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COMMUNICATION

Biopolymer capsules bearing polydiacetylenic vesicles as colorimetric sensors of pH and temperature†

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We report the creation of biopolymer capsules that show a colorimetric response to changes in pH and temperature. Polymerized diacetylenic (PDA) vesicles are embedded within capsules formed by complexing the cationic biopolymer, chitosan, with an anionic surfactant. The PDA vesicles impart their colorimetric properties to the capsules while remaining embedded within the capsule lumen. Accordingly, the capsules show a blue color at low pH (~6) or temperature (~25 °C) whereas the color changes to purple at medium pH (~8) or temperature (~45 °C) and finally to red at high pH (~10) or temperature (~60 °C). These capsule-based sensors are easy to prepare, low-cost, and can be easily tailored for various applications.

Introduction

Chemical and biological experiments frequently require the constant monitoring of pH and temperature. Methods to sense pH are well-known even to high-school students, with the simplest one being the use of litmus paper, which offers a colorimetric response based on the pH of a test solution. A variety of pH-responsive indicator molecules are also known (*e.g.*, fluorescent dyes, polymer gels),[1] and some of these have been integrated onto optical fibers or other devices to create electronic pH sensors. Recently, there has been interest in developing miniaturized pH sensors based on soft materials.[2] One area where such soft sensors could be useful is in the monitoring of pH within tissue-engineered biomaterials (*i.e.*, cells cultured within a gel matrix). For example, one could imagine a soft sensor particle or capsule embedded amongst the cells.[1,2] Such a particle could then provide a real-time indication of pH based, for instance, on pH-dependent changes in color or fluorescence. The soft nature of the sensor could allow it to integrate within the matrix. Moreover, if the sensor could be moved from one location to another in the matrix (*e.g.*, by magnetic or optical manipulation), it could be used to sense the *local* pH at various spots within the 3-D environment. In a similar vein, soft motile microsensors could also find use within microfluidic (lab-on-a-chip) devices to sense pH at various locations on the chip.[2]

Vesicles based on polydiacetylene (PDA) amphiphiles are known to display a colorimetric response to various stimuli,[3,4] including pH and temperature,[5,6] as well as to a range of analytes including solvents,[5] cations,[7] and membrane-active peptides.[8] The tails in the bilayers of such vesicles have diacetylenic groups, which can be polymerized by UV irradiation. The polymerization involves a 1,4-addition reaction and results in the bilayer being converted into a conjugated polymer with an alternating ene–yne sequence. PDA vesicle solutions accordingly exhibit a blue color. When these vesicles are exposed to environmental perturbations, such as changes in pH or temperature, the solution changes color from blue to red. This is believed to be due to the strain induced within the conjugated bilayers.[3–5] PDA-based vesicles and Langmuir–Blodgett (LB) films are widely used for colorimetric sensing of various analytes. Typically, sensing by PDA vesicles is accomplished in solution, *i.e.*, the analyte of interest is added to a solution of the vesicles and the ensuing colorimetric response is recorded. Some analytes, however, induce aggregation of PDA vesicles, which hampers their applicability as sensors. Note that adding the PDA vesicles to a test solution is often not possible because the vesicles would mix with and alter the solution. These limitations can be overcome if PDA vesicles can be encapsulated or immobilized within other solid or soft structures.[9–13] For example, in a recent study, PDA vesicles have been embedded within alginate gel fibers, which in turn showed a colorimetric response to temperature.[12]

In this paper, we demonstrate the creation of biopolymer capsules loaded with PDA vesicles and the use of these capsules as pH and temperature sensors. To our knowledge, such capsules have not been described previously in the literature. By encapsulating PDA vesicles within a capsule, we ensure that the vesicles remain localized—*i.e.*, they are too big to diffuse through the capsule shell into the external solution. On the other hand, analytes (small molecules) can freely diffuse from the solution through the capsule shell and into the lumen of the capsule. This allows PDA vesicle-bearing capsules to act as colorimetric sensors of pH and temperature; in essence, the capsules are endowed with the properties of the embedded vesicles. The key utility of vesicle-bearing capsules is that these can be placed in a test solution of a given analyte without affecting the solution in any way. After the sensing has been performed, the capsules can be removed from the solution. This approach is simple, inexpensive and versatile. All the ingredients are commercially available. The capsules are

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formed by the electrostatic complexation of chitosan and an anionic surfactant (Fig. 1), and the capsule size and properties can be easily tailored for different applications.

