Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supporting Information

Polyelectrolyte hydrogel capsules as stabilizers for reconfigurable complex emulsions

Rebecca V. Balaj, Seung Wook Cho, Prachi Singh, and Lauren D. Zarzar[*]

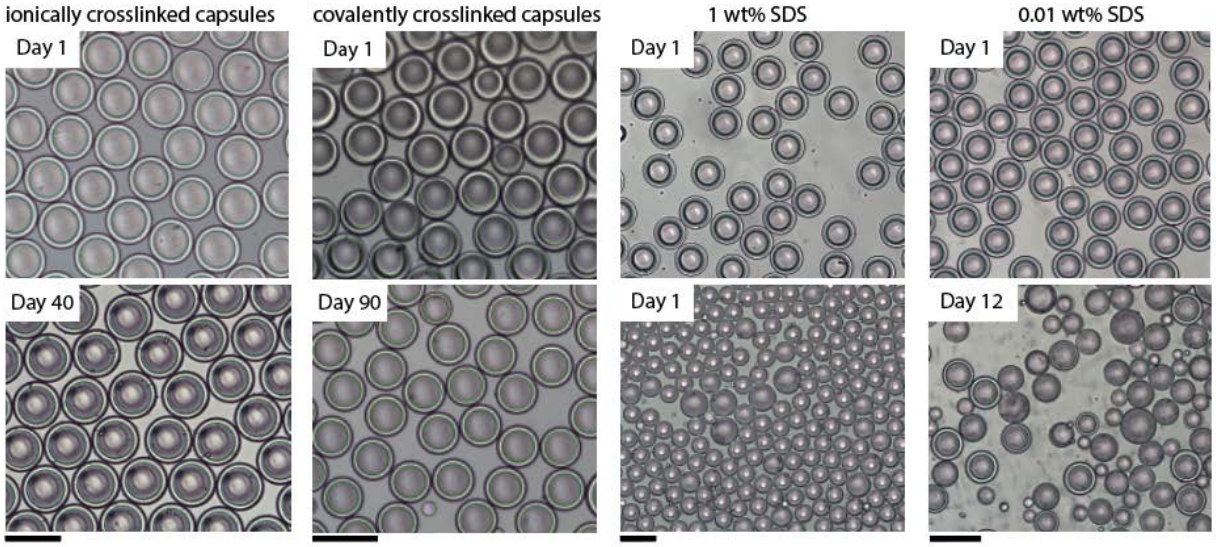

Figure S1. Droplets stabilized by calcium alginate capsules exhibit greater stability than droplets in a commonly used surfactant, sodium dodecyl sulfate (SDS). Double emulsions of perfluorooctane-in-hexane-in-water stabilized by ionically crosslinked calcium alginate capsules, covalently crosslinked calcium alginate capsules, 1 wt% SDS (above the critical micelle concentration), and 0.01 wt% SDS (below the critical micelle concentration) over time. Shown are the longest time periods over which we observed. Droplets stabilized with the alginate capsules in the absence of any surfactant exhibited superior long-term stability compared to the SDS-stabilized droplets. Scale bars, 100 μm.

