Two-Component, Ultrathin Microcapsules Prepared by a Core-Mediated Layer-by-Layer Approach

Ajay J. Khopade[†,‡] and Frank Caruso*[,§]

Max Planck Institute of Colloids and Interfaces, D-14424 Potsdam, Germany, and Centre for Nanoscience and Nanotechnology, Department of Chemical and Biomolecular Engineering, The University of Melbourne, Victoria 3010, Australia

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We report the preparation of two-component microcapsules by a method termed core-mediated layer-by-layer (LbL) growth, which utilizes a core particle to mediate the formation of thin multilayer coatings on particles, followed by core removal. In the first (model) system to demonstrate the process, multilayers were prepared on spherical, decomposable melamine formaldehyde (MF) particles by the alternating deposition of poly(styrenesulfonate) (PSS) from solution and MF originating from partial decomposition of the MF core (in-situ acid-decomposed MF, dMF) in a pH 4.0 buffer. The core-mediated LbL method was also applied to a purely biocompatible system, consisting of a sodium alginate (Alg) coating on calcium phosphate (CaP) cores. Ultrathin, two-component PSS/dMF and Ca^{2+}/Alg microcapsules were prepared by decomposing the multilayer-coated cores using 0.1 M hydrochloric acid. Confocal laser scanning microscopy and transmission electron microscopy verified the formation of ultrathin multilayer capsules. Binding of a positively charged fluorescent molecule (doxorubicin) to the multilayer capsules showed that the fluorescence intensity of the capsules regularly increased with increasing capsule wall thickness, confirming that the core mediates PSS/dMF and Ca^{2+}/Alg multilayer formation. Unlike conventionally prepared polyelectrolyte capsules from sacrificial cores, which are three component systems due to the presence of decomposed core material in the oppositely charged polyelectrolyte multilayers, the microcapsules reported here are two-component, as the core material itself forms one of the multilayer components.

Introduction

Microcapsules represent an important class of materials because of their application in the encapsulation and controlled release of diverse substances (e.g., drugs, cosmetics, dyes, and inks), catalysis, waste removal, and functional materials processing.[1] Their preparation has largely relied on three main routes, namely nozzle reactor processes, emulsion/phase separation procedures (often combined with sol−gel processing), and sacrificial core techniques.[2] Recent studies have shown that a new class of ultrathin capsules with well-defined physical and chemical properties can be obtained by the layer-by-layer (LbL) assembly of oppositely charged species[3] onto particles, followed by removal of the sacrificial core.[4] Most of these studies employ highly monodisperse, decomposable melamine formaldehyde (MF) resin particles as core materials. Several investigations have used polystyrene beads,[4a,5a] or crystals of small organic molecules[5b] or macromolecules (enzymes)[5c] as template cores for forming multilayered ultrathin capsules. The decomposable MF resin particles used consist of partially cross-linked MF resin that is soluble in dilute hydrochloric acid (HCl). This is an unstable material that becomes insoluble depending on the storage conditions or various other treatments.[6] Hence, optimization of the MF core removal conditions is critical to obtain polyelectrolyte multilayer capsules.[7] Nonetheless, the presence of the MF core decomposition products (acid soluble oligomers and/or monomers, herein denoted dMF) in the multilayer capsules is largely unavoidable because the charges in the polyelectrolyte multilayers can entrap small charged molecules (from solution),[8]

* To whom correspondence should be addressed. Fax: +61 3 8344 4153. E-mail: fcaruso@unimelb.edu.au.

† Current address: Sun Pharma Advanced Research Centre, Akota Road, Baroda 390 020, India.

‡ Max Planck Institute of Colloids and Interfaces.

§ The University of Melbourne.

(1) *Hollow and Solid Spheres and Microspheres: Science and Technology Associated With Their Fabrication and Application*; Wilcox, D. L., Berg, M., Bernat, T., Kellerman D., Cochran J. K., Eds.; Materials Research Society Proceedings Vol. 372; Materials Research Society: Pittsburgh, PA, 1995.

(2) Caruso, F. In *Nano-Surface Chemistry*; Rosoff, M., Ed.; Marcel Dekker: New York, 2001; pp 505−525.

(3) (a) Decher, G. *Science* **1997**, *277*, 1232 and references therein. (b) Decher, G.; Hong, J. D. *Macromol. Chem. Macromol. Symp.* **1991**, *46*, 321.

(4) (a) Caruso, F.; Caruso, R. A.; Möhwald, H. *Science* **1998**, *282*, 1111. (b) Donath, E.; Sukhorukov, G. B.; Caruso, F.; Davis, S. A.; Möhwald, H. *Angew. Chem., Int. Ed.* **1998**, *37*, 2202.

