Externally Triggered Dual Function of Complex Microcapsules

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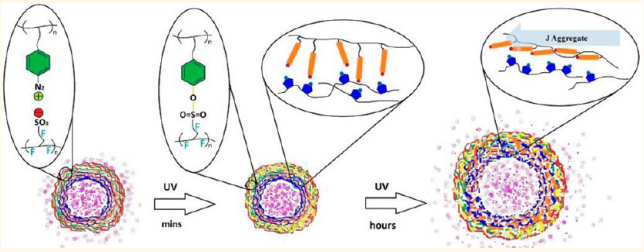
ABSTRACT By introducing UV-sensitive chemical groups causing different potential response as building blocks, fabricated LbL capsules can be endowed with dual UV-responsive properties in specific layers. One block is responsible for fast capsule sealing and the other for longer term capsule swelling and rupture. Therefore, the multifunction of these capsules could be activated selectively when exposed to external UV light with suitable wavelengths. In this work, dual-functional complex microcapsules (PDADMAC/PAZO-)$_4$-(DAR/Nafion)$_2$ containing both diazonium and aozbenzene groups were proposed as clear examples to realize a time-dependent UV response for successive encapsulation and release. Upon exposure to UV light, the DAR/Nafion layers underwent a rapid *in situ* cross-linking and hence to seal the capsule shells through diazonium-related photolysis. Then further gradual shell swelling was followed by realignment of azobenzene molecules in PDADMAC/PAZO layers. Fluorescent polymers were consequently studied as cargo substances. Results indicated that continuous UV light triggered rapid cargo encapsulation over minutes time scale and gradual release with continuous irradiation over hours.



KEYWORDS: diazonium · azobenzene · microcapsule · encapsulation · release

Vesicles carrying active substances which can enable response to multiple external stimuli have been widely studied and developed as attractive candidates for delivery systems, micro/nanoreactors, and sensors, *etc.*[1,2] One of the most successful approaches to tailor various functions in one entity is layer-by-layer (LbL) assembly, which provides an optional technique to engineer a series of functional capsules due to its simplicity and versatility.[3] In particular, a delivery system based on LbL capsules has attracted increasing interest during the past decades for its promising applications in different fields ranging from biotechnology and pharmaceutics to chemical synthesis and catalysis perspectives.[4−6] Generally speaking, different external stimuli have been employed to realize the functionalities of fabricated LbL capsule systems, to which functional layers of responsive polymers or nanoparticles are introduced, in order to meet their various requirements for capsules to respond to triggering stimuli, including remotely.[1,7] Typically, light-addressable capsules represent a series of the fast developed stimuli-responsive vesicles for potential use, as their micro/nanostructures can be simply adjusted by the external light. Optical treatment allows the functionalization of these fabricated capsules with accompanied changes in their morphologies, shell stability, as well as permeability, benefiting from underlying chemical transitions.[8−10]

Previous investigations have developed light-responsive capsule systems for delivery use, with their emphasis on either encapsulation or release. In order to achieve two or more functions in one carrier system, different stimuli are normally required. For example, UV irradiation is applied to facilitate cargo substance encapsulation, whereas external pH adjustment is used to adjust shell permeability and hence to trigger release;[11] cargo substances are encapsulated first, and then pulsed light or near-infrared laser is used to rupture capsule shells for release.[12] Actually, rupture of the capsule could be also wavelength-selective if capsule composition involves nanostructures with different absorption spectra.[13] As the essential part of light-responsive capsules, UV-responsive capsules whose functionalities can be accomplished by the external UV light make their contributions

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to the applications in surface science and environmental areas. With regard to the practical use, sometimes the continuous UV light (*e.g.*, sunlight) would be the only one stimulus available to functionalize these systems. Therefore, the great importance and challenge here would be the development of multifunctional capsule systems, which would integrate multifunctionalities such as both encapsulation and release in one system, simply triggered by only one external stimulus. However, to the best of our knowledge, no such UV-responsive LbL capsules have been developed yet for possible applications.

Considering the stepwise capsule fabrication procedure, it is possible to achieve the goal by introducing functional UV-responsive chemical groups (components) in one capsule system whose reactivity leads to variable response. Strategically, the encapsulation and further release could be activated through successive UV-triggered chemical transitions of these functional groups. Ideally, with proper control over the balance of capsule shell structures, UV light with continuous wavelengths (containing the effective working wavelengths for corresponding functional groups) could push forward a UV-exposure-dependent progress of capsule shell sealing and breakage (swelling and/or disruption). On one hand, in order to realize encapsulation, a fast and efficient sealing effect, which decreases shell permeability by making the shell dense, eliminating voids, and expelling water from multilayers, is therefore favored. The building blocks containing UV-sensitive diazonium groups, such as diazo-resin (DAR), could be used as optimal candidates to seal capsule shells by converting the paired ionic groups into covalent bonds rapidly.[14] On the other hand, release of the encapsulated substance can be accomplished by continued UV exposure to induce shell breakage, in either instant or sustained way. Typically, a slow release over an extended period can be achieved by incorporating the robust azobenzene molecules into multilayers. These molecules could undergo a UV-triggered molecule realignment in the form of end-to-end (J-styled) or face-to-face (H-styled) aggregates, resulting in phase separation in microdomains and further destruction of shell integrity.[15] Therefore, cargo substance encapsulation and further release triggered by a single UV source can be integrated in one complex capsule system, benefiting from opposite UV response of diazonium and azobenzene groups differing by time interval.

In this work, we are aiming to fabricate a unique dual-functional complex microcapsule system by introducing two different UV-sensitive chemical groups in multilayer shells. In order to achieve the desired fast shell sealing and gradual breakage triggered by UV light, capsules with PDADMAC/PAZO and DAR/Nafion layers were studied as typical examples here. Under a given UV irradiation condition, the fast shell sealing effect could be completed by *in situ* cross-linking of DAR/Nafion multilayers through a short-term UV-induced photolysis (*e.g.*, minutes). Further gradual breakage would be accomplished by realignment of azobenzene molecules in PDADMAC/PAZO multilayers triggered by long-term UV irradiation (*e.g.*, hours). Promisingly, these fabricated capsules would be able to encapsulate cargo substances due to the charge elimination and resulting hydrophobic structures and then realize controlled release through shell breakage originating from local azobenzene molecular self-reorganization. The morphology and stability changes induced by UV irradiation of these complex capsules were studied. Moreover, UV-triggered cargo substance encapsulation and further release were also investigated.

RESULTS AND DISCUSSION

Gradual Capsule Breakage Based on Realignment of Azobenzene Molecules. Exposure of $(PDADMAC/PAZO)_4$ microcapsules to UV light led to a gradual capsule swelling and further disruption process, as shown in Figure 1. Before UV irradiation, these capsules exhibit flat and intact images under SEM observation. After 10 min of UV irradiation, capsules were observed as swollen ones with an average size increase from 5.12 to $\sim$7 μm (Figure 1b). With the improved size increase effect, the integrity of shell formations was destroyed, as most of the capsules were found to be broken when the UV exposure duration reached 1 h (Figure 1c). Extending the UV irradiation to 2 h, no intact capsules could be found, with most of them torn into lamellar- and needle-like formation (Figure 1d).

