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Photolysis Triggered Sealing of Multilayer Capsules to Entrap Small Molecules

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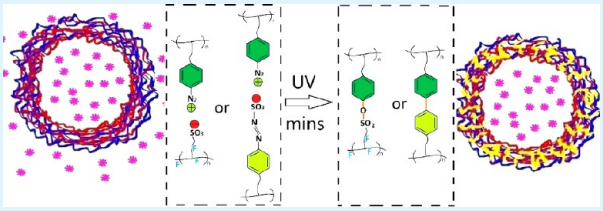
Supporting Information

ABSTRACT: Novel microcapsule systems containing UV-responsive diazonium groups were fabricated as microcontainers for cargo substance encapsulation by using a layer-by-layer (LbL) assembly technique. Upon direct exposure to UV light with a wavelength of approximately 380 nm, the diazonium groups of diazoresion (DAR) rapidly reacted with sulfonate or diazo-sulfonate groups of counterpart polyelectrolytes, which converted electrostatic interactions to covalent bonds, demonstrating an effective in situ cross-linking within multilayers via photolysis. Such chemical transition eliminated the paired ionic groups, therefore generating more hydrophobic multilayer shells, offering a unique approach to seal the porous polyelectrolyte capsule shells. Fluorescent molecule rhodamine B (RhB) was consequently studied as a typical example for small molecule encapsulation. Results indicated that the dye was remarkably retained within the microcapsules after UV-triggered capsule shell sealing.



KEYWORDS: *diazonium, capsule, encapsulation, photolysis, layer-by-layer*

1. INTRODUCTION

Layer-by-layer (LbL) self-assembled polyelectrolyte capsules, formed by sequential deposition of oppositely charged polyions, have been extensively studied and developed as effective delivery systems for various applications.[1−3] The LbL approach offers a rapid and experimentally efficient way to produce such multilayer capsules with precise control over layer architecture and properties.[4,5] Furthermore, the stepwise polymer deposition procedure facilitates the modification and functionalization of the capsule formations.[6,7] These fabricated capsules engineer numerous solutions to meet the diverse requirements in the fields ranging from biotechnology and pharmaceutics to chemical synthesis and catalysis perspectives.[4,8−10]

Molecule encapsulation into polymer based capsules with controlled release properties has attracted increasing interest during the past few decades. Ideally, such encapsulation should provide efficient loading and essential protection for cargo substances together with time and site specific release. Most importantly, effective encapsulation should offer a way to modulate substance entrapment within the capsules which could improve storage time and offer desired release properties.[11] Generally, polymeric micro- and nanocapsules made of LbL assembly have been widely explored to achieve substance encapsulation.[12,13] Many efforts have been devoted to provide solutions for the problems of how to encapsulate large molecules effectively into microcapsules without losing their activities. Previous approaches such as heat-treatment[14] and cross-linking[15] within the multilayers have been applied to decrease the shell porosity and strengthen the capsule shells and thus to decrease shell permeability. However, small molecule encapsulation remains to be a bottleneck, because these small molecules such as drugs, dyes, and other bioactive substances that have a molecular weight below 1 kD are small in size and relatively difficult to be encapsulated by the porous polyelectrolyte capsule shells.[16] Therefore, the encapsulation for such small molecules remains to be a challenge due to the demand for delivery of small drug molecules as well as various microreactor applications.

To encapsulate a desired cargo substance with small molecular weight in a LbL capsule requires a denser multilayer structure with fewer, small pores as well as low permeability. In other words, ideally, the polyelectrolyte shell should become intrinsically hydrophobic to the water-soluble substances. A robust and reliable approach to seal capsule shells via mild, preferentially externally induced, chemical modification is required to achieve this goal. For example, after capsule fabrication, a controllable method is employed to modify the assembled polyelectrolytes via chemical reactions of functional groups and hence avoid charges and expel water voids in between layers. Elimination of charges should be followed by sealing the pores on capsules shells. A kind of novel reactive polyelectrolyte that fulfills this requirement is the diazoresion (DAR; see Scheme 1). DAR has a strong UV absorption around 380 nm which could be assigned to the $\pi-\pi^*$ transition in the diazonium group ($-N^+\equiv N$).[17] Upon exposure to a 380 nm UV light, the diazonium group could be activated to form phenyl cation and then be substituted by nucleophilic groups, offering a novel external light triggered in situ cross-linking based on photolysis.[17,18] This outstanding advantage makes

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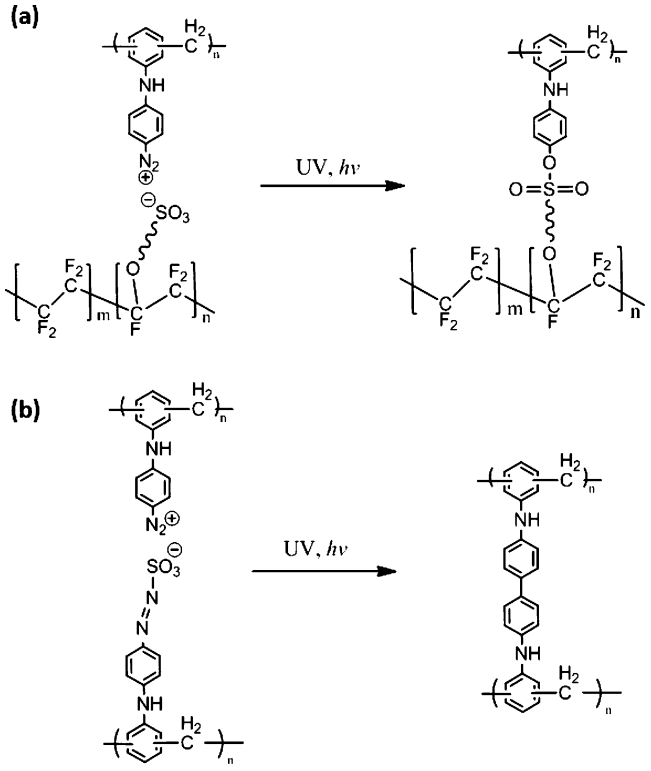
Scheme 1. Photolysis Reactions of Nafion/DAR (a) and DAR Single Component (b) Multilayer Systems



DAR an ideal candidate to achieve UV-responsive microcapsules for encapsulation. However, it should be pointed out here that several research groups have investigated the feasibility of molecule encapsulation in capsules containing diazonium groups, although no successful work was committed to realize encapsulation of small molecular substances, to the best of our knowledge.[19,20]

