Langmuir
Article
pubs.acs.org/Langmuir

Polymeric Microcapsules Assembled from a Cationic/Zwitterionic Pair of Responsive Block Copolymer Micelles

Timothy Addison,*,† Olivier J. Cayre,† Simon Biggs,† Steven P. Armes,‡ and David York§

†*Institute of Particle Science & Engineering, University of Leeds, Leeds LS2 9JT, United Kingdom,* ‡*Department of Chemistry, University of Sheffield, S10 2TN, United Kingdom, and* §*Procter and Gamble, Newcastle Technology Centre, Newcastle, United Kingdom*

Received October 26, 2009. Revised Manuscript Received November 24, 2009

Using a layer-by-layer (LbL) approach, this work presents the preparation of hollow microcapsules with a membrane constructed entirely from a cationic/zwitterionic pair of pH-responsive block copolymer micelles. Our previous work with such systems highlighted that, in order to retain the responsive nature of the individual micelles contained within the multilayer membranes, it is important to optimize the conditions required for the selective dissolution of the sacrificial particulate templates. Consequently, here, calcium carbonate particles have been employed as colloidal templates as they can be easily dissolved in aqueous environments with the addition of chelating agents such as ethylenediaminetetraacetic acid (EDTA). Furthermore, the dissolution can be carried out in solutions buffered to a desirable pH so not to adversely affect the pH sensitive micelles forming the capsule membranes. First, we have deposited alternating layers of anionic poly[2-(dimethylamino)ethyl methacrylate-*block*-poly(2-(diethylamino)ethyl methacrylate)] (PDMA-PDEA) and cationic poly(2-(diethylamino)ethyl)methacrylate-*block*-poly(methacrylic acid) (PDEA-PMAA) copolymer micelles onto calcium carbonate colloidal templates. After deposition of five micelle bilayers, addition of dilute EDTA solution resulted in dissolution of the calcium carbonate and formation of hollow polymer capsules. The capsules were imaged using atomic force microscopy (AFM) and scanning electron microscopy (SEM), which shows that the micelle/micelle membrane is sufficiently robust to withstand dissolution of the supporting template. Quartz crystal microbalance studies were conducted and provide good evidence that the micelle multilayer structure is retained after EDTA treatment. In addition, a hydrophobic dye was incorporated into the micelle cores prior to adsorption. After dissolution of the particle template, the resulting hollow capsules retained a high concentration of dye, suggesting that the core/shell structure of the micelles remains intact. Finally, thermogravimetric analysis (TGA) of dried capsules confirmed complete removal of the sacrificial inorganic template. As far as we are aware, this is the first demonstration of LbL assembled capsules composed entirely from responsive block copolymer micelles. The results presented here when combined with our previous findings demonstrate that such systems have potential application in the encapsulation and triggered release of actives.

Introduction

Over the past decade or so, the layer-by-layer (LbL) technique has been demonstrated to offer a versatile method for producing novel polymeric coatings onto a number of substrates;[1] thus allowing the development of highly functional surface coatings with a broad range of applications. The technique was initially demonstrated on planar surfaces[2] and subsequently on colloidal substrates.[3] More recently, emulsion droplets,[4,5] liquid crystals,[6,7] and biological cells[8] have also been shown to be suitable templates for LbL deposition. Many strategies for encapsulation have been proposed whereby *sacrificial* particles are used as templates, which can be dissolved to form hollow capsules.[3] There have been several reports in the literature demonstrating the encapsulation of drug-like molecules within microcapsules using three different strategies. Briefly, the first strategy involves preforming capsules with pores that can be reversibly opened in response to an environmental trigger such as pH, thus allowing the active to diffuse into the capsule interior.[9] Although this method is relatively versatile in that it allows the loading of a range of molecules, the final loading of the capsule is usually low since it is limited by the solubility of the active in solution. The second strategy relies on direct deposition of the polymeric multilayer film onto a crystalline template.[10] This method typically offers very high loadings, as the polyelectrolytes are deposited directly from aqueous solutions. However, it is limited to hydrophobic crystalline actives. The final strategy involves employing a porous sacrificial core for the template, onto which an active material can be deposited prior to adsorption of the different polymeric layers forming the capsule membrane. The pores of the sacrificial template offer a large surface area for the active to adsorb onto, hence facilitating the encapsulation of larger amounts. Subsequent dissolution of the template results in capsules that are loaded with the active material.[11] A more detailed account of these strategies can be found in a recent review by Caruso et al.[12]

*To whom correspondence should be addressed. E-mail: pre1twa@leeds.ac.uk. Telephone: +44 113 343 2392. Fax: +44 113 343 2781.