A typical feature of azobenzene molecules under UV exposure is their changes in dipole moment, although the azobenzene molecular conformation change in-plane is the most well-known phenomenon. Depending on the mutual orientation of the interacted dipole moments between the counterpart molecules, azobenzenee moieties are conducted to form end-to-end or plane-to-plane aggregates, also known as J- or H-aggregates, respectively.[16] Such styled aggregates can easily be monitored by UV−vis spectroscopic measurements technically, exhibiting as maximum absorption peak shift, red shift for J-aggregates and blue shift for H-aggregates. As shown in Figure 2a, a spectral change was visualized as a time-dependent shift toward the longer wavelength region. Generally, exposing the $(PDADMAC/PAZO)_4$ microcapsule suspension to the UV source led to a red shift by 17 nm and subsequently receding to a constant maximum absorption peak located at 383 nm. Compared to the limited decrease of maximum absorbance, which represented the possible photoisomerization of azobenzene, the red shift was found to be much pronounced. Therefore, it was believed that the J-aggregate formations of azobenzene were favored in the PDADMAC/PAZO capsule system.

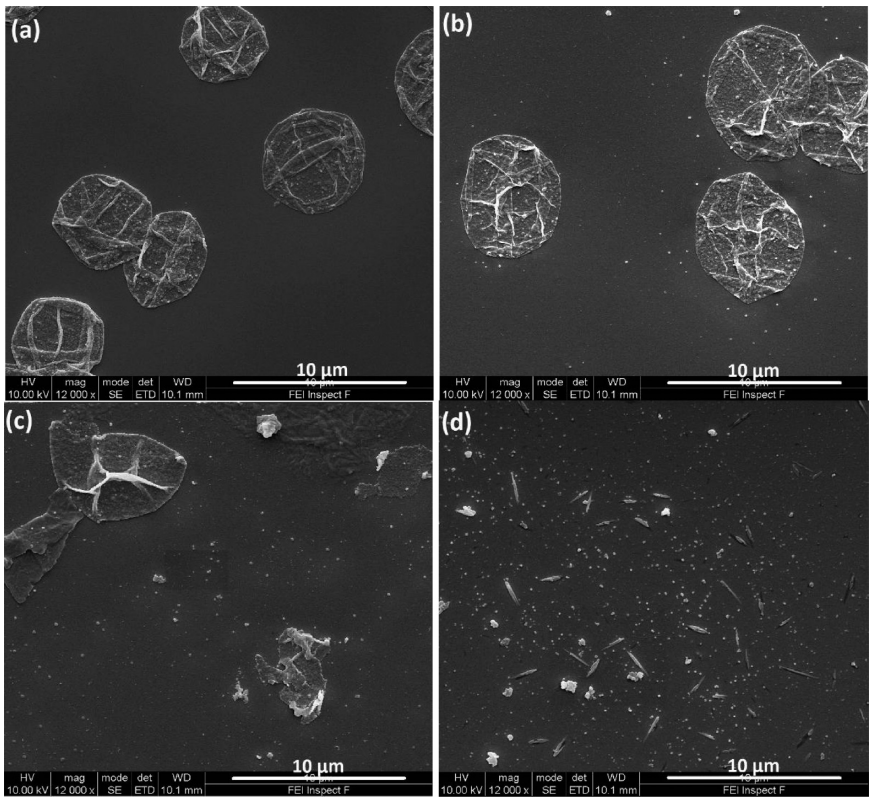


Figure 1. SEM images of $(PDADMAC/PAZO)_4$ capsules after UV irradiation of 0 (a), 10 min (b), 60 min (c), and 120 min (d).

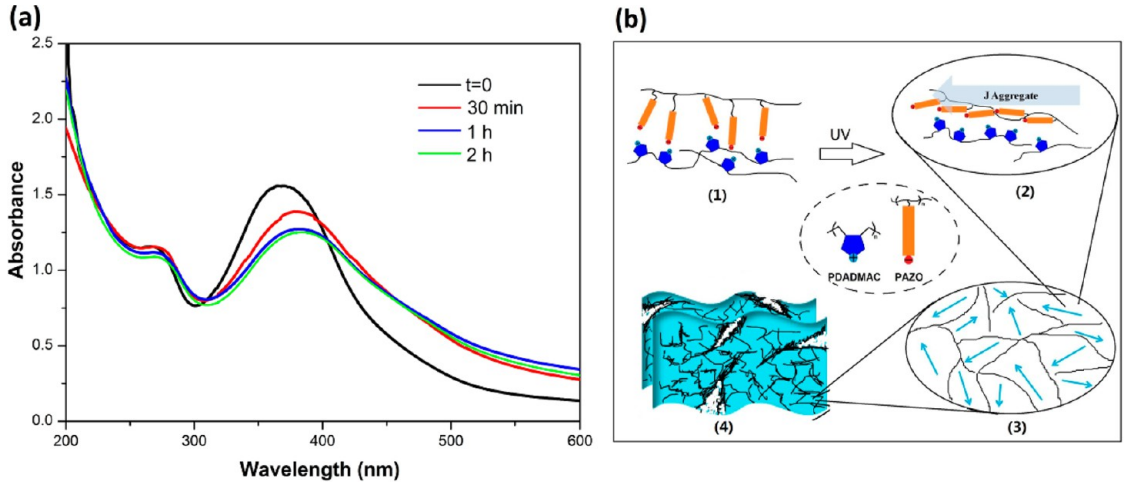


Figure 2. UV−vis spectra of $(PDADMAC/PAZO)_4$ microcapsule suspensions before and after UV irradiation (a), and schematic illustration of $(PDADMAC/PAZO)_4$ microcapsule disruption induced by UV irradiation (b).

In this work, UV irradiation was able to cause irreversible disruption of PDADMAC/PAZO microcapsules. However, another polyelectrolyte microcapsule system $(PAH/PAZO)_3PAH/PVS$ composed of PAZO, poly(allylamine hydrochloride) (PAH), and poly(vinylsulfonate) (PVS) demonstrated an opposite phenomenon after UV irradiation, where the capsules were shrunk significantly accompanied by apparent shell permeability decrease, as reported by Bédard and co-workers.[8] As proposed in their work, the UV-induced *trans* to *cis* isomerization of the azobenzene molecules was attributed to the main reason that caused capsule shrinkage. Thus one would raise the hypothesis that the different UV-induced capsule morphology changes could be originated from different chemical transitions occurring in these multilayers, where structural conformation change in-plane or molecular realignment locally plays the dominant role.

Whether to undergo a structural conformation change or a molecular realignment mainly depends on the interactions of azobenzene derivatives and their polymeric counterparts. When a polymer PAZO bearing

azobenzene groups on its side chains is used as the polyanion for microcapsule preparation, the interplay of PAZO with its polycation is very important. Polycations with different chain flexibilities and charge distributions show different influence on fabricated multilayers with PAZO. For example, when a linear polyethylenimine (PEI) was used as the polyanion, a blue shift toward shorter wavelength in a UV−vis spectroscopy was observed, and this tendency became predominant when increasing the number of deposited PEI/PAZO layers.[17] Combination of PAZO with relatively flexible PAH and PVS polymers led to conformational alteration of azobenzene molecule accompanied by a length reduction of this group from 9.0 to 5.5 Å.[8] Contrarily, when the polymer PDADMAC bearing five-membered heterocycles and ionic charges along the backbone structure was used as the polycation, deposition of PDADMAC/PAZO was not as regular as that of other paired polyelectrolytes; sometimes the interdigitation of PDADMAC and PAZO polymer chains was preferred instead of proper deposition in the corresponding multilayer films.[17] Such irregular polyelectrolyte deposition normally led to closely packed formation of 60 nm aggregates, exhibiting as patchy structures of the films.[17] Formed aggregates demonstrated steric hindrance to restrict the photoisomerization of azobenzene molecules and consequently facilitated the molecular realignment.[18,19]

