Controlled Permeability of Polyelectrolyte Capsules via Defined Annealing

Gemma Ibarz,[‡] L. Dähne,*[,||] Edwin Donath,[§] and Helmut Möhwald[‡]

Max-Planck Institute for Colloid and Interfaces, D-14424 Potsdam, Germany, Institute of Medical Physics and Biophysics, Liebigstrasse 27, D-04103 Leipzig, Germany, and Capsulution NanoScience AG, Geiselbergstrasse 69, D-14476 Golm, Germany

Received December 10, 2001. Revised Manuscript Received May 31, 2002

The influence of thermal treatment on the permeability of polyelectrolyte multilayer capsules is studied via confocal fluorescence microscopy of individual microcapsules. For the specific most frequently used example, capsules made of poly(styrenesulfonic acid) and poly(allylamine hydrochloride), the penetration of fluorescein is shown to be reduced by 3 orders of magnitude on heating at 80 °C. The data show that holes formed during preparation may be annealed in a predictable way.

Introduction

Multilayers made by consecutively adsorbing polyelectrolytes of alternating charge[1] are an increasingly active area of research because of many promising applications and the ease of manufacture. Although not all the details of the formation of these films are completely understood, e.g., nonequilibrium features are important, it has been possible to use the method to prepare supramolecular structures, notably hollow capsules with walls prepared by the technique in a similarly well-defined way.[2] For this control of permeability is a most important issue, but we often encountered problems reproducing results by measuring at different times after capsule fabrication. It turned out that samples age in a way that they become less permeable with time. We also observed that structural changes accelerated with increasing temperature,[3] and this calls for a more systematic study of the permeability to extrapolate to times and temperatures experimentally inaccessible. Although there exist many papers on encapsulation via much larger capsules,[4,5] we are here able to report on permeability of individual small capsules and its control using temperature treatment.

Our strategy in this paper is analogous to that frequently applied for polymeric glasses where relaxation processes are described by phenomenological equations with parameters depending on time and temperature.[6]

Establishing these parameters in an experimentally accessible range (e.g., at higher temperatures), one can then conclude on other conditions (e.g., on room temperature where the relaxation could take years). The analogy of these systems with glasses seems appropriate since probe diffusion appears similarly slow[7] and a recent paper[8] took the analogy even further with good arguments for salt-controlled glass transitions.

Materials and Methods

Materials. Sodium poly(styrenesulfonate) (PSS, $M_w \sim 70\,000$), poly(allylamine hydrochloride) (PAH, $M_w \sim 70\,000$), and fluorescein, sodium salt ($M_w = 376.28$) were purchased from Aldrich.

Weakly cross-linked melamine formaldehyde particles (MF particles), 6 μm in diameter, were obtained from Microparticles GmbH, Germany. All chemicals were used as received. The water used in all experiments was purified by USF Deutschland GmbH Purelab Plus purification system and had a resistivity higher than 18.2 MΩ·cm.

Capsule Preparation. The membrane filtration technique was applied[9] to adsorb consecutively PSS and PAH onto MF particles. The adsorption of polyelectrolytes (1 mg/mL) was carried out in 0.5 M NaCl solution for 5 min followed by three washings with H_2O. Then, the oppositely charged polyelectrolyte species was added. After eight layers, the melamine resin core was dissolved in hydrochloric acid at pH 1.1. The complete decomposition of the cores required about 20 s,[10] and the core degradation products were washed off with purified water several times until a neutral pH was achieved. The outermost layer in this study is always the positively charged PAH.

Optical Measurements. The permeability of the wall of an already formed hollow capsule was investigated by means of confocal laser scanning microscopy (CLSM). Confocal micrographs were taken with a Leica TCS NT confocal system

* To whom correspondence should be addressed.
[†] Max-Planck Institute for Colloid and Interfaces.
[‡] Institute of Medical Physics and Biophysics.
[§] Capsulution NanoScience AG.
[||] Current address: Capsulution NanoScience AG, Volmerstr. 7b, D-12489 Berlin, Germany.