(5) (a) Pastoriza-Santos, I.; Schöler, B.; Caruso, F. *Adv. Funct. Mater.* **2001**, *11*, 122. (b) Caruso, F.; Yang, W.; Trau, D.; Renneberg, R. *Langmuir* **2000**, *16*, 8932. (c) Caruso, F.; Trau, D.; Möhwald, H.; Renneberg, R. *Langmuir* **2000**, *16*, 1485.

(6) (a) Jahromi, S. *Macromol. Chem. Phys.* **1999**, *200*, 2230. (b) Jahromi, S. *Polymer* **1999**, *40*, 5103.

(7) (a) Gao, C. Y.; Moya, S.; Lichtenfeld, H.; Casoli, A.; Fiedler, H.; Donath, E.; Möhwald, H. *Macromol. Mater. and Eng.* **2001**, *286*, 355. (b) Gao, C. Y.; Moya, S.; Donath, E.; Möhwald, H. *Macromol. Chem. Phys.* **2002**, *203*, 953. (c) Gao, G.; Donath, E.; Möhwald, H.; Shen, J. *Angew. Chem., Int. Ed.* **2002**, *41*, 3789.

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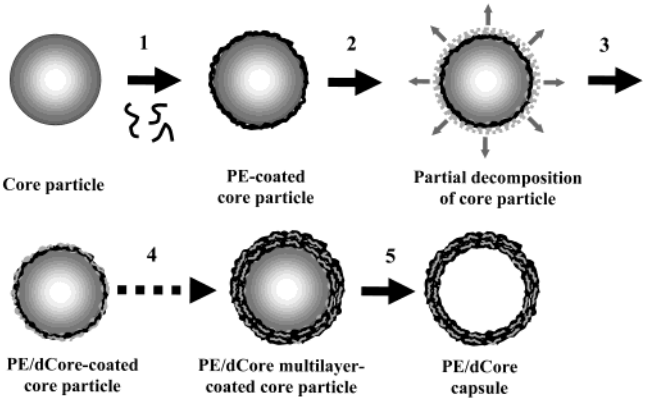
Scheme 1. Schematic Illustration of the Core-Mediated LbL Formation of Two-Component Ultrathin Multilayer Capsules[a]



[a] (1) Polyelectrolyte deposition onto a decomposable core particle; (2) partial decomposition of the core particle; (3) particle coated with polyelectrolyte and decomposed core material; (4) repetition of the cycles 1−3 yielding a multilayer-coated core particle; and (5) a two-component capsule, obtained after decomposition and removal of the core particle. Residual, unadsorbed materials are removed by repeated centrifugation/wash cycles after each adsorption step. PE = polyelectrolyte, and dCore = dissolved core components.

such as the amino derivatives of MF, which are positively charged in acidic media.[6] Therefore, multilayer capsules derived from MF cores may be regarded as three component systems, consisting of the two oppositely charged polyelectrolyte multilayer building blocks and dMF bound in the multilayers as a result of the core decomposition step. In some cases, the presence of dMF in the polyelectrolyte capsules may be undesirable, as it may prevent their reliable utilization in areas such as loading and release, as well as in crystallization studies.

In the current work, we describe the preparation of two-component multilayer microcapsules via a core-mediated LbL approach (Scheme 1). The reported approach involves first the adsorption of a polyelectrolyte layer onto the core particle and the removal of excess polyelectrolyte by centrifugation/washing cycles. Partial decomposition of the core, induced by a pH change, then results in adsorption of the oppositely charged core material onto the polyelectrolyte-coated particle. The excess core-generated products are then removed prior to adsorption of the next polyelectrolyte layer. This procedure is repeated several times to yield thin coatings on the core particle. Ultrathin multilayer capsules are obtained following complete decomposition and removal of the cores. It is noted that the possibility of complexation of the added polyelectrolyte with the core material in solution prior to adsorption, which may also adsorb and form thin layers on the cores, is avoided, and the process remains purely a core-assisted LbL technique, with the thickness of each bilayer controllable with nanoscale precision. Thus, the current process is significantly different from previous work that exploits the adsorption of preformed complexes onto particles through a method termed "controlled precipitation".[9] In that work,[9] one adsorption step yields coatings that are several tens to a hundred nanometers in thickness.