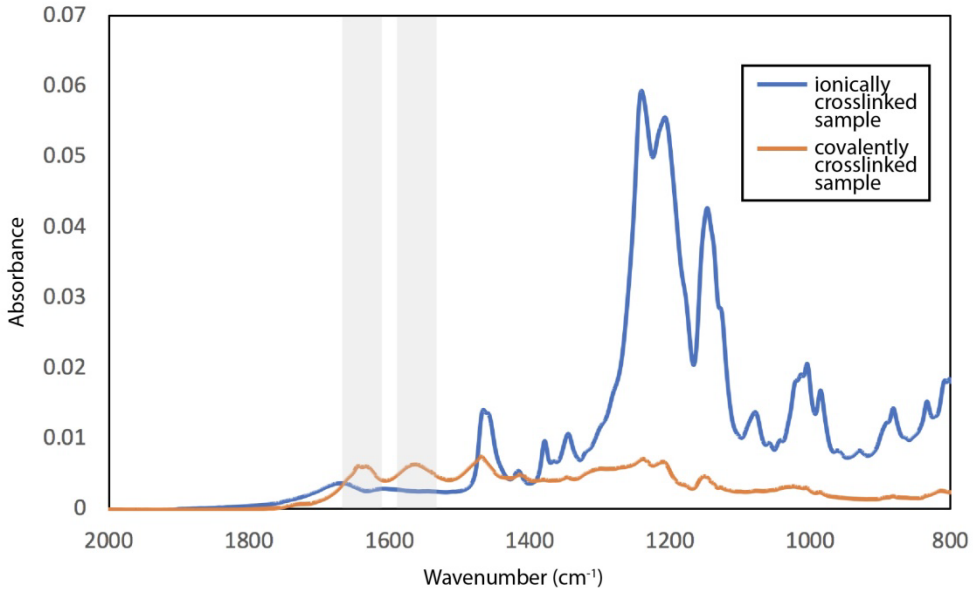

Figure S2. ATR-FTIR characterization of both ionically and covalently crosslinked alginate capsules. Double emulsions of perfluorooctane-in-hexane-in-water stabilized by ionically crosslinked calcium alginate capsules and covalently crosslinked calcium alginate capsules were characterized using a diamond attenuated total reflectance (ATR) accessory and a germanium ATR accessory respectively. Grey highlighted regions signify amide peaks from covalent crosslinking by EDC-NHS crosslinking chemistry, which are not present in the ionically crosslinked capsule sample.

Video S1. Perfluorohexane-in-hexane droplets encapsulated by ionically crosslinked alginate shells reconfigure rapidly upon addition of fluorinated surfactant. Double emulsions of perfluorooctane-in-hexane-in-water stabilized by ionically crosslinked calcium alginate capsules are shown to quickly reconfigure to the Janus morphology upon addition of Capstone FS-30, a fluorinated surfactant that preferentially reduces the interfacial tension at the perfluorohexane-water interface. Video is shown at real time. Scale, 25 µm.

Video S2. Oil droplets encapsulated by ionically crosslinked shells are immediately destabilized upon contact with a hydrophobic surface. Double emulsions of perfluorooctane-in-hexane-in-water encapsulated by ionically crosslinked calcium alginate capsules are shown to immediately destabilize and wet to an octyltriethoxysilane-modified hydrophobic glass surface (water contact angle, 60°) upon settling. Video is shown at 2x speed. Scale, 100 μm.

Experimental Methods

Chemicals. Alginic acid sodium salt (low viscosity, 4-12 cP), 6-aminofluorescein (95%), poly-L-Lysine FITC labeled (M_w 30-70k), sodium dodecyl sulfate (98%), and Capstone FS-66 were purchased from Sigma Aldrich. Both *N*-hydroxysuccinimide (NHS, 99.5%) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC, 99.9%) were purchased from Chem-Impex International Inc. 4-morpholinoethanesulfonic acid (MES, >99%) was purchased from TCI Chemicals. Calcium carbonate nanopowder (50 nm, 98%) was purchased from US Research Nanomaterials. Perfluorooctane (PFO) was purchased from TMC Industries Inc. Hexanes and acetone were purchased from Fisher Scientific. Capstone FS-30 was purchased from Synquest Laboratories. Triton X-100 was purchased from Alfa Aesar. Hydrochloric acid (HCl) (1M), reagent alcohol (94-96% ethanol and methanol) and hydrogen peroxide (30%) were purchased from VWR. Polyethyleneimine (PEI, M_n 60k, 50 wt% aq. solution), Sudan Red 7B, and n-octyltriethoxysilane (97%) were purchased from Acros Organics. Sulfuric acid (95-98%) and acetic acid (glacial, >99.7%) were purchased from Millipore Sigma. Ethylenediaminetetra-acetic acid disodium salt dihydrate (>99.5%) was purchased from IBI Scientific.