(1) Decher, G. *Science* **1997**, *277*, 1232.
(2) Donath, E.; Sukhorukov, G. B.; Caruso, F.; Davis, S. A.; Möhwald, H. *Angew. Chem., Int. Ed.* **1998**, *37*, 2202; *Angew. Chem.* **1998**, *110*, 2324.
(3) Leporatti, S.; Gao, C.; Voigt, A.; Donath, E.; Möhwald, H. *Eur. Phys. J. E* **2001**, *5*, 13.
(4) Brissova, M.; Lacik, I.; Powers, A. C.; Anilkumar, A. V.; Wang, T. *J. Biomed. Mater. Res.* **1988**, *39*, 61.
(5) Gaserod, O.; Sannes, A.; Skaj-Braek, G. *Biomaterials* **1999**, *10*, 773.
(6) Buchenau, U. *Glaszustand und Glasübergang*; Institut für Festkörperforschung, Forschungszentrum Jülich GmbH: Jülich, 1991.
(7) von Klitzing, R.; Möhwald, H. *Macromolecules* **1996**, *29*, 6901.
(8) Kovacevic, D.; van der Burgh, S.; de Keizer, A.; Cohen Stuart, M. A. *Langmuir* **2002**, *14*, 5607.
(9) Voigt, A.; Lichtenfeld, H.; Sukhorukov, G. B.; Zastrow, H.; Donath, E.; Bäumler, H.; Möhwald, H. *Ind. Eng. Chem. Res.* **1999**, *38*, 4037.
(10) Gao, C. Y.; Moya, S.; Lichtenfeld, H.; Casoli, A.; Fiedler, H.; Donath, E.; Möhwald, H. *Macromol. Mater. Eng.* **2001**, *286*, 355.

10.1021/cm011300y CCC: $22.00
Published on Web 09/19/2002

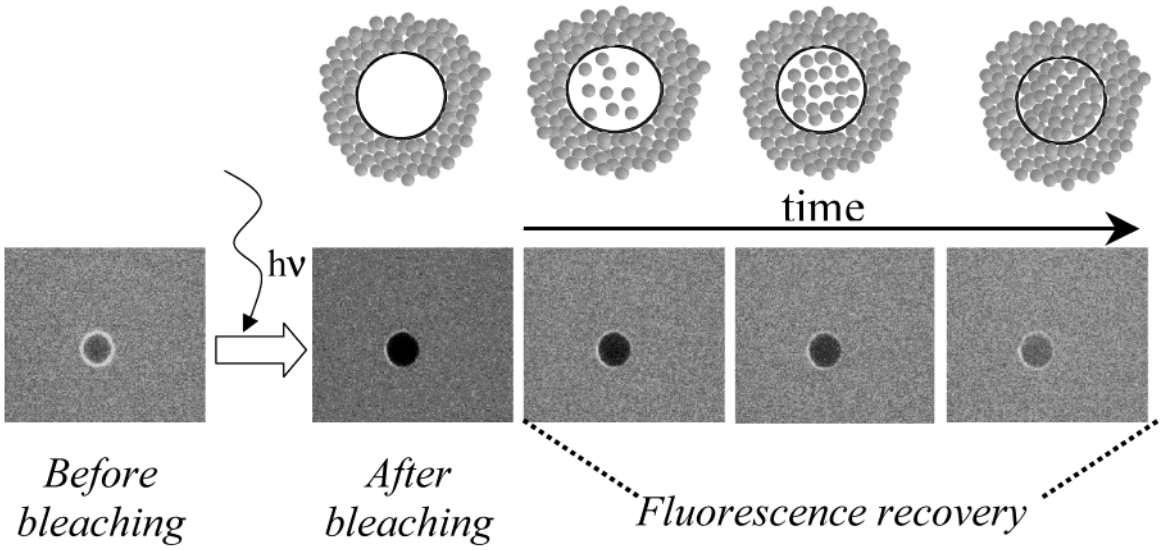

Figure 1. Typical CLSM measurement where the capsule permeability is quantified by means of fluorescence recovery after photobleaching (FRAP). The permeant molecule is the fluorescent dye fluorescein.

(Leica, Germany), equipped with a 100× oil immersion objective with a numerical aperture of 1.4. As the fluorescent label fluorescein was used, labeling was conducted by directly mixing the fluorescein with the capsule solution on a glass cover slip approximately 2 min before the observations started.

Quantification of Permeability. The capsule permeability was quantified by means of fluorescence recovery after photobleaching (FRAP). To follow the diffusion of fluorescein across the wall, we performed photochemical bleaching of the capsule interior. This was effected using the 488 nm line of the CLSM ArKr laser. The laser beam was focused onto a spot inside the capsule at 100% intensity. The time of bleaching was large enough to ensure that almost all fluorescein molecules in the capsule diffused through the laser focus. Imaging was typically performed at about 20% of the maximal laser intensity. The interval between image scans varied depending on the duration of recovery established in an initial pilot experiment. Recovery was considered complete when the intensity of the photobleached region stabilized (that is, the curve flattened). For quantitative analysis, the fluorescence intensity was integrated by tracing a closed area in the interior (ROI analysis provided by the CLSM software), giving an intensity value for each time point.