We first report the core-mediated multilayer buildup of dMF and the polyanion poly(styrenesulfonate) (PSS) onto decomposable MF particles, and the subsequent formation of PSS/dMF ultrathin microcapsules. The PSS/dMF combination was chosen as a model system to illustrate the viability of the core-mediated approach in the preparation of two-component multilayer capsules. PSS/dMF microcapsules obtained after core removal may have improved stability and mechanical properties over conventionally LbL prepared polyelectrolyte multilayer capsules due to the possibility of post-cross-linking reactions occurring between MF oligomers.[6,7] Second, we demonstrate that the core-mediated method can be applied to biocompatible materials (both the cores and coating materials). Sodium alginate (Alg) and core particles of calcium phosphate (CaP) were employed, as these materials have a long history of use in food and pharmaceutical industries.[10] Although the preparation of Ca^{2+}/Alg capsules by a number of different techniques is well-known in the literature,[11] this is among the first example concerning the nanoscale formation of multilayers of these materials on particles. As exemplified by the Alg/CaP system, the core-mediated LbL approach offers the possibility of avoiding the use of positively charged polyelectrolytes as components for forming microcapsules. This points to a significant advantage because positively charged polyelectrolytes are often toxic and therefore undesirable for use in drug delivery applications. Additionally, the components can be easily sterilized, which opens up the possibility to use capsular colloids of these materials as drug delivery systems by almost any route of administration, including critical routes such as injections. Furthermore, both the core and coating polyelectrolyte can be recycled through a simple filtration−purification process, thus potentially making the core-mediated approach highly suitable for industrial scale-up.

Experimental Section

Materials. Poly(styrenesulfonate) (PSS, $M_w \sim 70\,000$) and poly(ethyleneimine) (PEI, $M_w \sim 15\,000$) were used as obtained from Fluka. Sodium alginate (Alg), low viscosity grade, was purchased from FMC-Biopolymers. The weakly cross-linked MF resin particles (1 or 3.5 μm diameter) were obtained as 10 wt % dispersions from Microparticles GmbH, Berlin. The PS spheres of diameter 640 nm were synthesized in house according to the method of Furusawa et al.[12] The calcium phosphate (CaP) cores (10 wt %) and hydrochloric acid (HCl) were obtained from Sigma-Aldrich. Doxorubicin hydrochloride (DOX) was a kind gift from Sun Pharma, Baroda, India. The water used in all experiments was prepared in a Milli-Q system and had a resistivity higher than 18.2 MΩ cm.

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

(9) Dudnik, V.; Sukhorukov, G. B.; Radtchenko, I. L.; Möhwald, H. *Macromolecules* **2001**, *34*, 2329.

(10) Sodium alginate and calcium phosphate are classified as generally recognized as safe (GRAS) by the United States Food and Drug Administration. See www.cfsan.fda.gov/~dms/eafus.html.

(11) See, for example, the following references: Yang, H.; Wright, J. R., Jr. In *Cell Encapsulation Technology And Therapeutics Part II: Encapsulation Systems*; Kühtreiber, W. O., Lanza, R. P., Chick, W. L., Eds.; Springer-Verlag: New York, 1999; Section 1 Microencapsulation, Chapter 7 Calcium Alginate, pp 79−89. (b) Nagaich, S.; Khopade, A. J.; Jain, N. K. *Pharm. Acta Helv.* **1999**, *73*, 227.

(12) Furusawa, K.; Norde, W.; Lyklema, J. *Kolloid−Z. Z. Polym.* **1972**, *250*, 908.

Preparation of Solutions. PSS or PEI solutions (1.0 mg mL^{-1}) were prepared by dissolving the respective polyelectrolytes in Millipore water. The ionic strength of the PSS and PEI solutions was adjusted to 0.04−0.05 M with NaCl, which was equivalent to the NaCl produced in the dMF solution because of pH adjustment. The pH values of the PSS and PEI solutions were 6.5 ± 0.3 and 6.9 ± 0.2, respectively. The dMF solution preparation was a critical step as the reactions associated with melamine resin formation and degradation are complex, with many of the reactions being reversible.[6] The MF resin particle dispersion (10 wt %, 1 mL) was added to a stirred 75 mL 0.1 M HCl solution. The MF particles immediately dissolved, and a clear solution was obtained. The solution consisted of acid-decomposed products of MF (both monomers and/or oligomers, dMF). The dMF solution was kept for 24 h at room temperature (∼25 °C). The pH of this solution (pH 2.0−2.2) was adjusted to pH 4.0 ± 0.1 using 0.1 M NaOH solution, and the volume was made up to 100 mL with Millipore water (specific resistance > 18 MΩ cm) to obtain a 1.0 mg mL^{-1} dMF solution. The solution was kept for another 24 h at room temperature and filtered through a 0.2 μm Millipore filter before being used for film build up. Solutions that became turbid during any of the above processes (dissolution, pH adjustment, or storage) were discarded.