(1) Caruso, F. *Adv. Mater.* **2001**, *13*, 11.
(2) Decher, G. *Science* **1997**, *277*, 1232.
(3) Donath, E.; Sukhorukov, G. B.; Caruso, F.; Davis, S. A.; Möhwald, H. *Angew. Chem., Int. Ed.* **1998**, *37*, 2202.
(4) Lu, G.; An, Z.; Li, J. *Biochem. Biophys. Res. Commun.* **2004**, *315*, 224.
(5) Wackerbarth, H.; Schon, P.; Bindrich, U. *Langmuir* **2009**, *25*, 2636.
(6) Priest, C.; Quinn, A.; Postma, A.; Zelikin, A. N.; Ralston, J.; Caruso, F. *Lab Chip* **2008**, *8*, 2182.
(7) Tjipto, E.; Cadwell, K. D.; Quinn, J. F.; Johnston, A. P. R.; Abbott, N. L.; Caruso, F. *Nano Lett.* **2006**, *6*, 2243.
(8) Donath, E.; Moya, S.; Neu, B.; Sukhorukov, G. B.; Georgieva, R.; Voigt, A.; Baumler, H.; Kiesewetter, H.; Möhwald, H. *Chem.—Eur. J.* **2002**, *8*, 5481.
(9) Sukhorukov, G. B.; Antipov, A. A.; Voigt, A.; Donath, E.; Möhwald, H. *Macromol. Rapid Commun.* **2001**, *22*, 44.
(10) Caruso, F.; Yang, W.; Trau, D.; Renneberg, R. *Langmuir* **2000**, *16*, 8932.
(11) Yu, A.; Wang, Y.; Barlow, E.; Caruso, F. *Adv. Mater.* **2005**, *17*, 1737.
(12) Johnston, A. P. R.; Cortez, C.; Angelatos, A. S.; Caruso, F. *Curr. Opin. Colloid Interface Sci.* **2006**, *11*, 203.

The LbL technique was initially based on the use of homopolyelectrolytes, but more recent studies have shown that one (or both) of the polyelectrolytes can be replaced with charged particulate systems.[13] This has greatly increased the number of potential applications for the technology. For example, enzymes and vesicles[14] can be incorporated into these nanostructured films, suggesting possible uses as biosensors[15−17] and microreactors.[18−22] Other materials such as quantum dots,[23] nanoparticles,[24] and organic actives[25] have been successfully incorporated to LbL films with the aim of providing additional functionality. Furthermore, "soft" particles such as block copolymer micelles have also been used as part of the constructed multilayers, providing further ways of encapsulating materials within these films. The use of *stimulus-responsive* block copolymers that can be tailored to provide reversible aggregation in response to an environmental trigger such as temperature,[26] pH,[27] or ionic strength[28] is particularly interesting, since it can open new opportunities for triggered release of encapsulated actives. Indeed, it has been shown that water-soluble block copolymer micelles can provide a suitable local environment for the loading of hydrophobic actives such as dyes and drugs.[29] Such systems have been shown to have potential applications in drug delivery,[23] agrochemical formulations,[30] and personal care products.[31] In addition to the strategies reviewed by Caruso et al.,[12] incorporating copolymer micelles into multilayer films and capsules has the potential to offer further scope for the encapsulation and release of actives along with providing additional functionality. For example, the work of Hammond and co-workers initially demonstrated encapsulation and release from films comprising linear-dendritic block copolymer micelles.[32] More recently, these authors have showed pH-triggered release of hydrophobic drugs conjugated to block copolymer micelles within LbL assembled films.[33,34] Additionally, Cho and co-workers have demonstrated novel multilayer films composed of charged block copolymer micelles.[35,36] By incorporating quantum dots and organic dyes within the cores of the micelles, they have been able to impart optical functionality into such films.[35] In our laboratories, we have previously reported the pH-induced uptake/release of material from a monolayer of surface-adsorbed block copolymer micelles[37] and, more recently, from micelle layers incorporated within polyelectrolyte multilayers.[38]

Typically, block copolymers only form micelles above a certain critical micelle concentration (cmc). If dilution results in the copolymer concentration dropping below the cmc, this can result in micelle dissociation and premature release of the active(s). This problem is likely to occur in the case of drug delivery methods based on the use of these systems. However, it can be mitigated by surface immobilization of the micelles. We have shown that surface-adsorbed pH-responsive diblock copolymer micelles can resist dissociation upon large dilution conditions.[39] Additionally, in this particular case, micelles were only formed in bulk solution above pH 8. At low pH, where the micelles normally dissociate in bulk solution, the structure of the surface-adsorbed micelles was disrupted but relatively little desorption occurred. Upon replacing the bulk solution with an aqueous alkaline solution (thus providing suitable conditions for micelle formation), partial reformation of the original micelle morphology was observed at the solid surface. This behavior has been reported by us in the past using *in situ* atomic force microscopy (AFM) studies,[40] and it is likened to an "open−close" mechanism which highlights the potential of such micelle monolayers for targeted release of actives.

Our work here is concerned with the development of an alternative strategy for the encapsulation of hydrophobic materials within polymeric capsules composed of block copolymer micelles, with the individual cores of the micelles solubilizing hydrophobic actives. By encapsulating material within surface-adsorbed micelles that respond to specific changes in bulk conditions such as pH, we aim to develop a versatile system that can selectively capture and release molecules in response to specific environmental triggers. Previous research in this area has shown that one,[32] or more,[35] type of block copolymer micelles can be incorporated within *planar* LbL-assembled films. Furthermore, hollow polymeric capsules containing one type of block copolymer micelle have been demonstrated.[41] Previous studies have shown that it is possible to incorporate such micellar structures within the polyelectrolyte multilayer films adsorbed on sacrificial particle templates. Our previous efforts attempting to produce capsules composed exclusively of block copolymer micelles have been hampered by the conditions required for dissolution of the colloidal support irreversibly disrupting the capsule membranes, and as far as we are aware, no studies have yet examined the combination of anionic and cationic micelles as unique building blocks for the formation of such capsules. Here, we wish to demonstrate that, upon dissolution of the colloidal template, the micelle layers forming the membrane of the resulting capsules retain their core/shell morphology and thus retain any encapsulated material. In addition, we examine whether all residual core material can be removed from the capsules without adversely perturbing the structuring of the micelles present within the membrane.