The properties of microcapsules mainly depend on the shell composition. Therefore, the selection of counterpart polyelectrolyte for DAR is critical. It was suggested that very hydrophobic multilayers would carry less water and should therefore be less permeable for water-soluble substances than the hydrophilic ones.[13] Thus, Nafion, which consists of a perfluorinated backbone and contains sulfonic acid groups in short side chains,[21] would be the optimal polyelectrolyte representing a class of hydrophilic polyanions to build up multilayer capsules with DAR. On the other hand, polyelectrolytes having diazonium groups, such as positively charged DAR discussed here, can react with Na_2SO_3 to form diazo sulfonate ($-N{=}N-SO_3^-$) under mild conditions, resulting in charge reversed DAR to form negatively charged polymer.[22,23] Another new strategy to seal the porous capsule multilayer systems is therefore inspired by externally triggered reactions within the DAR single component microcapsules made of both positively and negatively charged forms of DAR. Practically, after UV induced photolysis, interacted ion charges, such as diazonium and diazo-sulfonate here, would be eliminated, leaving the cross-linked diazo-resin backbones to form rigid and more internally hydrophobic phenyl ring-rich multilayers. Strategically, exploring the possibilities to reduce polyelectrolyte capsule permeability could be achieved via UV-induced ionic group modification. The use of DAR components with both charges as well as the polyanion Nafion as layer constituents for capsule fabrication would endow the prepared capsules with minimal shell permeability.

In this work, we are aiming to encapsulate a small molecule in LbL microcapsules via an external UV triggered capsule shell sealing effect. Promisingly, these sealed capsules could serve as potential microcontainers or reactors for small molecular substances. To achieve the goal, two UV responsive microcapsule systems were proposed; their steady shell sealing and accompanied permeability decrease could be accomplished through diazonium-related photolysis, originating from rapid in situ chemical transitions from ionic bonds of counterpart charges to covalent bonds. This fast sealing effect not only should harden the capsule shells but also could provide a novel way for cargo substance encapsulation in the resulting hydrophobic structures. To determine the feasibility, rhodamine B (RhB), a low molecular weight dye, was studied as a typical example. Moreover, controlled release behavior of the encapsulated small molecules was also studied.

2. EXPERIMENTAL SECTION

2.1. Materials. Nafion perfluorinated resin (10 wt % in H_2O), poly(4-styrenesulfonic acid) sodium salt (PSS, M_w = 70 kDa), rhodamine B (RhB, M_w = 479), paraformaldehyde, diphenylamine-4-diazonium salt (Variamine Blue RT Salt), sodium sulfite (Na_2SO_3), sodium carbonate (Na_2CO_3), and other chemicals were purchased from Sigma-Aldrich. All the chemicals were used as received without further purification.

2.2. Preparation of Diazo-resin. Diazo-resin (DAR) was synthesized through a polycondensation reaction of diphenylamine-4-diazonium salt with paraformaldehyde, following an electrophilic mechanism.[18] After purification and drying in vacuum, a yellow-green powder was obtained and used for capsule preparation.

2.3. Capsule Preparation. Polyelectrolyte multilayers were assembled on the SiO_2 particles (4.99 ± 0.22 μm, Microparticles GmbH) by using the LbL assembly technique as described elsewhere.[24] For $(Nafion/DAR)_4$ microcapsule preparation, negatively charged Nafion was used as the first layer. For the DAR_8 single component microcapsules, a negatively charged DAR solution was first prepared through a chemical activation, as reported by Laschewsky.[23] Briefly, DAR charge reversal was carried out by adding DAR solution (positively charged) into equal moles of an ice-cooled mixture of Na_2SO_3 and Na_2CO_3 (0.25 M each). Then, the polymer assembly was carried out by alternative deposition of positively and negatively charged DAR. Prior to the assembly, SiO_2 templates were first treated with a mixture of 25% NH_3/30% H_2O_2/H_2O (1:1:5) for 15 min at 75 °C to ensure a better attachment of the first polymer layer to the particle surface[14] and then washed 3 times with pure water (resistivity 18.2 MΩ·cm). To avoid aggregation, polymer coated particles were sonicated for 10 s after each wash step. After template removal with treatment of 0.1 M HF solution, hollow microcapsules with four bilayers were obtained.

Corresponding multilayer films containing DAR were deposited on quartz slides by hand. The slides were alternately immersed in oppositely charged counterpart polyelectrolyte solutions for 8 min each, followed by 3 min wash steps.

2.4. Instrument and Measurement. A UV lamp (OmniCure 2000, Lumen Dynamics Group Inc.) with effective working wavelength ranging from 320 to 500 nm was used to irradiate the samples. The hollow capsule suspensions were placed in quartz cuvettes (S10C, Sigma), continuously stirred with magnetic stirrers, and exposed to UV lights directly. The power used to irradiate the samples was measured to be approximately 55 mW/cm^2.

A UV−visible spectrophotometer (LAMBDA 950, Perkin-Elmer) was employed to investigate the UV absorption of the polyeletrolytes and capsule suspensions discussed here. Aqueous solution measurements were made using quartz spectrophotometer cuvettes (S10C, Sigma).

