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Sustained Release Properties of Polyelectrolyte Multilayer Capsules

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Layer-by-layer (LbL) assembly of oppositely charged polyelectrolytes was used to coat fluorescein particles. These particles, with a size of 4−9 μm, were prepared by precipitation of fluorescein at pH 2. Polystyrensulfonate (PSS) and polyallylamine (PAH) were used to form a polyelectrolyte shell on the fluorescein core. The permeation of fluorescein molecules through the polyelectrolyte shell during core dissolution was monitored at pH 8 by the increasing fluorescence intensity as a result of dequenching. The number of polyelectrolyte layers sufficient to sustain fluorescein release was found to be 8−10. Increasing the number of layers prolonged the core dissolution time for minutes. The permeability of polyelectrolyte multilayers of the thickness of 20 nm for fluorescein is about 10^{-9} m/s. The features of the release profile and possible applications of the LbL method for shell formation in order to control release properties for entrapped materials are outlined.

Introduction

A major challenge in the development of advanced drug formulations is the elaboration of delivery systems capable of providing sustained release of bioactive materials. Mostly, these systems comprise polymer particles in the size range of 10^2 to 10^5 nm. The drug molecules are embedded in polymer matrices[1−3] or in core−shell structures.[4−6] In the latter, the shell degradation rate determines the release rate of the bioactive core material. In principle, a shell around the active core can be fabricated by adsorption of polymers or biopolymers onto the drug particle surface[7] or by adsorption of the monomers with subsequent polymerization at the interface.[8−10] The composition of the shell may additionally provide certain functionalities. It may be adjusted to facilitate the interaction of the core with the solvent or to add certain desired chemical properties. The shell may also have magnetic, optical, conductive, or targeting properties for directing and manipulating the core containing bioactive material.

Recently, a novel type of shell structure has been introduced.[11−13] Capsules were fabricated by layer-by-layer adsorption (LbL) of oppositely charged species[14] onto the surface of colloidal particles. Various cores, i.e., organic, inorganic, biological cells, drug crystals, emulsion droplets, ranging in size from 0.1 to tens of microns have been utilized as templates for multilayer formation by the LbL technique. Up to now a variety of different substances, such as synthetic and natural polyelectrolytes, proteins, nucleic acids, magnetic and fluorescent inorganic nanoparticles, lipids, etc., have been employed as layer constituents. The thickness of the shell walls depends on the conditions of its preparation. It can be tuned in the nanometer range. It was established[15,16] that the capsule walls have semipermeable properties. They are permeable for small molecules such as dyes and ions while they exclude compounds with a higher molecular weight. The exclusion of macromolecules can be tuned by environmental conditions. Capsules composed of polyelectrolytes, the charge of which depends on the pH, can be used for a pH-controlled uptake and release of macromolecules. The potential use of polyelectrolyte capsules for drug release, however, would require more quantitative data on the permeation of small molecules through polyelectrolyte walls. In light of sustained release, it would be advantageous to be able to decrease the layer permeability for small polar molecules once they are encapsulated. One possible way to approach this goal is the use of lipids as a layer constituent.[16−17] The formation of thicker capsule walls might be another straightforward way to decrease permeation.[18] It is thus expected that increasing the layer number will decrease dye penetration.

To verify this approach, fluorescein microparticles were covered with a different number of polyelectrolyte layers. Dyes such as fluorescein can be considered as model substances for a large class of drugs. Afterward, core dissolution was initiated by a pH change and monitored by the increasing fluorescence in the bulk.

Experimental Section

Materials. *Polyelectrolytes*. Sodium poly(styrene sulfonate) (PSS, Mw ~70 000), poly(allylamine hydrochloride) (PAH, Mw 50 000), and fluorescein, sodium salt, were obtained from Aldrich. Ethanol, sodium chloride, boric acid, and hydrochloric acid were purchased from Sigma. All materials were used without further purification.

The water used in all experiments was prepared in a three stage Millipore Milli-Q Plus 185 purification system and had a resistivity higher than 18.2 MΩ cm.

