Electronic Supplementary Material (ESI) for Chemical Communications


**Supplementary Information:**

# Encapsulation and release of aqueous components from sonochemically produced protein microspheres.

Emily K. Skinner[a] and Gareth J. Price[a]*

[a]*Department of Chemistry, University of Bath, Claverton Down, Bath, BA2 7AY, UK.*
**G.J.Price@bath.ac.uk, +441225 386504*

**Contents:** Pg.

**Experimental:**

All chemicals were purchased from Sigma Aldrich and were used as received. A Sonics and Materials Vibra Cell VC600 generator fitted with a 20 kHz horn and a microtip attachment was used for the microsphere synthesis and release studies. Ultrasound intensities were measured calorimetrically. Dynamic light scattering measurements were performed using a Malvern Nano-S instrument. Optical micrographs were captured using a GX optical L3001 microscope fitted with an Infinity 2 camera. Laser scanning confocal microscopy experiments were carried out using a Zeiss LSM 510 META microscope. Fluorescence experiments were carried out using a Spectrostar Omega plate reader (BMG Labtech) with NUNC 12 plates, the cycle time was 299s, 20 flashes per cycle with double orbital shaking at a frequency of 300 rpm for 289 s after each cycle, excitation filter; 485 nm, emission filter; 520 nm. A Mettler Toledo Seven Easy conductivity meter was used to carry out the conductivity measurements together with a Techne Tempette Junior TE-8J water bath.

**Emulsion preparation**: An oil phase of tetradecane containing span 80 and an aqueous phase of pure water or sodium chloride solution were sonicated with the horn tip at the oil : water interface using an intensity of 45 $Wcm^{-2}$. Placement of the tip in other positions e.g. in the bulk oil or aqueous phase results in less effective mixing and reduced formation of microspheres. After initial experiments covering a variety of conditions, the following optimised conditions were used for later experiments: oil phase: 4 % w/w Span 80 in tetradecane, aqueous phase: 1 M $NaCl_{(aq)}$, emulsion: 40% aqueous phase, sonication time: 5 minutes. The water droplet size distribution in the emulsion was characterised using dynamic light scattering after dilution with bulk tetradecane/span 80 (matching the composition of the continuous phase) to achieve 4 % w/w of aqueous phase in the sample.

**Emulsion-containing microsphere synthesis:** In a 15 mL plastic centrifuge tube, 50 mg of lysozyme was dissolved in 1 mL of 50 mM pH 8 Tris buffer and left to stand for 1 hour. 30 mg of DTT was charged to the lysozyme solution and left to stand for 2 minutes. 100 µL of freshly prepared emulsion was layered on the surface of the lysozyme solution. The horn tip was placed at the oil water interface and the system was sonicated for 30 s at 14 W $cm^{-2}$. After sonication the suspension was diluted to 15 mL with deionised water and left to stand overnight. If required, the suspension of microspheres was washed, to remove excess oil and protein fragments, by sequential re-dilution of the creamed suspension in clean deionised water. Samples for confocal microscopy were prepared using a w/o emulsion in which the tetradecane phase was saturated with nile red and the aqueous phase contained 1 mM 5(6)-carboxyfluorescein.

**Release of 5(6)-carboxyfluorescein**: Microsphere samples were prepared according to the protocol detailed above using an aqueous phase containing 50 mM 5(6)-carboxyfluorescein. Release was monitored using a fluorescence plate reader; each well contained 50 µL of microsphere suspension in 2.0 mL of HEPES buffer with the prescribed amount of DTT. Fluorescence was measured at 5 minute intervals over a 6 hour time period, during which time the

plate was incubated at 37 °C. Each DTT concentration was run in triplicate. In the case of mechanical breakdown by ultrasound, triplicate samples were taken from the microsphere suspension at chosen time points and diluted for measurement on the plate reader as described above.

**Release of Sodium Chloride:** Microsphere samples were prepared according to the protocol detailed above using a w/o emulsion containing a 6.1 M solution of NaCl. For each experiment the microsphere suspension was diluted to exactly 10 mL using MilliQ water (18.2 MΩ cm). The suspension temperature was equilibrated to 25 ± 0.5 °C using a water bath prior to each conductivity measurement. The conductivity of the freshly prepared suspension was measured, the microspheres were then sonicated for 2 minutes at 45 $Wcm^{-2}$ to cause rupture. The conductivity of the degraded suspension was then measured. Control samples were treated in an identical manner. Experiments were carried out on each sample type in triplicate.