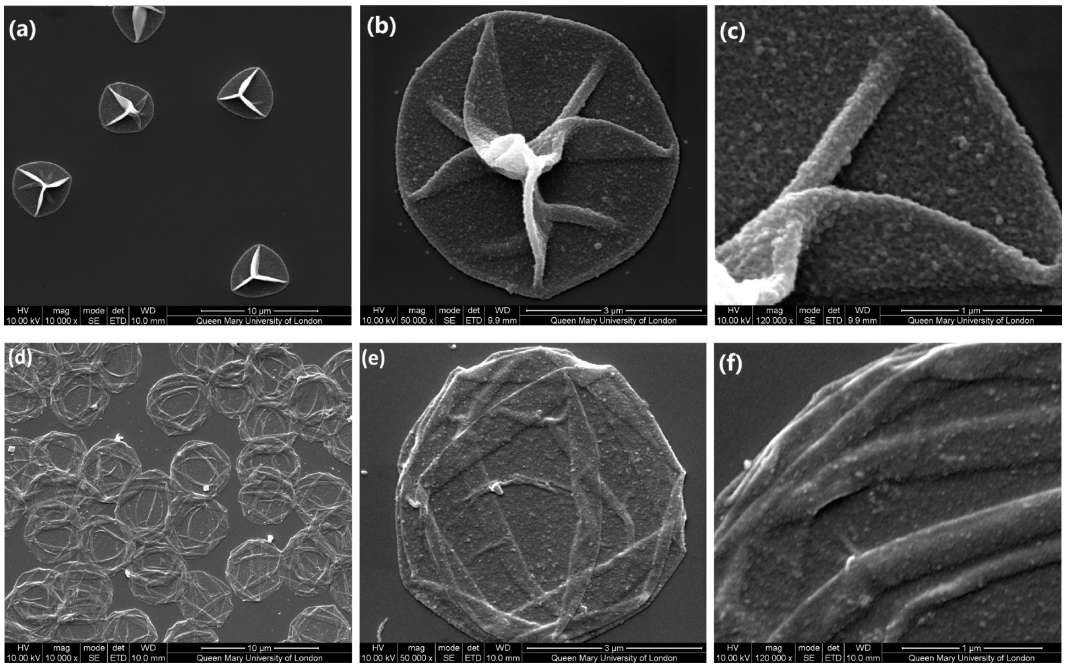


Figure 1. SEM images of DAR containing microcapsules at various magnifications. Top panel: $(Nafion/DAR)_4$ microcapsules; bottom panel: DAR single component microcapsules.

Typical morphologies of dry capsules after gold coating were provided by scanning electron microscopy (SEM) (FEI Inspect-F). Samples were observed using an accelerating voltage of 10 kV, a spot size of 3.5, and a working distance of approximately 10 mm.

Fourier transform infrared spectroscopy (FTIR) spectra of vacuum-dried capsule samples with and without irradiation were obtained using infrared spectroscopy (FTIR spectrometer 100, Perkin-Elmer). All data were collected at a spectral resolution of 4 cm^{-1}.

Confocal laser scanning microscopy (CLSM) graphs were captured with a Leica TS confocal scanning system (Leica, Germany) equipped with a 63×/1.4 oil immersion objective. Quartz crystal microbanlance technique (QCM) was applied to monitor the stepwise assembly process. Briefly, the crystal resonator (#151218-10, International Crystal Manufacturing CO, INC.) was immersed alternatively in polyelectrolyte solutions for a period of 15 min, carefully washed, and air-dried; then, the frequency shifts were recorded using the experimental setup described by Krause.[25] All the measurements were carried out at 30 °C in a thermal stable incubator (Digi Therm, Tritech Research, Inc.) in the duration of approximately 17 min for 200 scans in total.

Contact angle measurements (based on the static sessile drop method) of water (15 μL) on multilayer films containing DAR were captured using a Drop Shape Analysis System (Krüs, DSA100, Germany).[26] The measurement was repeated for several times. The water contact angles were expressed as mean ± SD of the obtained data.

2.5. Dye Encapsulation. To detect the feasibility of molecule encapsulation via DAR-related photolysis within multilayers, RhB (M_w = 479) was studied as small molecular cargo. Generally, 150 μL of capsule suspension (containing 1.5 × 10^7 capsules) was incubated in 1.5 mL dye solution (100 ug/mL) for 2 h with shaking. The RhB-capsule mixtures were exposed to UV light (50 mW/cm^2) directly for 10 min. After irradiation, capsules were collected and washed at least 5 times with water. Then, 400 μL of water was added to redisperse the dye encapsulated capsules. The RhB-encapsulated capsule suspension was split into several equal portions (50 μL for each) and kept in the dark for further studies. For CLSM measurement, capsules were centrifuged and washed twice with water to remove free dyes before each observation. For quantification of the encapsulated RhB amount, one portion (50 μL) of a capsule suspension containing RhB was used for dye retention study at a set experimental time point. The capsule suspension was centrifuged, and the resulting supernatant was carefully collected. The precipitated capsules were resuspended in the same volume of water and were broken after 5 freeze−thaw cycles incorporated with 5 min of ultrasonic treatment.[27] The fluorescence intensity of each sample was determined with a fluorescence spectrometer (Perkin-Elmer LS 55).

3. RESULTS AND DISCUSSION

3.1. Fabrication of DAR Contained Microcapsules. After LbL assembly driven by electrostatic interactions of polyions, monodisperse hollow $(Nafion/DAR)_4$ and DAR single component microcapsules (DAR_8) with four bilayers were obtained, respectively, as shown in Figure 1. Under SEM observation, these capsules were flat with creases and folds. In particular, a unique pattern of creases and folds that were different from other ordinary microcapsules were observed in the $(Nafion/DAR)_4$ microcapsule system. Compared with the SEM images of DAR single component microcapsules, these special patterns should be attributed to the presence of Nafion component, which made capsules more hydrophobic internally (will be discussed in Section 3.2) and exhibit a shrinking state once collapsed upon drying.

The stepwise assembly process was monitored by using QCM. The mass increase due to the polyelectrolyte adsorption was estimated from the QCM frequency shift according to the Sauerbrey equation[28] as follows:

$$\Delta f = \frac{-2{f_0}^2}{A\sqrt{\mu\rho}}\Delta m \quad (1)$$

where the area of the gold coated crystal (A) was 0.205 cm^2, the density of the crystal (ρ) was 2.648 g/cm^3, the shear modulus (μ) was 2.947 × 10^{11} g/cm·s^2, and the resonant frequency of the crystal (f_0) used was 10 MHz. Thus, the mass change due to the polymer adsorption on the electrode can be estimated as follows:

$$\Delta m\ (\text{ng}) \approx -0.905468\Delta F\ (\text{Hz}) \quad (2)$$

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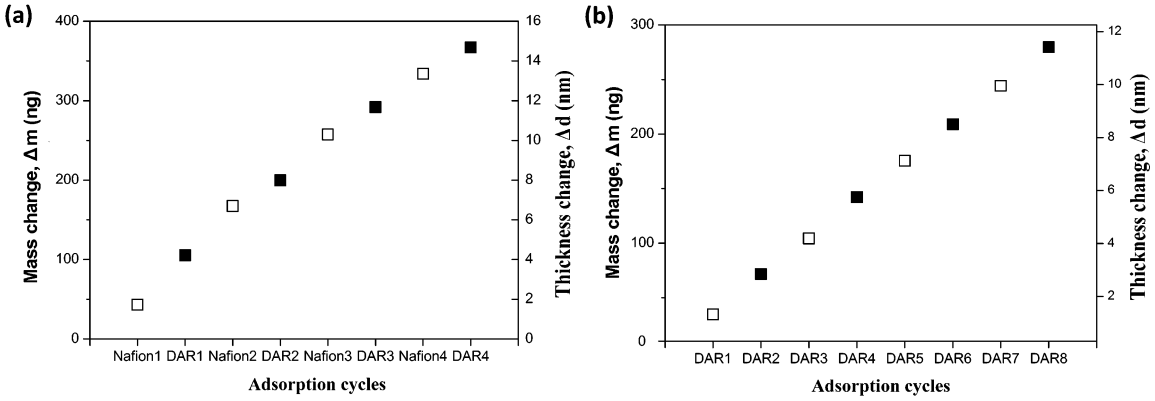

Figure 2. Estimated film mass (Δm) and thickness (Δd) changes of $(Nafion/DAR)_4$ (a) and DAR_8 (b).