Core-Mediated Multilayer Buildup and Capsule Formation. PSS or Alg (0.5 mg mL^{-1}) layers were adsorbed onto MF and CaP cores (0.1 mL of a 10 wt % dispersion), respectively, by incubation for 15 min. The 10 wt % MF dispersion is available commercially. A 10 wt % dispersion of CaP core particles was initially prepared by dispersing a weighed quantity of commercially available CaP powder (or recycled powder) in water, followed by centrifugation (200*g*) and washing three times with water. This step removed excess ultrafine CaP particles and soluble CaP, thus allowing formation of a thin film of the first alginate layer. The excess polyelectrolyte was removed by three centrifugation (1200*g*)/wash cycles. The PSS-coated MF cores and Alg-coated CaP cores were then exposed to pH 4.0 phosphate buffer (PBS, 0.04 M) for 3 min, followed by washing with pure water. The pH 4.0 exposure step generates the following: dMF from MF cores, which then adsorbs onto the preabsorbed PSS layer; and calcium ions from CaP cores, which bind to preadsorbed Alg. Polyelectrolyte (PSS or Alg) adsorption and pH 4.0 exposure steps were repeated to deposit PSS/dMF multilayers on the MF particles and Ca^{2+}/Alg multilayers on CaP particles, respectively, with intermittent washing steps. The Alg solutions after the adsorption steps were recovered and reused. The multilayer-coated particles were exposed to 0.1 M HCl for 10−15 min to remove the remaining cores and washed several times with Millipore water by centrifuging at 3000*g* for 5 min for the MF particles, and at 1500*g* for 3 min for the CaP particles, respectively. The CaP supernatants were recovered and reused. Multilayer growth was observed qualitatively by measuring the electrophoretic mobility of the coated particles using a Malvern Zetasizer 4.[8] Multilayer coating of the particles and the formation of ultrathin multilayer capsules were verified by transmission electron microscopy (TEM, Philips CM12 microscope operated at 120 kV). CLSM (Leica TCS SP, equipped with an 100x oil immersion objective) of the coated particles and multilayer capsules with bound DOX were also taken.

Conventional LbL Formation of PSS/dMF Multilayers. Bare, negatively charged 640 nm diameter PS particles (30 μL of a 10 wt % dispersion) were incubated with 1 mL of a 1.0 mg mL^{-1} PEI solution for 10 min, followed by three centrifugation (3200*g* for 5 min)/wash cycles, and the material was finally dispersed in 0.5 mL of water. PSS solution (1.0 mL, 1.0 mg mL^{-1}) was then added to the PEI-coated PS particle dispersion, with 10 min allowed for adsorption, and three centrifugation/wash cycles were performed (as above). The dMF adsorption step was performed similarly, except that the adsorption time was 3 min. The PSS and dMF adsorption steps were repeated to deposit multilayers. The coating experiments were performed on the decomposable MF particles (particle sizes: 1.0 and 3.5 μm, 0.1 mL of a 10 wt % dispersion) following a similar protocol, except that the centrifugation speed was kept at 1200*g* for 1 min for washing cycles and by omitting the first PEI adsorption step. Formation of the PSS/dMF layers on PS latices (640 nm) was observed qualitatively and quantitatively by microelectrophoresis (Malvern Zetasizer 4)[8] and quantitatively by using a home-built SPLS instrument.[13]

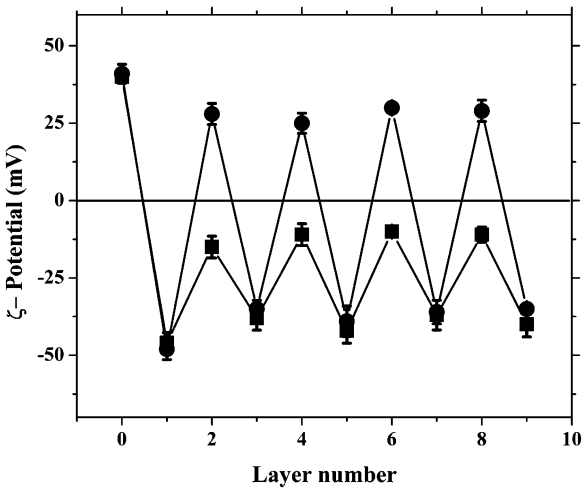


Figure 1. Alternating ζ-potentials for the core-mediated formation of PSS and dMF multilayers on 1.0 μm diameter MF particles: ■, pH 6.0; ●, pH 4.0. The even layer numbers correspond to dMF deposition, and the odd layer numbers correspond to PSS adsorption.

DOX Binding Experiments. Multilayer-coated particles or capsules were treated with a solution of the positively charged fluorescent compound DOX (10 μg mL^{-1}), which binds to free negative charges in PSS and Alg. This was verified by CLSM images. Fluorescence measurements were performed by using a Spex Fluorolog 2 (model FL2T2) spectrophotometer with excitation and emission bandwidths set at 1 nm and using an excitation wavelength of 480 nm. The interference from the scattered light due to the capsules was normalized. The typical peak at 596 nm between the shoulder peaks at 554 and 648 nm is due to the DOX molecule.

