studied comprehensively in the context of immunoassay applications in which different hydrophobic dyes were physically incorporated within a matrix of a hydrophobic PS-NP. CAPH was employed because it provided both hydrophobic alkyl- and aryl ester moieties as well as reactive carboxyl groups. Basic principles on how the hydrophobicity and molecular structure of both the dye and the PS matrix affect the nano-self-assembling process were evaluated. Moreover, it was possible to immobilize antibodies to the dye PS-NP by conventional EDC/NHS activation (see also chapter 5.1.). The antibody conjugates were evaluated in a lateral flow immunoassay. The detection limits that were achieved when using PS-NP with incorporated Sudan black indicated a higher sensitivity compared to commonly employed gold NP. Further improvement and a broader applicability might be achieved by using PS-NP with activated NHS esters that readily react with antibodies.  

However, specific mechanisms are required to trigger the intracellular release of the drug payload. Stimuli-responsive PS-NP that react to the change of specific conditions within the cell (e.g., pH value, oxidative conditions, hydrolytic enzymes) are required (see chapter 5.4.). PS-NP with protonatable groups such as the imidazole moiety in urocanate could swell upon decreasing the pH value. Thus, physically incorporate materials derived from acetlated dextran that degrade at low pH values around 5. Cell specificity of the PS-NP can be increased by attaching bioligands such as sugar moieties to the particle surface.
5.4. Smart and Stimuli-Responsive Nanomaterials

For many applications, stimuli-responsive PS-NP are desired that change their physical properties (e.g., size, swelling/dissolution behavior, morphology) or chemical structure (e.g., cleavage of groups, crosslinking) depending on external parameters (e.g., temperature, pH value, ionic strength, and irradiation). As an example, these particles might release drugs locally in a specific target tissue, cell type, or cellular compartment.

Irradiation is a convenient way to induce stimuli responsiveness. Several photochemically active carboxylic acids (2-methoxycinnamic acid, [(4-methyl-2-oxo-2H-chromen-7-yl)oxy]acetic acid, and azobenzene-4-carboxylic acid) were esterified with dextran.\textsuperscript{[114,181]} Depending on the DS values, these photoactive derivatives formed NP with diameters in the range of 115–215 nm either directly or after perpropionylation. By UV irradiation of the PS derivatives in a dissolved state, it was possible to induce structural changes as result of photochemical conversions; E-/Z-isomerization (2-methoxycinnamates, azobenzene derivatives) and photo crosslinking (chromene derivatives). It can be speculated that this approach can be employed to alter the properties of PS-NP derived from these photoactive derivatives.

In an approach to create stimuli-responsive nanomaterials, PS-NP with thiol moieties were prepared by dropping technique using a 3-mercapropionate ester of cellulose that was additionally functionalized with rhodamine B methacrylamide.\textsuperscript{[182]} Depending on the initial polymer concentration, particle sizes of 180 to 360 nm were achieved and the NP could be switched reversibly from fluorescent (low pH and/or irradiation at 365 nm) to nonfluorescent (high pH and/or heating to 130 °C). Moreover, it was demonstrated that the PS-NP are partly stabilized by formation of intermolecular disulfide bonds. Thus, it was possible to reversibly “dissolve” the particles in water by adding reducing agents (e.g., thioglycolate, dithiolthreitol) and to reform them by adding oxidation reagents (e.g., 1,3-propanedithiol; Figure 23). PS-NP were also prepared from dextran derivatives that were...
modified with carbonate groups bearing an oxidation sensitive arylboronic ester moiety.\textsuperscript{[183]} These NP fully degraded to dextran within 2 h upon addition of hydrogen peroxide.

PS-NP changing their properties depending on the pH value is interesting for controlled release of drugs within a particularly acidic or basic environment. A series of functional cellulose derivatives was synthesized by photoinduced thiol-ene reaction of cellulose 10-undecenoyl ester (DS $\approx$ 3) with thiol bearing molecules including 2-(dialkylamino)ethanethiol hydrochlorides (alkyl: methyl or ethyl).\textsuperscript{[184]} The hydrophobic derivatives were shaped into spherical PS-NP with diameters in the range of about 100 to 200 nm. In particular particles with diethylamino groups showed a reversible pH-dependent swelling (low pH/protonated ammonium groups) and deswelling (high pH/deprotonated amino groups; Figure 23).