Results and Discussion

In a previous study, we demonstrated the deposition of alternating layers of anionic and cationic micelles onto a colloidal

(13) Caruso, F.; Caruso, R. A.; Möhwald, H. *Science* **1998**, *282*, 1111.
(14) Stadler, B.; Chandrawati, R.; Goldie, K.; Caruso, F. *Langmuir* **2009**, *25*, 6725.
(15) Constantine, C. A.; Gattas-Asfura, K. M.; Mello, S. V.; Crespo, G.; Rastogi, V.; Cheng, T.-C.; DeFrank, J. J.; Leblanc, R. M. *Langmuir* **2003**, *19*, 9863.
(16) Yu, A. M.; Caruso, F. *Anal. Chem.* **2003**, *75*, 3031.
(17) Cassagneau, T.; Caruso, F. *Adv. Mater.* **2002**, *14*, 1837.
(18) Daehne, L.; Leporatti, S.; Donath, E.; Möhwald, H. *J. Am. Chem. Soc.* **2001**, *123*, 5431.
(19) Yu, A. M.; Gentle, I.; Lu, G. Q.; Caruso, F. *Chem. Commun.* **2006**, 2150.
(20) Ghan, R.; Shutava, T.; Patel, A.; John, V.; Lvov, Y. *Mater. Res. Soc. Symp. Proc.* **2004**, *782*, 241.
(21) Lvov, Y.; Caruso, F. *Anal. Chem.* **2001**, *73*, 4212.
(22) Caruso, F.; Schuler, C. *Langmuir* **2000**, *16*, 9595.
(23) Lavasanifar, A.; Samuel, J.; Sattari, S.; Kwon, G. S. *Pharm. Res.* **2002**, *19*, 418.
(24) Kim, B.-S.; Taton, T. A. *Langmuir* **2007**, *23*, 2198.
(25) Soo, P. L.; Luo, L.; Maysinger, D.; Eisenberg, A. *Langmuir* **2002**, *18*, 9996.
(26) Ishida, N.; Biggs, S. *Langmuir* **2007**, *23*, 11083.
(27) Bütün, V.; Billingham, N. C.; Armes, S. P. *Chem. Commun.* **1997**, 671.
(28) Ishida, N.; Biggs, S. *Macromolecules* **2007**, *40*, 9045.
(29) Bo, Q.; Zhao, Y. *J. Polym. Sci., Part A: Polym. Chem.* **2006**, *44*, 1734.
(30) Manteca, Z. I.; Chrisstoffels, L.; Berghaus, R.; Stierl, R. Preparation of block copolymer adjuvants for agrochemical formulations. WO 2005018321, **2005**.
(31) Rodriguez-Hernandez, J.; Checot, F.; Gnanou, Y.; Lecommandoux, S. *Prog. Polym. Sci.* **2005**, *30*, 691.
(32) Nguyen, P. M.; Zacharia, N. S.; Verploegen, E.; Hammond, P. T. *Chem. Mater.* **2007**, *19*, 5524.
(33) Kim, B.-S; Park, S. W.; Hammond, P. T. Abstracts of Papers, 235th ACS National Meeting, New Orleans, LA, United States, April 6−10, **2008**, PMSE.
(34) Kim, B.-S.; Park, S. W.; Hammond, P. T. *ACS Nano* **2008**, *2*, 386.
(35) Cho, J.; Hong, J.; Char, K.; Caruso, F. *J. Am. Chem. Soc.* **2006**, *128*, 9935.
(36) Cho, J.; Quinn, J. F.; Caruso, F. *J. Am. Chem. Soc.* **2004**, *126*, 2270.
(37) Schatz, C.; Smith, E. G.; Armes, S. P.; Wanless, E. J. *Langmuir* **2008**, *24*, 8325.
(38) Addison, T.; Cayre, O. J.; Biggs, S.; Armes, S. P.; York, D. *Langmuir* **2008**, *24*, 13328.
(39) Webber, G. B.; Wanless, E. J.; Armes, S. P.; Biggs, S. *Faraday Discuss.* **2005**, *128*, 193.
(40) Webber, G. B.; Wanless, E. J.; Armes, S. P.; Tang, Y.; Li, Y.; Biggs, S. *Adv. Mater.* **2004**, *16*, 1794.
(41) Zhu, Y.; Tong, W. J.; Möhwald, H. *Langmuir* **2008**, *24*, 7810.

silica template. Furthermore, we showed that the micelles within the multilayer film retained their core/shell structure with multiple adsorption cycles and could thus be used to encapsulate an increasing amount of hydrophobic dye as more layers were added.[42] However, one obvious disadvantage of using silica as a template is that its dissolution typically requires the use of conditions (either buffered ammonium fluoride or 20 wt % NaOH) that are sufficiently disruptive to the multilayer film to hinder the formation of stable capsules. More recently, we demonstrated that stable capsules can be formed by depositing alternating layers of cationic block copolymer micelles of poly[2-(dimethylamino)ethyl methacrylate-*block*-poly(2-(diethylamino)ethyl methacrylate)] (PDMA-PDEA) and anionic poly(sodium 4-styrenesulfonate) [NaPSS] onto a polystyrene latex.[38] Subsequent dissolution of the latex core is achieved by exposure to tetrahydrofuran (THF), which results in precipitation of the copolymer chains and thus loss of the core/shell structure of the micelles and hence loss of functionality. Other exploited colloidal templates for LbL deposition are organic melamine formaldehyde (MF) particles and inorganic calcium carbonate sols. Spherical MF particles can be produced in sizes ranging from 0.5 to 12 μm with samples typically being monodispersed. Weakly cross-linked MF can be readily degraded using 0.1 M HCl; however, it has been demonstrated that the dissolution products have a strong affinity for the inner walls of the polyelectrolyte capsules, rendering complete core removal improbable.[43,44] Micrometer-sized $CaCO_3$ can be precipitated by double decomposition resulting in spherical particles which have a significant degree of surface roughness.[45] $CaCO_3$ dissolves in the presence of chelating agents such as ethylenediaminetetraacetic acid (EDTA), which complexes with free calcium ions in solution. This allows dissolution to be conducted under a range of solution pHs, including alkaline conditions, which in our case could prevent any conformational changes of the PDMA-PDEA micelle layers within the adsorbed film.

