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Reverse-Phase LbL−Encapsulation of Highly Water Soluble Materials by Layer-by-Layer Polyelectrolyte Self-Assembly

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We report on a novel method for the encapsulation of highly water soluble materials by using layer-by-layer (LbL) polyelectrolyte self-assembly. State of the art polyelectrolyte self-assembly LbL coating and encapsulation methods are only applicable to insoluble or poorly water soluble template materials, because the process is performed in water causing dissolution of the solid template. Our method extends the material spectrum to highly water soluble template materials by using non-ionized polyelectrolytes in an organic phase (reverse-phase) instead of polyelectrolyte salts in an aqueous environment. By using the reverse-phase layer-by-layer (RP-LbL) technique, we have demonstrated the direct encapsulation of proteins, glucose, vitamin C, and inorganic salts in the solid state. Multilayer deposition was proven, layer thickness was determined by AFM, and the advantage of the method to prepare powders of encapsulated materials was demonstrated. The method is simple, robust, and applicable to a broad range of substances with potential applications in several industries.

1. Introduction

In state of the art layer-by-layer (LbL) polyelectrolyte self-assembly encapsulation, salts of anionic and cationic polyelectrolytes carrying negative and positive charges are dissolved into an aqueous solution and are deposited onto an oppositely charged template material driven by Coulomb interaction. Alternating deposition of positively and negatively charged polyelectrolytes is performed until the desired layer number is achieved, whereby the entire process takes place in an aqueous phase. Therefore, the process is limited to insoluble or poorly water soluble template materials. Early work on LbL polyelectrolyte self-assembly was performed by using different types of polymeric particles as a template.[1,2] Later, the process was extended to poorly water soluble enzyme crystals dispersed in buffers with high salt concentration,[3] non-water-soluble hydrophobic substances,[4] and cells.[5−7] LbL technology has promising applications in a range of industries including drug delivery[8] and diagnostics.[9,10]

However, the LbL polyelectrolyte self-assembly method remained limited to insoluble or poorly water soluble template materials, polymeric microspheres, hydrophobic crystalline materials, and some biological templates.

Here, we report an LbL polyelectrolyte self-assembly encapsulation method to extend the template material spectrum for the first time into highly water soluble materials, such as glucose, vitamin C, proteins, and inorganic salts by using non-ionized polyelectrolytes in an organic phase (reverse-phase) instead of polyelectrolyte salts dissolved in water.

The "reverse-phase-LbL" (RP-LbL) process is depicted in Figure 1. The highly water soluble template material is first dispersed in an organic solvent, e.g., glucose in ethanol. This can be done by dispersing the solid substance or by precipitation of the dissolved substance with an organic solvent. To achieve sufficient solubility of the polyelectrolyte in the organic solvent, non-ionized polyelectrolytes (niPolyelectrolyte) obtained by protonation of anionic polyelectrolytes and deprotonation of cationic polyelectrolytes were used. In comparison to the "water phase LbL" wherein the ionized salts of polyelectrolytes are used, the presented method uses neutral or non-ionized polyelectrolytes. Typical examples of non-ionized polyelectrolytes and organic solvent pairs are as follows: poly(methacrylic acid) in ethanol; modified poly(diallyldimethylammonium chloride) in ethanol; polyethylenimine in chloroform, Poly(ethylene/acrylic acid) [92:8] in ternary solvents like perchlororthylene and toluene; poly(ethyl acrylate/acrylic acid) [50:50] in ethanol; poly(2-dimethylaminoethyl methacrylate) in *tert*-butanol; poly(2-vinylpyridine) in *tert*-butanol or dimethylformamide (DMF); poly(*N*-vinylpyrrolidone) in alcohols or chloroform. Very few studies exist to use non-water-based LbL deposition; recently, *N*,*N*-dimethylformamide (DMF) was used to dissolve polyelectrolyte salts, and LbL-like deposition was demonstrated.[11]

For the encapsulation process, the highly water soluble template substance is suspended into an organic solvent, and the first

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(1) Sukhorukov, G. B.; Donath, E.; Lichtenfeld, H.; Knippel, E.; Knippel, M.; Möhwald, H. *Colloids Surf., A* **1998**, *137*, 253.

(2) Donath, E.; Sukhorukov, G. B.; Caruso, F.; Davis, S. A.; Möhwald, H. *Angew. Chem., Int. Ed.* **1998**, *37* (16), 2201−2205.

(3) Caruso, F.; Trau, D.; Möhwald, H.; Renneberg, R. *Langmuir* **2000**, *16* (4), 1485−1488.

(4) Caruso, F.; Yang, W.; Trau, D.; Renneberg, R. *Langmuir* **2000**, *16* (23), 8932−8936.

(5) Donath, E.; Moya, S.; Neu, B.; Sukhorukov, G. B.; Georgieva, R.; Voigt, A.; Bäumler, H.; Kiesewetter, H.; Möhwald, H. *Chem.−Eur. J.* **2002**, *8* (23), 5481−5485.

