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Title: pH-responsive layer-by-layer films of zwitterionic block copolymer micelles

A strategy to incorporate zwitterionic block copolymer micelles into layer-by-layer films was developed. Multilayers were capable of releasing functional molecules at acidic pH. The upper critical solution temperature of zwitterionic coronal chains was critical to the pH-response of the multilayers.

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pH-responsive layer-by-layer films of zwitterionic block copolymer micelles

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We report a strategy to incorporate micelles of poly[3-dimethyl (methacryloyloxyethyl) ammonium propane sulfonate]-*block*-poly[2-(diisopropylamino)ethyl methacrylate] (β PDMA-*b*-PDPA) into electrostatic layer-by-layer (LbL) films. We obtained micelles with pH-responsive PDPA-cores and zwitterionic β PDMA-coronae at pH 8.5 through pH-induced self-assembly of β PDMA-*b*-PDPA in aqueous solution. To incorporate β PDMA-*b*-PDPA micelles into LbL films, we first obtained a net electrical charge on β PDMA-coronae. Negative charges of the zwitterionic units were screened through complexation of β PDMA-*b*-PDPA micelles and poly(allylamine hydrochloride) (PAH). Positively charged micellar complexes were then self-assembled at the surface using poly(sodium 4-styrenesulfonate) (PSS). The pH-stability of the multilayers was examined by exposing the films to decreasing pH values. Despite the disintegration of micellar complexes below pH 7, no change in film thickness was recorded between pH 8.5 and 3.5. Only ~20% of the film released at pH < 3.5. Moreover, we demonstrated that when multilayers were exposed to decreasing pH at a temperature below the upper critical solution temperature (UCST) of betainized coronal chains, the film thickness increased by 20% between pH 4.5 and 3. This is probably due to phase separation of the corona block and formation of voids within the film, probably entrapping water molecules within the multilayers. Decreasing temperature also shifted the critical disintegration pH (onset of multilayer dissolution) to a slightly lower pH. By taking advantage of the pH-responsive cores, we showed that multilayers were capable of releasing functional molecules under moderately acidic conditions. Important biological properties of polybetaines, higher loading capacity of zwitterionic block copolymer micelles than linear polybetaines and pH-response of the multilayers at physiologically related pH values make such films promising for biomedical applications.

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1. Introduction

Functionalization of surfaces *via* a layer-by-layer (LbL) self-assembly technique – sequential adsorption of interacting polymers or species at the surface – has gained increasing importance in recent years due to its wide application areas.^{1–5} In addition to its simplicity and low cost, the use of aqueous polymer solutions makes this technique promising specifically for biomedical applications, *e.g.* preparation of biofunctional surfaces and/or controlled release of drugs, proteins or other biological molecules from surfaces. The properties of LbL films

are primarily determined by the chemical nature of the polymers. Polyzwitterions are neutral macromolecules accommodating both positive and negative charges on the same repeating unit. They are named “polybetaines” if the positive charges are quaternary ammonium cations. Depending on the source of negative charges, they are named polysulfobetaines, polycarboxybetaines or polyphosphobetaines. In addition to their interesting chemical structures resembling phosphorylcholine, a zwitterionic phospholipid found on the cell membrane,⁶ polybetaines have also important biological properties such as biocompatibility,^{7–11} anti-fouling,¹² and cell resistant behaviours.⁶ Although being investigated by several research groups,^{13–20} understanding the solution behaviour of homopolybetaines and their interactions with polyelectrolytes, surfactants and salts in solution still keeps its complexity. This is due to unusual behaviour of polybetaines in solution, the so-called ‘antipolyelectrolyte effect’, *i.e.* chain expansion and increased solubility in water in the presence of salt ions decreasing the intra- and inter-molecular electrostatic interactions between oppositely charged functional units on the polymer chains. There are also studies which focused on the

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synthesis and aqueous solution behaviour of block copolymers containing polybetaine blocks.^{21–27}

Self-assembly of polybetaines at surfaces is of interest to mimic biological surfaces or prepare biofunctional coatings due to the chemical structure and biological properties of polybetaines. However, despite their potential use in biomedical applications, the number of studies related to polybetaine based LbL films is limited and they involve mostly linear polybetaines. The major concern in the preparation of polybetaine containing LbL films is the tendency of polybetaine–polyelectrolyte complexes towards desorption from the surface during film deposition. Unlike traditionally grown LbL films in which the sequential polyelectrolyte complexation occurs between a polyanion and a polycation, polybetaine–polyelectrolyte complexes are not electrically neutral. Thus, desorption of the complexes from the surface is likely and results in inhibition of LbL growth.²⁸ The first multilayer film of a polybetaine was obtained through chemical activation of the polybetaine layer using small, charged molecules. In this way, the charge was reversed after every polybetaine layer making the LbL growth possible.^{29,30} The first studies on preparation of polysulfobetaine (PSB) and polycarboxybetaine (PCB) multilayers using the traditional layer-by-layer technique were demonstrated by Schlenoff and Sukhishvili, respectively. Both groups used the following strategies to grow the polybetaine films: (i) self-assembly of PSB³¹ or PCB²⁸ with a polyanion under strongly acidic conditions when the sulfo or carboxyl groups were either protonated or partially ionized and the polybetaines carried a net positive charge; (ii) self-assembly of PSB³¹ or PCB²⁸ at neutral pH values with a polyanion and a polycation in an alternating manner resulting in hybrid films. In addition to these, a few other studies have been reported afterwards.^{32–34} These studies presented successful incorporation of polybetaines into multilayers. However these films were either not stable at physiological pH or stable but showed no response at physiologically related pH values. Indeed, it is generally of interest to prepare dually functional surfaces for biological applications. For example, a polymer film coated surface can exhibit biopassive properties due to the chemical nature of the film components and at the same time show response to local pH changes caused by infection and release antimicrobial agents from surfaces.