In this study, LbL films are prepared using cationic diblock copolymer micelles of PDMA-PDEA combined with zwitterionic poly(2-(diethylamino)ethyl)methacrylate-*block*-poly(methacrylic acid) (PDEA-PMAA) micelles. The weakly basic PDMA-PDEA copolymer is molecularly dissolved at low pH, but it forms well-defined PDEA-core micelles in alkaline solution.[46] Bütün et al. showed that the PDMA blocks of such copolymers can be selectively quaternized by reaction with simple alkyl halides to produce permanent cationic blocks.[47] The unimer-to-micelle transition of PDMA-PDEA occurs over a relatively narrow pH range (pH 7−9), which depends on the molecular weight, block composition, and degree of quaternization of the copolymer. The zwitterionic AB diblock copolymer PDEA-PMAA is known to form micelles with an anionic coronal PMAA block and hydrophobic PDEA core in solution above pH 8.[48]

Initially, a quartz crystal microbalance (QCM) experiment was performed in order to determine the effect of the environmental conditions required for core dissolution on a multilayer film of block copolymer micelles. A stable baseline was established for 20 min before a solution of 50qPDMA-PDEA copolymer micelles (at pH 9) was injected and given 30 min to adsorb. Following a 10 min rinse with an electrolyte solution, the second PDEA-PMAA copolymer micelle solution (at pH 9) was injected and allowed to adsorb and subsequently rinsed in the same way. After 100 min, the films were exposed to one of two different solutions; in one instance, the bilayer of adsorbed micelles was washed using 0.1 M HCl with an approximate pH of 1.1 (conditions required for MF dissolution), and in the other with 0.02 M EDTA at a pH of 9 (chelating solution typically used to dissolve $CaCO_3$). In both cases this was followed by a final rinse at pH 9. The reason for buffering the pH of the EDTA solution is that it will provide conditions which should prevent conformational changes to the micelles within the polymer membrane. The frequency shifts associated with the different steps of the experiment are presented in Figure 1. Frequency shifts recorded for adsorption of the bilayer are typical of multilayer micelle films and are in good agreement with our previous studies in this area.[49] In comparison to a bilayer comprising a binary pair of homopolyelectrolytes, the frequency shift and thus mass sensed by the crystal according to the Sauerbrey equation[50] is very large; this is indicative of a highly hydrated film produced by structuring of the copolymer chains. Figure 1 shows that exposure of the micelle bilayer to strong acidic conditions results in a very large decrease in the mass associated with the crystal. This is most likely caused by rearrangement of the copolymer chains, resulting in the loss of micelle structure and thus loss of entrapped water within the layer. Furthermore, the conformational changes within the layer may also result in a significant amount of the copolymer desorbing from the crystal surface. The frequency of the crystal does not return to zero, which indicates that some of the copolymer chains are still adsorbed onto the crystal. When the pH is returned to 9.0 after several washing steps, a gradual reduction in frequency is observed which may be caused by a slow reorganization of the

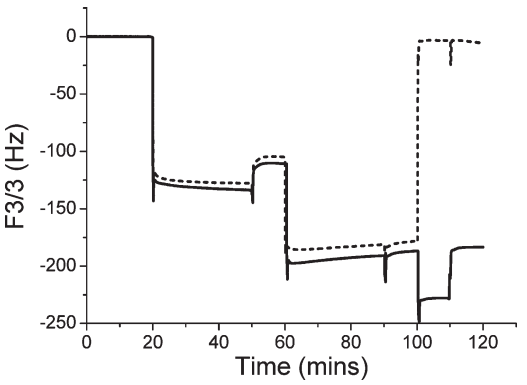

Figure 1. Frequency response of a quartz crystal to adsorption of a PDMA-PDEA/PDEA-PMAA bilayer The graph shows two separate experiments. In both cases, an initial baseline is established for 20 min, followed by injection of the PDMA-PDEA which is given 30 min to adsorb. Following adsorption, the cell was purged with excess electrolyte solution (pH 9) and given 10 min to equilibrate. Next, an injection of PDEA-PMAA was made which was given 30 min to adsorb followed by a 10 min rinse with electrolyte solution (pH 9). Immediately after this rinse and at a time of 100 min from commencement of data collection, an injection of 0.02 M EDTA solution (solid line) or 0.1 M HCl (dotted line) was made. Ten minutes later, a final rinse was performed by purging the cell with excess electrolyte solution at pH 9.

