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Note

A novel method for encapsulation of poorly water-soluble drugs: precipitation in polyelectrolyte multilayer shells

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Abstract

A novel method to include poorly water-soluble substances into the polyelectrolyte capsules of defined size, stability, composition and affinity properties is proposed. Encapsulation explores the polarity gradient across the capsule wall. Capsules creation makes use of electrostatic interaction and can involve many substances as layer constituents, such as synthetic polyelectrolytes, proteins, nucleic acids, lipids and multivalent dyes. Using capsules made of synthetic polyelectrolytes as a model system was demonstrated how to prepare, to measure and to use this gradient for low molecular weigh materials encapsulation.

Keywords: Drug delivery systems; Microcapsules; Encapsulation; Layer-by-layer; Biocompatibility; Polymers

Preparation of microcontainers for controllable drug delivery is an actual task for research in biotechnology, nutrition and medicine, and a new class of structures that can play a leading role in this field—hollow polyelectrolyte capsules—has recently, been introduced (Donath et al., 1998). Their creation makes use of electrostatic interaction and can involve many substances as layer constituents, such as synthetic polyelectrolytes, proteins, nucleic acids, lipids, inorganic nanoparticles and multivalent dyes (Decher, 1997; Kotov, 1999; Sukhorukov, 2001a). Up to now different colloidal cores of submicron and few-micron size were used to template Layer-by-layer polyelectrolyte assembly on their surfaces, for instance, on organic latex particles, inorganic particles (Caruso et al., 1998), dye and drug (Antipov et al., 2001a) nanocrystals, compact forms of DNA (Trubetskoy et al., 1999), protein aggregates (Balabushevitch et al., 2001), gel beads and even biological cells (Neu et al., 2001).

Basically, large macromolecules cannot penetrate polyelectrolyte multilayers while small solutes like ions or dye/drug molecules readily can. As a result the presence of macromolecules only inside the capsules leads to a difference in physico-chemical properties between bulk and

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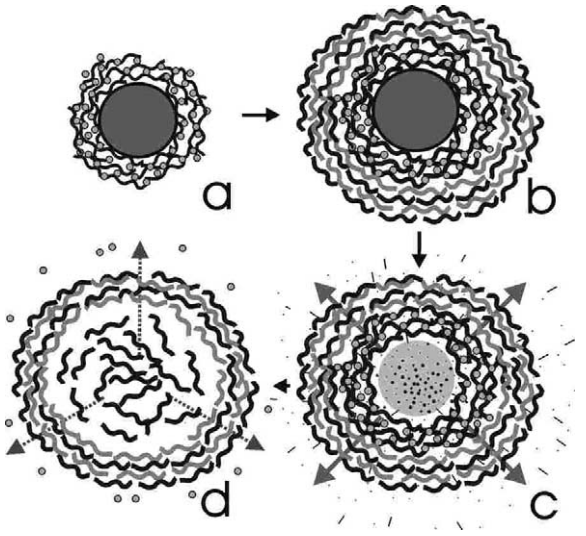
capsule interior (Sukhorukov et al., 1999; Radtchenko et al., in press) and gives an opportunity to establish a polarity gradient across capsule wall which could be used to precipitate poorly water-soluble materials (like most drugs) in their interior. Sustained release of entrapped material is achievable by controllable shell wall permeability (Sukhorukov et al., 2001b). The approach used to deliver macromolecules into the capsule interior to be kept there consists in double shell structure formation where the inner shell, made by the surface controlled precipitation method (Dudnik et al., 2001) can be later decomposed and release polymer components into the capsule interior. The outer shell remains stable and provides polymer capturing (Radtchenko et al., in press), Scheme 1.

For model experiments PSS (sodium Poly(Styrene Sulfonate), MW $\sim$ 70 000) was chosen as constituent of the inner shell because of its strongly charged groups and high stability. The inner PSS-based shell on colloidal MF-particles was obtained by PSS complexation with Yttrium^{3+}-ions (Metal^{3+}/PSS complexes are not absolutely stable—they could be destroyed by high ionic strength and a Metal^{3+} capturing agent (Radtchenko et al., in press) and controlled precipitation of the formed PSS^{-}/Y^{3+} complex



Scheme 1. Schematic illustration of the preparation of loaded by polyelectrolyte capsules.

onto the surface of the MF-particles[1] (1a). The final amount of complex reached a certain value that, as estimated, is sufficient to form approximately 80 monolayers on top of MF-particles. The MF-particles covered with the PSS/Y^{3+} complex were observed by confocal microscopy and show rather smooth coverage; hence, the complex is homogeneously distributed on their surface.