Experimental section

Materials

Chitosan of medium molecular weight (190 to 310 K) and Brookfield viscosity of 286 cps was obtained from Sigma-Aldrich. As stated by the manufacturer, the degree of deacetylation was *ca.* 80%. Chitosan is soluble only under acidic conditions, *i.e.*, at a pH <6.5, and here it was dissolved in 0.2 M acetic acid. Sodium dodecyl benzene sulfonate (SDBS) (hard type) was obtained from TCI America. The diacetylenic surfactant, 10,12-pentacosadiynoic acid (PCDA), was obtained from GFS Chemicals.

Sample preparation

Diacetylenic vesicles were created by adding the compound to deionized (DI) water, followed by sonication using a tip sonicator at 70 °C for 60 min. To polymerize the vesicles, the solution was irradiated at room temperature for 1 min using UV light at 254 nm from a low-pressure Oriel Hg pen lamp, with a light intensity of roughly 10^{-4} W cm^{-2}. The polymerized vesicle solution was stored in a refrigerator until needed. The procedure for preparing capsules with PDA vesicles is described in the Results and discussion section. For pH assays with the capsules, solutions at several discrete pH values were obtained by mixing sodium phosphate buffer solutions (pH 4.2, 8.8, and 9.1). Solutions at pH greater than 9 were prepared by adding 1 M NaOH to the pH 9.1 buffer. The pH of each solution was verified by a Denver Instruments UB-10 pH meter.

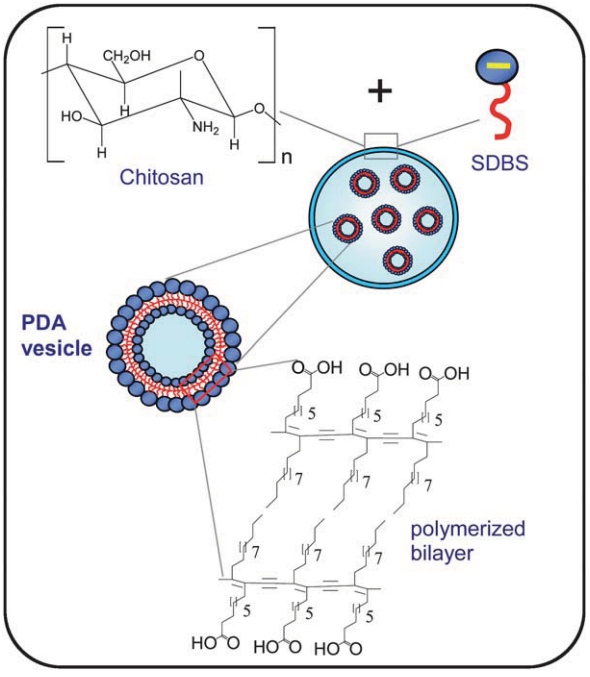

Fig. 1 Encapsulation of PDA vesicles in chitosan–SDBS capsules.

UV-Vis spectroscopy

A Varian Cary 50 UV-Vis spectrophotometer was used to monitor the color transitions of PDA vesicle solutions in response to changes in pH.

Dynamic Light Scattering

To measure the sizes of vesicles, a Photocor-FC light scattering instrument was used at a scattering angle of 90°. The instrument was equipped with a 5 mW laser source at 633 nm and a logarithmic autocorrelator.

Optical microscopy

A Zeiss Axiovert 135TV inverted microscope equipped with the Motic ImagePlus imaging system was used for high-quality transmission microscopy. Capsules were imaged with a 2.5× objective.

Results and discussion

First, we formed vesicles in DI water using the single-tailed diacetylenic surfactant, 10,12-pentacosadiynoic acid (PCDA) at a concentration of 1 mM.[5] The resulting vesicles are expected to be unilamellar structures (Fig. 1) and their diameter was measured to be ~80 nm by DLS. The vesicles were then polymerized by UV light at 254 nm, whereupon the solution turned a deep blue due to the bilayer being converted into a conjugated PDA polymer.[3–5] The PDA vesicles were stable in solution and were stored in a refrigerator.