UV-induced aggregates have been used as useful triggers to modify fabricated thin films; typical applications are electrical conductivity switching[20] and liquid crystal alignment.[21] In the case of shell-like formations, it was reported that the aggregates or assembled aggregates of azobenzene derivatives induced by UV irradiation can lead to "catastrophic" destruction of the shell integrity, which has been developed as a strategy to build up photosensitive liposomes[22] and polymersomes[23] for drug delivery use. Here, the gradual capsule breakage process in our $(PDADMC/PAZO)_4$ capsule system could be explained as the influence of these end-to-end J-aggregates as in-line effected by PDADMAC, as shown in Figure 2b. PAZO polymers with anisotropic azobenzene moieties were deposited on spherical templates together with PDADMAC to fabricate microcapsules (Figure 2b, stage 1). When exposed to UV light, the azobenzene molecular motion locally was found predominantly in a PDADMAC/PAZO multilayer system, where the rigid PDADAMC chains restricted the azobenzene molecular *trans* to *cis* conformation change and favored formations of J-styled aggregates (Figure 2b, stage 2). Assembled aggregates generated steady formations as mosaics or lattices of the small "unit" aggregates with a surface area of ~20 Å *via* strong noncovalent aromatic−aromatic interactions in the presence of water (Figure 2b, stage 3).[24,25] The numerous formations of such mosaic aggregates made the thin spherical shells lose their flexibility. Once the capsules became not flexible enough to keep the spherical shell structures, their integrity was consequently lost; then these capsules gradually started to be spilt locally (Figure 2b, stage 4), demonstrating capsule swelling phenomena and further disruption. Thus, at given composition of 4 PDADMAC/PAZO bilayers, the destruction effect would be hours long.

Rapid Capsule Sealing Induced by Diazonium-Related Photolysis. DAR (Figure 4, inset) is a well-known UV-sensitive polymer bearing diazonium groups on the side chains, which could be decomposed fast and readily when exposed to UV light with a wavelength at ~380 nm. Therefore, the UV irradiation here predominantly activated the diazonium groups to form a phenyl cation and then to be substituted by nucleophilic groups,[14] specifically the paired sulfonate groups of the polyanion involved in LbL assembly. In this work, the multilayer capsules composed of DAR and Nafion were prepared; then postpreparation UV treatment allowed an *in situ* chemical transition from weak ionic interactions to covalent bonding in multilayers. Different from the $(PDADMAC/PAZO)_4$, UV irradiation did not cause obvious morphology changes on the (DAR/Nafion) microcapsules; neither notable shell shrinkage nor breakage could be found, as shown in Figure 3. As per the spectroscopic study shown in Figure 4a,b, after 10 min of UV irradiation, the absorption peaks at 2222, 2169, and 1580 cm^{-1}, which represented the stretching of $-N_2^{+}$[26] and −C≡C− in the phenyl group conjugated with the diazonium group,[27] disappeared completely. This could be explained as the fast decomposition of diazonium. However, due to the existence of broad $C-F_2$ stretching of Nafion,[28] a new peak at 1162 cm^{-1} representing the generation of sulfonate coupled with the phenyl group[26] was overlapped. This UV-induced photolysis occurred very rapidly, and it could be completed within 1 min.[14] As found in our work, 10 min of UV irradiation at 55 mW cm^{-2} was adequate, extending UV treatment duration would lead to no remarkable chemical change. Unlike other UV-induced chemical transitions within multilayer capsules, this DAR-related change required no polymer chain rearrangement[29] or molecular conformation isomerization,[8] thus no obvious capsule size change could be found in this work and in similar systems.[30]

Introducing the diazonium groups into multilayers offers a novel approach to seal the fabricated capsules, where the *in situ* cross-linking is triggered by UV light. This strategy provides the possibility to encapsulate cargo substances. An example has been reported by Zhu and co-workers. In their work, the microcapsules composed of DAR/poly(styrene sulfonate) (PSS) were able to encapsulate macromolecules with molecular weight from 9.5 to 186 kDa.[30] However, one must notice, for the porous LbL assembled polyelectrolyte capsule shells, it is still a challenging task to realize small molecule encapsulation, especially for these

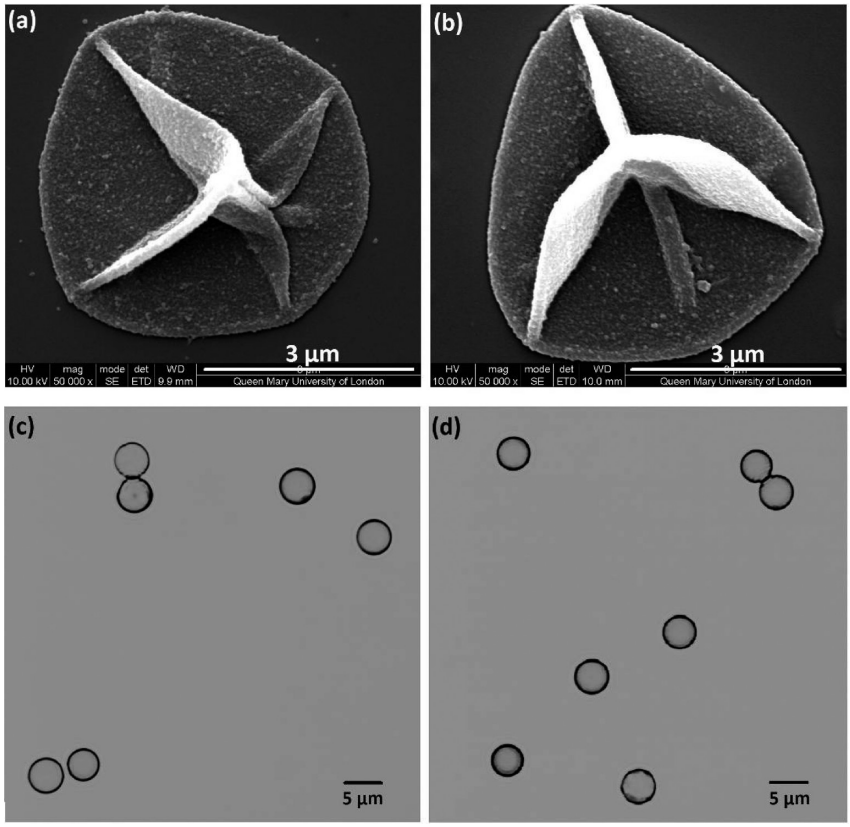


Figure 3. SEM (top panels) and CLSM (bottom panels) images of (DAR/Nafion)$_4$ microcapsules before (a,c) and after (b,d) UV irradiation.

