

Entrapment of a Weak Polyanion and H^+/Na^+ Exchange in Confined Polyelectrolyte Microcapsules

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Received May 11, 2005

An approach for the entrapment of a polyanion by polyelectrolyte microcapsules is reported. It is based on a reversal changing of microcapsule wall permeability from neutral to basic pH. Polyelectrolyte microcapsules were templated on latex (polystyrene) particles by the layer-by-layer adsorption of oppositely charged polymers of sodium poly(styrene sulfonate) and poly(allylamine hydrochloride), followed by core removal using tetrahydrofuran. In alkaline conditions, the microcapsules swell and become permeable for polymers. During encapsulation, the addition of salt ions increases the amount of the polymer encapsulated and contributes to its protonation because of redistribution of H^+ ions across a semipermeable microcapsule wall. The redistribution of small ions across the microcapsule wall was tuned by adding salt according to the Donnan equilibrium and was characterized by H^+ sensitive dyes.

INTRODUCTION

Studies of materials in small confined volumes are actual tasks for research, and they can find many applications in the pharmaceutical industry, biotechnology, the food industry, and so forth as microcontainers or microreactors. Many biological systems as micelles or liposomes are used as small confined systems.¹ Microcapsules prepared by a layer-by-layer (LbL) technique on functionalized colloidal particles by the adsorption of oppositely charged polyelectrolytes can be used as microcontainers.² Basically, they are permeable for small ions and molecules, whereas molecules above 5 kDa³ are excluded. Many different techniques have been introduced to incorporate materials into microcapsules⁴ via defined annealing,⁵ pH control,⁶ swelling, and shrinking.⁷

Polyelectrolyte multilayers are materials that can be used for storage and the delivery of substances, dependent on a function of pH, ionic strength, or solvent, by affecting interactions between polyelectrolytes.^{8,9} The pH-dependent pseudo-degradation of multilayer film is used for the release of dyes and macromolecules from polyelectrolyte film structures.¹⁰ Polyelectrolyte microcapsules made of poly(allylamine hydrochloride) (PAH) and poly(styrene sulfonate) (PSS) on latex (polystyrene; PS) particles undergo a pH response by changing size and permeability.^{11,12} The present study is based on the entrapment of polyanion poly(acrylic acid) (PAA) by changing the permeability of the microcapsule wall by means of reversibly adjusting the pH to the alkaline region and the redistribution of small ions between the microcapsule exterior and interior. After re-establishing

a neutral pH value, the capsule retrieves its former shape and permeability. An encapsulated polymer and a semipermeable wall determine the well-known Donnan distribution of small ions. Concomitantly, different values of pH in the external and inner solutions are attained. A H^+ -sensitive dye was used to monitor pH changes, and experiments were compared by the simple Donnan model. A simple Donnan view turns out to be a relevant description.

EXPERIMENTAL DETAILS

Materials. Sodium poly(styrene sulfonate) (PSS) (MW = 70 kDa) and poly(allylamine hydrochloride) (PAH) (MW = 70 kDa), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 5-aminofluorescein (AF), and 2-morpholinoethanesulfonic acid monohydrate were purchased from Sigma-Aldrich (Germany). Tetrahydrofuran (THF) and dimethylformamide were obtained from Roth (Germany); NaOH, HCl, and NaCl were obtained from Merck (Germany). Poly(acrylic acid) (PAA) (MW = 50 kDa) was purchased from PolyScience (U. S. A.). All chemicals were used as received. Solutions were prepared with water from a three-stage Millipore Milli-Q Plus 185 purification system. Polystyrene (PS) particles, diameter = $10.25 \pm 0.09 \mu\text{m}$, were obtained from Microparticles GmbH (Berlin).

Capsule Preparation. (PAH/PSS)₈ hollow microcapsules were prepared by the alternative adsorption of PAH and PSS on PS microparticles, followed by the subsequent dissolution of the template in THF, as described previously.¹¹ Polyelectrolyte aqueous solutions were prepared at 4 mg/mL in NaCl 0.5 M. The pH of the solutions was 4 for PAH and 7 for PSS.