(6) Mak, W. C.; Sum, K. W.; Trau, D.; Renneberg, R. *IEE Proc.: Nanobiotechnol.* **2004**, *151* (2), 67−72.

(7) Kreft, O.; Georgieva, R.; Bäumler, H.; Steup, M.; Müller-Röber, B.; Sukhorukov, G. B.; Möhwald, H. *Macromolecules* **2006**, *27* (6), 435−440.

(8) Johnston, A. P. R.; Cortez, C.; Angelatos, A. S.; Caruso, F. *Curr. Opin. Colloid Interface Sci.* **2006**, *11* (4), 203−209.

(9) Toellner, L.; Fischlechner, M.; Ferko, B.; Grabherr, R. M.; Donath, E. *Clin. Chem.* **2006**, *52* (8), 1575−1583.

(10) Chan, C. P. Y.; Haeussler, M.; Tang, B. Z.; Dong, Y. Q.; Sin, K. K.; Mak, W. C.; Trau, D.; Seydack, M.; Renneberg, R. *J. Immunol. Methods* **2004**, *1−2*, 111−118.

(11) Kamineni, V. K.; Lvov, Y. M.; Dobbins, T. A. *Langmuir* [Online early access]. DOI: 10.1021/la700465n. Published Online: May 31, 2007.

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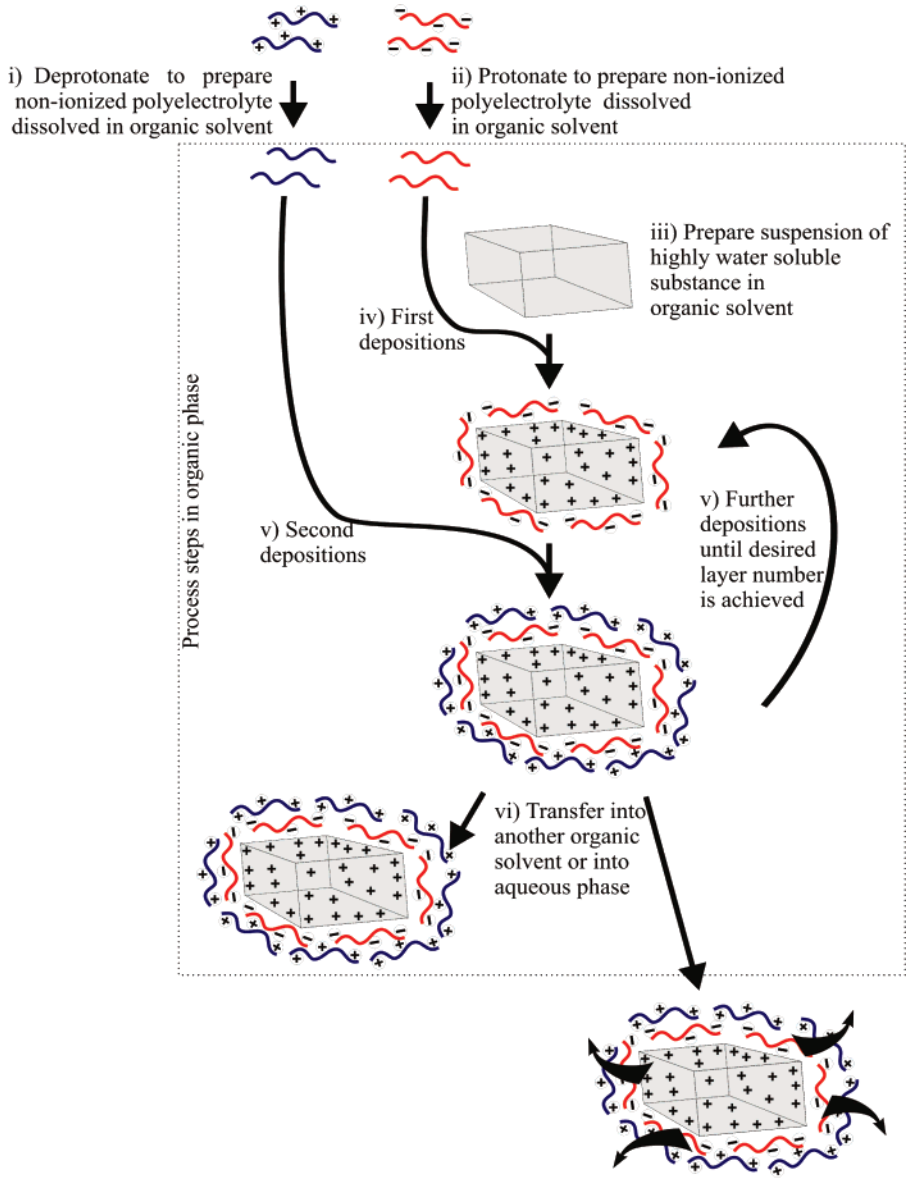