Encapsulation of PSS into polyelectrolyte capsules consists in assembling the stable 4 PSS/Poly(Allylamine Hydrochloride) (PAH), MW $\sim$ 50 000) layer pairs above the core/shell structures obtained by means of the Layer-by-layer technique (1b) (Voigt et al., 1999; Donath et al., 1998). After colloidal core decomposition in 0.1 M HCl (part c) the capsules are composed of two shell structures. To decompose the inner shell the capsules were treated by 2 M NaCl and 0.1M EDTA (condition where the outer shell is still stable) (1d). At normal conditions the capsule wall is permeable for water, hydrogen ions, hydroxyl ions, small salt ions and small organic molecules (Antipov et al., in press). Multivalent ions are small enough to penetrate through polyelectrolyte multilayers forming the outer shell and to be removed from the capsule suspension whilst the polymer used for inner shell build-up cannot be expelled due to its high molecular weight. Thus, this polymer is captured inside the capsule as freely floating molecules. A typical confocal fluorescence image of the capsules filled with free PSS after inner shell decomposition is shown on Fig. 1 (polyanion was labelled with rhodamine 6G).

The concentration of free polyanion molecules inside the capsules was estimated by dye binding and yielded a 0.1 monoM polymer concentration, i.e. a few pikograms of polymer per capsule. Actually, the amount of loaded polymer is determined by the ratio of polymer and colloidal particle concentrations at the controlled precipitation stage.

[1] Dispersions of monodispersed weakly cross-linked melamine formaldehyde particles (MF-particles) with a diameter of 5.6 μm were purchased from Microparticles GmbH (Berlin, Germany). These particles dissolve at pH values less than 1.6 and have been used as templates for the production of hollow polyelectrolyte capsules (Donath et al., 1998).

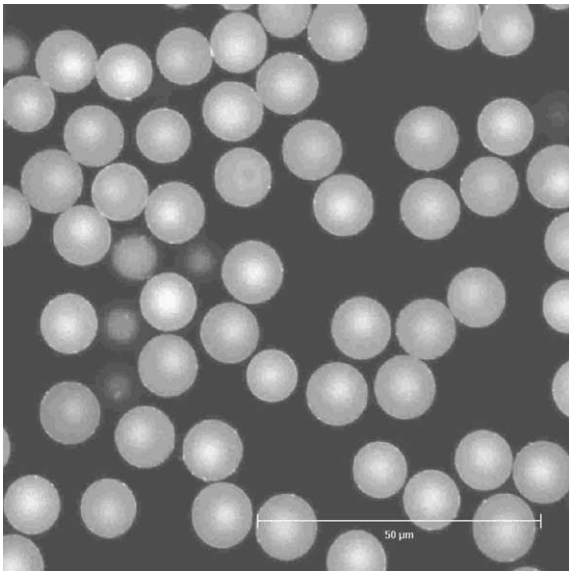


Fig. 1. Fluroscent confocal microscopy image of the polyelectrolyte capsules loaded with PSS by means of inner shell decomposition. Fluorescence comes from dye electrostatically bounded to PSS.

Polyelectrolytes have the ability to bind water molecules around their charged groups that's why, placed in mixture of water with nonpolar solvents, they will increase the water content in their vicinity. This effect was used to establish the polarity gradient between bulk and interior of capsules filled with polyelectrolytes. Indeed loaded shells exposed to organic solvents/water mixtures contain 'extra' water inside (bounded to loaded material) what effectively means more water molecules in their interior and a shift of the organic solvents/water ratio in their inner volume—as result of the polarity gradient through the capsule wall. To measure the gradient filled with PSS-Rhodamine (PSS was copolymerised with rhodamine (Dahne et al., 2001)) copolymer capsules were exposed to different water/acetone mixtures and their fluorescence was analysed (rhodamine derivates have pronounced sensitivity to polarity of the solvent, insert in Fig. 2 shows spectra of PSS-Rhodamine exposed to pure water (solid) and acetone 90% (dash) solutions). The resulting dependence of the acetone concentration inside the capsules versus one in the bulk is presented in Fig. 2. As clearly seen from the graph the amount of acetone inside the loaded capsules has a tendency to increase with increasing its bulk concentration, but even exposed to pure acetone they still keep about 50% of water in their interior.