The procedure for preparing chitosan–SDBS capsules involves electrostatic complexation.[14–17] This generally requires two polymers of opposite charge, or one polymer and a surfactant of opposite charge. Here, we employ chitosan as the cationic polymer and SDBS as the anionic surfactant.[14,17] A 1.3 wt% solution of chitosan (pH ≈ 4.5) is added dropwise to a vial containing a 5 wt% solution of SDBS. Contact between the chitosan and the SDBS at the drop interface leads to electrostatic complexation, thereby forming a shell around the drop. The shell increases in thickness with increasing incubation time in the vial.[14,17] For an incubation time of around 1 min, capsules with good mechanical integrity are formed, and these can be transferred and stored in buffers. In this way, capsules of given size (equal to the size of the generating drops; typically about 1 to 5 mm) can be created by a simple, mild process at room temperature.[14,17] Capsule size can be decreased to ~10 to 100 microns by using either finer nozzles to create smaller drops or by spraying the chitosan into the SDBS solution as a fine mist.[16] Also, instead of SDBS, a variety of anionic surfactants or anionic polymers like alginate or gellan gum can be used to form chitosan capsules.[16]

To prepare PDA-vesicle-loaded capsules, the 1 mM solution of PDA vesicles was combined with a solution of 2 wt% chitosan at a volume ratio of 1 : 2. The vesicles turned a slightly deeper blue upon addition of chitosan, but the mixed solution was otherwise stable and homogenous. This chitosan–PDA mixture was then added dropwise into a solution of 5 wt% SDBS and incubated for ~1 min to form spherical capsules. The capsules were then removed and stored in a pH 6 buffer until needed.

For studies as a function of pH, it is useful to compare the results for PDA vesicle-bearing capsules with those for the PDA vesicles alone. Towards this end, we prepared the PDA vesicle solution in various phosphate buffers. Samples were monitored by visual

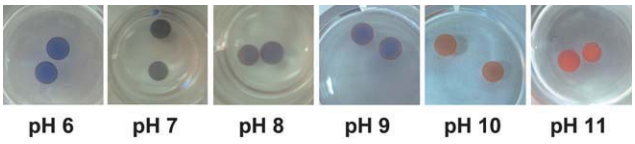

Fig. 2 Colorimetric response to pH of PDA vesicle-bearing chitosan–SDBS capsules at ambient temperature.

observation and UV-Vis spectroscopy (see Fig. S1, ESI†). As expected,[5] the vesicles show a colorimetric transition with increasing pH—from blue (pH 6) to purple (pH 8) and then to red (pH 10). Correspondingly, the spectra show a decrease in the absorbance peak at 640 nm relative to the peak at 539 nm. For a pH of 12 or above, the vesicles rapidly precipitate out of solution—in fact, some precipitation was also observed at pH 11. The molecular basis for these colorimetric transitions is that higher pH disrupts the hydrogen-bonds between PDA chains in the bilayers, causing the conjugated bilayers to become disordered.[3–5]

Next, we studied the effect of solution pH on chitosan–SDBS capsules bearing PDA vesicles. For these experiments, buffers at different pH values were first made. The capsules were then transferred manually into individual buffer solutions. Following an equilibration period (see below), the capsules assumed a characteristic color at each pH. As shown by Fig. 2, the capsules have a blue color at pH 6, a purple color at pH 8, and a red color at pH 10 and beyond. Thus, a colorimetric response to pH is seen with the capsules much like for the vesicles. In fact, the colorimetric transitions occur at about the same pH as was observed for the vesicle solutions (compare the above capsules and Fig. S1† for the vesicles). The above response of the capsules was reproducible, and moreover, it was identical for different capsule sizes.