Figure 1. Schematic representation of the reverse-phase LbL encapsulation process. (i) Deprotonation of cationic polyelectrolyte and (ii) protonation of anionic polyelectrolyte to form non-ionized polyelectrolyte dissolved in an organic solvent. (iii) Preparation of a suspension of highly water soluble material in an organic solvent. (iv) Deposition of the first non-ionized polyelectrolyte onto the highly water soluble template material. (v) Deposition of alternating layers of non-ionized cationic and anionic polyelectrolytes, most likely driven by a solid-phase acid−base reaction between the preceding layer on the template surface and the non-ionized polyelectrolyte to form a multilayer capsule. (vi) Transfer of the encapsulated material from the organic phase into another organic solvent (left) or into an aqueous phase (right).

non-ionized polyelectrolyte is added (preferentially in the same solvent or solvent mixture). After incubation, any excess of polyelectrolyte is removed by washing, centrifugation, and redispersion cycles in organic solvent. The working procedure is very similar to common LbL methods with the difference that the entire process is carried out in organic solvents (box in Figure 1). Then, deposition of alternating layers of non-ionized cationic and non-ionized anionic polyelectrolytes is performed to form a multilayer capsule until the desired number of layers is achieved.

In "water-phase" LbL, the polyelectrolyte salts are almost 100% ionized, and the transport of the polyelectrolyte to the template and the deposition process is mainly driven by Coulomb forces. In "reverse-phase" LbL, depending on the organic solvent, polyelectrolytes are non-ionized or only slightly ionized, and we believe the process is therefore different. Because there are no or much smaller coulomb forces between the non-ionized polyelectrolyte and the template, the transport of the non-ionized polyelectrolyte to the template surface is most likely driven by diffusion. Then, the deposition of the non-ionized polyelectrolyte onto the preceding surface layer is most likely driven by a solid phase acid−base reaction between the template surface and the non-ionized polyelectrolyte to form a nanometer-thick layer of ionized polyelectrolyte. Thereby, the non-ionized polyelectrolyte is removed from the solvent, causing a concentration gradient which further drives the transport by diffusion. Because the solid-phase acid−base reaction leads to an ionized template surface, electrostatic repulsion between particles takes place to stabilize the suspension and cause the typical reversal in zeta potential with every deposited layer.

2. Experimental Section

2.1. Materials. Poly(diallyldimethylammonium chloride) (PDADMA) MW100 000 to 200 000 Da, bovine serum albumin (BSA), ascorbic acid, sodium chloride, glucose, and fluorescein isothiocyanate (FITC) were obtained from Sigma Aldrich. Poly(methacrylic acid) sodium salts (PMA), MW 100 000 Da; polyethylenimine, linear