Results and Discussion

We first examined the core-mediated LbL adsorption method for the deposition of PSS from solution and dMF originating from the controlled and partial decomposition of the MF core. This involved the consecutive adsorption of the PSS layer onto 1.0 μm MF cores and exposure of PSS-coated MF cores to pH 4.0 phosphate buffer (see Experimental Section). Each pH 4.0 treatment step results in the partial decomposition of MF into its oligomers that complex with the preadsorbed PSS layer, generating a surface favoring deposition of the next PSS layer. Multilayer growth was followed by microelectrophoresis,[8] specifically by measuring ζ-potential values after each adsorption step. ζ-Potential values for the coated particles obtained at pH 6.0 alternated from −35 ± 3 mV (PSS adsorption, from layer number = 3) to −14 ± 3 mV (dMF deposition) (Figure 1). These values are indicative of multilayer growth, as they are in close agreement with those measured for PSS/dMF-coated 1.0 μm diameter MF particles prepared by the conventional LbL approach, i.e., by adsorption of PSS and predecomposed MF from solution (control experiments): in this case, alternating

(13) (a) Lichtenfeld, H.; Knapshinsky, L.; Sonntag, H.; Shilov, V. *Colloids Surf., A* **1995**, *104*, 313. (b) Lichtenfeld, H.; Knapshinsky, L.; Duerr, C.; Zastrow, H. *Prog. Coll. Polym. Sci.* **1997**, *104*, 148.

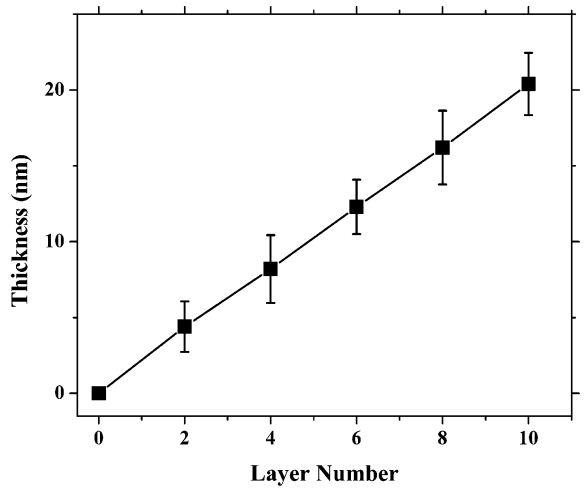
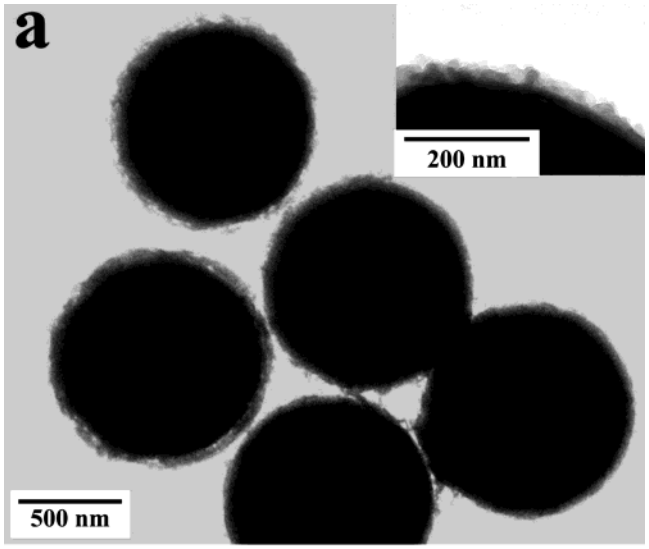


Figure 2. PSS/dMF layer thickness on 640 nm PS spheres, as determined by SPLS. The multilayers were deposited via the alternate deposition of PSS and dMF via the conventional LbL approach.

ζ-potentials of −38 ± 3 and −10 ± 2 mV for the PSS and dMF adsorption steps, respectively, were obtained at pH 6.0 (data not shown). It is noted that the dMF layer is weakly charged in pure water (pH ∼ 6.0); hence, charge reversal is not observed. This finding is similar to the LbL assembly of small molecular weight species (e.g., dyes or polycations) with oppositely charged polyelectrolytes;[8,14] however, it is in contrast to studies involving conventional oppositely charged polyelectrolyte adsorption, where charge reversal is primarily observed.[2,4,5] However, on adjusting the pH of the dispersion medium to 4.0 (i.e., where dMF is positively charged) before the measurements, positive ζ-potentials (+28 ± 3 mV) were observed for the dMF deposition steps (Figure 1).