Herein, we focused on construction of polybetaine based multilayer films which were stable at physiological pH but also showed pH-response when exposed to moderately acidic conditions. In this way, the surfaces would be of interest for biomedical applications due to not only pH-responsive properties of the multilayers but also exciting chemical structure of the polybetaines. Such films were prepared through incorporation of zwitterionic block copolymer micelles with betainized coronal chains and pH-responsive cores into multilayer films. Block copolymer micelles were obtained through pH-triggered self-assembly of a selectively betainized diblock copolymer in aqueous solution above neutral pH. To drive the LbL electrostatic self-assembly, a net electrical charge was imparted on the micellar corona through preparation of micellar complexes of zwitterionic block copolymer micelles and a polycation. In this

way, negative charges of the zwitterionic units were screened and the micellar coronae carried a positive electrical charge. Micellar complexes were then incorporated into the multilayers using an oppositely charged polyelectrolyte counterpart. Despite the disintegration of the micellar complexes below the critical demicellization pH of ~ 7 , films remained deposited at the surface when exposed to decreasing pH values (between pH 8.5 and 3.5). Only $\sim 20\%$ of the film was lost when the films were exposed to strongly acidic conditions ($\text{pH} < 3.5$). We have also found that lowering temperature below the UCST of the betainized coronal chains slightly shifted the critical pH (onset of multilayer dissolution) to a lower value. Moreover, by taking advantage of the pH-responsive hydrophobic micellar cores, we showed that these multilayers were capable of releasing functional molecules under moderately acidic conditions.

As far as we are aware, this study is the first demonstrating a strategy to incorporate zwitterionic block copolymer micelles whose coronal chains accommodate both positive and negative charges on the same repeating unit, into layer-by-layer films. Different from the studies reported on multilayers composed of linear polybetaines,^{28,31–34} we showed that important features such as higher loading capacity due to hydrophobic micellar cores, stability at physiological pH and pH-response under moderately acidic conditions could be imparted to polybetaine multilayers. This work contributes to the fundamental understanding of the preparation and structure–property relationship of multilayers containing zwitterionic block copolymer micelles. Our work also differs from studies on block copolymer containing multilayers^{35–43} due to the chemical nature of the zwitterionic micelles which is of importance for biological applications and use of an unconventional strategy enabling the incorporation of zwitterionic micelles into layer-by-layer films. Considering the biological potential of polybetaines, higher loading capacity of the zwitterionic block copolymer micelles than linear polybetaines and pH-response of the multilayers, such films may be promising for future biomedical applications.

II. Experimental part

II.I. Materials

Branched poly(ethylenimine) (BPEI; M_w 25 000), poly(allylamine hydrochloride) (PAH; M_w 58 000), poly(sodium 4-styrenesulfonate) solution (M_w 70 000, 30 wt% in H_2O), sodium hydroxide, dibasic sodium phosphate and hydrochloric acid were purchased from Sigma-Aldrich Chemical Co. Monobasic sodium phosphate (NaH_2PO_4) and dibasic sodium phosphate (Na_2HPO_4) were purchased from Merck Chemicals. All chemicals were used as received. The deionized (DI) H_2O was purified by passage through a Milli-Q system (Millipore).

II.II. Synthesis of poly[3-dimethyl (methacryloyloxyethyl) ammonium propane sulfonate]-block-poly[2-(diisopropylamino)-ethyl methacrylate] (BPDMA-*b*-PDPA)

A precursor PDMA-*b*-PDPA diblock copolymer having 61 mol % PDMA content and a molecular weight of $15\,800\text{ g mol}^{-1}$

(M_w/M_n : 1.10) was synthesized *via* group transfer polymerization as described previously.⁴⁴ Selective betainisation of DMA residues in the precursor block copolymer (2 g) was carried out in THF (100 mL) using a 10 mol% excess of 1,3-propane sultone based on DMA residues at room temperature as reported before.²⁵ The solution was stirred for 2 days and gelation occurred within 20 h. The resulting β PDMA-*b*-PDPA block copolymer was purified by Soxhlet extraction with THF to remove the excess betainising reagent. The resulting selectively betainised block copolymer, β PDMA-*b*-PDPA, was dried in a vacuum oven at 55 °C for at least 2 days. The extent of selective betainisation of the PDMA block was assessed by ¹H NMR spectroscopy. The molecular weight of the selectively betainised diblock copolymer was calculated assuming 100% betainisation and determined to be 22 360 g mol⁻¹.