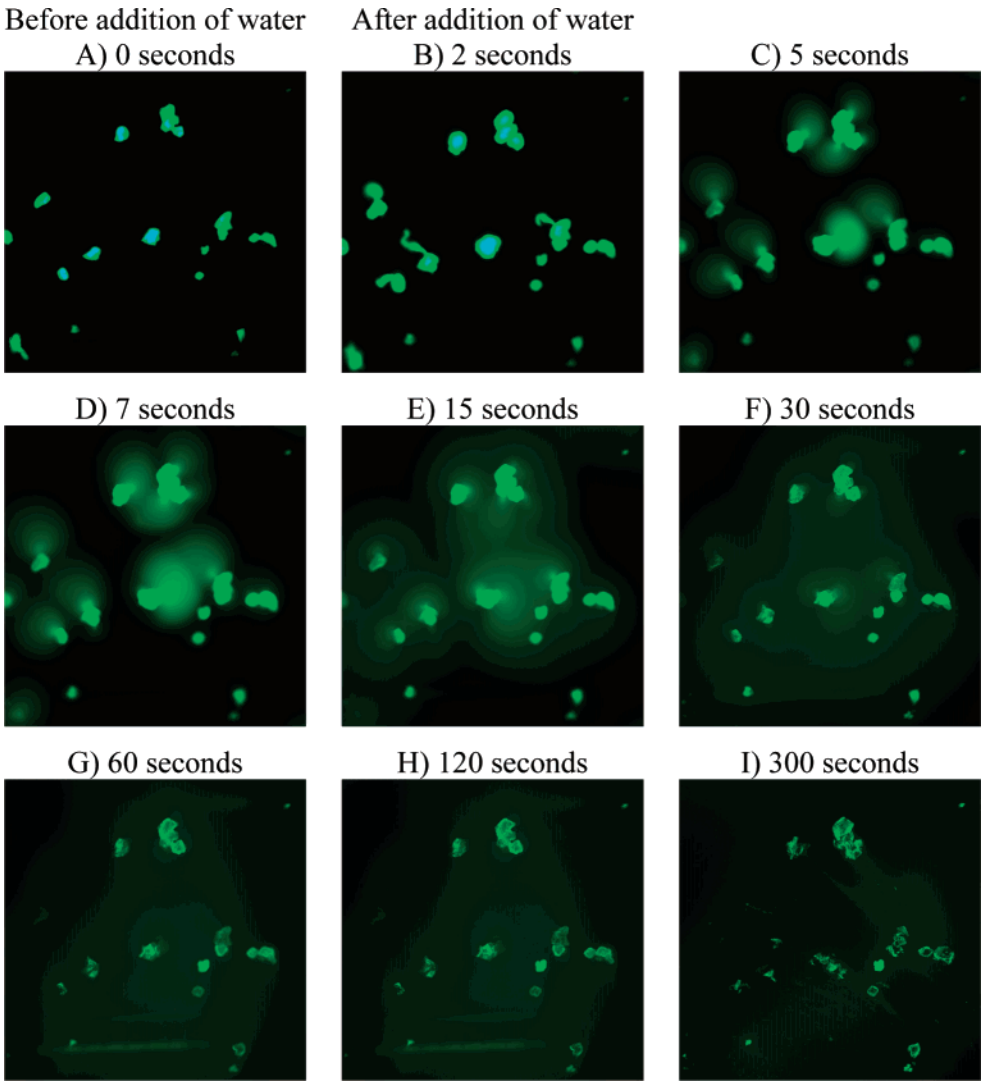

Figure 2. Fluorescent micrographs demonstrating the reverse-phase LbL encapsulation of the highly water soluble protein bovine serum albumin (BSA): (A) before addition of water. (B) Capsule volume increases due to buildup of osmotic pressure in capsules. Some capsules show "small jets" of releasing BSA. (C to H) Further release of BSA forming "clouds" and diffusion of BSA away from capsules. (I) Remaining capsule material. Fluorescent labeled BSA (BSA-FITC) was used to demonstrate release. The particle size was 5−20 μm, and the capsule was formed by 8 layers (poly(methacrylic acid), modified poly(diallyldimethylammonium chloride)). Nonencapsulated BSA dissolves immediately in the first 2 s.

(PEI) MW 250 000 Da; and poly(ethyl acrylate/acrylic acid) [50:50] copolymer were purchased from Polysciences Inc. Ethanol was obtained from Merck and sodium hydroxide and hydrochloric acid from Fluka. Chloroform was purchased from EM Sciences.

2.2. Preparation of Non-Ionized Polyelectrolyte Solutions. Non-ionized polyelectrolyte (niPolyelectrolyte) solutions in organic solvent were prepared by protonation of polyacid or deprotonation of polybase polyelectrolytes, followed by purification by dialysis against water and drying at room temperature under vacuum. The dried niPolyelectrolytes were then dissolved in organic solvents. For PMA, protonation was carried out with 25% HCl; polyelectrolytes purchased as the free base or acid were only purified by dialysis followed by drying. For PDADMACl, the substance was first modified by dissolving 1 g in 5 mL water, mixed with 10 mL of 25% sodium hydroxide solution, and allowed to react overnight at 55 °C while stirring. The non-ionized modified PDADMA (nimPDAMA) was extensively dialyzed against distilled water and dried under vacuum at 105 °C. Then, 0.272 g nimPDADMA was dissolved in 25 mL pure ethanol.

2.3. Reverse-Phase LbL-Encapsulation Process. For the encapsulation process, the template materials (BSA/BSA-FITC, glucose, ascorbic acid, and sodium chloride) were extensively milled together with ethanol for 5 min by hand in a small agate mortar. The suspension was transferred into a 1.5 mL vial and centrifuged at 500 rpm for 30 s; the supernatant containing a fraction of very small particles was discarded. The particles were redispersed in ethanol and allowed to precipitate for a few minutes. To obtain particles of almost the same size, the supernatant was collected for the RP-LbL encapsulation experiments, and the precipitate was discarded. Typically, LbL polymer layers were assembled onto the template particles by the sequential deposition of niPMA and nimPDADMA. The first layer was deposited by adding a 50 μL aliquot of nimPDADMA (10 mg/mL in ethanol) to 100 μL ethanol particle suspension, occasionally shaking the suspension, and allowing 15 min for deposition. The excess nimPDADMA was removed by two repeated centrifugation and redispersion cycles in ethanol. The next layer, niPMA (10 mg/mL in ethanol), was deposited by using the same procedure described for nimPDADMA. The process was repeated until the desired number of layers was deposited. Other combinations of polymers and/or solvents can be used. For the encapsulation of glucose with PEI/PMA layers, the glucose particles were ground and suspended in chloroform. By replacing the nimPDADMA solution with a solution of PEI in chloroform (saturated at room temperature) and performing first a washing step with chloroform followed by washing with ethanol, the encapsulation