**Results:**

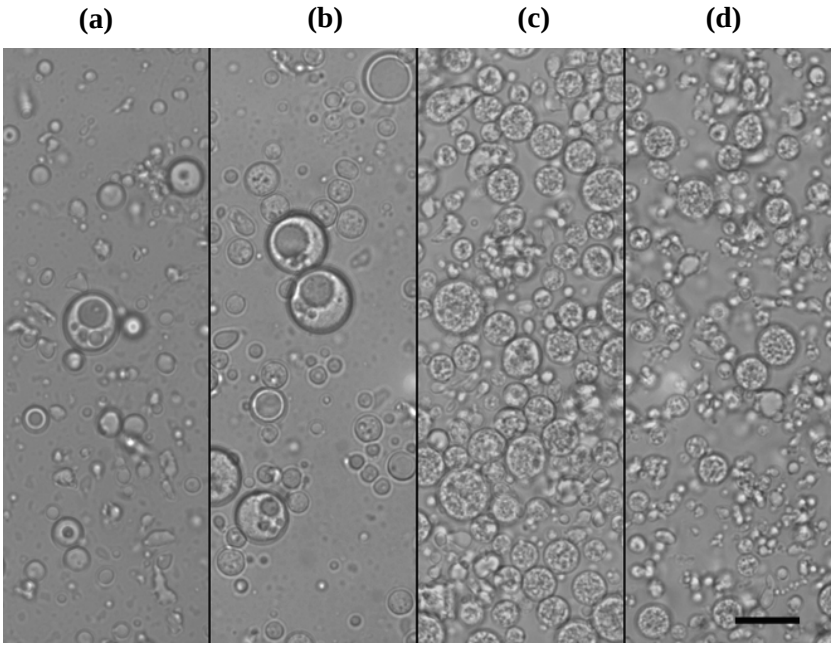


**Figure S1:** Emulsion containing microspheres formed using different salt concentrations in the internal phase; (a) pure water, (b) 0.06 M, (c) 0.5 M, (d) 3 M aqueous sodium chloride respectively. Scale bar: 10 μm.