II.III. Preparation of (β PDMA-*b*-PDPA) micelles

β PDMA-*b*-PDPA was dissolved in 0.001 M NaH₂PO₄ buffer at pH 3.0 with a final concentration of 1 mg mL⁻¹ and then diluted to 0.1 mg mL⁻¹ using 0.001 M NaH₂PO₄ buffer at pH 3. Micellization was triggered by gradually increasing the solution pH above pH 7.0. The pH was adjusted using 0.1 M NaOH or 0.1 M HCl solutions. The concentration of β PDMA-*b*-PDPA micelles (prepared at pH 8.5) was 0.75 mg mL⁻¹ only for the experiment at which hydrodynamic size was followed as a function of temperature.

II.IV. Preparation of (β PDMA-*b*-PDPA micelles + PAH) complexes

β PDMA-*b*-PDPA was dissolved in 0.001 M NaH₂PO₄ buffer at pH 3.0 with a final concentration of 1 mg mL⁻¹ and then diluted to 0.1 mg mL⁻¹ using a mixture of 0.001 M NaH₂PO₄ buffer and DI water with a volume ratio of 5 : 4. β PDMA-*b*-PDPA micelles were obtained by gradually increasing the pH to 8.5 using 0.1 M NaOH. PAH was dissolved in DI water at acidic pH with a concentration of 2 mg mL⁻¹. The pH of the PAH solution was adjusted to 8.5 prior to addition into the micelle solution. PAH was then gradually added into the solution of β PDMA-*b*-PDPA micelles at a volume ratio of 0.12 : 5. The pH was readjusted to 8.5 after mixing the solutions.

II.V. Dynamic light scattering and zeta-potential measurements of β PDMA-*b*-PDPA micelles and (β PDMA-*b*-PDPA micelles + PAH) complexes

Hydrodynamic size and zeta-potential measurements were performed using Zetasizer Nano-ZS equipment (Malvern Instruments Ltd.). Particle sizes were obtained by cumulants analysis of the autocorrelation data. Zeta-potential values were obtained from electrophoretic mobility values using the Smoluchowski approximation. Micelles and the micellar complexes were prepared as explained in Section II.III and II.IV.

II.VI. Transmission electron microscopy (TEM) imaging

TEM images were obtained using an FEI Tecnai G2 Spirit Bio-Twin CTEM operating at an acceleration voltage of 20–120 kV. A

drop of β PDMA-*b*-PDPA micelles or (β PDMA-*b*-PDPA + PAH) complexes (both with a concentration = 0.1 mg mL⁻¹) was placed on the surface of a copper grid coated with a carbon substrate with 3 mm diameter. After deposition of the micelles or micellar complexes at the surface, samples were air-dried.

II.VII. Deposition of multilayers for ellipsometry and AFM imaging

Silicon wafers or glass slides were immersed in concentrated sulfuric acid for 1 hour and rinsed with DI water. After drying under a flow of nitrogen, wafers were immersed in 0.25 M NaOH solution for 10 minutes, thoroughly rinsed with DI water and dried again under nitrogen flow. A precursor of 1 bilayer BPEI and PSS was deposited from 0.5 mg mL⁻¹ solutions of BPEI at pH 5.5 and PSS at pH 8.5. Multilayers were prepared at pH 8.5, by immersing the silicon wafers or glass slides alternately into oppositely charged (β PDMA-*b*-PDPA micelles + PAH) complexes and PSS solutions for 15 minutes with 2 intermediate rinsing steps in between. LbL films of PAH and PSS were also prepared in the same way for control experiments. The pH-stability of the films was followed by exposing the multilayers to 0.01 M Na₂HPO₄ or NaH₂PO₄ solutions of decreasing pH for 30 minutes. Film growth and pH-stability were monitored by measuring the dry film thicknesses using a spectroscopic ellipsometer of Optosense, USA (OPT-S6000). AFM imaging of the multilayers was performed using an NT-MDT Solver P47 AFM in tapping mode using Si cantilevers.

II.VIII. Release of pyrene from multilayers

β PDMA-*b*-PDPA was dissolved in 7×10^{-8} M aqueous pyrene solution at pH 3.0 with a final concentration of 0.1 mg mL⁻¹. The pH was gradually increased to 8.5 by adding 0.1 M NaOH. Micellar complexes were prepared using pyrene loaded β PDMA-*b*-PDPA micelles in the same way as described in Section II.IV. 30-bilayers of the (β PDMA-*b*-PDPA micelles + PAH)/PSS film were deposited on both sides of a glass slide. Multilayers were first immersed in 0.01 M NaH₂PO₄ buffer solution at pH 7.5. Samples were taken from the solution to monitor the release of pyrene from the multilayers. Pyrene was excited at 335 nm and the intensity at 375.5 nm was monitored as a function of time using a fluorescence spectrophotometer (Hitachi, F-2500). Once pyrene release at pH 7.5 was completed, the pH of the solution was decreased to 5 and the release was followed as described above. Similarly, once the pyrene release at pH 5 was completed, the pH was decreased to 3 and the pyrene release was followed until the change in intensity at 375.5 nm becomes insignificant. The approximate amount of pyrene loaded within the multilayers was calculated using a calibration curve which was prepared using pyrene solutions with concentrations ranging between 7×10^{-17} and 7×10^{-8} M. The amount of pyrene loaded within 60-bilayers of the (30-bilayer, double sided) (β PDMA-*b*-PDPA micelles + PAH)/PSS film was estimated to be ~47 ng.

