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Graphical Abstract:

Single-step microfluidic fabrication of soft monodisperse polyelectrolyte microcapsules by interfacial complexation

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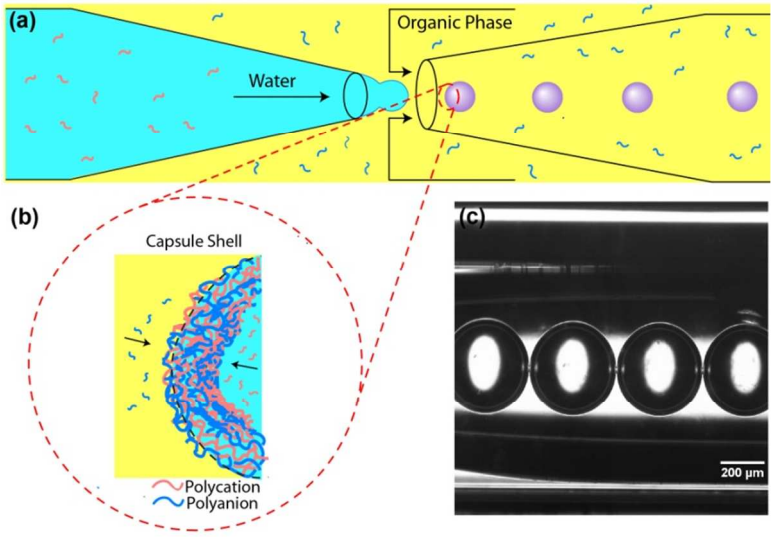
A single-step method is introduced to generate microcapsules with 1-2 μm thick shells and tunable mechanical properties based on polyelectrolyte complexation across a water/oil droplet interface.



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Common methods for fabrication of polyelectrolyte microcapsules rely on a multi-step process. We propose a single-step approach to generate polyelectrolyte microcapsules with 1-2 µm shell based on polyelectrolyte complexation across a water/oil droplet interface and study the effect of parameters controlling the polyelectrolyte complexation on shell thickness.

Microcapsules and controlled release of encapsulated substances represent active areas of fundamental and applied research. This is driven in part by compelling applications in a variety of areas including food science[1], drug delivery[2,3], and personal care products.[4,5] Several methods of microcapsule fabrication have been developed and optimized for particular needs, including, interfacial polymerization[6], double-emulsion templating[7-11], and layer by layer (LbL) assembly.[12,13] The LbL method relies on the ability of soluble polymers, often polyelectrolytes, to form insoluble inter-polymer complexes under appropriate conditions. Such complex formation can occur in bulk solution[14], across fluid interfaces,[15-17] and at solid interfaces by sequential adsorption.[13,14] Microcapsule fabrication by LbL typically involves sequential deposition of layers of selected polymers onto a sacrificial template followed by removal of the template material to provide a hollow, or fluid-filled, object with a multilayer shell.[12] The sacrificial templates are usually colloidal objects including solid particles[12], soft microgels[18] and emulsion drops.[19-21] Individual layers typically range in thickness from 1-10 nm and are built up using multiple adsorption steps, each with incubation times of a few (1-10) minutes, to provide final shell thicknesses of ~100nm.

Although appealing for its versatility, the LbL method has several non-trivial limitations. It is a time-consuming manual multi-step process which is ill-suited for generating microcapsules with thick (micron-scale) shells and the removal of the sacrificial template can pose a challenge for the shell's integrity.[12] Recent efforts have focused on automation as a means of accelerating LbL microcapsule synthesis.[18,22] The attendant device and process complexity are somewhat undesirable however and it remains the case that rapid fabrication of monodisperse mechanically robust polyelectrolyte-based microcapsules represents a challenging proposition and an unmet need.[7]

Here we propose a method to address this need, and demonstrate its viability. Our approach is based on the ability of polyelectrolytes to form complexes across fluid interfaces and leverages the finite solubility of an appropriately selected polyelectrolyte copolymer in a water-immiscible fluid. Water/oil or oil/water emulsions in which the water and oil contain polyelectrolytes of opposite charge will lead to the formation of a shell around the droplet as the species in the organic medium diffuses into the aqueous phase where it becomes charged and forms an ionic complex with its counterpart. Conversely, any partitioning of the aqueous polyelectrolyte into the oil phase is expected to be slow and should not result in complex formation as the polyelectrolytes are not charged in this phase. Polyelectrolyte complex formation by sequential adsorption against a solid surface is self-limiting in thickness on nm-length scales due to charge inversion. By contrast, assembly across a fluid interface does not suffer this limitation as the interior volume of the droplet provides a large reservoir for complexation with the exterior species as it adsorbs onto and diffuses across the interface of the droplet. We

anticipate therefore that the shell thickness will be greater than that of individual layers produced in LbL on solid supports, and that the shell thickness and integrity produced in such a single step may be sufficient to provide mechanically robust and stable capsules under appropriate circumstances.