We have to remark that there is a small amount of fluorescence loss owing to repeated scanning during recovery. We did not consider this effect for the results in this paper.

Results

Figure 1 shows a typical confocal fluorescence microscopy measurement from which we conclude on capsule permeation by a fluorescent dye probe. At low excitation intensity, we observe equal emission from inside and outside the capsule (left), and there appears to be some dye enrichment at the wall because of adsorption.[11] With increasing excitation intensity, the dye is bleached in the illumination area. However, fast diffusion of nonbleached dye in the external water phase still yields unaffected fluorescence from outside the shell in steady state. The inside, though, becomes dark because nonbleached dye penetrates the wall too slowly. With decreasing excitation intensity, one can then observe the fluorescence recovery in the capsule interior as a function of time and conclude on wall permeation.

(11) Caruso, F.; Lichtenfeld, H.; Donath, E.; Möhwald, H. *Macromolecules* **1999**, *32*, 2317.

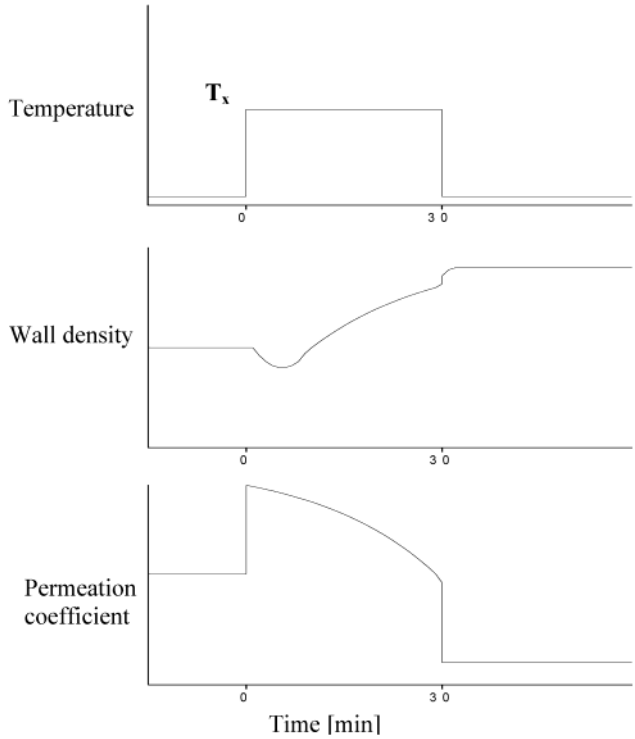

Figure 2. Temperature treatment protocol and expected variation of the wall density and permeation coefficient from PSS/PAH polyelectrolyte capsules as a function of time.

We will thus measure quantitatively the permeability of individual capsules.

The treatment protocol of our capsules is depicted in Figure 2. After preparation, we increase their temperature to a fixed value T_x, keep this for 30 min, and then perform permeation studies at room temperature. T_x varied between 50 and 80 °C.

Along this time sequence we expect the polymer segment density in the film to vary qualitatively as depicted in the middle of Figure 2: there may be some slight density decrease due to thermal expansion, but more prominently the density may become more homogeneous due to rearrangement in the matrix causing the elimination of voids. With return to room temper-

ature, the thermal expansion is gone and one obtains an overall dense wall. The permeability with time is expected to behave nonmonotonically, too. One expects a drastic increase because of thermal activation and a decrease with decreasing number of defects because of densification. With return to room temperature, one then expects less thermal activation but the structure relaxation will remain. It may be desirable to measure the permeability along this protocol, but present conditions enable only room-temperature experiments after the annealing protocol. Densification may also result in a decrease of layer thickness, but at the same time, the diffusion coefficient is expected to decrease as well. Overall, we assume that the major effect is brought about by the disappearance of voids. Hence, the permeation coefficient $P = D/h$, where D is the diffusion coefficient and h is the layer thickness, will decrease, as shown in Figure 2 (bottom)

Figure 3 displays the fluorescence intensity from the capsule interior as a function of time after a bleach pulse for different capsules, annealed at 80 °C and at 50 °C. One clearly observes a much faster recovery after annealing at the lower temperature and that then only partial bleaching was possible. One also realizes that the fluorescence intensities vary by up to ±25%. Reasons for this may be that the onset of recovery is not well-defined, that the permeability of individual capsules varies, and that some capsules had been partly affected by bleaching another capsule before. Irrespective of these fluctuations, the temperature dependence is so drastic that we can safely proceed with a more quantitative analysis. The recovery curve of the fluorescence intensity $I(t)$ as a function of time t is theoretically described as

$$I(\mathrm{t}) = I_\mathrm{o} + (I_\mathrm{s} - I_\mathrm{o})(1 - \mathrm{e}^{-S/VPt}) = I_\mathrm{o} + (I_\mathrm{s} - I_\mathrm{o})(1 - \mathrm{e}^{-3Pt/r}) \quad (1)$$

where I_s and I_o represent the fluorescence at $t \rightarrow \infty$ and $t = 0$, respectively. P is the permeability and S and V are in that order the surface and volume of the capsule assumed as a sphere.