The polarity gradient between capsule inner volume and bulk solution allowed to precipitate PWS materials exclusively inside them. The idea of precipitation is: a solution of PWS specimen in acetone is mixed with a water suspension of loaded capsules and diluted with acetone till total dissolution of the PWS material. Afterwards the mixture is exposed to air and acetone and let to evaporate. At a certain moment the amount of water inside the modified capsules will be high enough to force the PWS molecules to precipitate there while they will be still soluble in the bulk. Because of the gradient this precipitation will take place only inside the capsules if there is any free space and only with total filling and further acetone evaporation also outside the capsules. The key is to choose the right amount of PWS material, sufficient to just to fill the chosen amount of capsules. Experimentally this idea was proved with the model of capsules filled with PSS and Disperse Red 1 (DR1) as a PWS material. The resulting water suspension of capsules with pre-

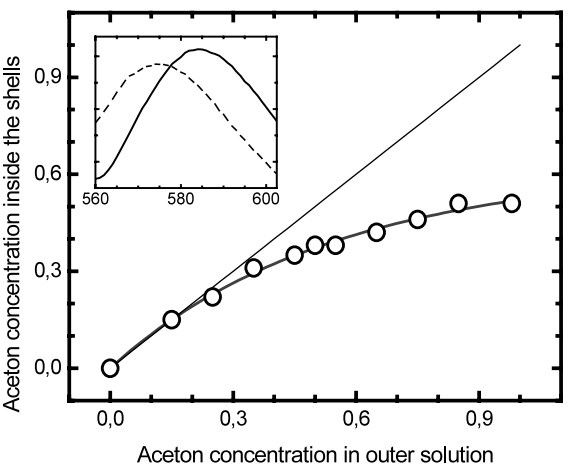


Fig. 2. Dependence of the acetone concentration inside the capsules versus one in the bulk. In the insert spectra of PSS-Rhodamine copolymer exposed to pure water (solid) and acetone 90% (dash) solutions.

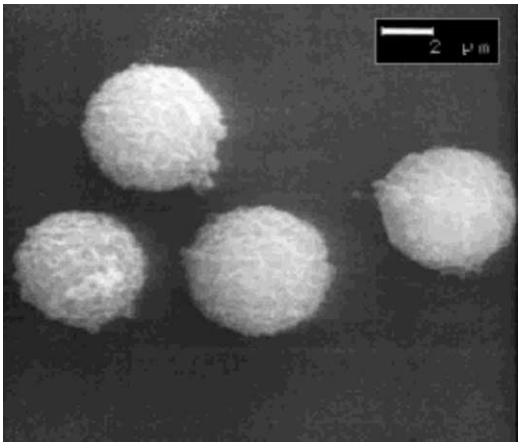


Fig. 3. Scanning electron microscopy image of the DR1 precipitated in modified with PSS capsules.

cipitated DR1 inside was observed by confocal fluorescence microscopy that reveal fluorescence from the capsule interior—an evidence of DR1 encapsulation. The concentration of loaded DR1 was estimated by mass spectroscopy and gave molar values. The dry state of capsules filled with DR1 was investigated by scanning electron microscopy (Fig. 3). The homogeneity of the capsules means controllable dosage of the PWS material. It should be noted that the same type of PWS precipitation was also obtained on another model system—capsules filled with a polycation (PAH).

Encapsulation of polyelectrolytes is a key process to control the physico-chemical properties of the interior of organic micro- and even nano-capsules. The presence of different polyelectrolytes only inside the capsules enables us to establish a polarity gradient across the capsule wall and as a result a mechanism to encapsulate a wide class of PWS materials. With the model system we demonstrated the controllability and high efficiency of the process. Precipitates were found only inside the modified capsules. A water suspension of capsules filled with PWS material, as a final product is easy to manage and to control the concentration and release. Such modified capsules could be used as microcontainers and suitable delivery system for a wide variety of drugs.

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