(42) Biggs, S.; Sakai, K.; Addison, T.; Schmid, A.; Armes, S. P.; Vamvakaki, M.; Bütün, V.; Webber, G. *Adv. Mater.* **2007**, *19*, 247.
(43) Gao, C.; Leporatti, S.; Moya, S.; Donath, E.; Möhwald, H. *Langmuir* **2001**, *17*, 3491.
(44) Gao, C.; Moya, S.; Donath, E.; Möhwald, H. *Macromol. Chem. Phys.* **2002**, *203*, 953.
(45) Antipov, A. A.; Shchukin, D.; Fedutik, Y.; Petrov, A. I.; Sukhorukov, G. B.; Möhwald, H. *Colloids Surf., A* **2003**, *224*, 175.
(46) Lee, A. S.; Gast, A. P.; Bütün, V.; Armes, S. P. *Macromolecules* **1999**, *32*, 4302.
(47) Bütün, V.; Armes, S. P.; Billingham, N. C. *Macromolecules* **2001**, *34*, 1148.
(48) Bütün, V.; Liu, S.; Weaver, J. V. M.; Bories-Azeau, Y.; Cai, Y.; Armes, S. P. *React. Funct. Polym.* **2006**, *66*, 157.
(49) Sakai, K.; Smith, E. G.; Webber, G. B.; Baker, M.; Wanless, E. J.; Bütün, V.; Armes, S. P.; Biggs, S. *Langmuir* **2006**, *22*, 8435.
(50) Sauerbrey, G. *Z. Phys.* **1959**, *155*, 206.

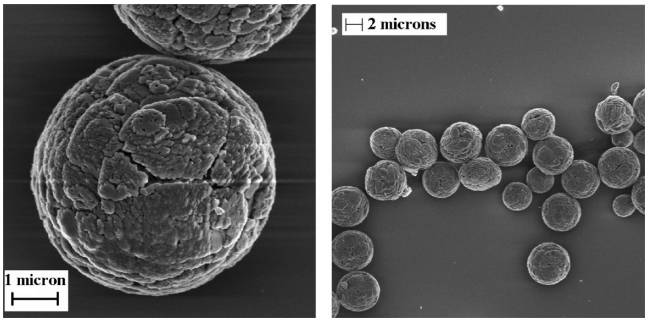

Figure 2. SEM micrographs of uncoated calcium carbonate particles.

copolymer chains. In comparison to these results, when the bilayer is washed with 0.02 M EDTA solution, an increase in the adsorbed mass is observed. This is most likely due to the sensitivity of the QCM technique: the dilute EDTA solution is denser than the electrolyte solution which it replaced, causing a decrease in the frequency of the crystal. After exchange of the EDTA solution with electrolyte, the frequency of the crystal returns to a value very close to that prior to addition of the EDTA. This suggests that the micelle structure within the film is still present and that the EDTA can be easily washed from the film. The data in Figure 1 indicate that dissolving the core of a sacrificial calcium carbonate colloidal template after deposition of a multilayer film of the copolymer micelles is likely not to disrupt the structure of the surface-adsorbed micelles.

Calcium carbonate particles were produced according to the protocol described by Antipov et al.,[45] as detailed in the Experimental Section. Scanning electron microscopy (SEM) analysis (Figure 2) revealed the formation of spherical particles ranging in size from 2 to 8 μm. The particles have a relatively high degree of surface roughness, but previous studies have shown that polyelectrolyte complexation will generally bridge over any underlying defects of the particulate template.[45] The strong affinity of PDMA coronal chains to anionic surfaces[51] means it is logical to expect that 50qPDMA-PDEA micelles will adsorb to rough carbonate surfaces. To obtain repeatable zeta potential data, it was necessary to remove the larger particles via sedimentation prior to taking measurements. It was found that these calcium carbonate sols exhibit a moderate degree of anionic surface charge, which is in good agreement with the literature.[45]

In order to determine if stable capsules can be formed from such carbonate particle templates, five bilayers of alternating 50qPDMA-PDEA and PDEA-PMAA were deposited onto the calcium carbonate templates, starting with a layer of cationic 50qPDMA-PDEA micelles. Following dissolution of the $CaCO_3$ particles (as described in the Experimental Section) and after a number of washing cycles to remove the EDTA, a droplet of the solution was placed on freshly cleaved mica and allowed to dry in air before observation with AFM and SEM. Figure 3 shows an AFM contact mode image of the resulting capsules. It is clear that the capsules have collapsed when drying. However, most capsules were observed as single objects which suggests that the capsules are colloidally stable rather than aggregated in suspension. Successful capsule formation also indicates that the adsorption of the initial micelle layers is not disrupted by the surface roughness of the calcium carbonate templates. There is no evidence that the dissolution of the carbonate core causes disruption or fracturing of the capsules. Also included in Figure 3 is an AFM phase

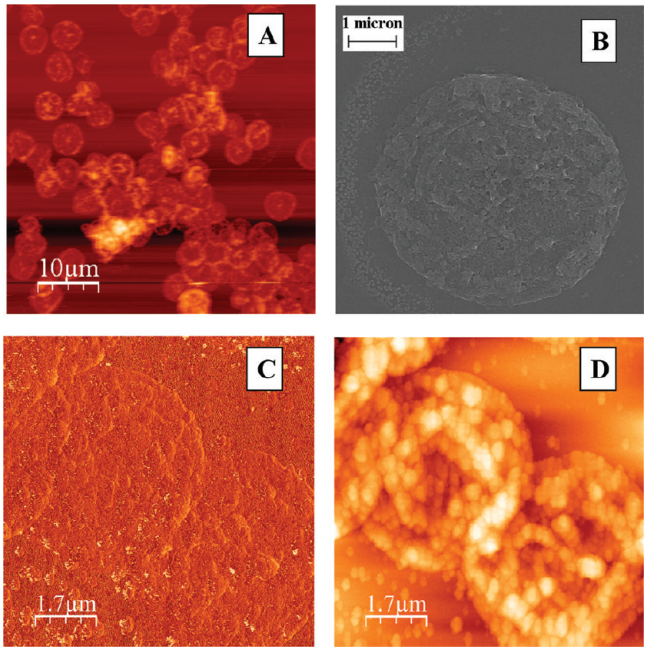

Figure 3. Large-scan AFM image taken in contact mode (A), SEM micrograph of a single capsule (B). AFM tapping-mode images of the same area, phase (C), and height (D).