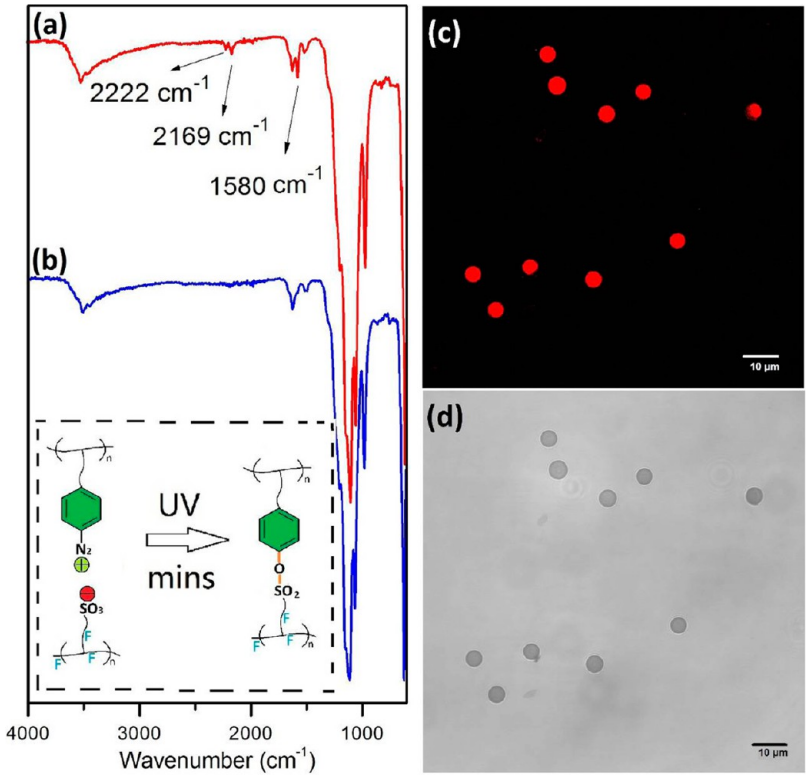


Figure 4. Left panel: FTIR spectra of (DAR/Nafion)$_4$ capsules before (a) and after 10 min of UV irradiation (b). Right panel: CLSM image of RhB encapsulation in irradiated (DAR/Nafion)$_4$ microcapsules (c,d). Inset shows the underlying photolysis of paired charges.

drugs, dyes, and other bioactive substances with molecular weight below 1 kDa.[31] To overcome this difficulty, Nafion, which consists of a perfluorinated backbone and contains sulfonic acid groups in short side chains,[32] is chosen for capsule fabrication together with DAR. After UV treatment, elimination of paired charges and resulting hydrophobic shell structures made these irradiated capsules become "sealed" with less water carrying ability and minimal permeability. As shown in Figure 4c,d, these sealed $(DAR/Nafion)_4$ capsules show good retention for the small molecule RhB (M_w = 479). Thus, appropriate sealing could be achieved in minutes.

Combination of Both Capsule Sealing and Breakage. As inspired by the completely opposite effect of UV-responsive multilayers, but at variable time points, UV-triggered cargo substance encapsulation and release abilities can be integrated in one complex capsule system. Ideally, with proper control over the balance of reactive chemical groups (diazonium and azobenzene), UV light with continuous wavelength can initiate a UV-exposure-dependent capsule sealing (which is attributed to diazonium-related *in situ* cross-linking) and shell breakage (which is due to realignment of local azobenzene molecules). Practically, the balance control could be achieved through adjustment of the ratio of two multilayer systems (DAR/Nafion and PDADMAC/PAZO), which might have different influence on built capsules. Regarding the strengthened shell structures of capsule systems containing Nafion after sealing, which required combination of freeze−thaw and sonication treatments to break them,[33] the multilayers containing a small percentage of cross-linking sites (diazonium of DAR) would be preferred. In this work, complex microcapsules $(PDADMAC/PAZO)_4$-$(DAR/Nafion)_2$ containing both azobenzene and diazonium groups were studied as typical examples, to which four PDADMAC/PAZO and two DAR/Nafion layers were introduced.

As shown in Figure 5 (first row), the prepared microcapsules were flat with creases and folds under SEM observation. Without UV irradiation, these capsules showed uniform size distribution, with an average diameter of 4.92 ± 0.32 μm. At high magnification (120k×), these capsules exhibited intact and relatively smooth surfaces (Figure 5c). With the constant UV irradiation intensity (55 mW cm^{-2}), complex capsules exhibited a time-dependent swelling process after exposure to UV light, as shown in Figure 5 and Figure 6. After the first 10 min of UV irradiation (Figure 5, second row), no obvious size change could be found when compared with the initial ones (Figure 5, first row), and the capsules possessed an average diameter of 4.95 ± 0.26 μm and the shell formations seemed intact; no obvious pore or crack could be observed. Therefore, the first 10 min of UV irradiation could be chosen as the treatment to seal the outmost DAR/Nafion layers, which will be discussed later. When the UV irradiation was extended to 20 min (Figure 5, third row), the average capsule size increased to 5.41 ± 0.61 μm. After exposure to UV for 30 min (Figure 5, fourth row), the capsules swelled to 5.57 ± 0.43 μm in diameter, and obvious pores in the size of 30−40 nm emerged on the shells. Further extending the UV irradiation duration, most of the capsules continued to increase their size and the numbers of small pores on their shells. After 3 h of UV irradiation (Figure 5, last row), almost all of the capsules were swollen, and some of them possessed a size of >8 μm; as a consequence of continuous UV irradiation, the capsule surfaces appeared much rougher due numerous pores with their size up to 100 nm formed on the shells. After that, further UV irradiation led to no visible effect on the capsule shell morphology and stability.

For the polyelectrolyte solutions, maximum absorption peaks were found at 358 and 372 nm for PAZO and DAR, respectively. In theory, due to the analogous UV absorption curves and neighboring maximum UV absorption peaks, the absorption of the complex capsules should exhibit as a compromise of azobenzene and diazonium groups. However, the prepared multilayer capsules showed an absorption shift slightly toward longer wavelength region when the two polymers were assembled with the other two polyelectrolytes (PDADMAC and Nafion). As shown in Figure 7, before UV irradiation, a strong absorption peak centered at 372 nm was found, which was contributed by the absorption of both azobenzene ($\pi-\pi^*$ transition at ~360 nm) and diazonium ($\pi-\pi^*$ transition at ~380 nm), and the weak peak detected at 270 nm was attributed to their parallel or concomitant absorption.[17,26] With exposure to UV irradiation, a fast absorbance decrease was observed. This result was attributed to photolysis of diazonium groups and possible photoisomerization reaction of azobenzene moieties if there was any. After 10 min of irradiation, the absorption intensity decreased to 50% of the initial absorbance. Considering the SEM image of complex capsules after 10 min of UV irradiation (Figure 5, second row), potential chemical changes occurring during this period would not cause capsule swelling.