Evidence for the LbL growth of PSS/dMF multilayers via the conventional LbL method was obtained by single particle light scattering (SPLS) measurements,[13] which were conducted on 640 nm diameter polystyrene (PS) particles. SPLS revealed a regularly increasing film thickness with the number of deposition steps, with an average thickness of 4.1 ± 0.3 nm calculated for each PSS/dMF bilayer (Figure 2).[15] These data show that although the exact composition of the dMF solution may vary with respect to molecular weight and branching of the decomposition products, regular layer growth is achieved, and that the bilayer thickness is similar to those reported for polyelectrolyte multilayers.[2,3−5] No significant decomposition (<5% decrease in absorbance) of the PSS/dMF multilayer films stored in water, 0.5 M NaCl, or 0.01 M HCl for 16 h indicated a high stability of these films due to inter- and intramolecular cross-linking of dMF.

Transmission electron microscopy was employed to directly visualize the PSS/dMF-coated MF particles (Figure 3a). The coated particles are characterized by increased surface roughness compared with the uncoated particles, which have a relatively smooth surface.[4a] Higher magnification of the coating revealed the grainy texture of the surface (Figure 3a, inset). A similar surface texture was obtained in our prior studies with polyelectrolyte−inorganic nanoparticle multilayers assembled onto PS spheres.[4a] Since the acid-decomposable MF resin consists of partially cross-linked melamine with formaldehyde, forming three-dimensional polymer resin particles,[6,7,16] MF oligomers (dMF) responsible for PSS layer formation may resemble polymeric nanoparticles, hence explaining the grainy texture of the PSS/dMF multilayered surface.



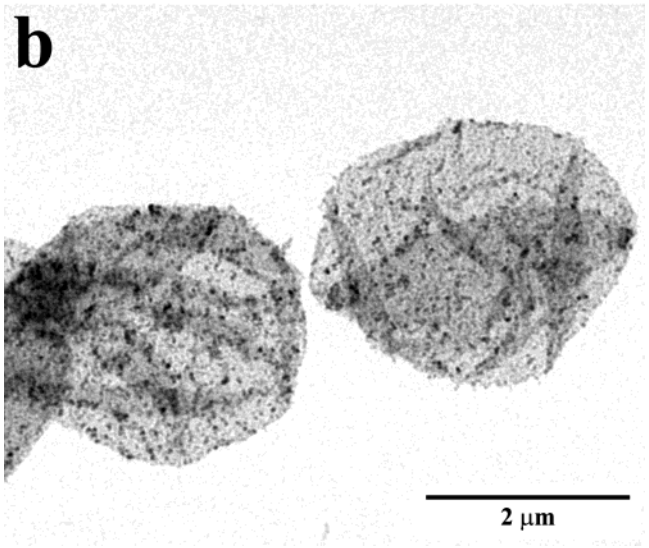


Figure 3. (a) TEM image of $(PSS/dMF)_4$-coated MF particles (1.0 μm diameter) obtained via the core-mediated LbL method, showing surface roughness due to deposition of the multilayer film. The inset shows the particle surface at higher magnification. (b) TEM image of the $[PSS/dMF]_3$ microcapsules prepared by the core-mediated LbL approach using 3.5 μm diameter MF particles. The MF core was removed with 0.1 M HCl.

Core-mediated PSS deposition was additionally proved by doxorubicin (DOX) binding experiments to the PSS/

(14) Radtchenko, I. L.; Sukhorukov, G. B.; Leporatti, S.; Khomutov, G. B.; Donath, E.; Möhwald, H. *J. Colloid Interface Sci.* **2000**, *230*, 272.

(15) Film thicknesses were calculated by using the Raleigh−Debye−Gans theory (Kerker, M. *The Scattering of Light and Other Electromagnetic Radiation*; Academic Press: New York, 1969) and by assuming a layer refractive index of 1.47.

(16) (a) Boh, B.; Kosir, I.; Knez, E.; Kukovic, M.; Skerlavaj, V.; Skvarc, A. *J. Microencapsulation* **1999**, *16*, 169. (b) Kage, H.; Kawahara, H.; Hamada, N.; Kotake, T.; Ogura, H. *Adv.Powder Technol.* **2002**, *13*, 265. (c) Sun, G.; Zhang, Z. *J. Microencapsulation* **2001**, *18*, 593.

dMF-coated MF particles (2, 3, and 4 PSS layer coatings ($[PSS/dMF]_2$, $[PSS/dMF]_3$, and $[PSS/dMF]_4$)). A linear increase in the fluorescence intensity of the positively charged DOX bound to the coatings with an increasing number of the PSS adsorption steps was observed (data not shown). The interaction of DOX with the sulfonate groups of PSS in the multilayer coatings was indicated by an increase in the intensity of the shoulder peak at 648 nm (compared with the main peak at 596 nm) with increasing PSS layer number.[17,18]