image obtained in tapping mode. The relatively low contrast of the SEM image shown in Figure 3 is most likely caused by the low conductive properties of the two copolymer samples; inorganic calcium carbonate would be expected to produce a greater contrast and would therefore be easily identifiable if present in significant quantity. Comparison of the AFM images in Figure 3 with an AFM image of capsules made from homopolyelectrolytes only suggests that the structuring seen within the capsules in Figure 3D is caused by presence of polymer aggregates within the dried capsules. This provides further evidence that the core/shell structure of the micelles is retained within the capsule membranes. An AFM image of capsules produced from an anionic/cationic pair of homopolyelectrolytes templated using the same calcium carbonate core is provided as Supporting Information. Our recent studies of the same diblock copolymer adsorption on silica and polystyrene latex templates have confirmed that the original core/shell morphology of surface-adsorbed micelles is retained even after the adsorption of a number of subsequent layers.[38]

The AFM and SEM images presented here demonstrate that block copolymer micelles can be employed as suitable building blocks for stable polymeric capsules, but an important remaining question is whether the copolymer micelles retain their functionality after dissolution of the inorganic template. The digital micrograph in Figure 4 shows a dispersion of capsules, produced from micelle solutions loaded with the moderately hydrophobic dye chrysoidine. These capsules have been washed multiple times, and, as would be expected, a small amount of dye leaches out of the micelles with each washing cycle. However, after several washing steps, it is clear from the intense orange/yellow color of the sediment that a significant quantity of dye still resides within the capsules. This gives strong evidence that the copolymer chains retain their structuring following dissolution of the particle template. Our previous studies,[38] and those by Schatz and coworkers,[37] have extensively shown that micelle multilayers which have been surface immobilized on particle surfaces can be induced to release model hydrophobes from the micelle cores. Thus, in principle, such capsules can be used to encapsulate and selectively

(51) Sakai, K.; Smith, E. G.; Webber, G. B.; Schatz, C.; Wanless, E. J.; Bütün, V.; Armes, S. P.; Biggs, S. *Langmuir* **2006**, *22*, 5328.

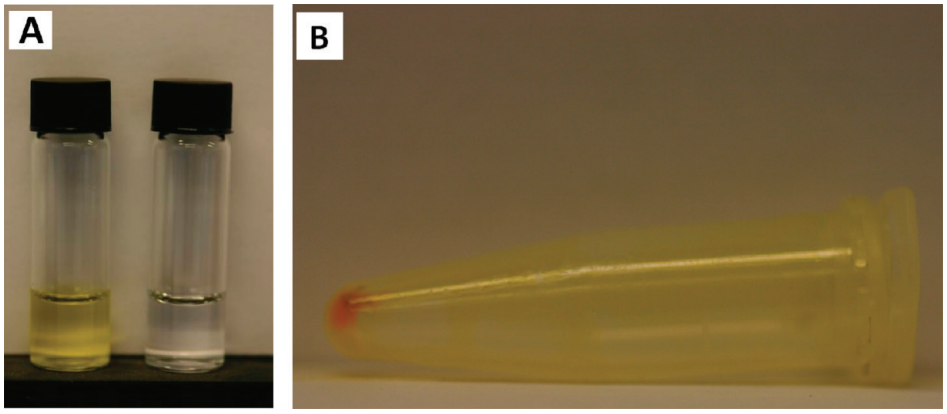

Figure 4. (A) Digital photograph showing a suspension of PDMA-PDEA/PDEA-PMMA capsules loaded with chrysoidine dye; for reference purposes a suspension of equivalent capsules containing no dye is provided in the photo. (B) Digital photograph showing the resulting capsules following two washing cycles, centrifugation, and removal of the supernatant; clearly a large amount of dye is still retained within the micelle cores of the capsule membrane.

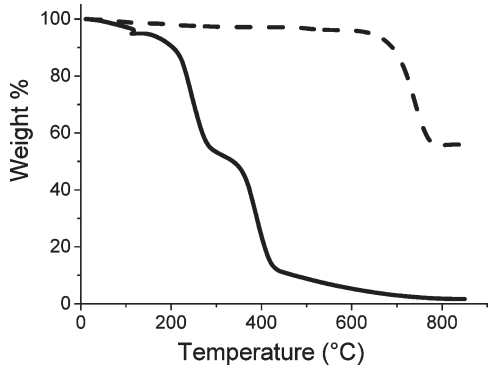

Figure 5. Thermogravimetric analysis of a sample of calcium carbonate particles (dotted line) and dried PDMA-PDEA/PDEA-MAA microcapsules (solid line) after selective dissolution of the $CaCO_3$ using EDTA.

release material from within the individual micelles that comprise the capsule walls.

Finally, to examine whether all of the calcium carbonate is removed from the capsules, thermogravimetric analysis was performed. Figure 5 shows a typical TGA curve obtained for the pristine calcium carbonate particles. A small initial mass loss is observed due to release of surface bound water. Between 650 and 750 °C, a much larger mass loss occurs as the calcium carbonate decomposes to give calcium oxide as a thermally stable residue. When TGA is conducted on a sample of dried hollow polymeric capsules, it is clear that only a very small amount of material does not decompose, suggesting that almost all the sample is organic, that is, polymeric. Additionally, it appears that only ~10% of the capsule sample weight remains at around 450 °C. As the temperature is further increased, the recorded weight is steadily decreasing with no clear drop in the weight of the sample being observed between 650 and 750 °C, where the template particles were found to decompose. It is thus reasonable to assume that the material detected at above 450 °C does not correspond to calcium carbonate but is most likely due to the presence of residual salt. Provided within the Supporting Information is a TGA profile of one of the block copolymers used within this work. These TGA data, when combined with the microscopy images, provide good evidence that little or no residual material is present within the capsules after core dissolution.