Taking into account the polyion film density adsorbed on the electrode, assumed to be 1.2 ± 0.1 g/cm,[29] the thickness of adsorbed film can be estimated as follows:

$$\Delta d\ (\text{Å}) \approx -0.368076 \Delta F\ (\text{Hz}) \quad (3)$$

Since there are two different microcapsule systems involved, the results were presented in two parts.

(Nafion/DAR)$_4$. QCM monitored a linear relationship (R^2 = 0.9895) between frequency shift, $-\Delta f$, and the number of polyelectrolyte deposition cycles, due to the alternate adsorption of aqueous Nafion and DAR. The average frequency decreases were found to be 56 Hz for Nafion and 45 Hz for DAR, which meant that 5/9 of the bilayer mass was composed of Nafion and 4/9 of DAR (Figure S1a, Supporting Information). After deposition of 4 bilayers, a total mass increase of 367.03 ng was found on a surface of 0.205 cm^2, which was accompanied with a thickness increase of 14.92 nm for 4 Nafion/DAR bilayers, as shown in Figure 2a. More specifically, an average thickness of one Nafion layer was estimated to be about 2 nm. Such relatively thin polyelectrolyte layers revealed that almost no free $-SO_3^-$ ions dissociated from Nafion were present in the internal multilayers, which would affect water uptake and proton conductivity of the multilayer system[30] (will be discussed later).

(DAR)$_8$. A better linear relationship (R^2 = 0.9998) between frequency shift, $-\Delta f$, and the number of polyelectrolyte deposition cycles was observed by QCM measurements, due to the alternate adsorption of aqueous DAR with two kinds of charges. The average frequency decreases were found to be 37.38 Hz for $DAR^{\oplus}$ and 39.56 Hz for $DAR^{\ominus}$, which implied that almost equal moles of oppositely charged DAR deposited on the crystal surface (Figure S1b, Supporting Information). After deposition of 4 bilayers, an average mass increase of 69.9 ng was estimated from one double layer ($DAR^{\oplus}/DAR^{\ominus}$), accompanied by a thickness increase of 1.4 nm for each single layer, as shown Figure 2b.

3.2. UV-Induced Shell Sealing. The diazonium group of DAR was reported to be a good leaving group, and it would be cleaved to form phenyl cation under UV irradiation with a suitable light source. Thus, upon irradiation, it could be substituted by nucleophilic groups present in polyanions, such as carboxylic, phosphate, and sulfonate groups.[18] In our work, UV irradiation led to photolysis within the interacted ion pairs of Nafion/DAR and $DAR^{\oplus}/DAR^{\ominus}$, which was exhibited as the decomposition of diazonium group (and formation of a sulfonate covalent bond), as shown in Scheme 1. In contrast to the other UV-related transitions, as reported by Katagiri[31] and Bédard,[32] this DAR related transition from ionic bonds to covalent ester bonds required no polymer chain rearrangement or reconformation within the multilayers; thus, no obvious capsule size decrease can be found in this work or somewhere else.[19]

The process of UV-induced DAR decomposition can be easily monitored by the observation of absorbance change in UV−visible spectroscopy. The original DAR aqueous solution (positively charged, $DAR^{\oplus}$) showed a strong absorption at 372 nm (Figure 3a, solid line), which originated from the $\pi-\pi^*$ transition of the diazonium group.[17] By treatment with a mixture of Na_2SO_3/Na_2CO_3 at low temperature, a charge reversed DAR solution (negatively charged, $DAR^{\ominus}$) was obtained, which caused a significant red-shift of maximum absorption by 28 nm located at 400 nm. This was accompanied by an absorbance decrease in UV−visible spectroscopy (Figure 3a, dot line). This pronounced change was attributed to formation of dizao-sulfonate groups, which gave a maximum absorption at a longer wavlength region.[22] Incorporating the DAR polymers with their counterpart polyions caused small red-shifts in the maximum absorption peaks of assembled capsules. As shown in Figure 3b,c, before UV irradiation, the maximum absorption peaks were found at 377 and 382 nm for $(Nafion/DAR)_4$ and DAR_8, respectively. In particular, compared with DAR aqueous solution (Figure 3a), assembled DAR_8 microcapsule suspension observed a red-shift by 10 nm (Figure 3c, solid line), which was attributed to the deposition of charge reversed DAR (diazo-sulfonate). However, this shifting toward longer wavelength was not as pronounced as that of the pure $DAR^{\ominus}$ solution, indicating that the $DAR^{\ominus}$ was only the partial component of the single component capsule system.[22] After 10 min of UV irradiation, for the two DAR containing microcapsule systems, remarkable absorbance decreases at ∼380 nm were noticed (Figure 3b,c, dot lines), indicating the reactivation of diazonium groups during UV irradiation. Meanwhile, a concomitant increase was observed at about 290 nm for the DAR single component system; similar results can be found elsewhere.[22,33,34]

In this work, the paired dizaonium/sulfonate and diazonium/diazo-sulfonate groups underwent a chemical transition process under UV irradiation. Specifically, one should notice that for a DAR single component system this photoreaction process in water is quite complex involving diazonium group decomposition followed by generation of cationic intermediates[33] and isomerization of diazo-sulfonate.[35] In general, the electrostatic interacted charges were eliminated, and new covalent bonds were generated. Technically, this UV induced photolysis within