Practically, the DAR-related photolysis took place very fast, and a previous report demonstrated a completed cross-linking effect within 50 s with treatment of a 80 W mercury lamp at a distance of 13 cm.[14] For the $(DAR/Nafion)_4$ capsules, we have already found that 10 min of UV irradiation was sufficient enough to decompose all the diazonium groups and to eliminate the maximum absorption with a UV intensity of 50 mW cm^{-2}. However, a corresponding peak did not disappear after 3 h of UV irradiation in this work. This result should be attributed to the existence of PDADMAC/PAZO layers. As suggested, the photoisomerization of the azobenzene molecule could not be completed in this system due to the steric hindrances

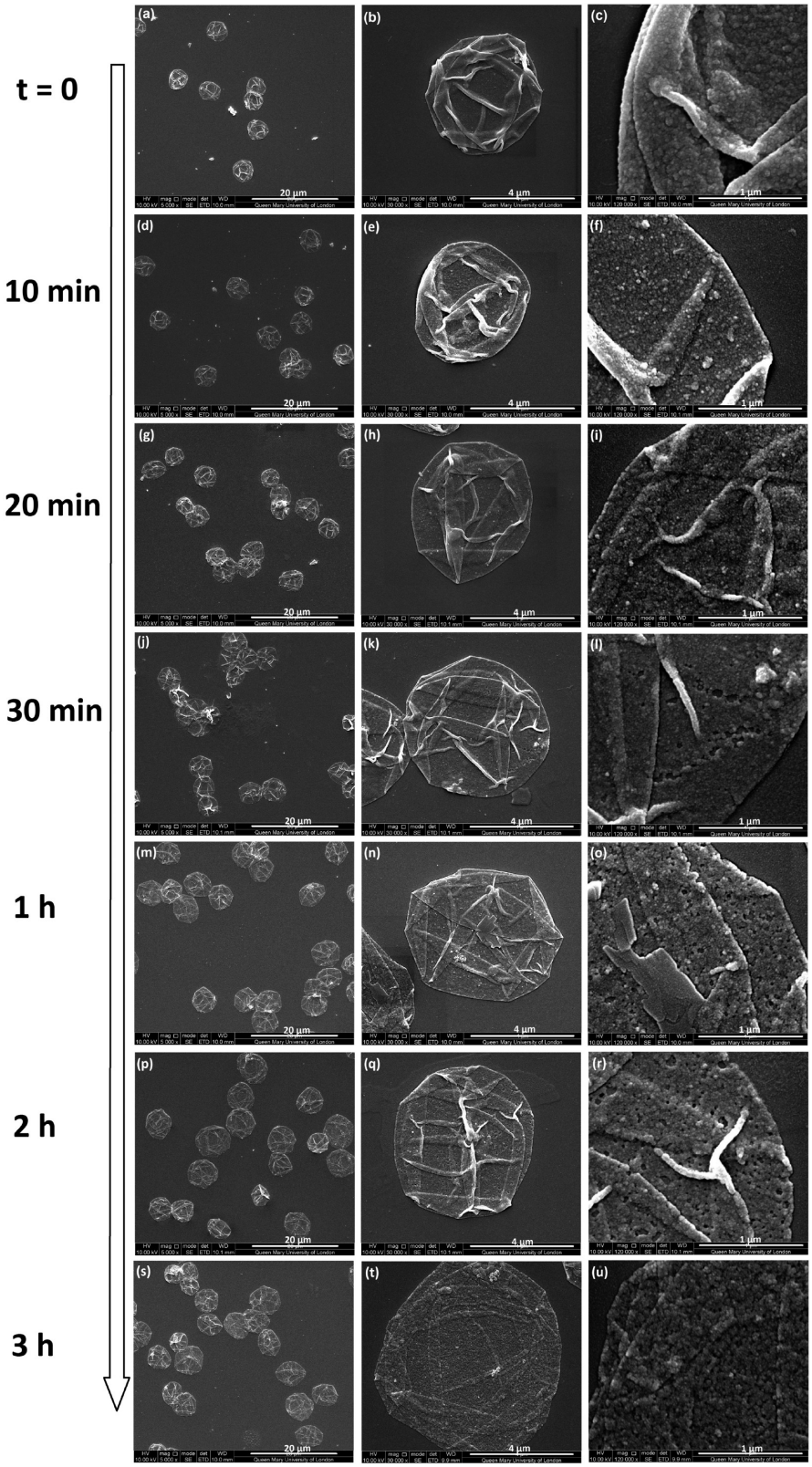


Figure 5. SEM images of complex microcapsules before (first row) and after UV irradiation of 10 min (second row), 20 min (third row), 30 min (fourth row), 1 h (fifth row), 2 h (sixth row), and 3 h (last row) at different magnifications.

against *trans* to *cis* conformation changes as well as possible hindrances of azobenzene aggregates, whose possible mechanism of formation has been discussed already.[17−19] Therefore, the remaining absorption was assigned to that of PDADMAC/PAZO layers, which cannot disappear in 3 h of UV irradiation.

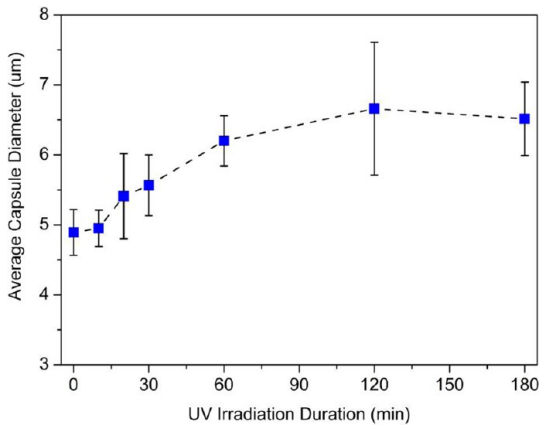


Figure 6. Size changes of complex capsules after UV irradiation. Capsule diameters and distributions were expressed as mean ± SD of at least 30 capsules per sample of random measurement of SEM images.

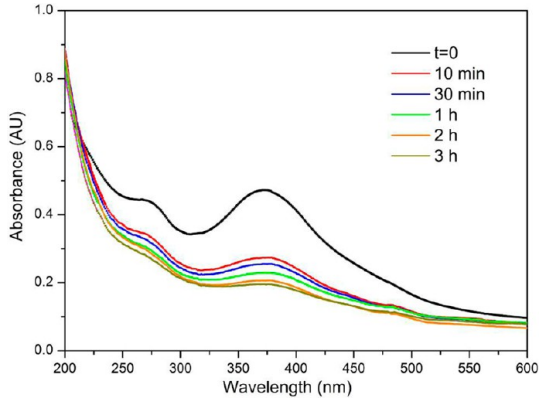


Figure 7. UV−vis spectra of complex microcapsules upon exposure to UV light.

As mentioned above, the UV irradiation would facilitate realignment of azobenzene molecules in the PDADMAC/PAZO system, resulting in formation of J-aggregates. The generation of such aggregates was also detected in the complex capsule system. As shown in Figure 7, a time-dependent shift of maximum absorption toward longer wavelength was found. Briefly, after 10 min of UV irradiation, the maximum absorption was shifted by 4 nm and located at 376 nm. Further exposing to UV light led to a slow red shift. When the UV irradiation reached 3 h, a total red shift by 8 nm was visible at 380 nm.