PDA vesicles are also known to exhibit colorimetric transitions in response to temperature[5] and this aspect was studied in the case of the capsules as well. Capsules in a pH 6 buffer solution were put in different vials, which were then placed in a water bath equipped with a Julabo immersion heater. The capsules were allowed to equilibrate in the bath for 30 min at a given temperature. They were then removed from the bath and photographed. As can be seen from the photographs in Fig. 3, the capsules again show a clear colorimetric response to temperature. The color varies from medium blue (20 °C) to dark blue (30 °C) to purple (45 °C) to red (60 °C) over the range of temperatures. Beyond 60 °C, the color remained red. These thermal transitions in color are believed to occur because heating disrupts the ordered arrangement of diacetylenic chains in the conjugated bilayers of PDA vesicles.[3–5] Note that the colorimetric transitions to both pH and temperature are irreversible for the case of the PDA vesicles used here.[3–5] In turn, the transitions of the capsules are also irreversible, *i.e.*, once their color changes in response to a change in pH or temperature, the original color cannot be restored. Reversible colorimetric transitions are possible, however, with modified diacetylenic surfactant vesicles.[18,19]

We have also studied the time-dependence of the colorimetric transition in the capsules at a given pH. For these experiments, a capsule of ~2 mm diameter that had been stored at pH 6 (at which point it had a transparent blue color) was placed in a pH 7 buffer at time $t = 0$. As shown previously by Fig. 2, the capsules reach a purple color at this pH. The micrographs in Fig. 4 show a purple front (dark color in the micrographs) developing within the capsule and moving inwards. This moving front envelops nearly the whole capsule by 260 s. From visual observations, we find that the purple color of the capsule continues to deepen for a few more minutes, and no further change in color is observed after about 10 min. Evidently, the characteristic time for the color change is dictated by diffusion of H^+ and OH^- ions through the capsule.[14,17] As expected,[14,17] this timescale depends on the capsule size, with smaller capsules changing color faster.

To reiterate, the key utility of the above vesicle-laden capsules is that they can be used as a non-invasive probe of a test solution. That is, the vesicles remain enclosed within the capsule shell and do not contaminate the external solution. Indeed, no "leakage" of color into the solution is evident in the above figures. Also, direct mixing of PDA vesicles with an analyte solution can be problematic—for example, we noted that PDA vesicle solutions show some precipitation at pH 11. However, when the capsule was placed in a pH 11 buffer (Fig. 2), the color change was reliably observed and any precipitation of the vesicles, if it occurred, was confined within the capsule and did not affect the solution. Our experience with the capsules has shown that they retain their robustness, stability, and colorimetric character for more than a month after preparation.

The above capsules can be further enhanced in a variety of ways. For example, we have previously shown that antibodies can be chemically conjugated to the external shells of these capsules.[17,20] The fact that chitosan is an aminopolysaccharide ensures that free amines are present on the capsule surface, which facilitates amine-based chemical conjugation schemes. Another useful aspect about the capsules is that in addition to vesicles, a variety of other payloads can be simultaneously encapsulated in them. Specifically, we have

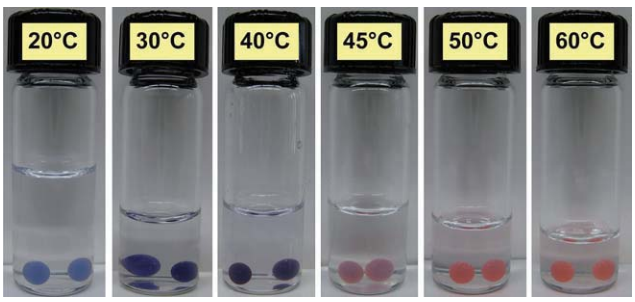

Fig. 3 Colorimetric response to temperature of PDA vesicle-bearing chitosan–SDBS capsules in a pH 6 buffer solution.