Adjusting pH. The pH of the solutions was adjusted by a digital, microprocessor-controlled pH meter, WTW pH 539 (Germany), using a combined glass/reference electrode, and calibrated with standard buffer solutions.

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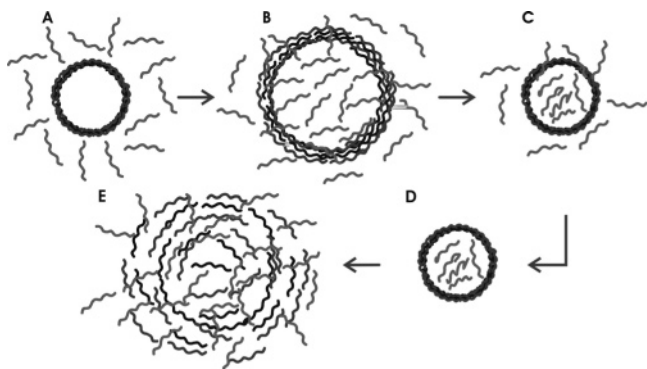


Figure 1. Scheme for entrapment and release of a polyanion. (A) Microcapsules are impermeable, (B) swollen and permeable at pH > 11.2, (C) shrunken with incorporated polyanion, and (D) washed containing loaded polyanion, and (E) the polyanion is released in alkaline conditions (pH > 12.5).

Fluorescence Spectroscopy. A Spexs Fluorog 1680 Dual spectrometer (Germany) was used for taking fluorescence spectra. The relation between the pH of the solution and the fluorescence intensity of the polymer solution was described by $I = A_1 \exp(\text{pH}/t_1) + A_2 \exp(\text{pH}/t_2) + A_3 \exp(\text{pH}/t_3) + I_0$. The parameters are $I_0 = -4.699$, $A_1 = 0.00105$, $t_1 = 0.95227$, $A_2 = 2.30122$, $t_2 = 68.31361$, $A_3 = 2.31809$, and $t_3 = 67.8305$.

Synthesis of Fluorescein-Labeled Poly(acrylic acid) (PAA_{AF}). Polyanion PAA was covalently labeled with aminofluorescein in order to apply fluorescence techniques as described in our previous work.¹²

UV–Vis Spectroscopy. A UV–vis spectrophotometer Cary 50 (Australia) was used to record absorption spectra. Spectra were recorded in a range of wavelengths from 400 to 600 nm.

Confocal Laser Scanning Microscopy (CLSM). Optical images of polyelectrolyte microcapsules in the solution were obtained on a Leica TCS SP confocal scanning system (Leica, Germany) equipped with a 40 \times , 10 \times /0.33, and 100 \times /1.4–0.7 oil immersion objective.

Entrapment of PAA_{AF}. A total of 100 μL of the capsule suspension at pH 7 was mixed with the same volume of PAA_{AF} solution at pH 7. The pH of the mixture was increased to 11.4–11.6 by adding 25 μL of NaOH (pH = 12) to make the microcapsules permeable to the polymer. After 2 min of incubation, 15 μL of aqueous HCl (pH = 1) was added to close the microcapsules; the final pH was 7. The suspension was then centrifuged and washed twice with water at pH = 7.

RESULTS AND DISCUSSION

Hollow (PAH/PSS)₈ microcapsules prepared on templates are permeable for small molecules and impermeable for macromolecules¹⁵ (Figure 1A). These microcapsules are sensitive to pH shifts in the alkaline region, and it is possible to reverse their permeability for a polymer. At pH > 11.2, a network of polyelectrolytes in the microcapsule wall changes its structure and porosity. This pH region may correspond to the formation of pores in the wall (by segregation of the polymeric network), related to a significant deprotonation of the PAH (pK_a of PAH in the PAH/PSS layers has been measured to be equal to 10.5^{16,17}). An additional increase of pH value above 11.6 induces swelling