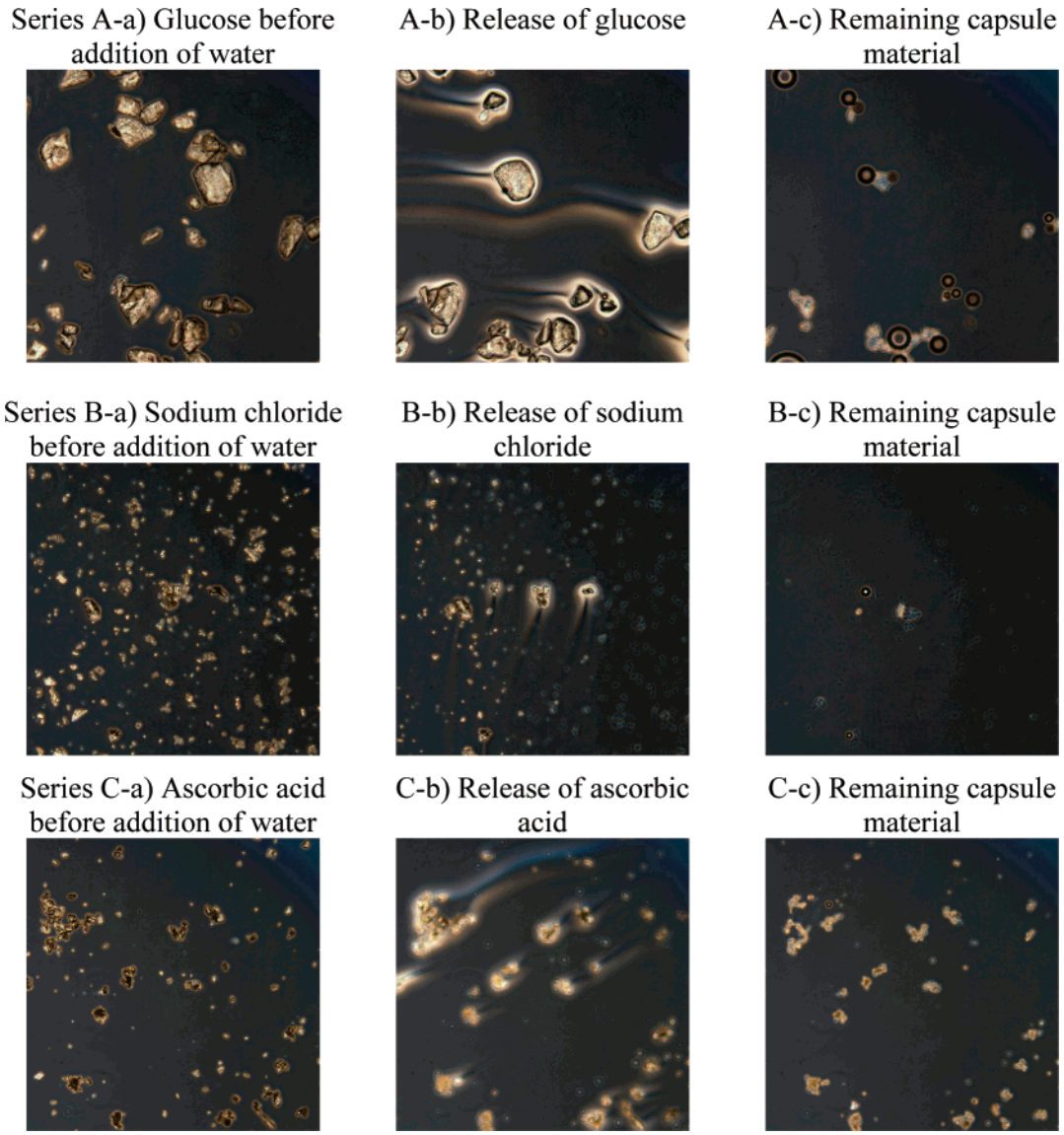

Figure 3. Light micrographs demonstrating the RP-LbL encapsulation of (A) glucose, (B) sodium chloride, and (C) ascorbic acid. Micrographs show (a) RP-LbL encapsulated crystals in ethanol before addition of water, (b) after addition of water, and (c) the empty capsule material after completed release. A fluorescein-labeled non-ionized polyelectrolyte was used in the RP-LbL process to demonstrate capsule formation (images not shown). In comparison to Figure 2, no increase in capsule volume was noticed, because low molecular weight templates can freely diffuse through the capsule and will not cause an increase of osmotic pressure in capsules' interior.

process can be conducted in the same way as that described previously for the mPDADMA/PMA capsules.