Scheme 1 Chemical structure of poly[3-dimethyl (methacryloyloxy-ethyl) ammonium propane sulfonate]-block-poly[2-(diisopropylamino)ethyl methacrylate] (β PDMA-*b*-PDPA).

III. Results and discussion

III.1. Aqueous solution properties of β PDMA-*b*-PDPA

Prior to incorporation of zwitterionic micelles into layer-by-layer films, we first studied aqueous solution behaviour of β PDMA-*b*-PDPA whose chemical structure is shown in Scheme 1.

β PDMA-*b*-PDPA is a block copolymer composed of polybasic PDPA and betainized β PDMA blocks. The PDPA block is pH-responsive due to its tertiary amino groups, while the β PDMA block is electrically neutral due to oppositely charged functional units residing on the same repeating unit. β PDMA-*b*-PDPA is soluble in water under acidic conditions and at room temperature. PDPA becomes insoluble in water at pH values above its pK_a of ~ 6 due to unprotonation of tertiary amino groups,⁴⁴ whereas the β PDMA block is soluble in water up to strongly basic pH values. Above pH 7, β PDMA-*b*-PDPA self-assembles into micellar aggregates with a PDPA-core and a β PDMA-corona structure. Fig. 1A shows pH-induced changes in the hydrodynamic size of β PDMA-*b*-PDPA and a schematic representation of the transition from β PDMA-*b*-PDPA unimers to β PDMA-*b*-PDPA micelles. The hydrodynamic size of β PDMA-*b*-PDPA micelles reached ~ 20 nm at pH 8.5 and did not vary significantly with increasing pH. Micellization was reversible but showed some hysteresis (hydrodynamic size of β PDMA-*b*-PDPA during demicellization was higher than that during micellization) due to intra- and inter-molecular hydrophobic interactions between the unprotonated PDPA chains in the core region suppressing the protonation of the PDPA-core block. Note that critical micellization of β PDMA-*b*-PDPA could be shifted down to pH 6.5 when the concentration of β PDMA-*b*-PDPA solution was increased from 0.1 mg mL^{-1} to 0.5 mg mL^{-1} .

We have also monitored the zeta-potential of β PDMA-*b*-PDPA as a function of pH (Fig. 1B). β PDMA-*b*-PDPA showed a positive zeta-potential between pH 4 and 6.5 which decreased gradually as the pH was increased due to unprotonation of the 3° amino

Fig. 1 pH-triggered micellization of 0.1 mg mL^{-1} β PDMA-*b*-PDPA at 25°C . Schematic shows transition from β PDMA-*b*-PDPA unimers to β PDMA-*b*-PDPA micelles (Panel A). Evolution of the zeta-potential of β PDMA-*b*-PDPA with increasing pH (Panel B).

groups on PDPA blocks. Between pH 6.5 and 8, which can be described as the transition region from unimers to micelles, the zeta-potential decreased significantly but it was still positive since both unimers and micelles coexisted together in this region and some positive charge might have remained at the surface of the micellar cores. Above pH 8, β PDMA-*b*-PDPA micelles were almost neutral.

Furthermore, we have examined the temperature-responsive behaviour of β PDMA-*b*-PDPA micelles. Intensity average particle sizes of β PDMA-*b*-PDPA micelles were measured at pH 8.5 when the temperature was lowered by 5°C steps from 25°C to 5°C . β PDMA-*b*-PDPA micelles were stable in aqueous solution at 25°C for a long time. However, an increase in the intensity of larger particles was observed at 20°C (data not shown) followed by aggregation of β PDMA-*b*-PDPA micelles at/below 15°C due to the loss of the solubility of β PDMA coronal blocks below its UCST. Fig. 2 shows the changes in size and the intensity (%) of β PDMA-*b*-PDPA micelles at 25°C and after 25 minutes at 15°C . Our observations are in good agreement with Armes and co-workers who reported on the thermoresponsive behaviour of the β PDMA block and the UCST of β PDMA to be around 15°C .⁴⁵

The UCST of polyzwitterions was associated with the inter-molecular interactions between the zwitterionic groups.^{46,47} Our

Fig. 2 Intensity (%) vs. size of 0.75 mg mL^{-1} β PDMA-*b*-PDPA micelles (prepared at pH 8.5) at 25°C and after 25 minutes at 15°C . Temperature was lowered by 5°C steps from 25°C to 15°C .

observations are also in good agreement with Laschewsky and co-workers who reported two different phase transition temperatures for the block copolymer of *N*-isopropylacrylamide (NIPAM) and 3-[*N*-(3-methacrylamidopropyl)-*N,N*-dimethyl] ammoniopropane sulfonate (SPP) due to the UCST of the SPP block and lower critical solution temperature (LCST) of the NIPAM-block. They found that the phase transition temperature at low temperatures varied between 8.5 and 19°C and highly depended on the SPP block length.⁴⁸

III.II. Preparation of (β PDMA-*b*-PDPA micelles + PAH) complexes

Electrostatic multilayer film assembly is based on sequential adsorption of polycations and polyanions at a surface leading to complexation between the oppositely charged polymer pairs.⁴⁹ However, unlike complexes of oppositely charged polyelectrolytes, complexes of polybetaines and polyelectrolytes are not electrically neutral and have greater tendency to dissolve in water. For example, Knoesel *et al.* reported on the formation of water-soluble complexes as a result of association between polysulfobetaines and polycations.⁵⁰ Therefore, desorption from the surface and formation of water-soluble complexes are highly possible during multilayer construction of a polybetaine and a polyelectrolyte resulting in inhibition of LbL growth.