of the microcapsules as a result of the deprotonation of PAH in the capsule wall.¹¹ It is well-known that the degree of protonation of a weak polyelectrolyte depends strongly on its environment, especially the nature of the polyelectrolyte with which it is associated. The degree of protonation of a complex PAH–PSS above pH 11 is almost zero.¹⁶ Fully ionized and negatively charged PSS induces swelling of the capsule wall as a result of repulsion between its monomeric charged groups. The swelling of microcapsules up to double size depends on pH shifts and is associated with changing the porosity of a polymer network. This mechanism is different from that reported for encapsulation at acidic pH in (PSS/PAH) microcapsules templated on weakly cross-linked melamine formaldehyde (MF)⁶ or CdCO_3 ¹³ particles. Reduction of the pH increased the charge density on PAH, leading to partial decomposition of the polymers and increased permeability of the shell.⁶ Oligomers resulting from the incomplete dissolution of the MF core can also be protonated at low pH and could contribute to the increase of permeability.¹⁴

Microcapsules in a polymer solution are permeable for a polymer that permeates inside the capsule (Figure 1B). When the pH is decreased below the permeability threshold of 11, the microcapsules recover their initial shape and are resealed (Figure 1C). The permeability of the microcapsules was checked under a confocal microscope. Microcapsules were added to different polymer solutions with and without salt (up to 0.5 M). They were impermeable for a polymer under these conditions for a pH kept below the critical value of 11.4. The microcapsules were washed with water, and the polymer inside the microcapsules appears homogeneously distributed (Figure 1D).¹²

The direct measurement of fluorescence inside the microcapsules is problematic because of the self-quenching of the label dye. The amount of entrapped PAA was determined by means of analysis of the material released from microcapsules by dissolving the polyelectrolyte microcapsule wall at a pH above 12.5^{12,18} (Figure 1E). In these conditions, the concentration of fluorescent PAA released in the cuvette is low enough to avoid any self-quenching, as the volume of 100,000 microcapsules (0.05 μL) contained in the sample is much smaller than the volume of the measured solution at pH = 12.5 (600 μL).

The fluorescence of a polymer depends on the degree of labeling, pH, and its concentration. An increase in polymer concentration and the degree of labeling contributes to intermolecular interactions and self-quenching. The fluorescence emission maximum (516 nm below 0.12 g dm⁻³) is shifted to higher values (522 nm at 2 g dm⁻³). In Figure 2, the emission spectrum at 516 nm depending on a PAA concentration at pH 12.5 is presented. This figure also serves as a calibration curve for determining the amount of released material from the microcapsules. At low concentrations of a polymer (below 0.12 g dm⁻³), fluorescence intensity is linearly proportional to polymer concentration.

Figure 3 represents the amount of encapsulated polymer as a function of the initial concentration of a polymer solution. At low concentrations of the initial external polymer solution, the amount of entrapped polyanions increases. For concentrations higher than 0.2 g dm⁻³, it reaches a plateau of 7 pg per capsule and, taking into account the diameter of the microcapsules, corresponds to a concentration of about

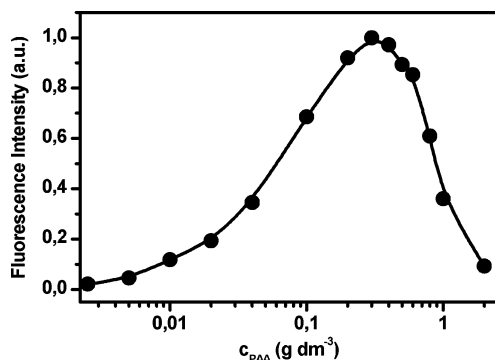


Figure 2. Fluorescence intensity as a function of polymer concentration at pH = 12.5.

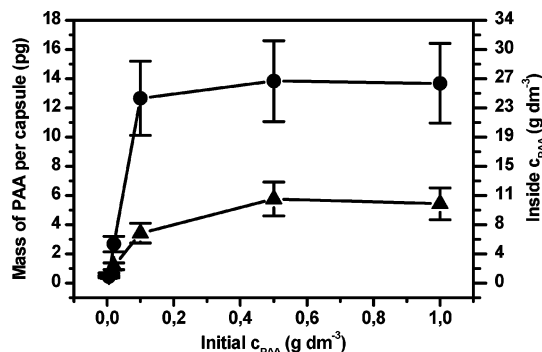


Figure 3. Amount of encapsulated PAA per capsule as a function of the initial polymer concentration in water (triangles) or in 0.2 M NaCl (circles).