In summary, we have demonstrated the formation of LbL-assembled microcapsules composed entirely of responsive block copolymer micelles using a calcium carbonate template while retaining the core/shell structure of the micelles within the capsule membrane. We showed that the conditions required for core dissolution are important in ensuring retention of the micelle structure within the capsule membrane. For the PDMA-PDEA/PDEA-PMAA diblock copolymer pair used here, this means working under mild alkaline conditions to prevent conformational changes within the PDEA-core micelles. In this work, we also show that the two types of micelles possessing the same pH-responsive PDEA core can be used to load the same hydrophobic active within a multilayered capsule membrane. The behavior of this encapsulated active within the micelles is likely complex with exchange between both the bulk solution and subsequent micelle layers occurring during the large number of processing steps required to produce the capsules. However, for future investigation, it is noteworthy that we are not restricted to using the same micelle cores; this may provide further opportunities to tailor the capsules and adsorb binary (or more complex) mixtures of actives within these cores. Alternatively, the flexibility of this method should provide the opportunity to load alternate (or fewer) layers, thereby allowing release profiles and loading capacities of the capsules to be further tuned. Finally, we have provided evidence to suggest that little or no residual carbonate is present following core dissolution, thus minimizing unwanted contamination within such systems.

Experimental Section

Materials. The chemicals were provided by the following sources: HCl (0.1 M), Sigma; ethylenediaminetetraacetic acid (ultra; 0.5 M in water), Fluka; ammonium bicarbonate (NH_4HCO_3, 98%), Alfa Aesar; sodium chloride (NaCl, ultra dry; 99.99%), Alfa Aesar; calcium chloride dihydrate ($CaCl_2$, 99%), Sigma; sodium hydroxide (NaOH, 99%; puriss pellets), Sigma; Chrysoidin G C.I. 11270 (Basic Orange 2), Aldrich. All chemicals were used as received. The water used in all experiments was prepared from a Millipore Milli-Q Ultrapure water purification system with a resistivity of 18.2 MΩ cm^{-1} (25 °C).

Copolymer Synthesis and Characterization. The PDMA-PDEA diblock copolymer was synthesized using group transfer polymerization (GTP). Full details of this synthesis may be found elsewhere.[52] The number-average molecular weight (M_n) and polydispersity index (PDI) of this copolymer were determined to be 17 700 g mol^{-1} and 1.08, respectively, as measured by gel permeation chromatography with a THF eluent at a flow rate of 1.0 mL min^{-1} using poly(methyl methacrylate) calibration

(52) Bütün, V.; Armes, S. P.; Billingham, N. C. *Polymer* **2001**, *42*, 5993.

standards. On the basis of the ^{1}H NMR spectra, the mean degrees of polymerization were calculated to be 97 for the PDMA block and 24 for the PDEA block. This precursor copolymer was then used to make further copolymers containing quaternized tertiary amine residues on the PDMA chains. This was achieved using a substoichometric amount of MeI, as described previously.[47] The mean degree of quaternization of the PDMA block was assessed using ^{1}H NMR and found to be 50% for the sample used within this study.

The synthesis of the PDEA-PMAA diblock copolymer was also carried out by GTP, as follows. The solid tetra-*n*-butylammonium bibenzoate (TBABB, 2 mol % based on 1-methoxy-1-trimethylsiloxy-2-methyl-1-propene (MTS) initiator) catalyst was added to a round-bottomed flask containing a magnetic stirring flea. The flask was then purged with nitrogen before THF (38 mL, depending on the reaction scale) was transferred into the flask via a double-tipped syringe. The MTS initiator (0.30 mL) was added next, followed by the dropwise addition of 2-(diethylamino)ethyl methacrylate (DEA) monomer (15.0 mL). The increase in temperature was measured using a contact thermometer attached to the side of the reaction vessel. When the reaction exotherm had abated, aliquots were extracted for gel permeation chromatography (GPC) and ^{1}H MNR analysis. The 2-tetrahydropyranyl methacrylate (THPMA) monomer (12.5 mL) was then added, and a second exotherm was observed. The reaction solution was stirred until the solution temperature had returned to room temperature before aliquots of the copolymer were withdrawn for GPC and ^{1}H MNR analysis. THF solvent was removed and the PDEA-PTHPMA diblock precursor was precipitated into excess *n*-hexane before drying in a vacuum oven at room temperature. The M_n and the PDI of this copolymer precursor were determined to be 21 900 g mol^{-1} and 1.24, respectively. Selective removal of the 2-tetrahydropyranyl (THP) groups was carried out at room temperature using a 1:1 THF/H_2O mixture in which the precursor copolymer was soluble. HCl (5 M, 1.5 mol excess relative to the THPMA and tertiary amine units) was added to the copolymer solution, and the reaction was stirred for 2−3 days at 20 °C. The THF was evaporated using a rotary evaporator, and KOH solution (2 M) was added to adjust the pH back to neutral. The deprotected copolymer was recovered by precipitation from its aqueous solution into excess acetone to remove KCl salt. Since deprotection of the THP groups was achieved under mild conditions, it was assumed that the final copolymer had the same narrow molecular weight distributions as the corresponding precursor. On the basis of the ^{1}H NMR spectra, the mean degrees of polymerization were calculated to be 50 for the PDEA block and 50 for the PMAA block.