To incorporate zwitterionic block copolymer micelles into LbL films, we focused on screening either of the zwitterionic charges and obtained a net electrical charge on the betainized coronal chains using homopolyelectrolytes. PAH was chosen as

a model weak polycation to screen the negative charges of β PDMA coronal chains. First, we have prepared micellar complexes of β PDMA-*b*-PDPA micelles and PAH at pH 8.5. In the rest of the manuscript, micellar complexes will be denoted as (β PDMA-*b*-PDPA micelles + PAH). The reason for preparing complexes at a pH which is well above the critical micellization pH was to minimize the amount of charge on the surface of the micellar cores and ensure the interaction of PAH with the betainized coronal chains. Importantly, the concentration of PAH in the final mixture was critical in the formation of stable micellar complexes. We could not avoid aggregation and uncontrollable increase in the particle size of micellar complexes at higher concentrations of PAH probably due to bridging of β PDMA-*b*-PDPA micelles by PAH chains. Table 1 shows changes in hydrodynamic size and the zeta potential of β PDMA-*b*-PDPA micelles upon complexation with PAH at pH 8.5. As expected, β PDMA-*b*-PDPA micelles which are electrically neutral at pH 8.5 showed a positive zeta-potential after addition of PAH and screening of negative charges on sulfo groups of

Table 1 Change in the zeta-potential and hydrodynamic size of β PDMA-*b*-PDPA micelles upon complexation with PAH at pH 8.5

	Zeta-potential (mV)	Hydrodynamic size (nm)
(β PDMA- <i>b</i> -PDPA) micelles	0.8 ± 0.2	19.8 ± 0.2
(β PDMA- <i>b</i> -PDPA micelles + PAH) complexes	11.3 ± 0.6	24.7 ± 1.1

β PDMA-coronae. We recorded ~ 5 nm increase in hydrodynamic size when β PDMA-*b*-PDPA micelles were complexed with PAH. We attribute this slight increase in particle size to a change in the conformation of β PDMA coronal chains as well as formation of an outer shell by PAH. It has been reported that polyelectrolytes expand in solutions with high salt concentration (antipolyelectrolyte behaviour)^{51–54} due to the decrease in the extent of inter- and intramolecular association of positive and negative charges. β PDMA coronal chains might have adopted more expanded conformation upon complexation with PAH due to screening of the negative charges resulting in a decrease in the degree of inter- and intra-molecular interactions among the β PDMA coronal chains. Moreover, the chain length of PAH is greater than that of β PDMA coronal chains. Thus, upon electrostatic adsorption of PAH on the coronae (PAH binds to sulfo groups of β PDMA), part of PAH chains may remain unadsorbed (uncomplexed) resulting in the formation of a charged outer shell. Schematic representation of complexation is shown in Scheme 2. We have also performed TEM imaging of bare β PDMA-*b*-PDPA micelles and (β PDMA-*b*-PDPA + PAH) complexes (Fig. 3). Particle sizes obtained from TEM imaging were in good agreement with the values obtained using dynamic light scattering (DLS).

Note that we have also tried preparing micellar complexes using PSS. There appeared small (~ 10 nm) and large (higher than 50 nm) micellar complexes together upon mixing β PDMA-*b*-PDPA micelles with PSS. Also, the size of the larger particles did not remain constant and increased with time. The difference between the behaviour of PAH and PSS during complexation can be correlated with the charge density of the polymers. PSS is fully charged at pH 8.5, while PAH is partially charged ($pK_a \sim 8.8$ (ref. 55)). The higher charge density of PSS might lead to: (i) strong association between quaternized amino groups of β PDMA coronae and PSS resulting in partial removal of β PDMA-*b*-PDPA chains from the micelles, leaving behind smaller aggregates; (ii) bridging of β PDMA-*b*-PDPA micelles by PSS and formation of larger micellar complexes due to more extended conformation of PSS.

Fig. 3 TEM images of β PDMA-*b*-PDPA micelles and (β PDMA-*b*-PDPA micelles + PAH) complexes at pH 8.5.

Fig. 4 Evolution of film thickness as a function of bilayer number for (β PDMA-*b*-PDPA micelles + PAH) complexes/PSS (sphere) films. Data for the PAH/PSS system (star) are plotted for comparison.

III.III. Incorporation of (β PDMA-*b*-PDPA micelles + PAH) complexes into LbL films

After obtaining net charge on the coronal blocks, we then focused on LbL self-assembly of (β PDMA-*b*-PDPA micelles + PAH) complexes at the surface using an oppositely charged homopolyelectrolyte. Positively charged (β PDMA-*b*-PDPA micelles + PAH) complexes were self-assembled with PSS at pH

Scheme 2 Schematic representation of complexation between β PDMA-*b*-PDPA micelles and PAH.