13 g dm^{-3} . With the addition of a 0.2 M NaCl concentration in an initial polymer solution, the plateau value is increased to 14 pg per capsule; one can calculate that this corresponds to a concentration of about 27 g dm^{-3} of PAA inside the microcapsules. The accumulation of polymers can be attributed, first, to capsule contraction (reducing the size by a factor of 2 leads to a volume reduced by a factor of 8). In addition, PAA probably also adsorbs on the wall of the microcapsules, increasing the final amount of polymer caught by the microcapsules. This may correspond to an increase in microcapsule wall permeability and a change of polyanion hydrodynamic volume. The hydrodynamic radius of PAA was initially 8 nm in the pure water and decreased to the pH-independent limit value of 5 nm by the addition of 0.1 M NaCl .¹⁹ The addition of salt in a polymer solution also decreases electrostatic repulsion between the negatively charged polyanion and microcapsule wall. There is no core residue in the interior to accumulate or adsorb PAA (this has been checked by Raman confocal microscopy¹¹).

Because of the pH sensitivity of aminofluorescein (its fluorescence intensity dramatically drops in acidic solutions) and the low pH inside the microcapsules (caused by the presence of the weak poly acid PAA), the interior appears dark when microcapsules are placed in water at pH 7. Adding NaOH to the external solution causes the microcapsule interior to become bright (Figure 4), corresponding to the redistribution of Na^+ and H^+ ions between the inner and external solutions.

To study the Donnan equilibrium and the redistribution of small ions across the microcapsule wall,^{20,21} NaCl was used. Semipermeable properties of the capsule wall allow the application of a mathematical model that can predict the

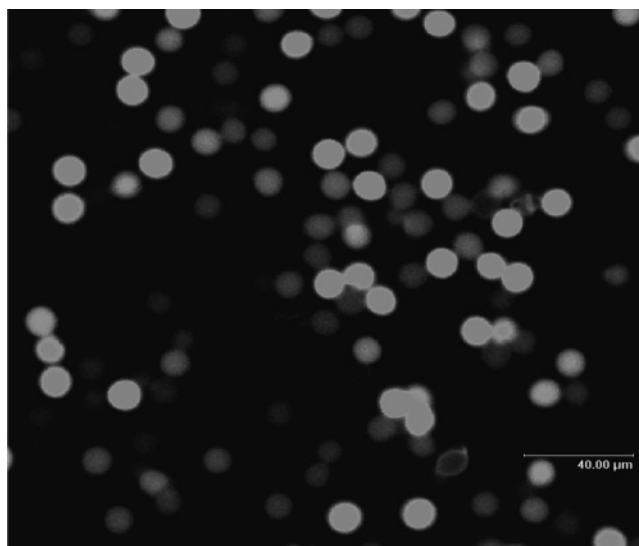


Figure 4. The CLSM image of encapsulated polyanion in microcapsules with the addition of NaOH (pH < 11.5).

behavior of a polymer solution in a confined microcapsule interior. Here, we introduce a simple model of the Donnan distribution of small ions across the semipermeable capsule wall. The small ions H^+ , OH^- , Na^+ , and Cl^- considered in the model are redistributed between the external and inner solutions of the microcapsules. The capsule wall is permeable to small ions and impermeable to encapsulated polyanions. The external solution serves as an infinite reservoir for ions. The volume asymmetry of the two solutions in the model corresponds to a low concentration of microcapsules in suspension, and in the accompanying experimental setup, the ratio between the number of microcapsules in the inner volume and that in the external reservoir was more than $1:10^6$.