This proposed approach to generate microcapsules is simple and fast compared to current sacrificial colloidal LbL methods, with the formation of monodisperse capsules immediately after emulsion formation enabled by using capillary microfluidics. We demonstrate the success of the method for a model polyelectrolyte pair (chitosan/sulfonated styrene copolymer) and study the effect of salt, pH, and polymer concentration on capsule formation and shell thickness. We observe the formation of stable capsules over a wide range of salt and polymer concentrations, and aqueous pH considered.

Aqueous-core microcapsules were made by forming droplets of an aqueous solution of chitosan in a toluene solution of s-SEBS. Chitosan is a water-soluble weak polycationic biopolymer, commonly used for biomedical applications[23], and s-SEBS is a partially sulfonated triblock copolymer, of styrene and ethylene/butylene, poly(styrene-*b*-ethylene/butylene)-*b*-styrene (29 wt.% styrene, 56% sulfonation). The non-sulfonated styrene content along with the ethylene/butylene segments provide a finite solubility in toluene, despite the presence of sulfonated species along the polymer. The microcapsule shells are formed by ionic complex formation between the positively charged protonated amines of the chitosan and the negatively charged sulfonate groups of s-SEBS, in the near-interface region within the aqueous interior of the droplet.

Droplet formation was performed using a glass-capillary microfluidic device which was assembled using standard methods[24] as described in Supporting Information. The device and shell formation process are schematically illustrated in Figure 1. Polyelectrolyte complexation across the droplet interface results in shell formation as shown schematically in Figure 1b and by microscope images in Figure 1c, taken in the outlet capillary.

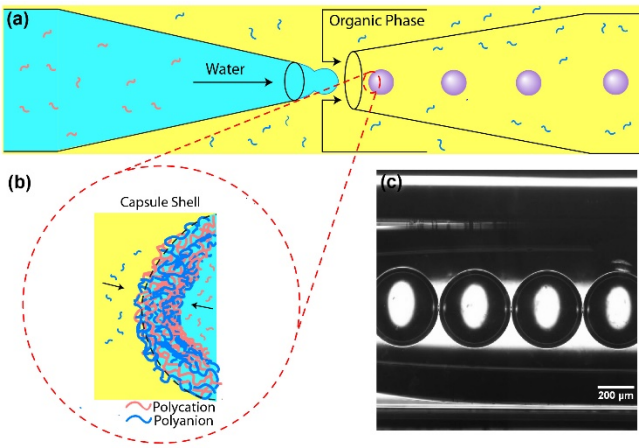

Figure 1. Schematic of the capsule fabrication method in a microfluidic device (a) Aqueous phase (turquoise) containing a water soluble polyelectrolyte and an oil phase (yellow) containing oil soluble polyelectrolyte are brought into contact to fabricate aqueous-core microcapsules with a polymeric shell. (b) Electrostatic interaction of the polycation (red) and polyanion (blue) across the droplet interface generates a robust shell with thickness in the range of 1-2 μm (c) Optical image of monodisperse stable capsules (diameter~200 μm) in the exit flow capillary.

The size of microcapsules produced was tunable in the standard fashion by varying the relative flow rates of the toluene and water solutions (Supporting Information, Figure S1). Monodisperse microcapsules ranging from ~150 to 400 μm in diameter were produced. Figure 2a shows a representative sample of capsules with diameter of 150 ±2.55 μm. Shell thickness was determined using confocal microscopy of capsules fabricated with fluorescently labeled chitosan. Images were recorded of capsule cross-sections as shown in Figure 2b. The shell thickness was determined by Gaussian fitting of the fluorescence intensity profile across the shell, with an internal control provided by the imaging of colloidal particles of known size (Supporting Information, Figures S2-S5). Figure 2b shows capsules made using 0.1 wt.% solutions at pH 2.7 in the absence of salt. The thickness measured by confocal microscopy is ~ 2.1 μm; this is 2 orders of magnitude thicker than the typical shells produced by a single adsorption step in solid-supported LbL assembly.[25] Scanning electron microscopy was used to provide a secondary measure of the shell thickness, albeit in an altered dry state. Capsules were deposited onto a flat surface and dried under vacuum at room temperature for 24 hours before imaging. Representative data are shown in Figure 2c for 0.1 wt.% solutions at pH 2.7 in the absence of salt confirming that the capsule shells are indeed around 2 μm thick.