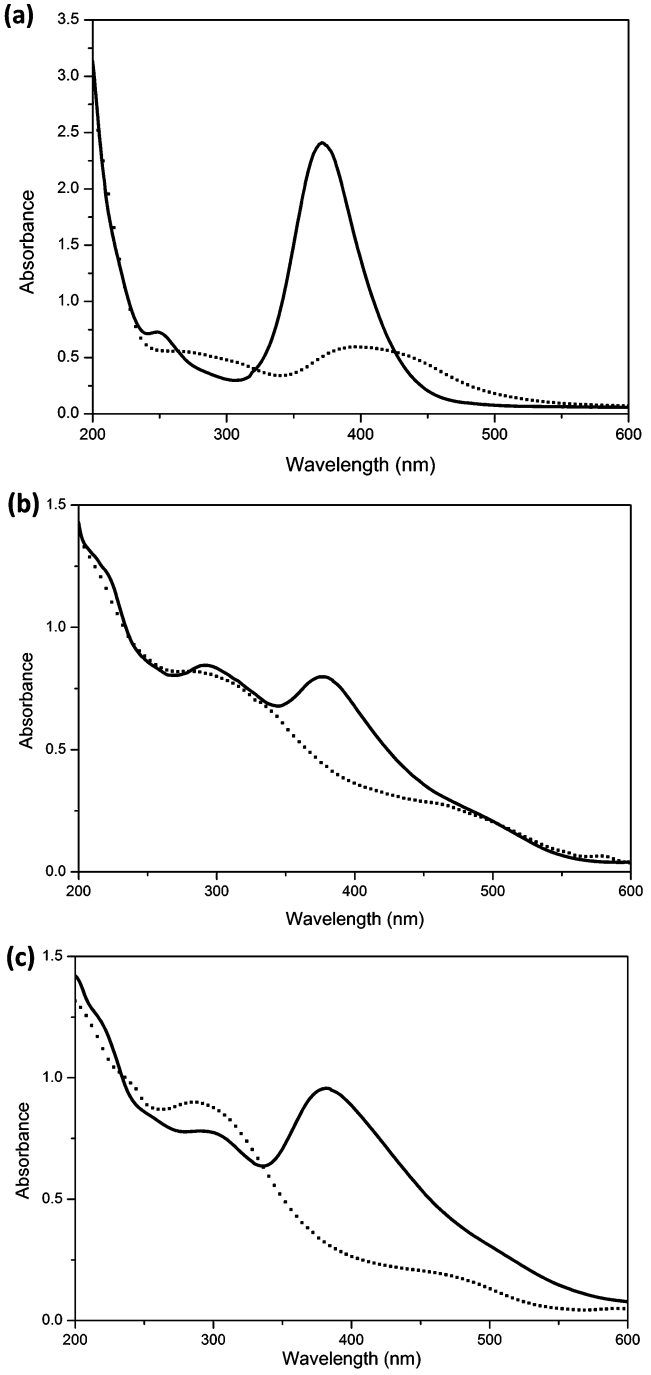

Figure 3. UV−visible absorption spectra of DAR solutions (a) before (solid line) and after (dot line) charge reversal and $(Nafion/DAR)_4$ (b) and DAR_8 (c) capsules before (solid line) and after (dot line) UV irradiation.

DAR containing microcapsules can also be confirmed by FTIR results. As shown in Figure 4, for both of the $(Nafion/DAR)_4$ and DAR_8 microcapsule systems, absorption peaks around 2222, 2169, and 1573 cm^{-1} were observed in the FTIR spectra before irradiation (Figure 4a,c), which originated from the asymmetric stretching of $-N_2^+$ and symmetric stretching of −C═C− in phenyl group conjugated with the diazonium group.[17,34]

For $(Nafion/DAR)_4$, after 10 min of irradiation, the absorption at 2222, 2169, and 1573 cm^{-1} disappeared completely (Figure 4b), indicating a fast decomposition of the diazonium groups. However, a new absorption peak at 1162 cm^{-1} corresponding to the generation of sulfonate coupled with the phenyl group[17] was overlapped by the strong $C-F_2$ stretching of Nafion.[36] Similar results were also found in the DAR_8 microcapsule system, as shown in Figure 4c,d, where the complete disappearance of the diazonium group and −C═C− in phenyl group signals was found. Meanwhile, the peak representing normal absorption of phenyl ring at 1595 cm^{-1} was observed due to the absence of a previously dominating peak (phenyl group linked with diazonium group) in the nearby region.[37] Besides, another peak at 1104 cm^{-1} corresponding to the N−O stretching of the complexes of diazonium and sulfonate groups $(-N_2^+ \rightarrow OSO_2^-)$[38] disappeared, which should be attrributed to the elimination of diazonium and diazo-sulfonate groups in the progress of photolysis. All these DAR-related chemical changes occurred very fast, e.g., could be almost completed within 50 s with a medium mercury lamp (80 W) at a distance of 13 cm.[18] In this work, no further change in FTIR results can be found when extending irradiation duration to 20 min.

UV induced chemical transitions not only converted the electrostatic interactions (diazonium/sulfonate, diazonium/dizao-sulfonate) into covalent bonding but also changed the hydrophilicity of prepared multilayers, which was indicated by the decreased wettability of these multilayer films. As shown in Figure 5, typical water droplet shapes and corresponding contact angles on the films were demonstrated. For $(Nafion/DAR)_4$ multialyers, an average water contact angle of 54 ± 3.0° was detected before UV irradiation, and then, the UV treatment resulted in a remarkable reduction of surface water wettability, exhibiting as an observed average water contact angle of 78 ± 1.0° (Figure 5a,b). Similarly, the UV irradiation decreased the surface water wettability of the DAR single component multilayer system, as a clear change of average water contact angle from 38 ± 4.0° to 49 ± 4.0° was detected before and after irradiation, respectively (Figure 5c,d).

3.3. UV-Triggered Small Molecule Encapsulation. Generally, UV exposure triggered a chemical reaction in the DAR containing capsule systems, converting electrostatic interactions to covalent bonds, where the polymer layers likely became more compacted and the pore size in the capsule shells was significantly reduced. Thus, the permeability of the multilayer shell was decreased upon UV irradiation, due to the covalent cross-linking between paired polyions, resulting in retention of encapsulated molecules. Our research results indicated that the externally sealed microcapsules under 10 min of UV irradiation can retain dye polymers (AF488, 10 kDa) for weeks, and the fluorescent signal of remaining dye polymers was found to be 80% of the initial encapsulated ones over 2 weeks of shell sealing (Figure S2, Supporting Information). However, the challenge is in encapsulation and secure storing of small active molecules. Many efforts have been devoted to altering the permeability of multilayer shells for the purpose of nanoscale encapsulation of biopolymers, drugs, and dyes. Strategies such as spontaneous deposition of water-soluble substances via bonding to charged oligomers[39] and controlled precipitation into capsules via variable solubility[40] were developed to achieve the goals.