2.4. Study of Multilayer Deposition by Fluorescent Intensity and Zeta-Potential Measurements. A nimPDADMA−FITC conjugate with a conjugation ratio of 1:10 (FITC molecules/polymeric monomer) and BSA−FITC with a conjugation ratio of 1:10 (molar ratio) were prepared (as described in Bioconjugate Techniques, Greg Hermanson, Academic Press, 1996), purified by dialysis, and dried at room temperature. To demonstrate increasing fluorescent intensity according to the number of fluorescent-conjugated polyelectrolyte layers, glucose particles were encapsulated as described, except instead of nimPDADMA a solution of nimPDADMA−FITC (10 mg/mL in ethanol) was used.

Zeta-potential measurements were carried out in ethanol by measuring the electrophoretic mobility of particles using a micro-electrophoresis experiments (Zeta Sizer Nano, Malvern, UK). A Henry factor of 1 was chosen to account for ethanol as a solvent instead of water.

2.5. LbL Capsule and Layer Thickness Studies by AFM. AFM measurements were carried out with an AFM/NSOM system (Nanonics, Israel). Encapsulated glucose crystals were first deposited onto freshly cleaved mica and the organic solvent evaporated. By adding very small amounts of water, the encapsulated glucose was dissolved and removed, leaving behind the hollow capsule. After air drying, the remaining (but now collapsed) hollow capsules were imaged in AFM tapping mode.

3. Results and Discussion

3.1. Reverse-Phase LbL Encapsulation of Various Template Materials and Release Studies. To demonstrate the versatility of the method, a range of highly water soluble model substances were encapsulated and studied: high molecular weight proteins (BSA, 65 kDa), low molecular weight organic substances (glucose, vitamin C, citric acid sodium salt), and inorganic salts (sodium chloride). In addition, combinations of different non-ionized polyelectrolytes were used.

Fluorescent-labeled BSA (BSA-FITC) was prepared to prove encapsulation and to study the release of the substance from the capsule by fluorescence microscopy. Figure 2 shows RP-LbL encapsulated BSA in ethanol before the addition of water (Figure 2A) and a series of images Figure 2B−I after the addition of water. A cloud of released BSA−FITC is clearly seen after 5 s;

the last image after 300 s shows the remaining capsule material (the capsule material is slightly fluorescent due to absorption of some BSA−FITC onto the polymeric capsule). In comparison, nonencapsulated BSA is completely dissolved in the first 2 s. A detailed inspection of the images in Figure 2 reveals the mechanism of release (Supporting Information Figure S1). Directly after the addition of water, the diameter of the encapsulated BSA particle increases about 50% (corresponding to a volume increase of more than 300%) caused by dissolution of the BSA and buildup of osmotic pressure in the capsule. At the point the capsule material can no longer withstand the osmotic pressure, a "jetlike" stream of BSA is clearly observed (Supporting Information Figure S1B), followed by further release through the opening by diffusion until only remains of the capsule are observed (Supporting Information Figure S1F). Encapsulation of low molecular weight substances is demonstrated in a series of images in Figure 3: Figure 3A, glucose; Figure 3B, ascorbic acid; and Figure 3C, sodium chloride. The first image (a) of the series shows the encapsulated substance in ethanol, the second (b), after addition of water, and the last (c), the remaining capsule material. Fluorescent-labeled non-ionized polyelectrolytes were used in some experiments to prove capsule formation and to study capsule morphology by fluorescence microscopy. The "jetlike" release was only observed for high molecular weight template materials but not for low molecular weight template materials. Low molecular weight template materials can diffuse through the capsule and will not cause buildup of osmotic pressure.

3.2. Proof of LbL Multilayer Deposition. The formation of multilayers was experimentally confirmed by the deposition of several layers of fluorescent-labeled non-ionized polyelectrolyte onto glucose templates in ethanol. Figure 4A demonstrates an increase of fluorescence intensity with deposition of each second layer (the fluorescent-labeled polymer). The slight decrease in fluorescence intensity with deposition of the non-fluorescent-labeled polymer was observed earlier[12] (the irregular shape of the glucose crystals causes a relatively large error in fluorescence intensity measurements; therefore, the experiment was repeated with polymeric spheres (Supporting Information Figure S2) leading to the same conclusion but a smaller error). In addition, zeta-potential measurements were used as a second independent method to confirm LbL multilayer deposition. BSA was encapsulated by six polymer layers, and zeta potentials were measured in ethanol (water cannot be used). Figure 4B demonstrates reversal of the sign of the zeta potential from initially −37 mV (zero layers of nonencapsulated BSA) to +40 to +62 mV for deposition of positive layers or −25 to −85 mV for negative layers, typical for LbL-like encapsulation. Encapsulation was performed by non-ionized polyelectrolytes, and the question arises, how do encapsulated particles exhibit the typical reversal in surface charge? The initial nonencapsulated BSA particles are charged, and we believe an acid−base surface reaction takes place during deposition, causing formation of charges. In any case, there will be a small number of groups ionized in ethanol, which also may contain residues of water causing the typical observation.