Methods. Fluorescein particles were prepared by addition of one part of ethanol and four parts of hydrochloric acid, pH 2,

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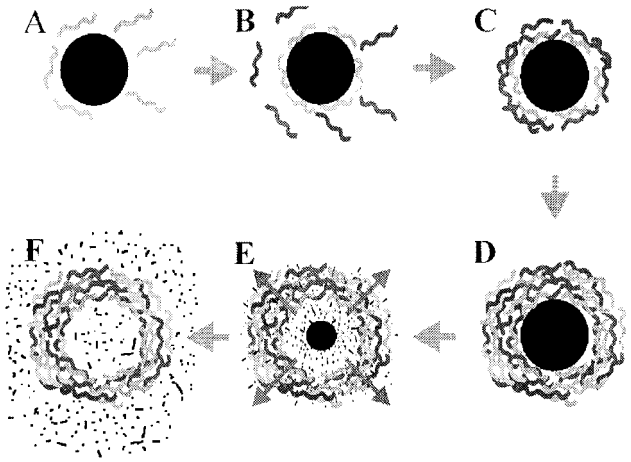

Figure 1. Scheme of the polyelectrolyte multilayer deposition process and of the subsequent core dissolution. The initial steps (A−D) involve stepwise shell formation on a fluorescein core. After the desired number of polyelectrolyte layers is deposited, the coated particles are exposed to pH 8 (E) and core dissolution with fluorescein penetration into the bulk is initiated, resulting finally in fully dissolved cores and remaining empty capsules (F).

to one part of 15 mg/mL aqueous fluorescein solution. The particle size was measured by optical microscopy. The 4−9 μm diameter fluorescein particles were washed in hydrochloric acid by five repeated centrifugation cycles at 450 g. To prevent preliminary core decomposition, all further multilayer deposition and washings were performed in hydrochloric acid at pH 2.

Polyelectrolyte Multilayer Assembly. The multilayer assembly was accomplished by adsorption of polyelectrolytes at a monomer concentration of 10^{-2} M in 0.5 M NaCl, pH2. Oppositely charged polyelectrolyte species were subsequently added to the suspension of fluorescein particles followed by repeated centrifugation cycles in hydrochloric acid.[13] Fluorescein particles were allowed to interact with polyelectrolyte solution for 15 min. Fluorescein particles were centrifuged at 700 g for 10 min. Gentle shaking followed by 1 min ultrasonication was used to disperse particles after centrifugation. PSS was used to form the first layer.

Fluorescence Spectroscopy. The core dissolving was conducted in H_3BO_3−NaCl−NaOH buffer, pH 8. The kinetics was followed by recording the time dependence of the emission at 522 nm. Excitation was set at 488 nm.

Confocal Microscopy. Confocal images of capsules after dissolving the core were obtained by means of a Leica confocal scanning system. A 100× oil immersion objective with a numerical aperture of 1.4 was used.

Results and Discussions

Figure 1 provides the scheme of fluorescein particle encapsulation and release. After LbL adsorption (Figure 1A−D), core dissolution is initiated by changing the pH from pH 2 to pH 8 (Figure 1E) and completed after a certain period of time (Figure 1F).

Fluorescein particles rapidly dissolve at pH 8. The idea was thus to slow the rate of core dissolving by covering the particles with a polyelectrolyte multilayer. Shell walls consisting of a different number of layers were fabricated and examined in regard to their fluorescein permeability behavior. Fluorescence spectroscopy is a convenient tool for the determination of the core dissolving rate because the fluorescence of the core is completely suppressed as a consequence of the self-quenching of the dye. Upon releasing the dye into the bulk, the fluorescence

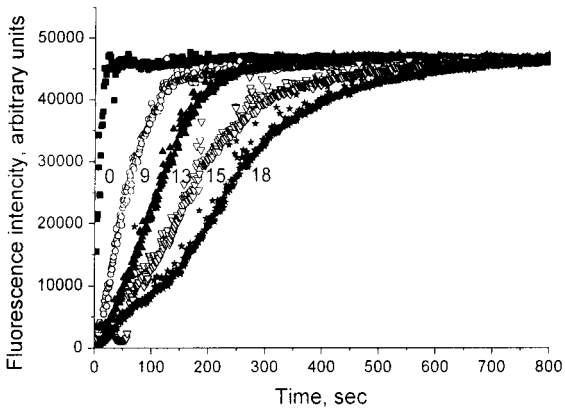

Figure 2. Fluorescence increase upon time, obtained by dissolving fluorescein particles covered with shells of different thickness (9, 13, 15, and 18 layers), compared with naked (0) fluorescein particles.