Here, in our work, special emphasis was focused on the development of LbL microcapsule systems to realize small molecule encapsulation without any chemical or physical bonding with cargo substances or help of external media adjustment such as solubility change, but using UV light only to

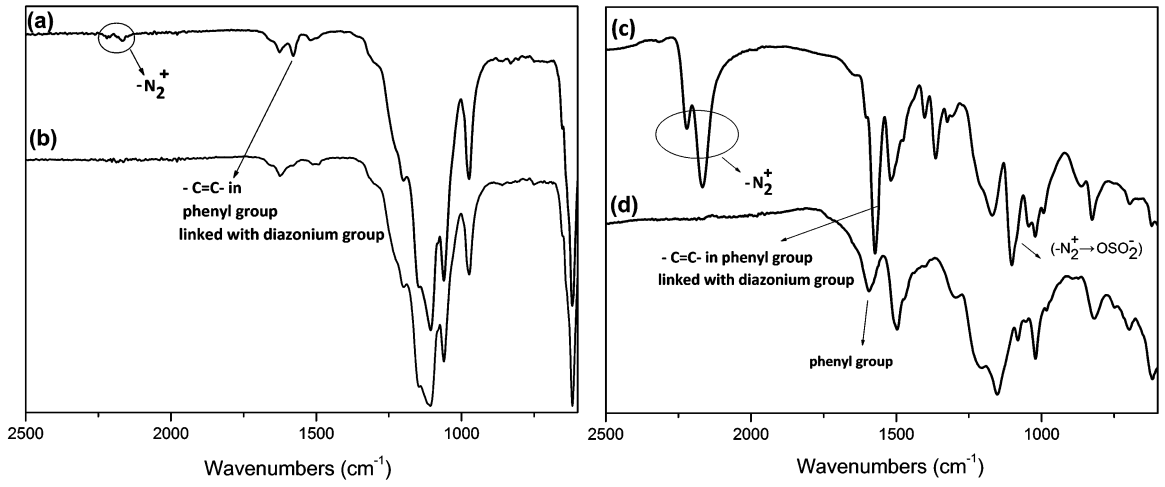

Figure 4. FTIR spectra of $(Nafion/DAR)_4$ (left) and DAR_8 (right) microcapsules before (a, c) and after (b, d) 10 min of UV irradiation.

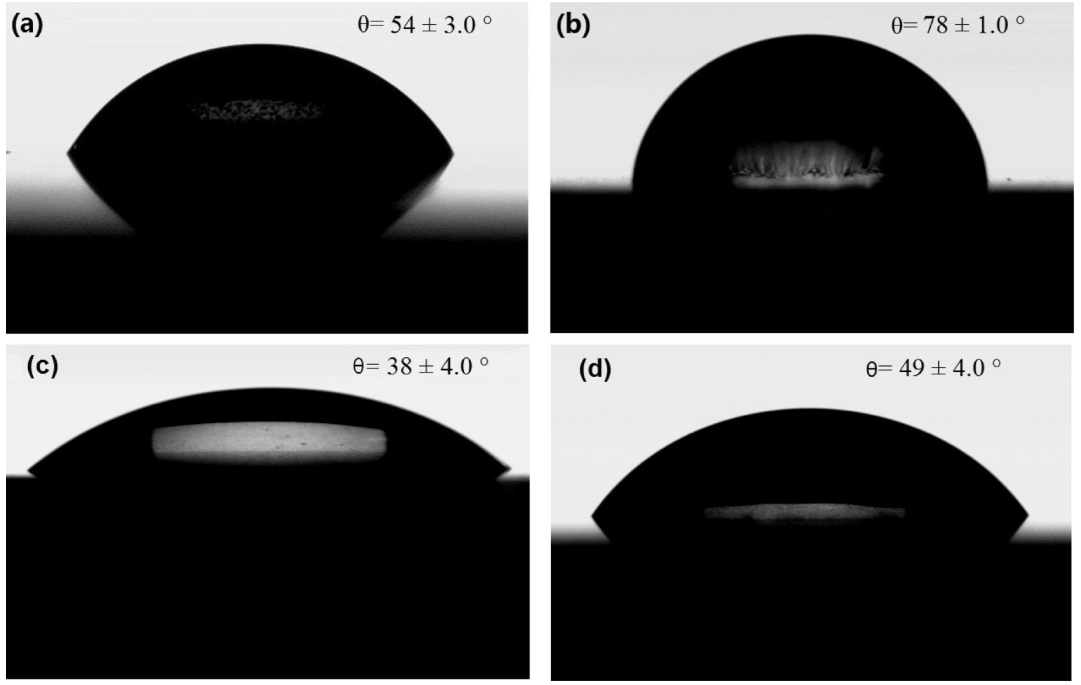

Figure 5. Photographs of water droplets on $(Nafion/DAR)_4$ (a, b) and DAR_8 (c, d) multilayers before (left) and after (right) UV irradiation.

trigger the capsule sealing remotely. Specifically, the fluorescent molecule RhB with a molecular weight of 479 was used as an appropriate example to illustrate encapsulation. Generally, after 10 min of UV irradiation, the diazonium-related photolysis covalently cross-linked the multilayers[19] and also changed the hydrophilic ionic groups to the hydrophobic ester groups (see Scheme 1).[41] Accompanied by the elimination of paired charges, the network-like shell structures became denser due to less water adsorption in the multilayers. Our hypothesis is that fewer charges after modification attract less water voids and their expelling results in close interaction of polymer segments in the multilayer with less and smaller pores; thus, capsule shell permeability can be greatly reduced. As shown in Figure 6, after 10 min of UV irradiation, the DAR containing microcapsules, both $(Nafion/DAR)_4$ and DAR_8, sealed small RhB molecules in their cavities, demonstrated as an average fluorescent signal intensity of ∼89 units inside capsules and near 0 unit outside capsules. These encapsulated RhB molecules can be precipitated together with their capsule carriers after centrifugation (Figure 6c). On the contrary, the capsule with defects cannot entrap any fluorescent molecule inside, as shown by an empty cavity under CLSM observation (Figure 6b, ∼0 units inside capsules, as pointed out by an arrow symbol). Remarkably, irradiated $(Nafion/DAR)_4$ exhibited a better encapsulation efficiency than DAR_8 after sealing. As shown in Figure 6d, a fluorescent signal in the UV-sealed $(Nafion/DAR)_4$ microcapsules over 2 months was still detectable, although the average fluorescent signal intensity was very low. Compared with the CLSM images of RhB encapsulated capsules at Day 0 (Figure 6a,b), this image was taken with an enhanced laser power, resulting in a low-contrast fluorescent signal and noisy background. In contrast, no fluorescent signal can be visualized from the other capsules (nonirradiated $(Nafion/DAR)_4$ and irradiated/nonirradiated DAR_8) (Figure S3e,g, Supporting Information), illustrating that almost all the encapsulated fluorescent RhB molecules were released from these micro-