We then fit the time dependence by a function

$$I = I_\mathrm{ini}(1 - \mathrm{e}^{-At}) \quad (2)$$

The coefficient A is related to the diffusion coefficient according to

$$A = 3P/r = 3D/rh \quad (3)$$

for diffusion through a spherical wall with radius r and thickness h.

The structure of eq 2 immediately follows as a solution to Fick's law

$$\mathrm{d}c/\mathrm{d}t = -A\,(c - c_\mathrm{o}) \quad (4)$$

with c_o and c being the concentrations outside and inside the capsules, respectively, and $c \sim I$. A typical fit from which we deduce A is given in the bottom of Figure 3. One realizes that the fit is reasonable considering the spread of data points.

The coefficient A derived from the data as a function of temperature is given in Figure 4, the central result

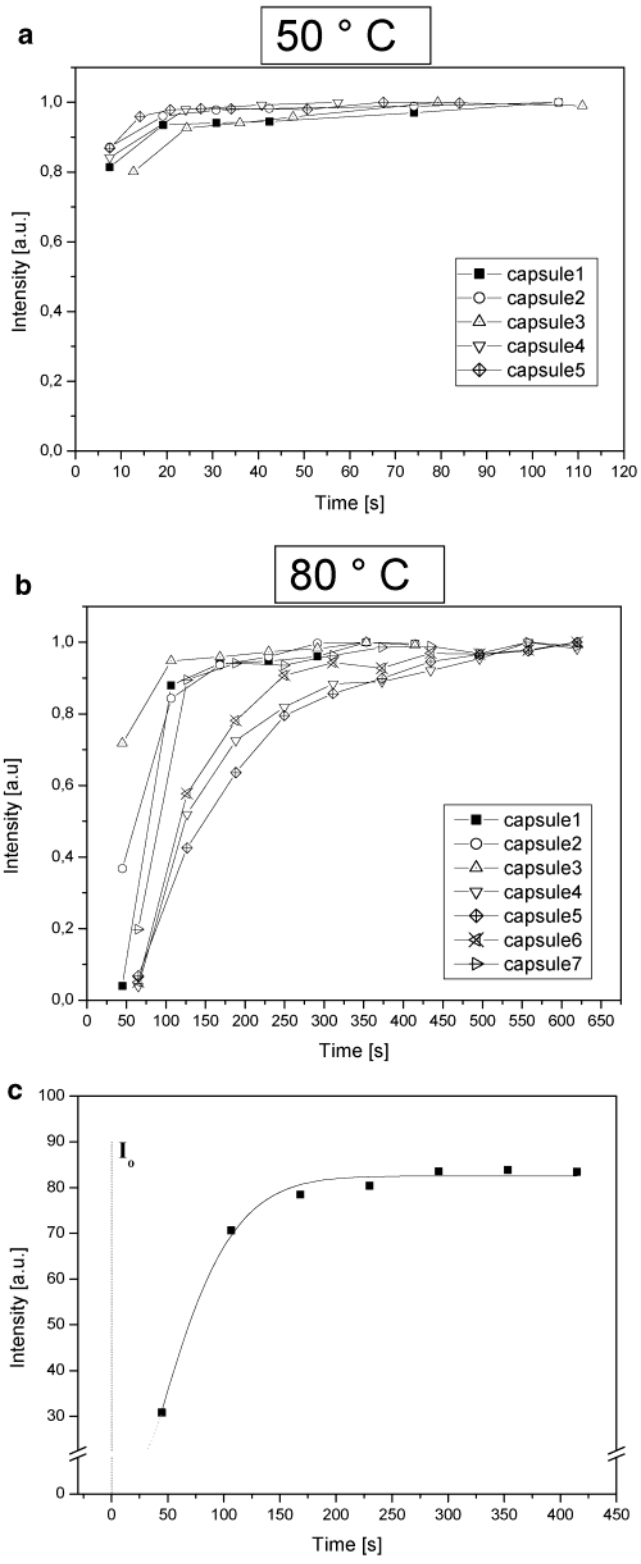

Figure 3. Fluorescence intensity recovery curves of PSS/PAH capsules treated at 50 and 80 °C, respectively. Bottom: Typical fit of a recovery curve using eq 2.