3.3. Atomic Force Microscopy Studies. Atomic force microscope (AFM) measurements were carried out to measure the layer thickness. RP-LbL-encapsulated glucose crystals were deposited onto a fresh mica surface and washed with water to remove the encapsulated glucose; the remaining capsule material was air-dried and imaged. Figure 5 shows representative hollow

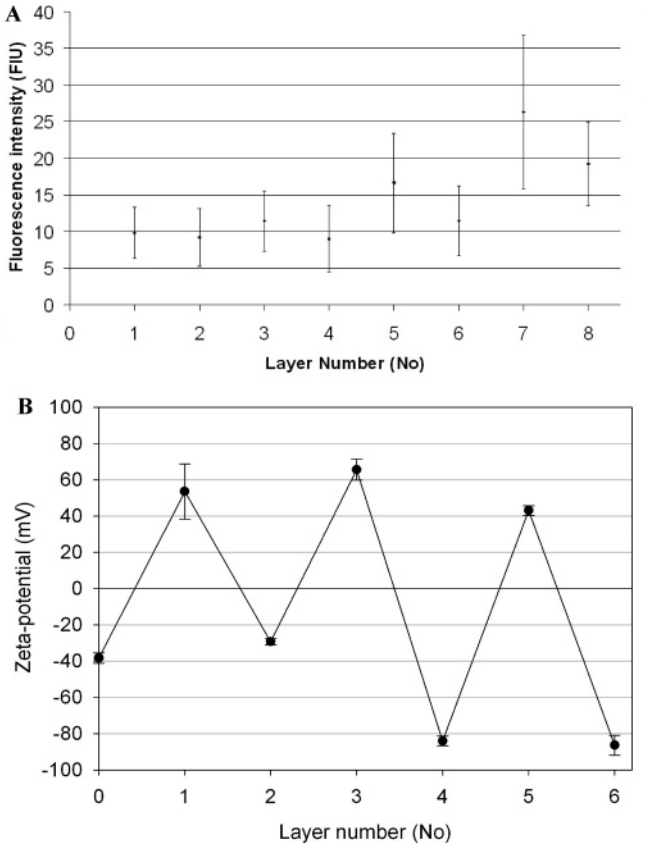

Figure 4. Proof of LbL layer deposition for the encapsulation of glucose and BSA particles. (A) Fluorescence intensity units (FIU) as a function of layer number (No) for encapsulation of glucose. Fluorescence-conjugated polymers were only used for odd-layered numbers; a slight decrease in fluorescence intensity is observed for even layers caused by quenching. (B) Zeta potential as a function of layer number (No) for encapsulation of BSA. Multilayer deposition is demonstrated by the alternating sign of the zeta potential.

capsules with Figure 5A height image and Figure 5B 3D plot. The layer thickness was determined by using Figure 5C cross section (lowest height between surface and capsule) and Figure 5D height histogram (difference in peak height of surface and capsule) studies of the capsules. The total thickness of the collapsed capsule, corresponding to 16 layers, was found to be 65−70 nm, resulting in a height of about 4 nm per single layer. This value is larger than reported for capsules made from an aqueous phase which are in the range 1.5−2.5 nm.[13] A possible explanation for the larger thickness is that the non-ionized polyelectrolyte has a more globular orientation during deposition, and therefore, the packing on the surface is denser. After transfer into water, the ionized and hydrated capsule wall expands, and thickness increases. This is further supported by the observation that capsules made in the aqueous phase will shrink if they are transferred into an organic solvent/water mixture.[14] In addition to AFM, the surface morphology was also studied by scanning electron microscopy (SEM). An increase in surface roughness was found for LbL films compared to the original template surface (Supporting Information Figure S4), which is in agreement with earlier studies on "water-based" LbL.

3.4. Manufacturing of Powders from Encapsulated Material. One advantage of organic solvents compared to water is

(12) Yang, W.; Trau, D.; Renneberg, R.; Yu, N. T.; Caruso, F. *J. Colloid Interface Sci.* **2001**, *234*, 356−362.

(13) Dong, W. F.; Ferri, J. K.; Adalsteinsson, T.; Schoenhoff, M.; Sukhorukov, G. B.; Möhwald, M. *Chem. Mater.* **2005**, *17*, 2603−2611.

(14) Kim, B. S.; Lebedeva, O. V.; Koynov, K.; Gong, H.; Glasser, G.; Lieberwith, I.; Vinogradova, O. I. *Macromol.* **2005**, *38*, 5214−5222.