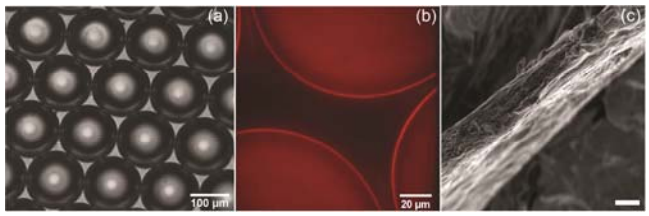

Figure 2. Microscope images of microcapsules (a) Bright field optical microscopy showing monodisperse chitosan/s-SEBS capsules with uniform diameter of 150 ± 2.7 μm. (b) Corresponding confocal image of the microcapsules showing core-shell structure. The shell appears as the bright line with ~ 2.1 μm thickness surrounding the capsule. (c) Scanning electron micrograph of dried microcapsules shows a robust shell film with micron scale thickness. The scale bar is 2 μm.

One expects that the thickness of the shell should be governed by the balance between the timescale for diffusion of s-SEBS across the droplet interface and the timescale for the complexation reaction. As the complex forms it impedes further diffusion of s-SEBS into the droplet and eventually halts the inward growth of the shell. The substantial shell thickness observed here (relative to molecular dimensions) suggests that diffusion is fast relative to complex formation or that for the range of compositions considered, diffusion is not quickly suppressed by the shell formation. This is consistent with recent reports of interfacial polyelectrolyte complexation studied by tensiometry.[15]

We further examined the shell thickness resulting from capsule formation in the presence of salt, at different polymer concentrations, pH, and equimolar concentrations of different salt species. Data are shown in Figure 3. The shell thickness was insensitive to these variations except for salt where a 20% reduction was observed in the presence of salt, but with thickness insensitive to the amount of salt

present. The lack of pH dependence is likely due to fact that chitosan (pKa ~ 6.5) is well protonated over the range of pH considered and the charge density of s-SEBS in the salt form used here is pH independent. As such the chitosan/s-SEBS ionic interactions are not sensitive to pH in our experiments. Likewise, the lack of thickness dependence on solution concentration signals no substantive change in diffusivity or chain conformation with concentration. This is reasonable given that all solutions were in the dilute regime, with concentrations less than the overlap concentrations expected for chitosan at similar molecular weights.[26]

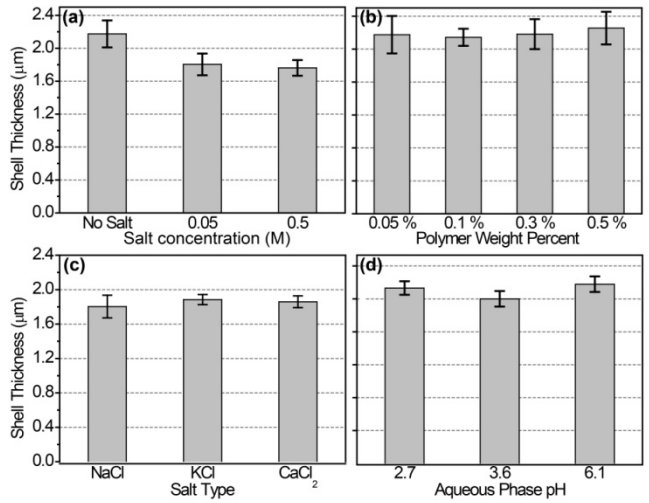

Figure 3. Microcapsule shell thickness as a function of: (a) NaCl salt concentration in the aqueous phase for polymer concentrations of 0.1 wt.% and aqueous pH 2.7 (b) Polymer concentrations. Aqueous phase pH is 2.7. (c) Cation identity for 0.05 M salt concentration. (d) Aqueous phase pH, for 0.1 wt.% polymer concentrations.

We investigated the mechanical properties of the microcapsules using a capillary micromechanics technique (Supporting Info, Figure S6). In this system, the pressure required to deform a microcapsule passing through a tapered capillary is determined by the Young modulus of the shell.[27,28] Here we record the pressure required to deform the capsules to dimensions sufficient for them to escape the tapered capillary. This transit pressure serves as a single point proxy for the stiffness of the capsule shell. Transit pressures were measured for capsules of 500 and 243 μm diameter using a capillary tapered from inner diameter of 580 to 122 μm at an angle of 7° ,Figure 4a. A clear decrease in the transit pressure in the presence of salt was noticed, Figure 4b, but an insensitivity of the transit pressure to the salt concentration. These results mirror the form of the shell thickness dependence in Figure 3. As expected, the reduction is more dramatic for larger capsules which are subjected to a larger degree of deformation in transiting the capillary. The capsules recovered their spherical shape on exiting the capillary or on reversal of the applied pressure while within the taper (Movies S1, Supporting information) demonstrating that the deformation of the shell during transit was reversible.