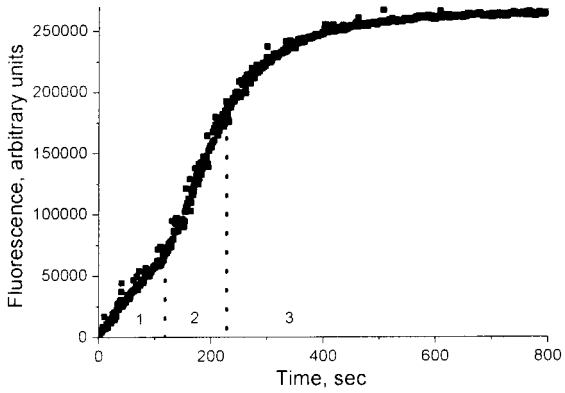

Figure 3. Three stages of dissolution of fluorescein core covered with 17 PSS/PAH layers.

intensity increases. Thus, the rate of cores dissolving can be followed directly by measuring the fluorescence increase in the sample.

In Figure 2 typical time-dependent fluorescence curves, which were obtained by switching the pH to 8, are shown. Fluorescein particles covered by layers of different thickness (9, 13, 15, and 18 layers) are compared with the control showing the fluorescence increase upon the dissolving of bare fluorescein particles. As shown in Figure 3, after a comparatively short induction period (1) the rate of dissolving becomes constant (2) before finally the fluorescence in the bulk levels off (3). The initially more slowly increasing fluorescence is related to the start of core dissolution. At this stage of the process, the structure of the polyelectrolyte multilayer may change because of the nascent osmotic pressure coming from dissolved fluorescein molecules. Shortly after the beginning of core dissolution, the concentration of fluorescein inside the capsules becomes saturated and hence stays constant, because a steady-state situation between progressing core dissolution and permeation is established. This situation is described by a constant concentration gradient between the shell interior and the bulk because the bulk solution can be assumed as being infinitely diluted. Therefore, the rate of fluorescein permeation through the polyelectrolyte layers becomes constant. Indeed, a linear increase of the fluorescence is observed (2). This state corresponds to the stage of dissolution depicted in Figure 1E. The slope of the linear region decreases with the number of polyelectrolyte layers. Obviously an increasing number of adsorbed layers reduces the fluorescein permeation. After the core is completely dissolved, the fluorescein concentration inside the shell decreases. Diffusion slows down and finally the release stops (3).

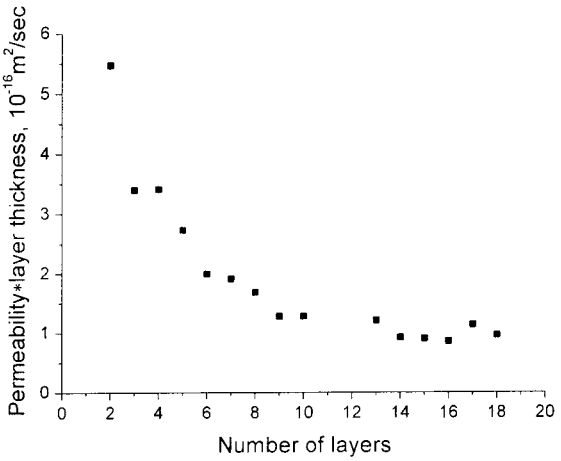

Figure 4. Fluorescein diffusion coefficient as a function of layer number.

Let us discuss the permeation as a function of time in quantitative terms. The flux of fluorescein through the shell wall is given by its permeability, multiplied by the concentration difference and the shell wall area. Equation 1 combines this flux (J) with the rate of change of fluorescein concentration, c_e, in the bulk. When this rate is constant, one may easily calculate the permeability (P) from the slope of the fluorescence increase (part 2 on Figure 3).

$$\frac{dc_e}{dt}V_0 = J = P(c_i - c_e)S \quad (1)$$

where V_0 is the volume of solution, J is the fluorescein flux through the capsule walls with the total surface area S, and ($c_i - c_e$) is the difference of the fluorescein concentration inside (c_i) and outside (c_e) the capsules.

The fluorescein concentration inside the capsule is constant (saturated) as long as the core is not fully dissolved. It is 250 mg/mL at 25 °C. The fluorescein concentration in the bulk c_e is much less. Hence, the concentration difference in the right side of eq 1 can be replaced by $c_i - c_e = c_s$, where c_s denotes the concentration of a saturated fluorescein solution. The capsules were assumed spherical with an average diameter 5 μm. The permeability thus becomes

$$P = \frac{dc/dt \cdot V_0}{c_s S} \quad (2)$$

where dc/dt is taken from the experimental slopes.

For 8 to 18 layers, the permeability value varied from 7×10^{-9} to 2×10^{-9} m/s. Assuming a thickness of 3 nm for each individual polyelectrolyte layer, the permeability can be converted into a diffusion coefficient (D) by means of multiplying the permeability with the shell wall thickness. The calculated diffusion coefficients ranged from 1.7×10^{-16} to 1×10^{-16} m^2/s.

