Supporting Information for

Formation and Characterization of Polyglutamate Core-Shell Microspheres

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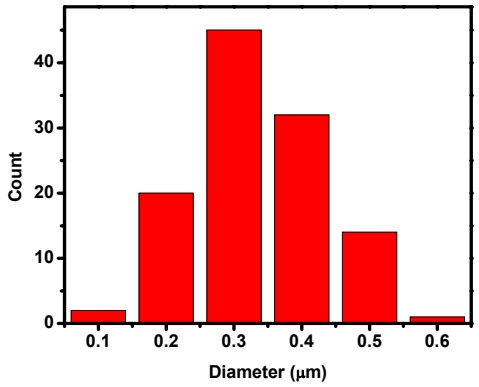
Experimental Conditions

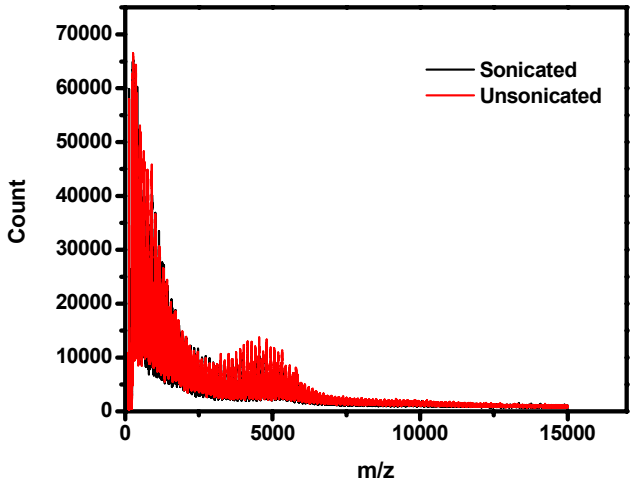
20 kHz ultrasound was applied at the oil/water interface with a Sonics and Materials VCX-750 ultrasonic system for three minutes with a 2 mm diameter horn at an acoustic power of 50 Watts/cm^2. The reaction vessel was immersed in a water bath kept at 20 °C which maintains a microsphere solution temperature of less than 27 °C during sonication. Microspheres were easily formed from SPG with MWs as low as 1500 and as high as 100,000. The vegetable oil used was Crisco® Pure Vegetable Oil.

Size Distribution



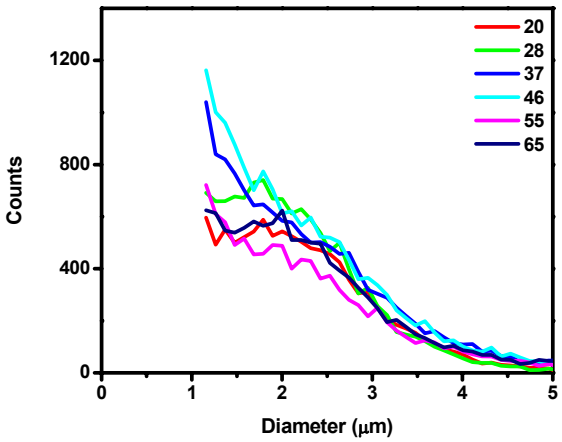
Supporting Figure 1. Size distribution obtained from SEM images of polyglutamate core-shell microspheres; 150 microspheres were sized from the micrographs. Spheres average 400 nm ±100 nm.