We can consider a polyanion having one charge per monomeric unit and undergoing the protonation reaction $c_P \rightleftharpoons c_{P^-} + c_{H^+}$

$$K_p = \frac{c_{H^+}c_{P^-}}{c_{P_i}} \quad (1)$$

with the dissociation constant K_a and $\text{p}K_a = -\log K_a$, where c_{P^-} and c_{P_i} indicate the monomeric concentration of charged polyanion and the total concentration of polyanion, respectively. The electroneutrality condition for the inner capsule solution reads

$$c_{H^+} - c_{OH^-} + c_{Na^+} - c_{Cl^-} - c_{P^-} = 0 \quad (2)$$

The symbols in the above expression denote concentrations of the ionic species considered. In the external solution, we have $\text{Na}^+ = \text{Cl}^-$ and a defined pH_0 . Then, we can easily apply the well-known relations of the Donnan equilibrium of ions for the ideal case

$$\frac{c_{H_i^+}}{c_{H_o^+}} = \frac{c_{OH_o^-}}{c_{OH_i^-}} = \frac{c_{Na_i^+}}{c_{Na_o^+}} = \frac{c_{Cl_o^-}}{c_{Cl_i^-}} \quad (3)$$

Indexes o and i refer to the external and inner solutions, respectively. The inner concentration of H^+ ions is obtained by solving the system of eqs 1–3 and is converted and

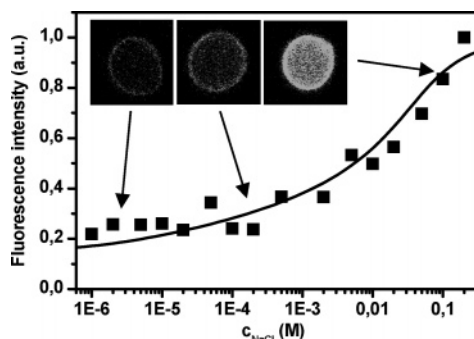


Figure 5. Fluorescence intensity of encapsulated polyanion as a function of NaCl in the external solution, measured with CLSM; a full line represents model predictions with the following values of the model parameters: $c_{\text{PAA}} = 0.1 \text{ M}$, $\text{p}K_{\text{a}} = 5$, and $\text{pH}_0 = 6.1$.

normalized to fluorescence intensity by the relation obtained by the experiment (see Experimental Details). The squares in Figure 5 represent increasing fluorescence intensity inside the microcapsule, and the respective decrease in concentration of the hydrogen ions is dependent on an increasing salt concentration. The full line in Figure 5 was calculated by using the following values of parameters: c_{PAA} , 0.1 M; $\text{p}K_{\text{a}}$, 5; and pH_0 , 6.1. Because of the electroneutrality condition and the accumulation of Na^+ ions inside the microcapsules, the concentration of H^+ in the inner solution is decreased and the pH is increased. The degree of polyanion protonation is changed contemporaneously with pH changes. The H^+ -sensitive fluorescence dye covalently bonded to a polymer was used to observe changes in the concentration of hydrogen ions inside the microcapsules. With the addition of NaCl, the microcapsules become brighter. A shift in brightness corresponds to a pH shift inside the microcapsules. A full line represents model predictions of fluorescence intensity for the inner capsule solution. A reasonable agreement between predictions and experimental results can be seen, indicating that the pH inside the microcapsules is tuned by the external salt solution. The pH difference between the external and inner solutions is influenced by polyanion concentration and $\text{p}K_{\text{a}}$ of the polyanion.¹⁶

CONCLUSION

The permeability of microcapsules is tuned by changing the pH from neutral to alkaline. Polyanions are encapsulated by the reversibly changing permeability of the microcapsule polyelectrolyte wall. In this way, microcapsules attain the function of a microcontainer and micro-pH sensor. The amount of encapsulated polyanion can be controlled by changing the initial concentrations of the polyanion and of NaCl in the external solution. The distribution of small ions between the external solution and the inner polyanion solution of the microcapsule contributes to a lower pH inside the microcapsule. The difference in pH between the external and inner solutions is decreased by the addition of salt to the external solution. Adding NaCl to the external solution also leads to an osmotic pressure difference between the external and inner solutions of the microcapsules. It was shown that, because of the established Donnan equilibrium, the inner pH can be controlled by conditions in the external solution.

ACKNOWLEDGMENT

This work was supported by the EU project "Nanocapsules for Targeted Delivery of Chemicals" STREP N001428 and the Sofja Kovalevskaja Program of the Alexander von Humboldt foundation, the Slovenian Ministry of Science, the German Ministry of Education and Research, Max-Planck Society. D.H. thanks DAAD for support (Referat 324, Number: A/04/21021).

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