Equipment. Brightfield optical micrographs and fluorescence micrographs were taken on a Nikon Ti-U inverted microscope using a Zyla 4.2p camera (Andor) for the fluorescence images and an Imaging Source 23UX249 color camera for brightfield. Confocal micrographs were taken using an Olympus FV1000 Confocal Microscope. Probe sonication was done using a QSonica 700 W sonicator with a ½-inch diameter replaceable tip probe. Fluid pressures for microfluidics were controlled using a Fluigent MC-FS EZ four-channel microfluidic flow control system. Contact angles were measured on a ramé-hart Model 295 goniometer. Lyophilization was done with a VWR FreeZone benchtop freeze dryer. FTIR measurements were completed using a Bruker V70 FTIR using both a Harrick GATR single reflection germanium ATR accessory (62-degree angle of incidence) and a Harrick MVP-Pro single reflection diamond ATR accessory. A liquid nitrogen cooled MCT detector was used, 400 scans were averaged at 6 cm^{-1}resolution.

Synthesis of fluorescein-modified alginate. To enable fluorescence and confocal microscopic visualization of the capsule, alginate was modified with fluorescein. Fluorescein-coupled alginate was synthesized utilizing EDC-NHS coupling chemistry to couple the amine in 6-aminofluorescein to the carboxylic acid of the alginate. Five different solutions were prepared: 7 grams of 3 wt% alginate, 2.1 mL of 100 mM MES buffer (pH 5) solution, 560 μL of 6-aminofluorescein (30 mg/mL of RO water) solution, 280 μL of EDC (50 mg/mL in 25 mM MES buffer) solution, and 280 μL of NHS (30 mg/mL of RO water) solution. All solutions were combined into a 20 mL vial and were stirred for 15 minutes. Afterwards, the reaction mixture was dialyzed against water with 3.5 kD MWCO cellulose membrane for 2 days at 5 °C in order to remove excess EDC, NHS, and aminofluorescein. After dialysis, the solution was frozen at -80 °C and lyophilized to produce dry powder. The reaction vessel was covered with aluminum foil at all times to minimize light exposure.

Fabrication of calcium carbonate coated complex emulsions. Bulk emulsification: A solution of 1 wt% calcium carbonate nanopowder in water was well-dispersed using probe sonication (amplitude of 50 for 4 minutes, pulse on time of 10 seconds and pulse off time of 10 seconds) just prior to use. Hexane and perfluorooctane then were heated until miscible (approximately 40 °C[1]) and emulsified by vortexing in the 1 wt% calcium carbonate dispersion and then cooled to room temperature to induce phase separation and the formation of double emulsion droplets[2]. Various amounts of fluorosurfactant Capstone FS-66 could be dissolved into perfluorooctane before emulsification as shown in **Figure 3** in order to tune the droplet morphology. Microfluidic emulsification: A two reagent, glass, droplet flow focusing chip containing four inlet channels and one outlet and a 100 μm junction (purchased from Dolomite) was used in conjunction with a Fluigent pressure controller. 1 wt% calcium carbonate nanopowder aqueous dispersion (probe sonicated as described above) was introduced into the microfluidic chip as the outer phase through tubing (0.005-inch ID, 1/16-inch OD, PEEK tubing of 26 inches in length) with applied pressures of 1000 mbar. Hexane and perfluorooctane were introduced to the microfluidic chip as the inner phases through tubing (0.0025-inch ID and 1/16-inch OD, PEEK tubing of 26 inches in length) and pressures of 600 and 1200 mbar respectively. For both emulsification procedures, droplets were collected and allowed to settle to the bottom of a glass vial. The calcium carbonate nanoparticle supernatant was removed and replaced with water. The supernatant was removed and replaced with water several times in order to wash away all of the calcium carbonate particles not adhered to the droplet surfaces. Emulsions were observed to be stable during the time periods used (on the order of days).