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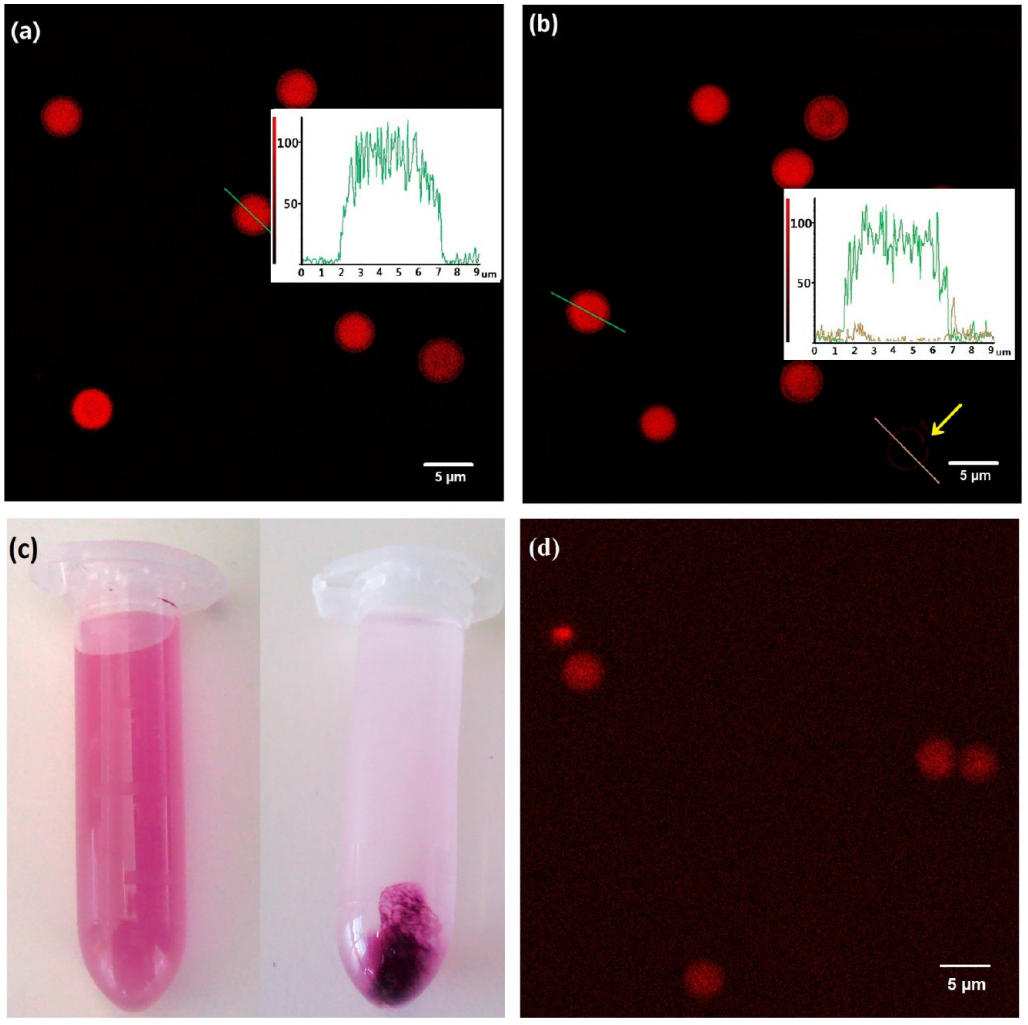

Figure 6. CLSM images of RhB containing $(Nafion/DAR)_4$ (a) and DAR_8 (b) microcapsules after 10 min of UV irradiation. Image c showed the RhB encapsulated DAR microcapsules before (left) and after (right) centrifugation at 4500 rpm for 5 min. Image d showed CLSM images of RhB containing $(Nafion/DAR)_4$ over 2 months after UV irradiation. The line scan insets showed relative fluorescent intensity in capsules; the arrow showed a broken capsule, in which no RhB could be retained.

capsules during 2 months. According to the previous research, RhB has a strong tendency to aggregate in aqueous solution, especially in concentrated solution, which would lead to self-quenching of RhB molecules. Specially, when the RhB concentration reached 5×10^{-4} M, a decrease of the fluorescence quantum yield became significant.[42] In our work, maximum encapsulated RhB amount was calculated to be 5.4×10^{-5} M (690 fg in one capsule, 1.875×10^6 capsules in each 50 μL sample solution). At this concentration, the RhB aggregation degree was low but unavoidable. Due to the possible quenching effect of RhB, it might not be precise for dye quantification over a long time. However, the CLSM images of RhB preservation in irradiated $(Nafion/DAR)_4$ over a long duration after shell sealing could provide qualitative proof to claim the contribution of a Nafion component, which might be considered as a key factor in the retardation of RhB release, especially after charge elimination. In addition, the non-irradiated $(Nafion/DAR)_4$ and DAR_8 capsules also showed their abilities to encapsulate RhB without UV irradiation, although the encapsulation efficiency was quite low when compared with the irradiated ones (Figure 7 and Figure S3a,d, Supporting Information). This result should be attributed to the fabricated hydrophobic multilayers of these two capsule systems, which facilitated retention of a small amount of RhB molecules.

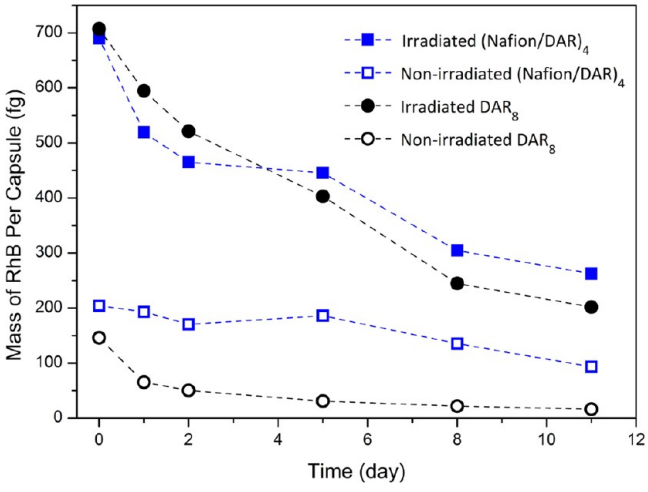

Figure 7. Mass of encapsulated RhB amount per DAR containing microcapsule with and without UV irradiation.