8.5. This film system will be denoted as (β PDMA-*b*-PDPA micelles + PAH)/PSS in the rest of the manuscript. Fig. 4 shows evolution of the film thickness as a function of bilayer number for (β PDMA-*b*-PDPA micelles + PAH)/PSS films. Multilayer growth of PAH/PSS at pH 8.5 is plotted for comparison. As seen in Fig. 4, (β PDMA-*b*-PDPA micelles + PAH)/PSS exhibited robust film growth. The driving force for multilayer growth was electrostatic interactions between quaternized amino groups of β PDMA-corona and sulfo groups of PSS. Note that PAH chains forming the outer shell of (β PDMA-*b*-PDPA micelles + PAH) complexes might have also contributed to the film growth. Micellar complexes were expected to be flattened (rather than being spherical) at the surface due to strong electrostatic interactions between the layers. Evidence is that the film grows slower than the spherical size of the micelle (~ 20 nm) per bilayer. This can be explained by the extended conformation of PSS and strong electrostatic interactions among the layers. PSS, which was fully charged at the deposition pH of 8.5, was adsorbed in the plane of the surface on the layer of micellar complexes in the extended form. Also, fully charged PSS can associate with quaternized amino groups of β PDMA coronae through multiple binding points resulting in an intense film structure. Data for the PAH/PSS film which does not contain β PDMA-*b*-PDPA micelles were also plotted for comparison. The difference in thickness between PAH/PSS and (β PDMA-*b*-PDPA micelles + PAH)/PSS films was insignificant until a bilayer number of 6 but became more pronounced at increasing bilayer numbers. This increase in difference with increasing bilayer number can be explained by a decrease in the flattening and spreading of micelles as moving away from the substrate as well as an increase in the surface roughness of multilayers of micellar complexes resulting in an increase in the amount of adsorbed polymer.

Note that we have also tried to self-assemble bare β PDMA-*b*-PDPA micelles at the surface using either PSS or PAH (data not shown). In contrast to multilayers of micellar complexes, we could not obtain robust film growth. For example, when PSS was used as a counterpart for LbL deposition of bare β PDMA-*b*-PDPA micelles at pH 8.5, we observed desorption of the film from the surface specifically during deposition of PSS layers. The interpolymer complexes between β PDMA-*b*-PDPA micelles and PSS probably formed but stripped off the surface during PSS deposition yielding a water-soluble complex due to high excess charge on PSS chains resulting in inhibition of LbL growth. PAH which is partially charged at pH 8.5 was not as effective as PSS to strip off the interpolymer complex from the surface. Yet, we could not obtain a robust growth of β PDMA-*b*-PDPA micelles and PAH multilayers. Only ~ 1.1 nm per layer increment was recorded after the 6th layer. This very small increase in film thickness also indicates the solubilization of a significant amount of β PDMA-*b*-PDPA micelle-PAH interpolymer complexes from the surface.

Schlenoff and co-workers also reported on the impossibility of LbL self-assembly of a polysulfobetaine with either a polyanion or a polycation at neutral pH. They could only self-assemble the polysulfobetaine with PSS under strongly acidic conditions (pH 0.8) when sulfo groups were protonated, thus

polysulfobetaine acted as a polycation. Interestingly, they were able to prepare multilayers under neutral conditions when the polysulfobetaine was sandwiched between a polyanion and a polycation resulting in a hybrid film system.³¹ In a different study, Kharlampieva and Sukhishvili also reported on the self-assembly of a polycarboxybetaine under neutral or basic conditions through construction of a hybrid multilayer system. Otherwise, the polycarboxybetaine could only be self-assembled at the surface under acidic conditions when carboxylic acid groups were in the protonated form and the polycarboxybetaine acted as a polycation.²⁸

III.IV. Surface morphology of (β PDMA-*b*-PDPA micelles + PAH)/PSS films

The surface morphology of a 10-bilayer (β PDMA-*b*-PDPA micelles + PAH)/PSS was investigated by AFM (Panel A). Again, the PAH/PSS film was also examined for comparison (Panel B). Fig. 5 shows the $5\ \mu\text{m} \times 5\ \mu\text{m}$ area AFM height images of the two films. The surface of the PAH/PSS film was rather smooth with a root-mean-squared (rms) roughness of ~ 3 nm, while the rms surface roughness of the (β PDMA-*b*-PDPA micelles + PAH)/PSS film was ~ 18 nm. On the surfaces of (β PDMA-*b*-PDPA micelles + PAH)/PSS films, circular islands having lateral sizes of ~ 100 – 150 nm and heights of ~ 30 – 50 nm are seen with a clear distinction. These circular islands are sparsely located on a smooth underlying film. The height histograms of the two films are seen in Fig. 5C. The histogram of the (β PDMA-*b*-PDPA micelles + PAH)/PSS film was asymmetric with respect to the peak position and showed larger spread towards larger height values indicating the presence of islands on a dense smooth film. PSS, which was fully charged at the deposition pH of 8.5, was expected to adsorb on the layer of micellar complexes in extended conformation and form many ionic crosslinks at their surface, smoothening the underlying layer of micellar complexes.

III.V. pH-induced dissolution of (β PDMA-*b*-PDPA micelles + PAH)/PSS multilayers

We monitored the stability of (β PDMA-*b*-PDPA micelles + PAH)/PSS multilayers (constructed at pH 8.5) against pH by exposing the films to 0.01 M phosphate buffer solutions of decreasing pH. Again, data for the PAH/PSS film were plotted for comparison (Fig. 6). We divided the pH range under consideration into three: (i) region I shows the pH range between pH 8.5 and pH 7; (ii) region II shows the pH range between pH 7 and pH 3.5 and (iii) region III shows the pH range between pH 3.5 and pH 2.