Dextran NP modified with acetal groups were found to be stable in aqueous media at physiological pH values of 7.4, but degrade readily at low pH values around 5.0 due to increased acetal hydrolysis.\textsuperscript{[78,128]} Mildly acidic conditions are found, e.g., in sites of inflammation, tumor tissues, or endocytic vesicles. Thus, PS-NP derived from dextran acetals are well-suited for targeted delivery of drugs. Following this approach, fluorescein-modified bovine serum albumin and plasmid DNA were incorporated into these dextran-based NP.\textsuperscript{[79]} The loaded particles were subsequently modified with a cell-penetrating peptide, which enabled uptake by nonphagocytic cells. The peptide was bound to the PS-NP surface by reaction of its alkoxyamino group with the dextran reducing-end forming an oxime linkage. By applying loaded and surface-functionalized particles, efficient uptake of fluorescein-modified bovine

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**Figure 23.** (Top) Schematic depiction of the reversible redox-controlled formation of nanoparticles derived from cellulose 3-mercaptopropionate that was previously converted with rhodamine B methacrylamide. Adapted with permission.\textsuperscript{[182]} Copyright 2014, The Royal Society of Chemistry. (Bottom) pH-dependent Z-average diameters and polydispersity indices (PDI) as well as electron microscopy images and schematic representation of nanoparticles derived from cellulose 10-undecenoyl ester (CUE) that was previously converted with either 2-(diethylamino)ethanethiol (DEAET) or 2-(dimethylamino)ethanethiol (DMAET) hydrochloride. Adapted with permission.\textsuperscript{[184]} Copyright 2016, The Royal Society of Chemistry.

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**Figure 24.** Confocal microscopy images (scale bar: 20 $\mu$m) of HeLa cells treated with dextran acetal nanoparticles (NP) that contained fluorescein-modified bovine serum albumin and were a) chemically modified with an alkoxyamine-functionalized cell-penetrating peptide (CPP $+$) or b) treated with an unfunctionalized (i.e., nonbinding) CPP$-$. c) Luciferase activity of nonphagocytic cells treated with dextran acetal NP that contained a luciferase-encoding plasmid DNA and were converted with CCP $+$ or CCP $-$. Adapted with permission.\textsuperscript{[79]} Copyright 2009, American Chemical Society.
serum albumin and transfection of DNA to HeLa cells was demonstrated (Figure 24). Furthermore, dextran acetal-based NP were intensively studied for encapsulation of other substances like ovalbumin, imiquimod, and siRNA.[125–128]

Thermo-responsive PS-NP were prepared from starch acetate (DSacetate = 2.41) decorated with graft-poly(N-isopropylacrylamide) groups (25.7% grafting ratio) using a dropping approach to induce self-assembling into unimolecular micelles.[185] Heating these particles at 60 °C for 24 h led to rearrangement into bilayer vesicles with a slightly increased size (Figure 25). At the early stages of the heating procedure, the thermo-responsive graft-co-polymer side chains shrank resulting in an initial decrease of the particle size. These micelles tend to aggregate due to hydrophobic interaction and attain a minimal energy state with a spherical bilayer morphology. Eventually, the vesicles collapse into irregular particles upon cooling to 20 °C. Vesicles could also be obtained by starch acetate palmitate (DSPalmitate = 0.005 to 0.20; DSacetate = 2.58 to 2.78).

6. Conclusion and Perspectives

It has been demonstrated over the last decade that hydrophobically modified PS derivatives can self-assemble into spherical NP with sizes in the range from about 50 to 500 nm. Different preparation techniques and various types of PS derivatives have been evaluated in this context. The PS-NP have been functionalized, either by physical incorporation of active compounds or by covalent binding of functional moieties, and were successfully tested in biomedical applications such as drug delivery and sensing. The intention of this review is to provide a comprehensive overview of the present state of knowledge in this area of nanotechnology. Some aspects can be identified that will play a crucial role in upcoming research and development activities.

Lab-scale quantities of up to about 100 mL of aqueous PS-NP dispersion are currently available with little experimental effort by the techniques described in this review, in particular dialysis and dropping approaches. However, in order to develop commercialized applications for PS-NP, larger quantities with reproducible batch quality are required. Upscaling strategies for the established procedures need to be studied and new continuous, circular, or large batch technologies need to be evaluated.

Advanced PS chemistry offers many possibilities for introducing functional groups into the PS backbone. These procedures will receive increasing relevance for functionalization of PS-NP including chemical modification of the particles as well as the synthesis of novel functional PS derivatives with self-assembling behavior. Modular synthesis approaches (e.g., “click-chemistry”) can be expected to become more frequent because they enable broad diversity and high reaction efficiencies. Finding novel approaches to introduce specific functionalities into PS-NP is directly tied to research and development efforts aiming for the use of PS-NP in advanced applications. Multifunctional and stimuli-responsive nanomaterials are highly demanded for drug delivery and sensing applications. Novel areas could also be explored (e.g., in the environmental or consumer products sectors), once functional PS-NP become accessible beyond lab-scale quantities. Overall, it can be expected that the focus will gradually shift in the future from fundamental science that aims to understand how PS-NP can be prepared to more applied research with a focus on the development of specific product solutions.

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Conflict of Interest

The authors declare no conflict of interest.

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