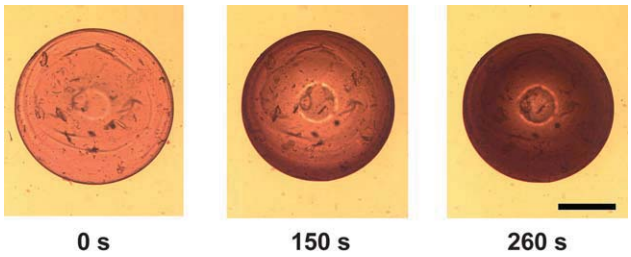

Fig. 4 Optical micrographs showing the progression of the colorimetric transition in a PDA vesicle-bearing capsule. The capsule is transferred from a pH 6 buffer to a pH 7 buffer at $t = 0$. At this initial stage, the capsule has a blue color and appears bright. With increasing time, a dark front (purple) moves through the capsule. The scale bar represents 1 mm.

embedded magnetic nanoparticles (MNPs) in these capsules and the resulting capsules show magnetic character,[17,20] *i.e.*, they can be manipulated with an external magnet and moved within a fluid to a desired location. Thus, the capsules can be easily equipped with other properties in addition to their capabilities for sensing pH and temperature.

Conclusions

In conclusion, we have shown that stimuli-responsive capabilities can be readily imparted to polymer capsules by encapsulating PDA vesicles within the interior of the capsules. The vesicles cannot leak out of the capsules but small molecules (such as ions) can pass through the capsule wall and thereby get sensed by the vesicles. The colorimetric response of the capsules to pH and temperature spans the full range seen with PDA vesicle solutions. The capsules can be used as non-invasive sensors in different analyte solutions, which is an advantage over the direct use of PDA vesicles. The low cost, ease of preparation, and the versatility of these capsules should make them an attractive solution in many sensing applications.

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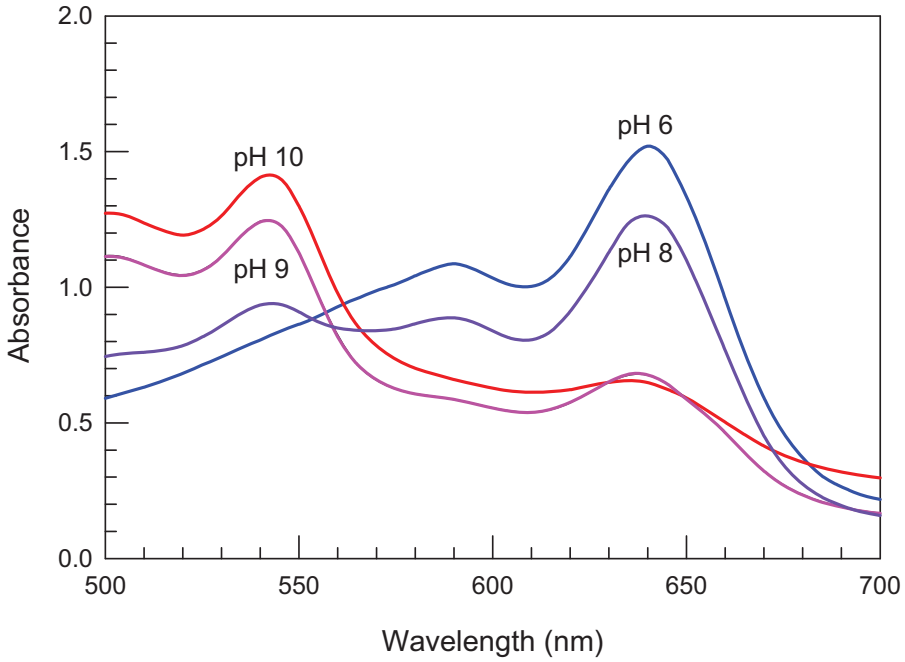

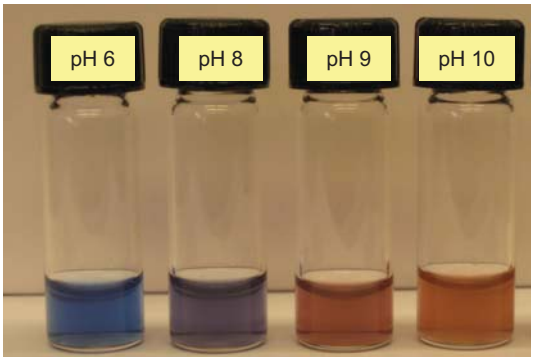

Figure S1. UV-Vis spectra (top) and photographs (bottom) of PDA vesicle solutions at different pH. The solutions each contain 0.33 mM of the diacetylenic amphiphile and the solution pH was varied by using phosphate buffers in different proportions.