As expected, no change in the film thickness was recorded for the (β PDMA-*b*-PDPA micelles + PAH)/PSS film in region I which corresponded to pH values above the critical demicellization pH.

In region 2, β PDMA-*b*-PDPA micelles were expected to open up (critical demicellization pH ~ 7) and deconstruct the multilayers, however we did not record any loss in the film thickness. Only, a slight increase in thickness between pH 6.5 and 5.5 was recorded, probably due to enhanced protonation of the tertiary amino groups of PDPA-cores near its pK_a resulting in

Fig. 5 $5 \mu\text{m} \times 5 \mu\text{m}$ area AFM height images of 10-bilayer (β PDMA-*b*-PDPA micelles + PAH)/PSS (Panel A) and PAH/PSS (Panel B) films. The height histograms of the two films as determined from $2 \mu\text{m} \times 2 \mu\text{m}$ area scans (Panel C).

Fig. 6 pH-triggered dissolution of (β PDMA-*b*-PDPA micelles + PAH) complexes/PSS (spheres) films. Data for the PAH/PSS system (stars) are plotted for comparison.

an increase in the hydrophilicity of the matrix and entrapment of water molecules within the multilayers. Note that this slight increment in thickness is attributed to protonation of PDPA-cores rather than PAH due to the fact that the amount of PAH is significantly lower than β PDMA-*b*-PDPA micelles within the multilayers and its pK_a (~ 8.8 (ref. 55)) is far above the pH range that is under consideration. The stability of the films in region 2 can be explained by the compensation of the excess positive

charge arising from protonation of PDPA-core blocks and PAH by the PSS counterpart as the acidity increased. In other words, micellar complexes opened up but β PDMA-*b*-PDPA unimers and PAH went into a restructuring with PSS (counterpart), and remained at the surface. We will also show the pH-induced disintegration of micellar complexes and release of a hydrophobic dye from the micellar cores under moderately acidic conditions in Section III.VII. In another study, Erel *et al.* observed a similar phenomenon for hydrogen-bonded multilayers of TA and micelles of poly[2-(*N*-morpholino)ethyl methacrylate]-*block*-poly[2-(diisopropylamino)ethyl methacrylate] (PMEMA-*b*-PDPA) which were constructed at pH 7.4 and exposed to decreasing pH conditions. Although PMEMA-*b*-PDPA micelles dissociated below the critical demicellization pH, they found that the film thickness was conserved between pH 7.5 and 4 due to a restructuring between the film components.⁴³

In region 3 (pH < 3.5), $\sim 20\%$ of the (β PDMA-*b*-PDPA micelles + PAH)/PSS film released from the surface. Further protonation of PDPA core blocks and PAH resulted in a pronounced increase in excess positive charge which could not be compensated by PSS as effectively as it was in region II. The charge imbalance within the multilayers led to electrostatic repulsion between the layers and an increase in osmotic pressure, and finally ended up in swelling and partial dissolution of the multilayers.^{56–58} However, considering the fact that the loss in film thickness

corresponded to only 20% of the total film even after exposure to pH 2, it can be concluded that the highly charged PSS played a critical role in the reassociation among the film components within the multilayers. Note that PAH/PSS control films did not show pH-response throughout the whole pH range.

The significant difference between the (β PDMA-*b*-PDPA micelles + PAH)/PSS film and the ones reported in the literature based on linear polybetaines is that multilayers of β PDMA-*b*-PDPA micellar complexes were stable under neutral and basic conditions but also showed response under acidic conditions. In another study, Schlenoff and co-workers were able to prepare hybrid films of poly(2-vinyl pyridine) sulphopropyl betaine (P2VZ), poly(diallyl dimethyl ammonium chloride) (PDAD) and PSS at neutral pH but those films were not responsive to pH variations.³¹ Another study by Kharlampieva and Sukhishvili demonstrated successful deposition of a hybrid film composed of a polycarboxybetaine, PMAA and 20% quaternized poly(4-vinylpyridine) at pH 4.5 but those films dissolved either above pH 7 or below pH 4.²⁸

III.VI. Effect of temperature on pH-induced dissolution of (β PDMA-*b*-PDPA micelles + PAH)/PSS multilayers

As discussed in Section III.I, β PDMA-*b*-PDPA micelles which were prepared at pH 8.5 aggregated below 15 °C due to the UCST of the β PDMA block. In this context, we wanted to further check the effect of temperature on pH-triggered disintegration of multilayers of micellar complexes. We monitored the change in film thickness as a function of pH at 10 °C and compared the data with that at 25 °C (Fig. 7). In region I, we did not observe any difference between 10 °C and 25 °C. However, in region II, the thickness of (β PDMA-*b*-PDPA micelles + PAH)/PSS multilayers increased significantly as the pH decreased from 5 to 3 (~20% at pH 3.5) at 10 °C. This increase in thickness can be explained by the phase separation and conformational rearrangement of β PDMA coronal chains below its UCST temperature resulting in formation of voids within the multilayers. These voids might have led to entrapment of more water molecules within the multilayers, and this was reflected as an increase in the film thickness. Different from the behaviour of the films at 25 °C, multilayers were still swollen (~20%) at pH 3

Fig. 7 pH-triggered dissolution of (β PDMA-*b*-PDPA micelles + PAH)/PSS multilayers at 25 °C and 10 °C.