If the permeability of the polyelectrolyte multilayer is provided by diffusion through the entangled polymer network, it should scale with the inverse of the layer thickness. The behavior of the permeability times thickness as a function of the number of layers is shown in Figure 4. As can be seen, the permeability decreases with increasing layer number much faster than expected from a straightforward thickness increase. Only from approximately 8 layers onward does the permeability multiplied by the shell thickness become a constant, indicating that the permeability is now controlled by the thickness increase; for example, the diffusion limiting region is the polyelectrolyte layer. This finding is consistent with the earlier observations[19] where it was shown that the conformation of the first eight layers differs from that of further assembled layers. These deeper layers are more dense, resulting in a 5-fold reduction of the estimated diffusion coefficient as can be inferred in Figure 4.

Let us discuss in more detail the permeation mechanism. One may distinguish between diffusion through water-filled pores and a "bulk" diffusion mechanism through the homogeneous phase of the polyelectrolyte multilayer shell. For thinner walls, the drastic dependence on shell thickness may be explained either by existence of pores that are successively closed by further layer deposition or by thickness-dependent diffusion coefficient. Diffusion dependence on layer depth was indeed observed when the diffusion of polar labels in planar polyelectrolyte films was studied. Diffusion coefficients ranging between 10^{-18} and 10^{-20} m^2/s were obtained.[19] To compare our results with those of the previous study, we should remark the following differences: (1) in ref 19 the films were prepared by drying after each adsorption step, which is not possible during particle coating and which is known to result in a denser film; (2 the measurement in ref 19 consisted of depositing a dye probe of a defined depth into the film and observing the time-dependent fluorescence changes due to quencher diffusion. For sufficiently small pore concentration, which is surely the case here, this technique is sensitive only to "bulk" diffusion and will not reflect the permeation through pores.

Thus one may expect that the diffusion coefficients that are derived from permeability data are larger than values measured in ref 19, and from this work we also know that they may vary by at least an order of magnitude with salt conditions. Still, this cannot explain the difference of 2−3 orders of magnitude, and therefore we conclude that it is highly probable that the fluorescein permeation occurs via diffusion through pores. Comparing the diffusion coefficient D obtained in this study with that in bulk water ($\sim 10^{-10}$ m^2/s), which is an upper limit for the diffusion in the pore volume, we may estimate the pore area in the shells wall. From this comparison it can be concluded that the pore area constitutes not more than 10^{-6} of the total surface.

Next, we address a possible mechanism of pore formation. It is quite conceivable that the hydrostatic pressure difference arising as a result of the water flux driven by the difference in osmotic pressure appearing upon fluorescein core dissolution creates a tension in the wall, which may widen existing or create new pores. As seen in Figure 3 (part 1), especially for a larger numbers of layers, in the beginning of dissolution the release is sustained. The pores are formed as a result of a rearrangement of polyelectrolyte molecules, which facilitate the permeation.

Concerning the third state of the fluorescein release curve, it can be expected that the time dependence of the release at this stage is exponential:

$$c_e(t) \sim (1 - e^{-P(\bar{S}/V)t}) \quad (3)$$

where $\bar{S}$ and V represent the surface and the volume of an average capsule. However, because the onset of this state is defined by complete core dissolution for polydisperse samples as in our case, eq 3 can only be used for permeability estimation. Similar values were obtained.

Conclusion

To summarize, it can be concluded that polyelectrolyte multilayer shells assembled around cores consisting of low

molecular weight compounds provide barrier properties for release under conditions where the core is dissolved. This finding is a novel approach for fabrication of systems with prolongated and controlled release properties. The release can be adjusted with the number of assembled polyelectrolyte layers. This system provides also an elegant mean for studying the permeability of polyelectrolyte multilayers.

A large variety of synthetic polyelectrolytes with different properties, lipids, and polysaccharides have been already used for multilayer assembly.[16,20] This provides many possibilities to tune the release properties of the shells together with ensuring biocompatibility and possibility of using various cores. The assembly of shells by the LbL technique opens new pathways for biotechnological applications, where controlled and sustained release of a substance is required. Many problems connected with drug formulation, release, and delivery, controlling the concentration in the organism and periodicity of its reception might be solved by the formation of shells on precipitates and nanocrystals.

We hope that the possibility of convenient measuring and controlling release through polyelectrolyte multilayer shells will stimulate further studies toward applications of core−shell structures fabricated by the LbL technique.

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