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Considering capsules as network-like structures formed by entangled polymers, a number of pores would be noticed in the shells. Sealing methods, e.g., DAR-related photolysis discussed here, eliminated the hydrophilic group and effectively decreased the pore size, making the shells less permeable and retaining loaded substances in the capsule interior. As seen from Figure 7, the RhB retention in the microcapsules with and without 10 min of UV irradiation normalized by capsule number has been provided. For the $(Nafion/DAR)_4$ capsules, 690 fg of RhB can be retained in one irradiated $(Nafion/DAR)_4$ capsule, while there was no more than 1/3 of RhB content (204 fg) detected in the nonirradiated one. This pronounced difference between the microcapsules with and without irradiation illustrated remarkable decreased shell permeability, which was attributed to the DAR-related sealing effect on the capsules. Over time, the RhB molecules gradually penetrated out the microcapsules. After a period of 11 days, there was only 93 fg of RhB found in one nonirradiated $(Nafion/DAR)_4$ capsule. A possible explanation of this could be that the charged dye molecules remain trapped in the shells via electrostatic interactions with oppositely charged Nafion. However, for irradiated $(Nafion/DAR)_4$, there was still 262 fg of RhB detected in one capsule, which was almost triple that of the nonirradiated capsule, confirming that most of the RhB molecules were retained by physical encapsulation instead of the bonding to shell components. In addition, the sum of the RhB content in capsules and supernatant roughly matched the total RhB amount encapsulated per sample (Figure S4, Supporting Information). From the two curves, it was confirmed that the diazonium-related photolysis greatly "sealed" the capsules and slowed down RhB molecule penetration. Similar results of the sealing effect can also be found in the DAR single component microcapsule system, where 707 fg and 202 fg of RhB were found in irradiated DAR_8 microcapsules at Day 0 and Day 11, respectively, showing continuous gradual leakage from potentially incompletely sealed capsules.

In particular, a result worth mentioning is the relatively small RhB amount found in the nonirradiated DAR_8 capsules when compared with the nonirradiated $(Nafion/DAR)_4$ capsules. As shown in Figure 7, at the beginning, there was 156 fg of RhB found in nonirradiated DAR_8 microcapsules, after which a fast release occurred in the duration of 11 days, resulting in 10% (16 fg) of RhB retained inside capsules over 11 days. In contrast, 11 days later, there was 45.6% (93 fg) of RhB found in the nonirradiated $(Nafion/DAR)_4$ capsules. The authors proposed these different release behaviors of the two nonirradiated DAR containing microcapsule systems as a result of the combination of high hydrophobicity and good film-forming property[43] of Nafion layers. In LbL assembled mulitlayers, the proton mobility of the Nafion/DAR system mainly depended on free $-SO_3^-$ groups in the multilayer.[44,45] In our work, QCM results revealed a thin layer structure of Nafion (~2 nm) in the Nafion/DAR multilayer system, demonstrating the shortage of free $-SO_3^-$ in the internal layers.[44] As restricted by the counterions of DAR, no hydrophilic domain (mainly the dissociated $-SO_3^-$ groups) can achieve water uptake and assist proton conductance, leaving the very hydrophobic Teflon-like backbone structure of Nafion to play a predominant role. As a consequence, the water mobility was greatly reduced in the multilayer system, demonstrated as low permeability of the Nafion/DAR shells. Therefore, after fabrication, the $(Nafion/DAR)_4$ system was more hydrophobic and less water permeable, providing a better cargo substance retention effect than DAR_8, even without UV irradiation (Figure 7). Furthermore, the nonirradiated $(Nafion/DAR)_4$ microcapsules found a good preservation ability for fluorescent macromolecules (AF488-Dextran, 10 kDa), while the nonirradiated $(PSS/DAR)_4$ microcapsules showed no such ability, as no fluorescent signal in their cavities can be visualized (Figure S5, Supporting Information).

4. CONCLUSIONS

In summary, we proposed two UV responsive polyelectrolyte microcapsule systems containing DAR for encapsulation. Upon direct exposure to UV light, the ionic bonds of counterpart ions were converted to covalent chemical bonds through DAR-related photolysis, which offered an externally controlled method to seal the multilayer capsules. These capsules were investigated as unique microcontainers for cargo substance encapsulation, which benefited from UV induced photolysis of the paired charges and resulting hydrophobic backbones. Simply triggered by UV light, both of the two kinds of microcapsules exhibited an excellent and efficient small molecule encapsulation effect. When compared with the nonirradiated ones, the UV-sealed microcapsules exhibited a much higher RhB preservation stored over a longer time (11 days) after UV irradiation due to physical entrapment in the capsule cavities. Specifically, without UV irradiation, the $(Nafion/DAR)_4$ microcapsules showed a better dye encapsulation efficiency than others. This effect could be attributed to the nature of Nafion bearing a hydrophobic backbone. These two DAR containing microcapsule systems would be very promising microcontainers/vesicles for medical or biotechnological applications. Remarkably, such microcapsules also showed great application for many environmental and photochemical uses, where UV light could be used as an efficient external trigger to drive these microsystems. In addition, this method could be used to accomplish remote guided entrapment of various substances into floating microcapsules in biological media, followed by capsule withdrawal and molecule analysis.

■ ASSOCIATED CONTENT

Supporting Information

Frequency shift ($-\Delta F$) due to alternatively polyelectrolyte adsorption (Figure S1), CLSM images of AF488-Dextran (10 kDa) encapsulation in irradiated capsules (Figure S2), CLSM images of RhB encapsulation in nonirradiated $(Nafion/DAR)_4$ and DAR_8 microcapsules (Figure S3), mass of RhB retained and released by $(Nafion/DAR)_4$ and DAR_8 microcapsules (Figure S4), and CLSM images of AF488-Dextran (10 kDa) encapsulation in nonirradiated capsules (Figure S5). This information is available free of charge via the Internet at http://pubs.acs.org/.

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Notes

The authors declare no competing financial interest.

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