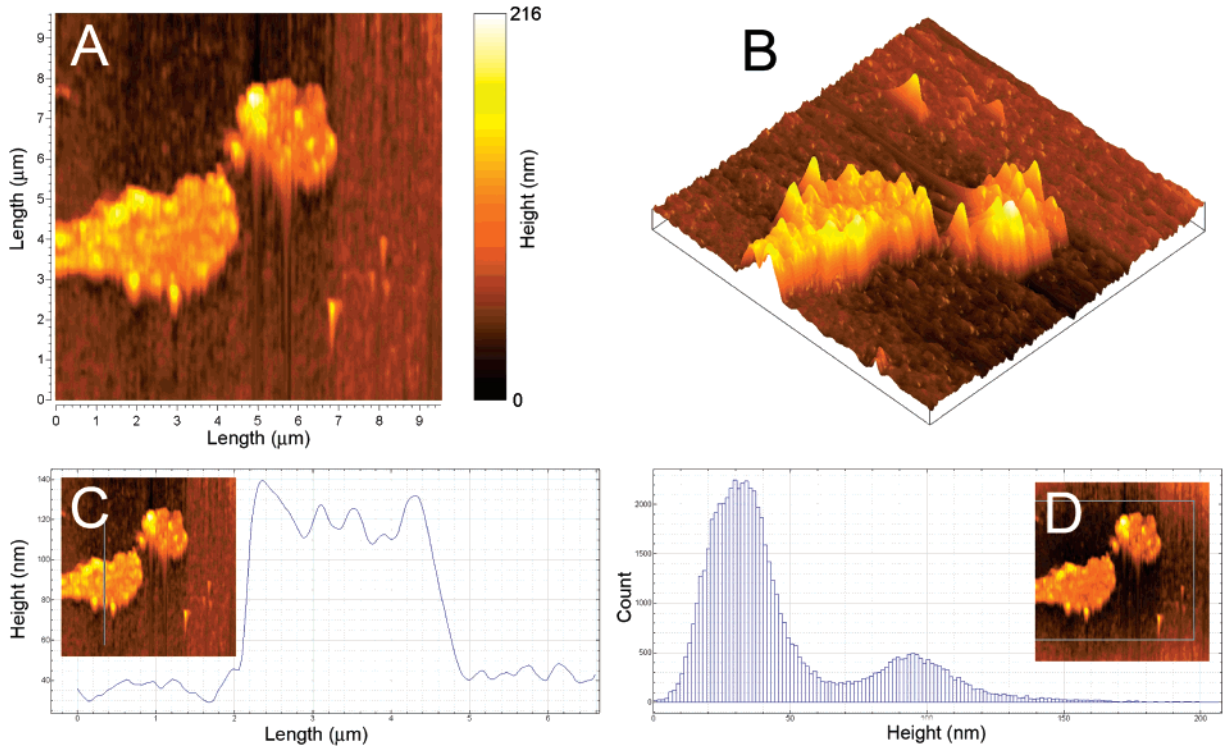

Figure 5. Representative AFM micrograph of hollow RP-LbL capsules (dried on mica) after removal of the encapsulated material (glucose) with water: (A) height image; (B) 3D plot. Capsules were made from 8 layers of poly(methacrylic acid) (PMA) and modified poly(diallyldimethylammonium chloride) (mPDADMA). (C) Cross section of capsule and (D) histogram to determine height distribution. The height of 70 nm from the cross section analysis and 65 nm from the histogram corresponds to 16 layers (of the collapsed capsule), indicating a thickness of about 4 nm per layer.

lower energy consumption and temperature for evaporation; also, boiling retardation is less likely. This makes the RP-LbL method interesting to fabricate powders of encapsulated materials. We demonstrated the fabrication of protein (BSA) powder encapsulated by 16 layers $(mPDADMA/PMA)_8$ in ethanol. A fine powder was obtained by vacuum evaporation at ~35 °C. Microscopic observation of the encapsulated BSA particles before evaporation and in the powder revealed that no agglutination or increase in particle size was observed (Supporting Information Figure S3); also, the powder could be immediately redispersed in ethanol.

4. Conclusion

An organic-phase LbL polyelectrolyte self-assembly process was developed, and direct encapsulation of highly water soluble substances used as a solid template was demonstrated. Multilayer deposition was confirmed by fluorescence intensity and zeta-potential measurements. A layer thickness of 4 nm was determined by AFM measurements. The manufacture of powders after the encapsulation process was demonstrated. Organic solvents are easy to evaporate, in particular, under vacuum, at low temperature, and with reduced energy consumption. Because the temperature is lower, sensitive substances or biomolecules are applicable, and the organic solvent can be returned into the process. The use of an organic phase also has other advantages; template particles can be formed by dissolving the material first in water and then adding organic solvent until the solubility is reduced and the material crystallizes or precipitates. The presented method may be relevant to constructing drug delivery systems for the next generation of drugs, which will be most likely peptide-, protein-, DNA-, or RNA-based. The presented method has potential applications in several fields of technology and manufacturing, for example, in the food industry to encapsulate fragrants and vitamins, in the cosmetic industry to encapsulate antioxidants, in the pharmaceutical industry for drug release, and in agriculture, pigment manufacturing, and the chemical industry.

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Supporting Information Available: Micrographs showing release from capsules, fluorescent intensity data, and micrographs showing particle morphology. This material is available free of charge via the Internet at http://pubs.acs.org.

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