Mass Spectra



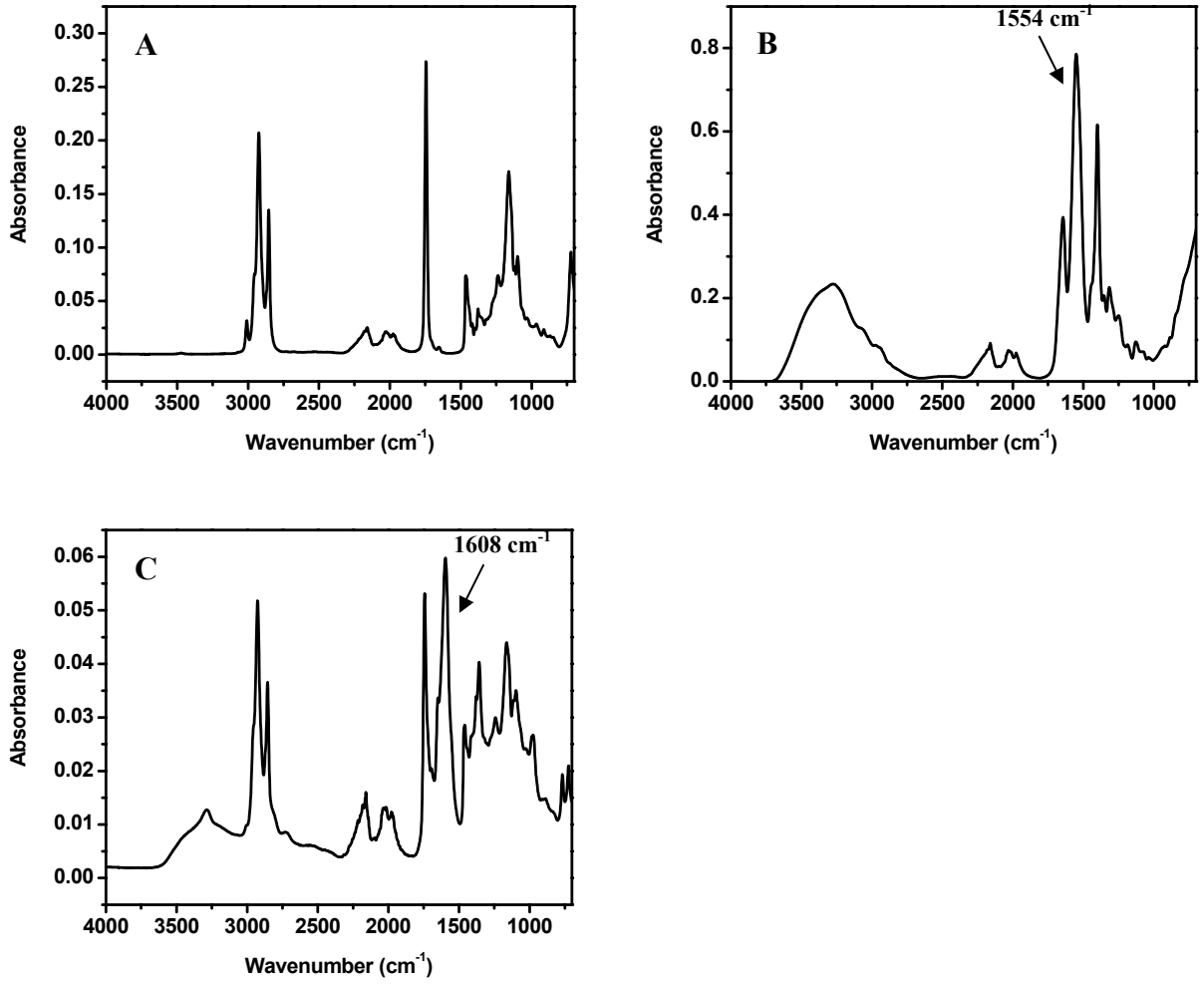
Supporting Figure 2. MALDI mass spectra of sonicated and unsonicated 1500-6000 MW SPG. No dimers, oligomers or other multimers were observed indicating that the spheres are not held together by covalent crosslinking.

Temperature Effect on Microsphere Stability



Supporting Figure 3. Effect of temperature on the stability of polyglutamate core-shell microspheres. A microsphere suspension was placed in a water bath for one hour at 20 °C, then one hour at 28 °C, etc., up to one hour at 65 °C. Over the six hours, no significant decomposition of the microspheres was observed within the experimental error.

