**pH-Sensitive Nanocontainers**

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1. Introduction

Materials with well-defined structures in the submicrometer region attract increasing interest. The main idea is to tailor composition and function of materials with precise control over size and morphology at the nanometer level. In this context, for example, the self-assembled superstructures of surfactants, lipids, and amphiphilic block copolymers have proven to be valuable tools.[1] They provide a compartmentalization at the nanometer level, which may be used as a structural template for the newly formed materials. This might lead then to new properties for well-known standard materials and, hence, also to new applications.

Hollow nanoparticles are particularly interesting for applications as confined reaction vessels (e.g., for (bio-) chemistry performed with single molecules[2] or biomimetic mineralization), drug carriers, or protective shells for enzymes or catalysts.[1,3] Similar and very effective nanometer-sized containers, e.g., micelles or vesicles, are used by nature in biological systems. However, their limited mechanical stability prevents many possible applications (e.g., in drug delivery).[4] Therefore a considerable effort has been devoted to the development of preparation techniques leading to polymer nanocapsules of higher stability. Form- and size-invariant nanocapsules were successfully prepared in lipid[5,6] and block copolymer[7] vesicles by polymerization or by multi-step branching reactions leading to dendrimers.[8] Surface-cross-linked hollow polymeric structures can also be obtained by crosslinking the shell of micellar diblock-[9] or triblock copolymer systems.[10] Another possibility for generating polymer hollow spheres is to form a polymer shell around a preformed template particle, which can subsequently be removed. This can be realized by layer-by-layer deposition of oppositely charged polyelectrolyte[11] or by applying emulsion-[12] suspension-[13] or mini-emulsion-[14] polymerization techniques. Despite these very promising synthetic routes to such polymeric nanocontainers their high stability and low permeability seems, however, to be also their major drawback limiting their potential applications, i.e., by preventing an effective loading of preformed capsules or releasing encapsulated material in a controlled way at the desired target. Very recently two promising first approaches have been presented to control the permeability of polymer capsules. This could be achieved by switching the structure of polyelectrolyte multilayer shells[15] or reconstituted membrane proteins in block copolymer membranes[16] reversibly from a closed to an open state with the help of external stimuli. In this article we summarize our recent efforts to provide a related, alternative route to control permeability of polymer nanocapsules.

2. Polyelectrolyte Nanocapsules as Mimetics of Virion Particles

This approach is largely inspired by a naturally occurring nanocontainer system with pH-controlled access to its inner cavity. The protein shells of the cowpea chlorotic mottle virus (CCMV) show a reversible, pH-induced structural transition. Increasing the pH from ca. 5 to 7 leads to a swelling of the protein coat by about 10%.[17] During this swelling gated pores are opened within these shells that allow free molecular exchange
between the virus’ interior and the bulk medium. Recently this gating transition of the virion particles has successfully been used for a host–guest encapsulation.[18] However, practical applications of such virions are not feasible due to the difficulties in their handling and producing them on a larger scale. Therefore our aim was to mimic the virion cages by a fairly simple synthetic system that shows a similar structural transition.

The conformation of polyelectrolytes is very sensitive towards the physicochemical conditions of their surrounding medium (e.g., ionic strength, pH, or presence of multivalent ions). Hence we selected water-soluble polymer hollow spheres formed by covalently crosslinked polyelectrolyte shells as a model system. The carboxylate groups of nanocapsules based, for example, on poly(acrylic acid) dissociate increasingly with increasing pH. As a result of the associated electrostatic repulsion between the increasing number of identically charged carboxylate anions along the polymer backbone the shells of such particles should swell considerably upon raising the pH. We expected this structural transition to influence considerably the permeability of polyelectrolyte shells similar to the CCMV (see Fig. 1 for a schematic representation).

Such polyelectrolyte capsules could successfully be synthesized via vesicular polymerization.[19] Here the hydrophobic part of a lipid bilayer is selectively swollen by hydrophobic monomers (e.g., styrene, alkyl(meth)acrylates). A subsequent crosslinking, free-radical polymerization leads to the formation of a two-dimensional polymer network in the interior of the lipid bilayer. Such polymeric scaffolds increase considerably the mechanical stability of their matrix membranes, without impeding the mobility of the lipids.[20]

Due to their crosslinked nature the polymers preserve their hollow sphere morphology after isolation from the matrix vesicles. While the size and shape of the resulting polymer particles are directly determined by the templating vesicles, the polymer scaffold can fairly easily be modified after its isolation from the vesicular matrix using conventional chemical reactions. As hydrophobic monomers we used mixtures of tert-butyl acrylate and ethylene glycol dimethacrylate as the crosslinking agent. After selective saponification of the tert-butyl ester groups the resulting poly(acrylic acid) spheres are dispersible in water.

The hollow sphere character of these particles could be proved by cryo-transmission electron microscopy (cryo-TEM) and combined static/dynamic light scattering investigations.[19] The behavior of a representative sample of poly(acrylic acid) hollow spheres in buffer solutions of varying pH is shown in Figure 2. As can directly be seen the particle radius increases from about 45 nm at pH < 4 to about 195 nm for pH > 9. This corresponds to an increase of the enclosed volume by a factor of 80! It has to be emphasized that this swelling is completely reversible. The extend of this expansion depends at given pH additionally on ionic strength of the buffer, presence of multivalent ions (e.g., Ca²⁺), crosslinking density of the polymer network structure within the spherical shells and the presence of hydrophobic co-monomers.[19] For example, in buffers of very low ionic strength a swelling of the particles up to a factor of 10 (i.e., an increase of the enclosed volume by a factor of 1000!) has been observed.

Preliminary experiments show that similar to the systems reported in the literature[15] these particles can be used to encapsulate water-soluble polymers and dyes and retain the encapsulated material at low pH values. Interestingly, the particles precipitate for pH < 3.5, which provides a convenient route to isolate and purify the loaded capsules. Subsequently their encapsulated contents could be released again at high pH, which allows local and temporal control on uptake and release of molecules.

Although this route via vesicular polymerization represents a rather elegant approach to achieve such responsive polymeric nanocapsules, potential technical applications of this process are still rather limited. The reason for this lies in the high preparative effort required to produce them in larger quantities (e.g., kilograms) and the obviously very delicate reaction conditions required. The latter is directly reflected in
3. Conclusions

It has to be emphasized that the described polyelectrolyte systems should be regarded as representative examples of such stimuli-responsive nanocontainers. The possibilities to incorporate additional design criteria (e.g., temperature sensitivity, targeting moieties, special surface characteristics) are straightforward. Particularly since our recently established preparation procedure allows the synthesis of such particles also on a technical scale we believe that the principle of using the protective ability of the nanocontainers in combination with controlled permeability will have many future applications in areas such as, e.g., drug delivery, sensor technology, diagnostics, and catalysis.

Fig. 3. TEM graph of hollow nanocapsules before (left) and after (right) loading with pyrene.