Quartz Crystal Microbalance. A Q-Sense QCM-D 300 instrument was used to assess the adsorption of a bilayer of copolymer micelles at the silica/water interface. A single sensor crystal with a silica coating was used. The cleaning protocol for the sensor and an O-ring that seals the cell/sensor assembly have been described earlier.[49] After assembling the QCM-D instrument, an electrolyte solution (0.1 M NaCl) was injected into the cell and allowed to equilibrate for 20 min. After a stable baseline had been established, a copolymer solution of PDMA-PDEA (500 ppm, pH 9.2) was injected and given 30 min to adsorb. The excess copolymer was washed out by flushing the cell with a copious amount of electrolyte solution (0.1 M NaCl, pH 9.2) before being given 10 min to equilibrate. The second copolymer was then injected, adsorbed, and rinsed in the same way as the first copolymer. Following the formation of the micelle bilayer, solutions of either 0.1 M HCl (pH 1.1) or 0.02 M EDTA (pH 9.0) were injected into the cell and allowed to equilibrate for 10 min. A final wash through with electrolyte solution was performed, after which the experiment was terminated.

Preparation of Micrometer-Sized Calcium Carbonate Particles. Calcium carbonate particles were formed by the rapid addition of 0.01 M $CaCl_2$ solution (500 mL) to 0.01 M NH_4HCO_3 solution (500 mL). The precipitation was carried out in a 1.2 L baffled vessel under high speed mixing (2500 rpm). After 10 min, the mixer was stopped and the vessel was covered and left for 24 h. The large volume of water was removed using siphoning and centrifugation, with the resulting particles being washed twice using deionized water. The particles were oven-dried (60 °C, 12 h) and stored in plastic tubes prior to use.

Preparation of PDMA-PDEA/PDEA-PMAA Multilayer Film. Copolymer micelles of PDMA-PDEA were deposited onto calcium carbonate templates using the LbL technique. An aqueous solution of PDMA-PDEA (1 mL, 1000 ppm) at pH 9.2−9.3 was added to an aqueous calcium carbonate dispersion (0.5 mL) containing 0.025 g of particles at the same pH. The sample was agitated and given 20 min to allow for full coverage of the particles to take place before being centrifuged (500 g, 2 min), and the supernatant removed. Washing of the particles was performed by redispersion in 1.5 mL of pH-adjusted 0.1 M NaCl solution (pH 9.3) followed by a further cycle of centrifugation and supernatant removal. For adsorption of a second layer, the particles were redispersed in 0.5 mL of pH-adjusted electrolyte solution before a solution of PDEA-PMAA (1.0 mL, 1000 ppm, pH 9.3−9.3) was added and given 20 min to adsorb. The separation/replacement/redispersion protocol was repeated in order to coat particles with the desired number of micelle layers.

Formation of Block Copolymer Capsules. In order to create hollow capsules, the calcium carbonate particles were coated with five PDMA-PDEA/PDEA-PMAA pairs. The coated particles were then washed three times with pH-adjusted electrolyte (0.1 M NaCl) at pH 9.3 to remove the excess copolymer. Dissolution of the calcium carbonate cores was achieved by addition of a small volume of 0.02 M EDTA (1.5 mL, pH 9.0) to the coated particle suspension (0.025 mg). After allowing 10 min for dissolution, the hollow capsules were centrifuged (3500 g, 10 min). The supernatant was removed, and the capsules were redispersed in pH-adjusted Milli-Q water. After a further two washing cycles, the capsules were redispersed in pH-adjusted Milli-Q water at pH 9.3.

Preparation of Chrysoidine-Doped Micelle Solution. The Chrysoidine-doped PDMA-PDEA and PDEA-PDMAA micelle solutions were prepared using degassed 0.1 M NaCl solution, which was adjusted to pH 9.3 using dilute aqueous NaOH. Excess Chrysoidine was added to the copolymer micelle solution, which was given 24 h to equilibrate under constant stirring. The resulting stock solution was filtered using a 0.2 μm disposable membrane filter prior to making application

Scanning Electron Microscopy. Scanning electron microscopy images were obtained using a LEO 1530 Field Emission Gun SEM instrument operating at 3 kV.

Atomic Force Microscopy. AFM samples were prepared by pipetting a drop of water-dispersed capsules onto a freshly cleaved mica surface. A Veeco Bioscope II instrument was used, and all images were collected in air using either contact or tapping mode. A POINTPROBE silicon SPM probe was used, with a typical resonance frequency of 320 kHz and a force constant of 42 N m^{-1}.

Thermogravimetric Analysis. TGA was performed on a Perkin-Elmer Pyris1 instrument using samples of approximately 5 mg which had been oven-dried (50 °C) for 24 h prior to analysis. Samples were heated under a nitrogen purge from room temperature to 850 °C at a heating rate of 10 °C/min.

Acknowledgment. The EPSRC, and Procter and Gamble are thanked for providing Timothy Addison with an Industrial Case Award. Dr C. V. Vo is thanked for the quaternization of the copolymer sample.

Supporting Information Available: Figure S1: AFM, tapping mode images of $CaCO_3$ templated capsules, produced from standard homopolyelectrolytes. The anionic/cationic polyelectrolyte pair used here was NaPSS/PDADMAC and the capsule membrane consisted of 5 bilayers. Figure S2: Thermogravimetric analysis of a sample of 50qPDMA-PDEA block copolymer. This material is available free of charge via the Internet at http://pubs.acs.org.