The FTIR spectra of complex capsules before and after UV irradiation are shown in Figure 8 in order to demonstrate possible chemical changes in detail. After 10 min of UV irradiation, the peaks at 2222 and 2162 cm^{-1} representing stretching of $-N_2^+-$ disappeared completely. With the disappearance of the peak at 1577 cm^{-1} (−C═C− in the phenyl group conjugated with the diazonium group), a new peak representing a normal absorption of the phenyl group, which was overlapped by the signal of the diazonium group before, was observed at 1590 cm^{-1}. All these

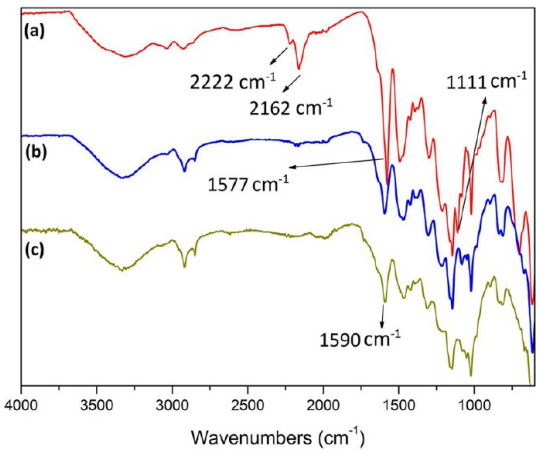
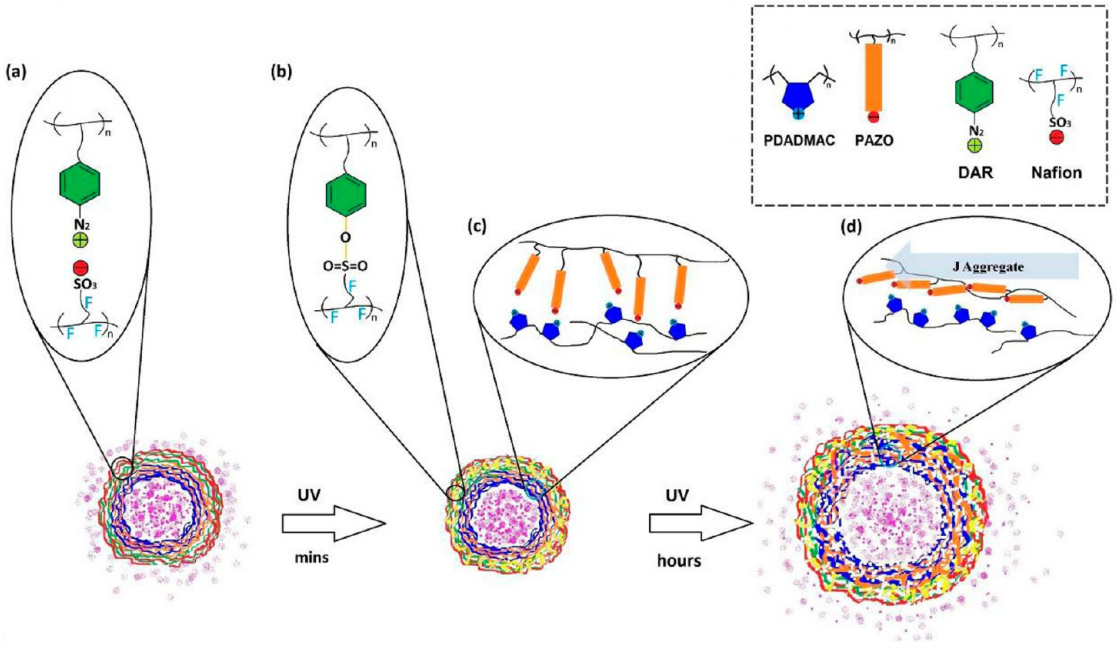


Figure 8. FTIR spectra of complex capsules before (a) and after UV irradiation for 10 min (b) and 3 h (c).

changes should be explained as the UV-induced decomposition of diazonium groups in the multilayers.[26,27] Meanwhile, another peak at 1111 cm^{-1} corresponding to the N−O stretching vibrational mode of complexes formed within DAR and the adjacent polyanion also disappeared. Considering the existence of both Nafion and PAZO polymers surrounding DAR, referring to the shell structure $(PDADMAC/PAZO)_4$-$(DAR/Nafion)_2$, this peak should be attributed to N−O stretching of complexes of diazonium and sulfonate groups (in Nafion) $(-N_2^+ \rightarrow OSO_2^-)$[34] and also the complexes of diazonium and carboxylate groups (in PAZO) $(-N_2^+ \rightarrow OCO^-)$.[35] The disappearance of this peak also confirmed decomposition of diazonium groups under UV irradiation.

Possible photoisomerization of azobenzene molecules changing from *trans* to *cis* could be easily monitored by FTIR, demonstrated by the disappearance of a *trans*-N═N stretching vibration at 1380−1400 cm^{-1} and generation of *cis*-N═N stretching at ~1460 cm^{-1} as reported in related research.[36,37] However, when the UV irradiation duration reached 3 h, no obvious changes due to azobenzene molecular conformation change in-plane could be observed in this work. Combining the found red shift effect in UV−vis spectroscopy (Figure 7), the J-aggregation was hence believed to be the predominant transition in this duration, although the degree of aggregation was found not to be as significant as that in the pure PDADMAC/PAZO system (Figure 2a).

Generally, the UV-induced chemical changes in these complex capsule systems are superimposed, involving *in situ* covalent bonding between paired diazonium and sulfonate groups (Scheme 1a,b) and J-styled realignment of azobenzene molecules which are mostly influenced by the interplay of PDADMAC polymers (Scheme 1c,d). One should notice, as presented in the $(PDADMAC/PAZO)_4$-$(DAR/Nafion)_2$ structure, the diazonium of DAR not only interacted with the sulfonate groups of Nafion but also paired with



Scheme 1. Schematic illustration of UV-induced complex capsule shell sealing and further swelling.

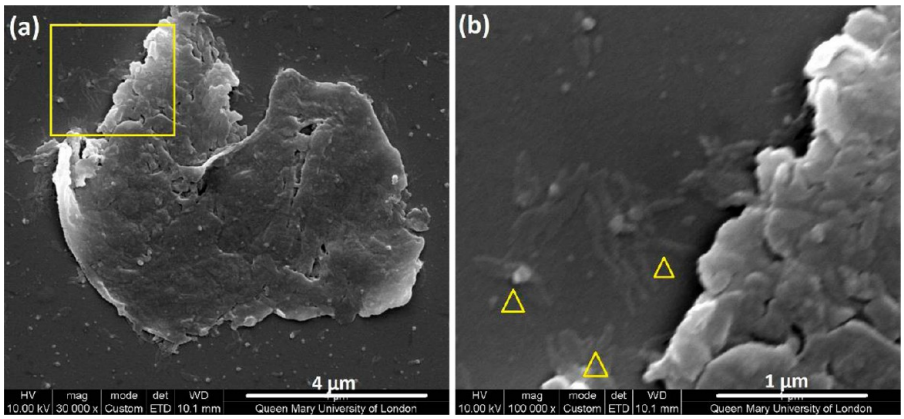


Figure 9. SEM images of a broken complex microcapsule (a) and lamellar-like formations (b) after UV irradiation at 3 h. Image b shows the magnified area of interest; the symbol Δ shows the lamellar-like formations.

carboxylate groups of PAZO. As a consequence, the interaction between the PDADMAC and PAZO was somewhat weakened, while the effect of DAR-related sealing was strengthened by the appearance of cross-linking between PAZO and adjacent DAR layers. Therefore, the UV-induced red shift in complex capsules was not as significant as that observed in the capsules composed of only PDADMAC/PAZO layers (Figure 2a). In return, in the complex capsule system of $(PDADMAC/PAZO)_4$-$(Nafion/DAR)_2$, the potential azobenzene molecular motion as J-aggregates occurred in the PDADMAC/PAZO layers was not powerful enough to cause obvious phase separation or patch-like structures in macroscale. Therefore, the intrinsic chemical transition here primarily led to swollen microcapsules (size change from 5 to 8 μm) and generation of nanoscale pores on the shell. Differently, similar UV-induced azobenzene realignment usually tore the shell-like formations into pieces, demonstrating lamellar- or crystal-like formations.[22] However, in this work, only very few broken capsules and lamellar-like formations (symbol Δ) can be found occasionally after 3 h of UV irradiation, as shown in Figure 9.