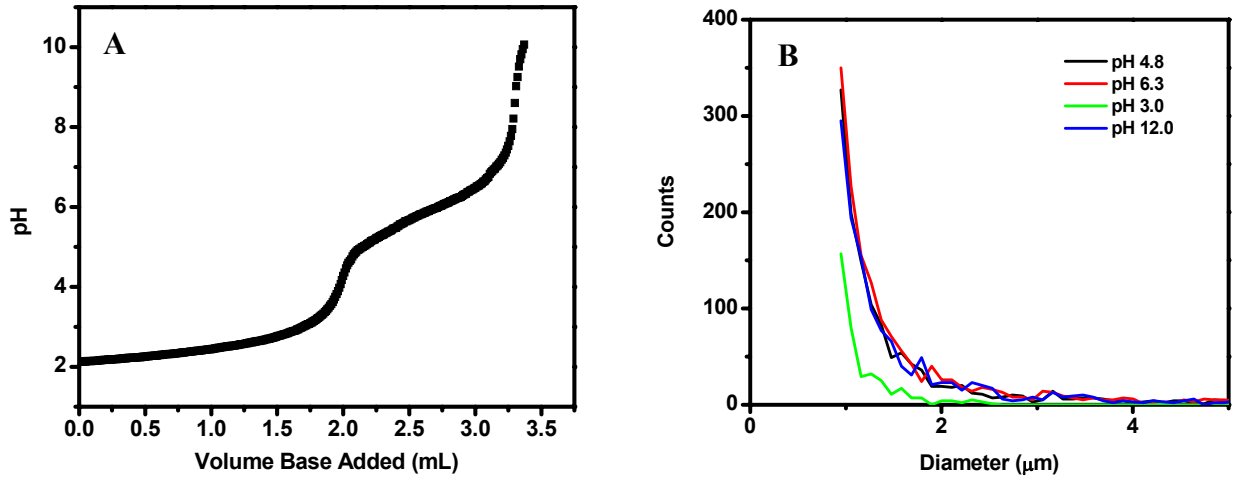
IR Studies



Supporting Figure 4. IR spectra of (**A**) pure vegetable oil, (**B**) sodium polyglutamate (SPG) solution (pH 7.2), (**C**) Sonicated PG spheres containing oil (pH 7.2). The asymmetric CO_2 vibration of SPG microspheres (1608 cm^{-1}) is 54 cm^{-1} higher in energy than that of a simple SPG solution (1554 cm^{-1}). This is consistent with an ion-paired or hydrogen bonded carboxylate in the microspheres (**C**) compared to a free carboxylate in the initial SPG solution (**B**)

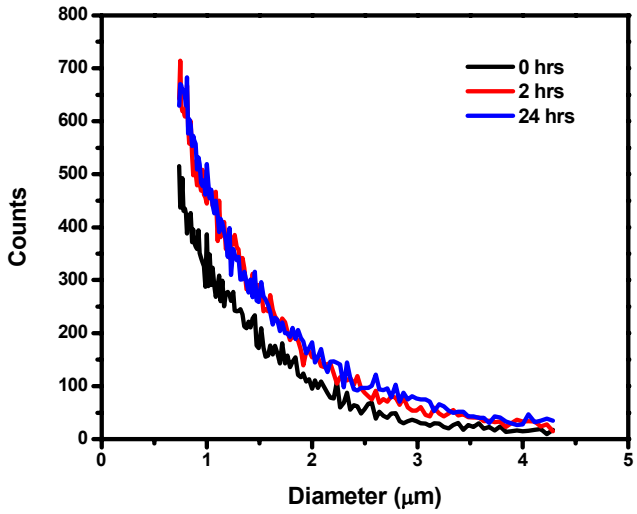
pH Effects on Microsphere Stability

While the pK_a of a single glutamic acid residue is 4.5, the pK_a of its polymer increases due to coulombic forces between the deprotonated residues. Titrating solutions of the 50,000-100,000 MW sodium polyglutamate, we found an apparent pK_a to be closer to 6 with deprotonation occurring over the broad range of pH 4.5-9.



Supporting Figure 5. (A) Titration curve of the addition of NaOH to SPG after the solution pH had been adjusted to 2 with HCl. **(B)** Microsphere stability vs. pH. Particle counting data (Coulter Multisizer IIE) of sodium polyglutamate microspheres after being exposed for 1 hr to solutions of different pHs.

Microsphere Stability in Plasma



Supporting Figure 6. Microspheres were stable (to within experimental error) for more than 24 hours when stored in bovine plasma. SPG microspheres were washed to remove excess salt and suspended in filtered bovine plasma. The solution was stored at 2 °C to delay bacterial growth.