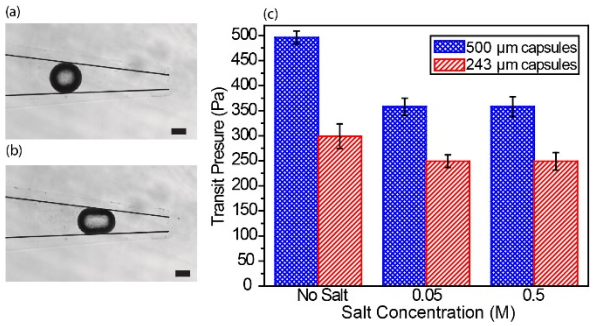

Figure 4. Optical image of pressure driven flow of a microcapsule through a tapered capillary: the microcapsule deforms as the pressure is increased from (a) 187 Pa to (b) 249 Pa. The scale bar is 100 μm. Black lines are added to outline the inner boundary of the capillary. (c) Transit pressure for chitosan/s-SEBS microcapsules prepared using 0.1 wt.% polymer concentrations and different concentrations of NaCl in the aqueous phase as indicated.

The results obtained here suggest that the structure of the polyelectrolyte complexes formed by interfacially-mediated assembly across water/oil interfaces may differ markedly from the familiar case of alternating LbL on solid surfaces. Indeed our expectation was that the presence of salt would result in thicker shells due to screening of the electrostatic interaction between s-SEBS and chitosan, resulting in a less-dense shell overall. This expectation was driven by prior reports of such polyelectrolyte multilayer swelling and exponential growth during assembly at high ionic strength.[25,29] The converse case has also been reported however, with dehydration and associated thickness decrease for the strong/strong polyelectrolyte pair of PDADMAC/PSS subjected to salt-containing rinse steps.[30] We considered the possibility is that the inter-penetration of chistosan/s-SEBS may be controlled by hydrodynamic effects during droplet formation, smearing out effects due to solution composition. Experiments performed across a range of droplet sizes produced using different relative flow rates however suggest that this is not the case (Supporting information, Figure S7). From the perspective of diffusivity and the balance of reaction-diffusion timescales, one should expect thicker shells at high salt concentration. This would be due to faster diffusion of s-SEBS in salt-containing droplets due to charge screening and the associated reduction of chain dimensions.[31]

At this point we speculate that the reduction in capsule thickness in the presence of salt may be driven by the reduction in the chain dimensions for both polymers under these conditions. The interaction between dense coils of chitosan and s-SEBS may lead to a reduction of the reaction time scale and therefore more quickly inhibit further diffusion of s-SEBS into the chitosan droplet. Another possibility is that hydrogen bonding between the sulfonic acid groups of SEBS and the hydroxyl and amine groups on chitosan is sufficient to compensate for the screening of electrostatic interactions in the presence of salt. We stress that these are simply speculations. It is apparent that the formation of polyelectrolyte shells droplet microfluidics is a complex process about which we currently understand little and that further investigation is warranted, particularly to bring understanding on par with that of polyelectrolyte multilayers (PEM) formation on solid substrates. It is nonetheless clear that the properties of the shell can be tuned

through variation of salt concentration, with thinner, more deformable capsules produced in the presence of salt.

Conclusions

We have presented a novel and facile method for rapid fabrication of monodisperse soft microcapsules by interfacially-mediated polyelectrolyte complexation in concert with droplet microfluidics. The resulting shells exhibit thicknesses that are well in excess of those observed in polyelectrolyte-surfactant complexation at fluid interfaces[16,17] or single steps of PEM assembly on solid supports.[25] This points to significant inter-diffusion of the oppositely charged polyelectrolytes during shell formation. The capsules produced were mechanically robust and their deformability was demonstrated to be sensitive to the presence of salt. Capsules fabricated in this manner may prove important for applications in synthetic biology, controlled release and fundamental studies of the flow behavior of deformable particles. There appear to be substantive differences relative to LbL PEM assembly that warrant additional consideration.