Complex Capsules for Encapsulation and Release Triggered by Continuous UV Light. Practically, 10 min of UV irradiation with an intensity of 55 mW cm^{-2} can accomplish the DAR-related photolysis reaction, which could convert the electrostatic interacted charges into covalent chemical bonds, as discussed above. This cross-linking within paired ionic groups provided adequate capability to seal the shells and therefore to entrap the cargo substance inside.[30] Here, efforts were devoted to encapsulate molecules in the complex capsules, in which the DAR/Nafion multilayers would serve as potential sealing layers to achieve the goal. As the SEM images presented in Figure 5, UV irradiation had a significant

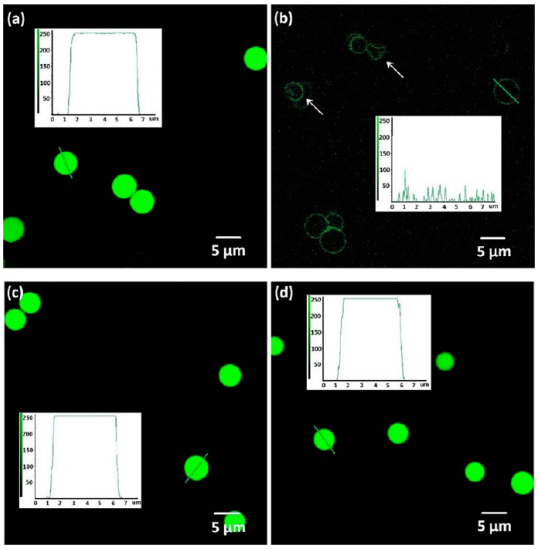


Figure 10. CLSM images of AF488-dextran encapsulation in complex (top panel) and (DAR/Nafion)$_4$ (bottom panel) capsules right after shell sealing (a,c) and after 7 h of additional UV irradiation (b,d). The line scan insets showed relative fluorescent intensity in capsules; the arrows represent collapsed capsules in water.

effect on the morphology changes of complex capsules, exhibiting a time-dependent swelling progress. In particular, no obvious capsule size increase or shell porosity change can be observed after the first 10 min of UV irradiation, thus this UV treatment duration was chosen here as a typical working duration for capsule sealing. On the other hand, considering the long UV treatment duration that would be applied to trigger release of encapsulated substances, fluorescent polymers with high photostability should be used here. Strategically, Alexa Fluor-labeled dextran (AF488-dextran, 10 kDa) was studied because it has been reported that the sulfonic acid substituents of Alexa Fluor could increase water solubility and inhibit dye−dye interactions, which made the Alexa Fluor dyes brighter and more stable than common dyes (*e.g.*, fluorescein and rhodamine), reducing quenching and bleaching.[38] Moreover, AF488-dextran has been used in other research works for fluorescent visualization and encapsulation study.[8,39]

After the first 10 min of UV irradiation, the complex capsules were sealed, and the AF488-dextran was therefore retained inside, visualizing as bright fluorescent image in capsule cavities; very strong fluorescent signal intensity (more than 250 units) was detected (Figure 10a). Further UV irradiation caused a gradual capsule swelling progress (Figure 5), resulting in the release of encapsulated AF488-dextran. After 7 h of UV irradiation, only hollow capsules with limited fluorescent signal can be observed (Figure 10b). Meanwhile, some of the swollen capsules were found to be collapsed in water (pointed out by the arrow). Contrarily, the capsules without the PDADMAC/PAZO layers, (DAR/Nafion)$_4$ to be specific, demonstrated a constant

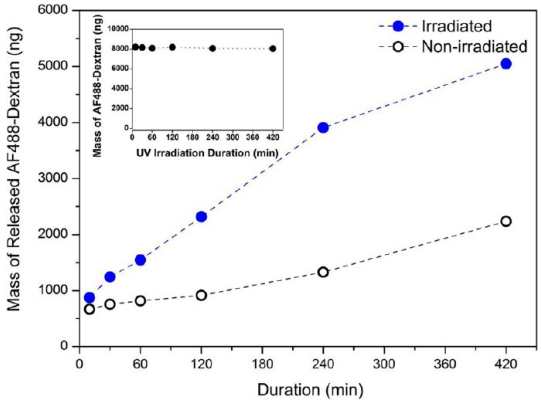


Figure 11. Mass of UV-triggered AF488-dextran release from complex capsules. The inset shows the detected mass of AF488-dextran of the control group after UV irradiation.

fluorescent signal intensity before and after 7 h of UV irradiation. This result was attributed to the UV-induced sealing effect of the DAR/Nafion layers, which can predominantly seal the multilayer shells and not break complex capsules at relevant time points.

The released mass of AF488-dextran from sealed complex capsules was then investigated, released either by UV irradiation or by diffusion. In the meantime, an AF488-dextran solution was also irradiated as the control group, in order to monitor possible UV-induced photobleaching effect under the same UV treatment condition. Its concentration was adjusted to be 20 μg mL^{-1}, or 8000 ng of AF488-dextran in 400 μL volume to be specific. This amount roughly matched the mass of encapsulated AF488-dextran in each portion of complex capsule sample, as determined from the results of preliminary experiments.

The mass of the encapsulated AF488-dextran was determined to be 7.2 mkg inside 6.7 $\times$ 10^6 capsules in this work, which meant that 1.1 pg of AF488-dextran was entrapped in one capsule. In the 7 h of UV treatment duration, the control group (irradiated pure AF488-dextran solution) showed good stability against UV light. After 7 h of UV treatment, there was still 7.8 mkg of AF488-dextran detected in each portion, exhibiting a roughly constant mass of AF488-dextran at each set experimental time point (Figure 11, inset). Therefore, it could be assumed that the influence of UV irradiation on dye photostability of AF488-dextran solution here (20 μg mL^{-1}) was negligible, and the data of UV-triggered AF488-dextran release could be reliable in this duration.

Generally, with the increase of UV irradiation time, the detected AF488-dextran tended to increase, as shown in Figure 11. For each sample containing 6.7 $\times$ 10^6 complex capsules, after 10 min of UV irradiation, 0.87 mkg of released fluorescent polymers was found. When UV irradiation was extended to 30 min, 1.24 mkg was then detected. When the UV irradiation duration reached 4 h, more than 50% of AF488-dextren (3.9 mkg) was released from the capsules.

After 7 h, 5.0 mkg of the AF488-dextran was found, which demonstrated a UV-triggered release efficiency of 70.5%. It should be noted here that not all the encapsulated AF488-dextran was released (as detected) even after 7 h of UV irradiation. Besides possible photobleaching effect, this result could be explained by the adsorption of the fluorescent polymers on capsule shells, as some green "rings" can be seen under CLSM observation (Figure 10b). On the contrary, for the capsules kept in the dark, the fluorescent polymer release was found to be quite slow when compared to that of the irradiated ones. At the beginning, 0.67 mkg of AF488-dextran was expelled from the diffusive shells. With an increase of incubation time, the release by diffusion through the multilayers showed a continuously increasing tendency. However, there was only 2.2 mkg of AF488-dextran detected after 7 h of incubation, 31% of the initial encapsulated amount.