Multilayer capsules were obtained after complete core decomposition using a 0.1 M HCl solution. A TEM image of the capsules is shown in Figure 3b. The microcapsules formed are similar in morphology to those of polyelectrolyte capsules prepared by conventional LbL assembly of two oppositely charged polyelectrolytes on sacrificial MF cores (and the core subsequently removed).[4b] A minimum of two and a maximum of five PSS deposition cycles were required to obtain capsules from the decomposable MF templates. Below two deposition cycles, the amount of PSS deposited was not sufficient to obtain stable capsules. More than 10 layers resulted in a lower PSS/dMF layer permeability, thus preventing permeation of the excess dMF (produced as a result of the final core removal step) from the core through the "membrane" film. In this case, broken capsules were obtained, which was confirmed by both CLSM and TEM measurements (not shown). The decrease in permeability may be attributed to chemical cross-linking reactions between MF oligomers produced as a result of exposure of MF particles to pH variations during the coating process[6] as well as increased film thickness.[7] This suggests that the core-mediated LbL approach could potentially be used to control the permeability properties of multilayer capsules.

To demonstrate the general nature of the core-mediated LbL approach and extend it to biocompatible systems, the process was applied to Alg coatings on calcium phosphate (CaP) core particles to produce Ca^{2+}/Alg microcapsules. This was based on the well-known chemistry in the formation of calcium−alginate gels[19] and the possibility of multilayer growth by the stepwise interaction of polyvalent ions with polelectrolytes.[8,14] The core-mediated Alg adsorption steps follow the general scheme illustrated in Scheme 1. After incubation of Alg with CaP core particles, the excess alginate is removed by washing with pure water by centrifugation cycles. A film formed as a result of Alg binding with surface calcium. pH adjustment erodes (dissolves) the core to produce calcium ions that saturate the binding sites on Alg through a "calcium jump" (calcium diffusion) process.[20] The excess calcium ions remaining on the surface are then used to build the next Alg layer. Repeating this process deposited multiple Ca^{2+}/Alg layers, providing a means to control the film thickness.

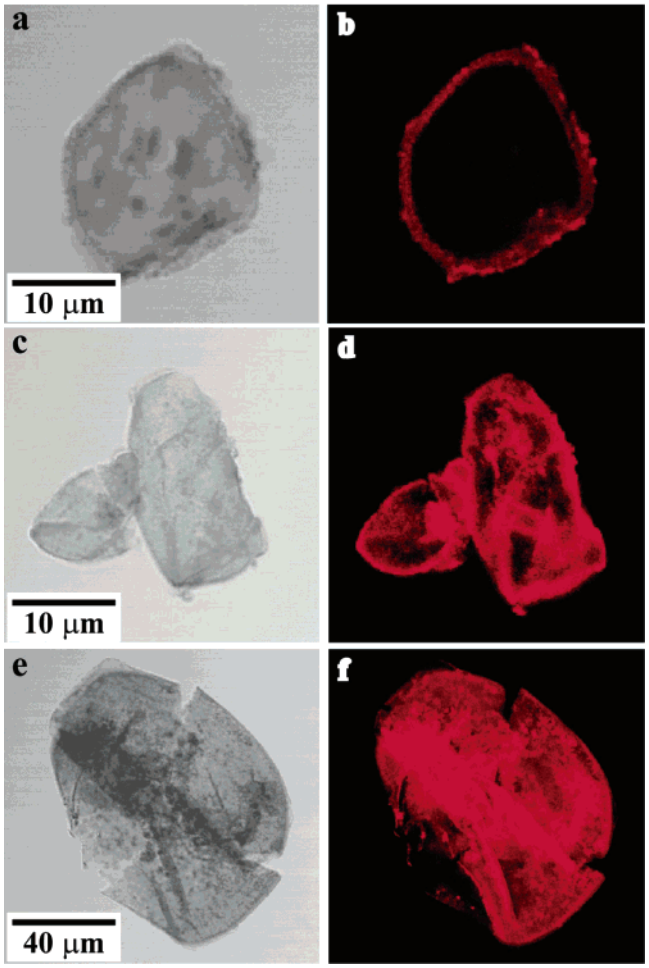


Figure 4. CLSM images of (a,b) $[Ca^{2+}/Alg]_3$-coated CaP particles and (c−f) $[Ca^{2+}/Alg]_3$ capsules prepared by the core-mediated LbL deposition of Alg and Ca^{2+}. The capsules were exposed to a fluorescent compound, DOX, which binds to Alg. Images a, c, and e are transmission images, and b, d, and f are fluorescence images. The scale bars correspond to both the fluorescence and transmission images.

Finally, the CaP core is removed by dissolution, resulting in Ca^{2+}/Alg capsule formation.