(region 3) at 10 °C. Decreasing the pH beyond 3 resulted in partial dissolution of the films, however the loss of the film at 10 °C was smaller than that at 25 °C. This difference was probably due to enhanced hydrophobic interactions between the coronal chains below the UCST of β PDMA, slightly suppressing the dissolution of layers from the surface.

Sukhishvili and co-workers also reported on the formation of voids within hydrogen-bonded films of polymethacrylic acid (PMAA)/poly(vinyl methyl ether) (PVME) or PMAA/poly(*N*-vinyl caprolactam) (PVCL) resulting in enhanced permeability of a dye from the multilayers when the temperature was raised above the lower critical solution temperature (LCST) of PVME and PVCL.⁵⁹ In another study, Erel *et al.* demonstrated an increase in the amount of pyrene released from the multilayers of poly[2-(*N*-morpholino)ethyl methacrylate]-*block*-poly[2-(diisopropylamino)ethyl methacrylate] (PMEMA-*b*-PDPA) micelles and TA at pH 7.4 when the temperature was raised above the LCST of PMEMA coronal blocks and correlated this fact also with formation of voids within the film due to phase separation of PMEMA.⁴³

III.VII. pH-induced release of pyrene from (β PDMA-*b*-PDPA micelles + PAH) complexes/PSS multilayers

We have also examined the potential of (β PDMA-*b*-PDPA micelles + PAH) complexes/PSS multilayers to be used as

Fig. 8 pH-triggered release of pyrene from (β PDMA-*b*-PDPA micelles + PAH) complexes/PSS multilayers. Panel A shows normalized intensity plotted as a function of time at pH 7.5, pH 5 and pH 3.5. Panel B shows the normalized total intensity of pyrene released at pH 7.5, pH 5 and pH 3.5.

platforms for controlled release of functional molecules from surfaces. The release of a hydrophobic dye (pyrene) from the multilayers was monitored as the acidity was increased (Fig. 8). First, we immersed the glass slides whose both sides were coated with 30 bilayers of (β PDMA-*b*-PDPA micelles + PAH) complexes/PSS multilayers into a 0.01 M phosphate buffer solution at pH 7.5. We collected samples every 10 minutes for fluorescence intensity measurements. Once the amount of pyrene released from the multilayers at pH 7.5 remained constant, we decreased the solution pH to 5 and continued collecting the samples every 10 minutes. The same procedure was repeated for the transition from pH 5 to pH 3.5. Note that samples were always returned to the original solution after the intensity measurements to avoid the change in concentration. We found that 35% of pyrene released from the micellar cores at pH 7.5. Considering the fact that micellar complexes were intact within the multilayers at pH 7.5, release can be explained by self-diffusion of pyrene from the micellar cores. When the multilayers were exposed to pH 5, we recorded a jump in the intensity of pyrene released into the solution, indicating the opening of PDPA-cores and intensive release of pyrene from the film surface. We also recorded a decrease in fluorescence intensity with time at pH 5 due to quenching as the amount of pyrene in the solution increased. Further decreasing the pH to 3.5 triggered release of some additional pyrene, however our results demonstrated that a significant amount of pyrene was released at pH 5.

IV. Conclusion

We have presented a strategy to incorporate block copolymer micelles with zwitterionic coronae and pH-responsive cores into electrostatic multilayers. Such block copolymer micelles could be incorporated within the multilayers when negative charges of the zwitterionic units were screened through complexation of the block copolymer micelles with a polycation. Positively charged micellar complexes were then self-assembled at the surface using a polyanion. Interestingly, when films were exposed to decreasing pH values, no change in film thickness was recorded between pH 8.5 and pH 3.5, although the zwitterionic block copolymer micelles disintegrated below pH 7 (critical demicellization pH). This is probably due to compensation of the excess positive charge arising from protonation of polybasic micellar cores by the polyanion counterpart, followed by a reassociation among the film components within the multilayers. Partial dissolution of the film was observed only under strongly acidic conditions. Moreover, we demonstrated that when multilayers were exposed to decreasing pH values at a temperature below the UCST of betainized coronal chains, the film thickness increased by 20% between pH 4.5 and 3. This is probably due to phase separation of the coronal chains and formation of voids within the film where the water molecules were entrapped. Decreasing temperature also shifted the critical disintegration pH (onset of multilayer dissolution) to a slightly lower pH probably due to enhanced hydrophobic interactions between the micellar coronae bringing additional stabilization to the film. Finally, we showed that multilayers containing

micellar complexes were capable of releasing functional molecules at moderately acidic pH. This study differs from other block copolymer micelle containing multilayers^{35–43} due to the chemical nature of the zwitterionic micelles which is of importance for biological applications and the use of an unconventional strategy enabling the incorporation of such nanoaggregates into layer-by-layer films. In addition, different from the multilayers of linear homopolybetaines,^{28,31–34} these multilayers had higher loading capacity due to hydrophobic micellar cores of zwitterionic micelles, were stable at physiological pH but showed pH-response under moderately acidic conditions. Considering the interesting biological properties of polybetaines, higher loading capacity of block copolymer micelles than linear polybetaines and pH-response of the multilayers, such films might be promising for the development of functional surfaces and/or controlled delivery applications from surfaces.

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