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Notes

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References

1 Je Lee, S.; Rosenberg, M. *LWT - Food Science and Technology* **2000**, *33*, 80.
2 Singh, M. N.; Hemant, K. S.; Ram, M.; Shivakumar, H. G. *Research in pharmaceutical sciences* **2010**, *5*, 65.
3 Ibarz, G.; Dähne, L.; Donath, E.; Möhwald, H. *Advanced Materials* **2001**, *13*, 1324.
4 Ammala, A. *International Journal of Cosmetic Science* **2013**, *35*, 113.
5 Miyazawa, K.; Yajima, I.; Kaneda, I.; Yanaki, T. *J Cosmet Sci* **2000**, *51*, 239.
6 Kobašlija, M.; McQuade, D. T. *Macromolecules* **2006**, *39*, 6371.
7 Datta, S. S.; Abbaspourrad, A.; Amstad, E.; Fan, J.; Kim, S.-H.; Romanowsky, M.; Shum, H. C.; Sun, B.; Utada, A. S.; Windbergs, M.; Zhou, S.; Weitz, D. A. *Advanced Materials* **2014**, *26*, 2205.
8 Tu, F.; Lee, D. *Langmuir* **2012**, *28*, 9944.
9 Abbaspourrad, A.; Datta, S. S.; Weitz, D. A. *Langmuir* **2013**, *29*, 12697.
10 Abbaspourrad, A.; Duncanson, W. J.; Lebedeva, N.; Kim, S.-H.; Zhushma, A. P.; Datta, S. S.; Dayton, P. A.; Sheiko, S. S.; Rubinstein, M.; Weitz, D. A. *Langmuir* **2013**, *29*, 12352.
11 Liu, L.; Wu, F.; Ju, X.-J.; Xie, R.; Wang, W.; Niu, C. H.; Chu, L.-Y. *Journal of Colloid and Interface Science* **2013**, *404*, 85.
12 Yan, Y.; Björnmalm, M.; Caruso, F. *Chemistry of Materials* **2013**, *26*, 452.
13 Decher, G. *Science* **1997**, *277*, 1232.
14 Petrov, A. I.; Antipov, A. A.; Sukhorukov, G. B. *Macromolecules* **2003**, *36*, 10079.
15 Monteillet, H.; Hagemans, F.; Sprakel, J. *Soft Matter* **2013**, *9*, 11270.
16 Monteux, C.; Williams, C. E.; Bergeron, V. *Langmuir* **2004**, *20*, 5367.
17 Monteux, C.; Williams, C. E.; Meunier, J.; Anthony, O.; Bergeron, V. *Langmuir* **2003**, *20*, 57.
18 Richardson, J. J.; Liang, K.; Kempe, K.; Ejima, H.; Cui, J.; Caruso, F. *Advanced Materials* **2013**, *25*, 6874.
19 Grigoriev, D. O.; Bukreeva, T.; Mohwald, H.; Shchukin, D. G. *Langmuir* **2007**, *24*, 999.
20 Priest, C.; Quinn, A.; Postma, A.; Zelikin, A. N.; Ralston, J.; Caruso, F. *Lab on a Chip* **2008**, *8*, 2182.
21 Shchukina, E. M.; Shchukin, D. G. *Current Opinion in Colloid & Interface Science* **2012**, *17*, 281.
22 Kantak, C.; Beyer, S.; Yobas, L.; Bansal, T.; Trau, D. *Lab on a Chip* **2011**, *11*, 1030.
23 Di Martino, A.; Sittinger, M.; Risbud, M. V. *Biomaterials* **2005**, *26*, 5983.
24 Utada, A. S.; Lorenceau, E.; Link, D. R.; Kaplan, P. D.; Stone, H. A.; Weitz, D. A. *Science* **2005**, *308*, 537.
25 Vidyasagar, A.; Sung, C.; Gamble, R.; Lutkenhaus, J. L. *ACS Nano* **2012**, *6*, 6174.
26 Jung, J.; Zhao, Y. *Bioorganic & Medicinal Chemistry* **2012**, *20*, 2905.
27 Kong, T.; Wang, L.; Wyss, H. M.; Shum, H. C. *Soft Matter* **2014**.
28 Wyss, H. M.; Franke, T.; Mele, E.; Weitz, D. A. *Soft Matter* **2010**, *6*, 4550.
29 Guzman, E.; Ritacco, H.; Rubio, J. E. F.; Rubio, R. G.; Ortega, F. *Soft Matter* **2009**, *5*, 2130.
30 Iturri Ramos, J. J.; Stahl, S.; Richter, R. P.; Moya, S. E. *Macromolecules* **2010**, *43*, 9063.
31 Zhou, K.; Li, J.; Lu, Y.; Zhang, G.; Xie, Z.; Wu, C. *Macromolecules* **2009**, *42*, 7146.