CLSM was employed to investigate the morphology of the CaP microcrystals and to verify their coating with Alg multilayers. Figure 4a,b displays a CLSM image of a Ca^{2+}/Alg-coated CaP microcrystal. It is evident from the transmission image (Figure 4a) that the microcrystal possesses a solid core. Direct evidence for polymer coating of the CaP core is provided in the corresponding CLSM fluorescence image (Figure 4b). This displays fluorescence due to DOX, which is bound to the outer layer of the coated CaP microcrystal (see later). The fluorescent ring shows a smoother inner surface than the outer surface. Coated CaP microcrystal suspensions were stable for days when stored in an aqueous medium at neutral pH (6.8−7.2), reflecting the stability of the adsorbed layers. CLSM micrographs of Ca^{2+}/Alg microcapsules, obtained by exposing Ca^{2+}/Alg-coated CaP microcrystals to acidic solution (0.1 M HCl) and then dispersing them in water, are displayed in Figure 4c,d. HCl solubilizes the core material (converts calcium phosphate to soluble calcium chloride), and the individual ions diffuse through the semipermeable polymer capsule walls. The Ca^{2+}/Alg-coated microcrystal suspensions lost their turbidity upon the addition of HCl. The

(17) Khopade, A. J.; Caruso, F. *Biomacromolecules* **2002**, *3*, 1154.
(18) Karukstis, K. K.; Thompson, E. H. Z.; Whiles, J. A.; Rosenfeld, R. J. *Biophys. Chem.* **1998**, *73*, 249.
(19) (a) Haug, A. *Acta Chem. Scand.* **1959**, *13*, 601. (b) Grasdalen, H.; Larsen, H.; Smidsrød, O. *Carbohydr. Res.* **1981**, *89*, 179. (c) Grant, G. T.; Morris, E. R.; Rees, D. A.; Smith, P. J. C.; Thom, D. *FEBS Lett.* **1973**, *32*, 195.
(20) Bergstrom, E.; Goodall, D. M.; Norton, I. T. In *Gums and Stabilizers for Food Industry*. Kinetics of ion-binding and structural change in alginates; Phillips, G. O., Williams, P. A., Wedlock, D. J., Eds.; IRL Press: Oxford, 1990, pp 501−505.

transmission image (Figure 4c) shows no evidence of a solid core, indicating dissolution and removal of the CaP microcrystal. The different morphologies observed are due to the diversity of the microcrystal shapes (near spherical to square and rectangular). Indentations and contours on the microcapsule walls are most probably due to the original structure of the microcrystal core particles. Similar observations were reported with oppositely charged polyelectrolyte capsules obtained from cores of small organic crystals.[5b] There was also evidence of rupturing of the microcapsule walls when the CaP particles were greater than about 50 μm (average of dimensions) (Figure 4e,f). This is likely a result of the sudden increase in osmotic pressure inside the capsule wall when the microcrystal core was treated with HCl due to formation of $CaCl_2$. Since the divalent calcium ions interact with negatively charged carboxyl ions, diffusion through the multilayer film may be slow.[20] The ruptured surface also confirms the hollow nature of the collapsed capsules. Rupture, however, could be avoided by a pH gradient dissolution process (incubating coated particles successively in pH 3.5, 2.5, 1.5, and 1.0 solutions for 5 min), followed by washing with water.

DOX treatment of the capsule dispersions imparted a red color to the capsules, which was evident when the capsules sediment on storage for a few hours. DOX binding studies with Ca^{2+}/Alg microcapsules also showed an increase in the fluorescence intensity of DOX bound to the capsular membrane. The intensity of the shoulder peak at 648 nm also increased with an increasing number of alginate adsorption steps. This finding is in qualitative agreement with the PSS/dMF capsules, confirming the linear growth of core (calcium ion)-mediated alginate layers. In terms of recycling, both Alg and CaP were easily recovered for further use. The Alg solution used for coating was recovered by filtration through 0.2-μm filter membranes, and the CaP was recovered from the mother liquor obtained after core-removal from the coated particles by filtration (to remove precipitates of calcium alginates), followed by treatment with sodium or ammonium phosphate solution. A gelatinous white precipitate formed and was allowed to settle overnight, and the supernatant was decanted. The precipitate was washed several times with water, filtered, and calcined to obtain pure calcium phosphate powder.

Conclusions

We have demonstrated that PSS/dMF or Ca^{2+}/Alg multilayers can be deposited on particles by making use of controlled decomposition of the core, which mediates layer growth. Subsequent removal of the core from the multilayer-coated particles yields two-component capsules. This work offers a general and novel approach that can be extended to other types of core materials with defined decomposition behavior and polyelectrolyte interaction capability, thus opening new pathways to the fabrication and application of ultrathin multilayer capsules.

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