of this paper. Although values measured for individual capsules vary by 50%, conclusions can safely be drawn because the values vary with temperature by a factor of 30. A function $A \sim \exp(-BT)$ appears to describe the permeability data well, where T denotes the annealing temperature and B depends on the annealing time, although this is not the only possibility and it is not based on any mechanistic model. Included in Figure 4

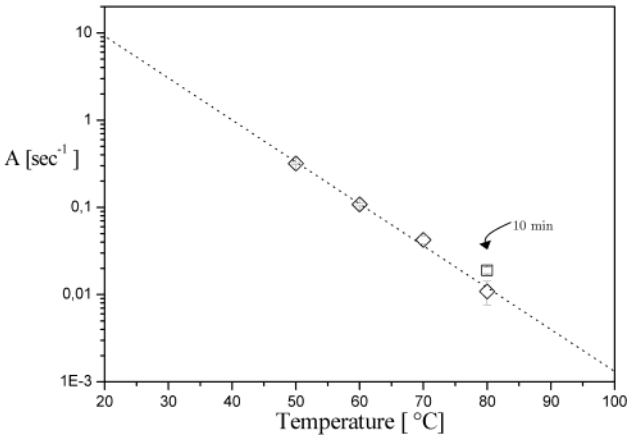

Figure 4. Variation of the coefficient *A* as a function of temperature.

is also the result of a measurement annealing for only 10 min. One realizes, as expected, a higher permeability.

Discussion

In this work, we have selected an example of a typical multilayer capsule to show systematically how temperature annealing decreases wall permeability. Because the control capsules were too permeable to enable any quantitative permeation studies, we had to anneal at temperatures above 50 °C. Having established a phenomenological relation we can now answer questions typical for an extrapolation according to Figure 4.

(i) Which lower level of permeability could reasonably be achieved by annealing, e.g., at 95 °C, i.e., close to the water boiling temperature and allowing some more time? From Figure 4 one deduces that $A = 10^{-3}$ s^{-1} corresponding to a lower limit.

(ii) Which permeability is expected keeping the sample at room temperature or storing at lower temperature? Extrapolation of Figure 4 to the left indicates that values of *A* around 10 s^{-1} are expected, and long storage times might reduce this toward 1 s^{-1}.

According to eq 3 we may convert measured values of *A* into a diffusion coefficient *D*. Taking the value at 80 °C ($A = 10^{-2}$ s^{-1}, corresponding to $P \approx 2 \times 10^{-8}$ m/s), the radius $r = 10^{-4}$ cm, and a layer thickness of $h = 10^{-6}$ cm, we obtain $D = 1.2 \times 10^{-12}$ cm^2/s.

This value is similar to that previously determined from drug release.[12]

In the drug-release experiments the samples were, however, not heat annealed and one therefore, according to Figure 4, would have expected diffusion coefficients 3 orders of magnitude larger. However, in this case, a drug particle was coated and there was no previous step of core removal. For this reason, presumably the core removal creates defects in the wall due to the osmotic pressure buildup.[10] These defects may be responsible for the comparatively large permeability of capsules. These defects have not been completely annealed by heat treatment and *D* may thus not be the microscopic diffusion coefficient but an effective one. *D* is also 2 orders of magnitude larger than values previously determined for planar films.[7] In the latter case, each adsorption step, in contrast to that of capsule fabrication, involves a drying step, and this may successively anneal any defect. Thus, this may cause a denser coating.

Experiments are now under way to discriminate between these mechanisms and to optimize for controlled permeation.

We should also mention that temperature-dependent structural rearrangements have been observed by confocal and scanning force microscopy, and these changes are very specific for different systems.[3,13] Therefore, the data derived here cannot be generalized to other systems, but the general procedure should be applicable to a broad range of systems and properties.

Conclusions

In this paper, we have shown that by applying defined annealing procedures the permeability of polyelectrolyte multilayer capsules can be tuned in a predictable way. Although the quantification is mostly phenomenological, it enables extrapolation on conditions that are difficult to achieve experimentally.

Acknowledgment. This work was partially supported by a grant from BMBF, 03C0293A1.

CM011300Y

(12) Antipov, A. A.; Sukhorukov, G. B.; Donath, E.; Möhwald, H. *J. Phys. Chem. B* **2001**, *105*, 2281.

(13) Gao, C. Y.; Leporatti, S.; Donath, E.; Möhwald, H. *J Phys. Chem. B* **2000**, *104*, 7144.