Fabrication of ionically crosslinked calcium alginate capsules. Calcium carbonate coated droplets fabricated as described in the section, “Fabrication of calcium carbonate coated

complex emulsions" above were used as the precursors to the calcium alginate encapsulated droplets and procedures to create the calcium alginate capsule are modified from literature references[3]. The supernatant from the calcium-carbonated coated droplets was removed and replaced with 2.5 wt% sodium alginate aqueous solution. If fluorescent capsules were desired, 1.25 wt% of fluorescent alginate was substituted in for the non-fluorescent alginate (see section, "Synthesis of fluorescein-modified alginate"). The solution was gently agitated to evenly disperse the droplet in solution, and the pH was adjusted to 4.5 via addition of 1M HCl. The low pH caused the calcium alginate to dissolve and the released calcium ions triggered ionic crosslinking of the alginate at the droplet surface, forming a capsule. Excess alginate was removed by allowing droplets to sediment and disposing of the supernatant. Droplets were further washed with water several times. Encapsulated complex emulsion droplets were stored in water at room temperature and were found to be stable for months.

Covalent crosslinking of alginate capsules. Covalently crosslinked droplets were fabricated by further modification of ionically crosslinked calcium alginate capsules produced as previously described in the section, "Fabrication of ionically crosslinked calcium alginate capsules". Crosslinking was accomplished by coupling carboxylic acid groups with primary amines using EDC and NHS. The following solutions were prepared: 10 mL of 1 wt% PEI in water, 250 μL of EDC (160 mg/mL) in 25 mM 4-morpholinoethanesulfonic acid (MES) buffer of pH = 5.21, and 250 μL of NHS (200 mg/mL) in water. Ionically crosslinked complex emulsion capsules were added to 500 μL of 1 wt% PEI solution followed by addition of the EDC and NHS solutions. An additional 80 μL of fluorescent poly-L-lysine (6 wt%) was added if fluorescent visualization of the capsule was desired. The reaction was allowed to gently shake on a shaker for 24 hours. Upon completion, droplets were allowed to settle in solution whilst the supernatant

was removed and replaced with RO water. This washing procedure was repeated several times to remove unreacted reagents.

Treatment of glass surfaces and patterned adherence of droplets. Glass coverslips were thoroughly washed with acetone, a reagent alcohol solution (largely ethanol), and water, then treated with piranha solution for 30 minutes (3:1 volume ratio of sulfuric acid to 30% hydrogen peroxide). To make PEI coated glass, the coverslip was rinsed with water, dried, and then dipped halfway into a 1 wt% PEI solution for ten minutes. The glass was rinsed with water and dried. A monolayer of ionically crosslinked double emulsion droplets was then added allowed to sit on a coverslip's surface for 30 minutes. Excess droplets were subsequently removed via a gentle rinse with water. Droplets were found to stick only to areas treated with PEI. To make hydrophobic glass as shown in **Figure 4**, surface activated glass coverslips (cleaning and activation procedure described above) were left sitting in a 2 wt% n-octyltriethoxysilane solution in RO water for 2 hours. The pH of this silane solution was adjusted to approximately 3.5 with addition of 10 μL acetic acid. Finally, the glass coverslip was washed well with both reagent alcohol and water before use.

References

[1] M. J. P. De Melo, A. M. A. Dias, M. Blesic, L. P. N. Rebelo, L. F. Vega, J. A. P. Coutinho, I. M. Marrucho, *Fluid Phase Equilib.* **2006**, *242*, 210.

[2] L. D. Zarzar, V. Sresht, E. M. Sletten, J. A. Kalow, D. Blankschtein, T. M. Swager, *Nature* **2015**, *518*, 520.

[3] J. Y. Leong, B. T. Tey, C. P. Tan, E. S. Chan, *ACS Appl. Mater. Interfaces* **2015**, *7*, 16169.