It is worth mentioning that the UV-triggered complex capsule successive sealing and disruption is based on UV absorption in the first instance. Generally speaking, we have two effects such as absorption of PAZO which occurs in the range of 330−450 nm and induced reactivity of the DAR component at 380 nm at maximum. Thus, these intervals that almost overlapped restrict the separation of both effects by tuning wavelength. Fine narrowing wavelength for UV irradiation, for instance, by using UV lasers instead of a lamp, would have a different effect on DAR and PAZO components, and hence, the two adverse effects in multilayers could not be interfered with timing. On the other hand, decrease of the UV intensity might affect the timeline of the process. In the present work, the UV irradiation was set at an intensity of 55 mW cm^{-2} to keep all conditions the same throughout the experiments. We did the experiment at half intensity of 27 mW cm^{-2}, and the release rate of cargo from PAZO/PDADMAC capsules was lowered, reflecting retention of PAZO block rearrangements in the multilayers. One would suggest that at lower intensities the sealing process might get longer, as well. However, the rupture effect may overlap with the sealing, considering that the capsules are not absolutely identical in population. Overall result might be the smearing effect of both shell sealing and disruption in the course of time.

Apart from the intensity and wavelength of UV irradiation, the multilayer shell architecture plays a key role in UV-induced capsule mechanical change. Likewise, the same UV irradiation time could have different effects on capsule with varied layer constituents. Speculatively, one would suggest that there must be a minimum number of each component layer required in order to realize the UV-stimulated effect such as either sealing or rupture. It is hard to anticipate what would happen if more functional polyelectrolyte layers are assembled. This might be a subject for future study. At present, careful considerations should be given to design of the dual-functional complex capsule systems, where the influencing factors (*e.g.*, UV dosage, capsule shell architectures, ratio of functional groups) would have subtle effects on the capsules' potential UV response.

CONCLUSIONS

UV triggered dual-functional complex microcapsules were fabricated in this work for the purpose of integrating both encapsulation and release in one capsule system. Two multilayer systems effecting differently, PDADMAC/PAZO and DAR/Nafion, were introduced to build up complex capsules by using LbL assembly technique. Exposure these capsules to 10 min of UV irradiation (at ∼380 nm) led to diazonium-related photolysis within DAR and adjacent Nafion or PAZO layers. This short time UV irradiation eliminated the paired charges and facilitated cargo substance encapsulation due to densification of multilayer shells. The successful encapsulation of fluorescent polymers such as AF488-dextran has been demonstrated. Later, exposure of these capsules to longer UV treatment duration (at ∼360 nm) caused an irreversible shell swelling progress, which was activated by the preferred J-aggregations of azobenzene molecules in PDADMAC/PAZO multilayers, providing a way to release the encapsulated substances. Generally, UV-triggered release of AF488-dextran was investigated as a typical example. Promisingly, these complex capsules containing two different functional multilayers would be useful microcontainers for various applications, for which their entrapment, retention, and controlled release could easily be trigged by continuous UV light. Hopefully, our strategy to fabricate such dual-function complex capsules, which could be activated by single external stimulus, would inspire the engineering of multifunction vesicles for potential applications.

MATERIALS AND METHODS

Materials. Alexa Fluor 488-labeled dextran (AF488-dextran, 10 kDa) was purchased from Invitrogen. Silicon dioxide microparticles (4.99 ± 0.22 μm) were purchased from microparticles GmbH (Germany). Nafion perfluorinated resin (10 wt % in H_2O), poly[1-[4-(3-carboxy-4-hydroxyphenylazo)benzenesulfonamido]-1,2-ethanediyl, sodium salt] (PAZO, M_w ∼ 100 kDa), poly(diallyldimethylammonium chloride) (PDADMAC, M_w 200−350 kDa, 20 wt % in H_2O), paraformaldehyde, diphenylamine-4-diazonium salt (Variamine Blue RT Salt), Rhodamine B (RhB), and other chemicals were purchased from Sigma-Aldrich. All the chemicals were used as received without further purification.

Methods. *Capsule Preparation.* Diazo-resin (DAR) was synthesized through a polycondensation reaction of diphenylamine-4-diazonium salt with paraformaldehyde, following an electrophilic mechanism,[14] and used as positively charged polyelectrolyte for

capsule fabrication. Polyelectrolyte multilayers were assembled on the SiO_2 particles by using the LbL assembly technique,[40] starting with positively charged polyelectrolytes (PDADMAC or DAR). In order to ensure a better attachment of first polymer deposition, these 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 prior to the assembly.[41] To avoid aggregation, polymer-coated particles were sonicated for 10 s after each wash step. After the polymer deposition steps, the sacrificial templates were removed by treatment with a 0.2 M NH_4F and HF buffer solution at pH = 4.5.[42] Microcapsules $(PDADMAC/PAZO)_4$, $(DAR/Nafion)_4$, and complex microcapsules $(PDADMAC/PAZO)_4$-$(DAR/Nafion)_2$ were obtained after several wash steps.

UV-Induced Encapsulation and Release. To detect the feasibility of encapsulation in complex capsules, fluorescent small molecule (RhB) and polymer (AF488-dextran) were used as model cargo substances. Briefly, fabricated microcapsules were redispersed in these fluorescent solutions (300 μg mL^{-1}) for 2 h with shaking. Then the mixtures were treated with UV irradiation for 10 min, washed several times to remove free fluorescent substances in water, and observed with a Leica TS confocal scanning system (Leica, Germany) equipped with a 63×/1.4 oil immersion objective.

For quantification of released fluorescent polymer (AF488-Dextran, 10 kDa) from complex microcapsules, 1 mL of capsule stock suspension (containing 1×10^8 capsules) was resuspended in 2.0 mL of AF488-dextran solution (300 μg mL^{-1}) for 2 h with shaking. The capsule−dye mixtures were exposed to UV light directly for 10 min. After shell sealing, capsules were collected and washed several times with water to remove free polymers. The resulting suspension was then split into two portions (diluted to 3 mL for each); one portion was treated with additional UV irradiation up to 7 h, and the other portion was kept in the dark. After a set UV experimental time point, 400 μL of capsule−dye mixture (containing 6.7×10^6 capsules) with/without additional UV treatment was taken out and centrifuged, the supernatant was carefully collected, and the precipitate was added with equal volume of pure water. The fluorescence intensity of each sample (in supernatant or in precipitate) was determined with a fluorescence spectrometer (Perkin-Elmer LS 55) and normalized with the standard fluorescent polymer solutions with known concentrations.

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. Continuously stirred capsule suspensions were exposed to the UV source directly. The UV intensity used in this work was detected to be approximately 55 mW cm^{-2}.

UV−visible (UV−vis) absorption spectra of polyeletrolytes and capsule suspensions were determined by using a spectrophotometer (LAMBDA 950, Perkin-Elmer). Aqueous solution measurements were performed using quartz spectrophotometer cuvettes (S10C, Sigma).

Capsule morphologies were provided by scanning electron microscopy (SEM) (FEI Inspect-F). Diluted capsule suspension was dropped on silicon wafer, air-dried, and coated with gold. SEM observation was carried out using an accelerating voltage of 10 kV, a spot size of 3.5, and a working distance of approximately 10 mm.

An infrared spectroscopy (FTIR spectrometer 100, Perkin-Elmer) was applied to detect the FTIR spectra of vacuum-dried capsule samples. All the data were collected at a spectral resolution of 4 cm^{-1}.

Confocal laser scanning microscopy (CLSM) images were captured with a Leica TS confocal scanning system (Leica, Germany) equipped with a 63×/1.4 oil immersion objective.

Conflict of Interest: The authors declare no competing financial interest.

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