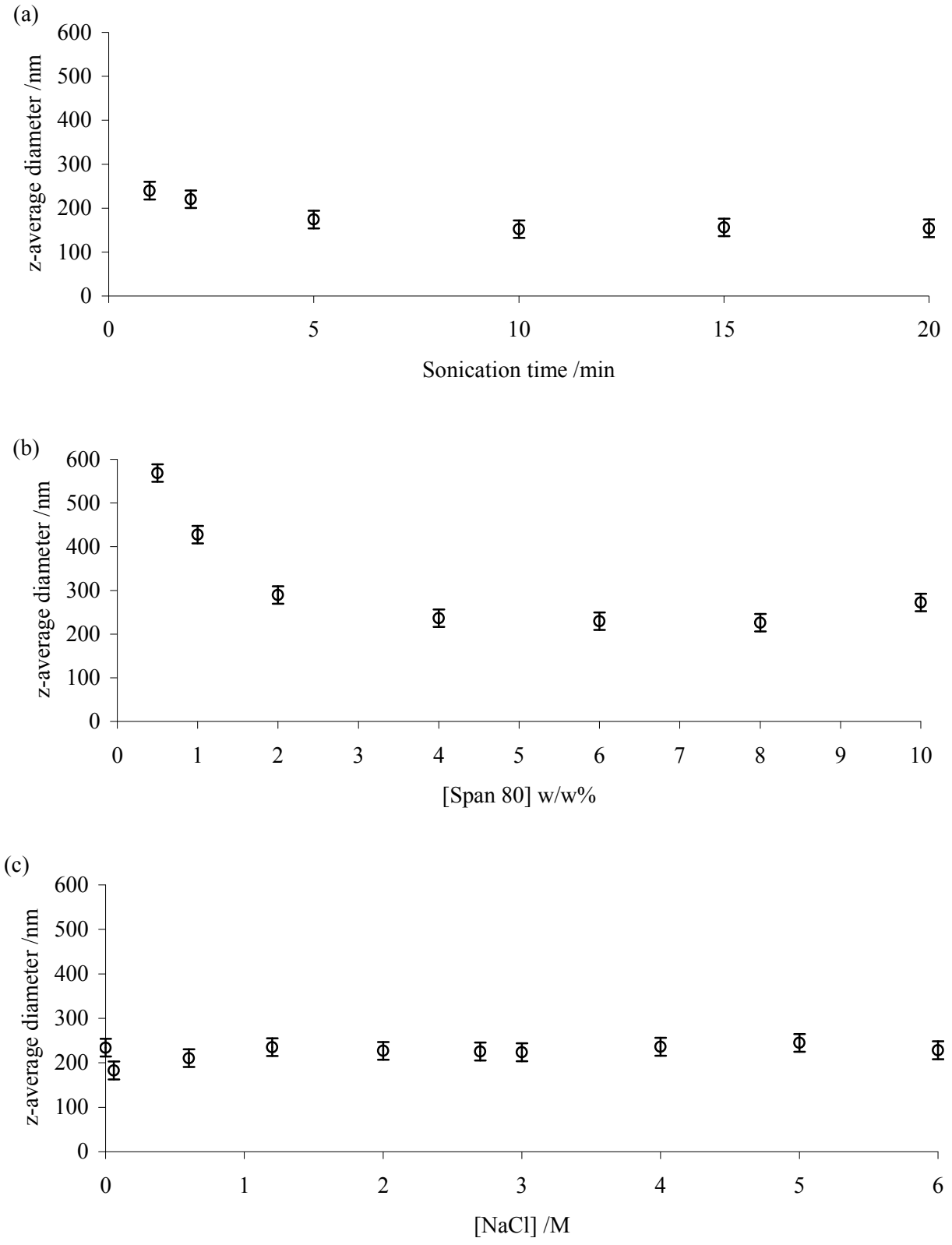


**Figure S2:** Effect of sonication time, surfactant concentration and salt concentration on the droplet diameter in a 40% v/v tetradecane emulsion measured by dynamic light scattering. (a) 4 w/w% Span 80, 0.06 M NaCl. (b) 6 M NaCl, 5 min sonication (45 $Wcm^{-2}$) (c) 4 wt% Span 80, 5 min sonication (45 $Wcm^{-2}$).

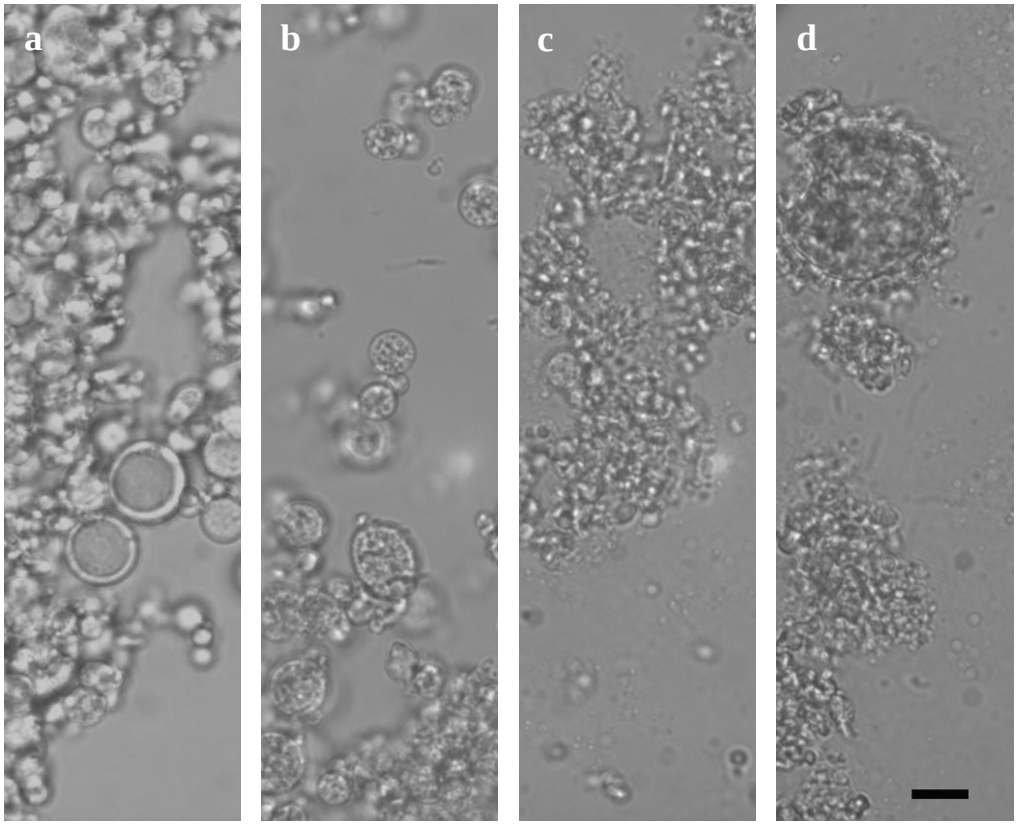


**Figure S3:** Lysozyme microspheres synthesised using: (a) 20% (b) 40% (c) 60% (d) 80% v/v aqueous phase of 1 M NaCl in tetradecane. (Scale bar 10 μm)

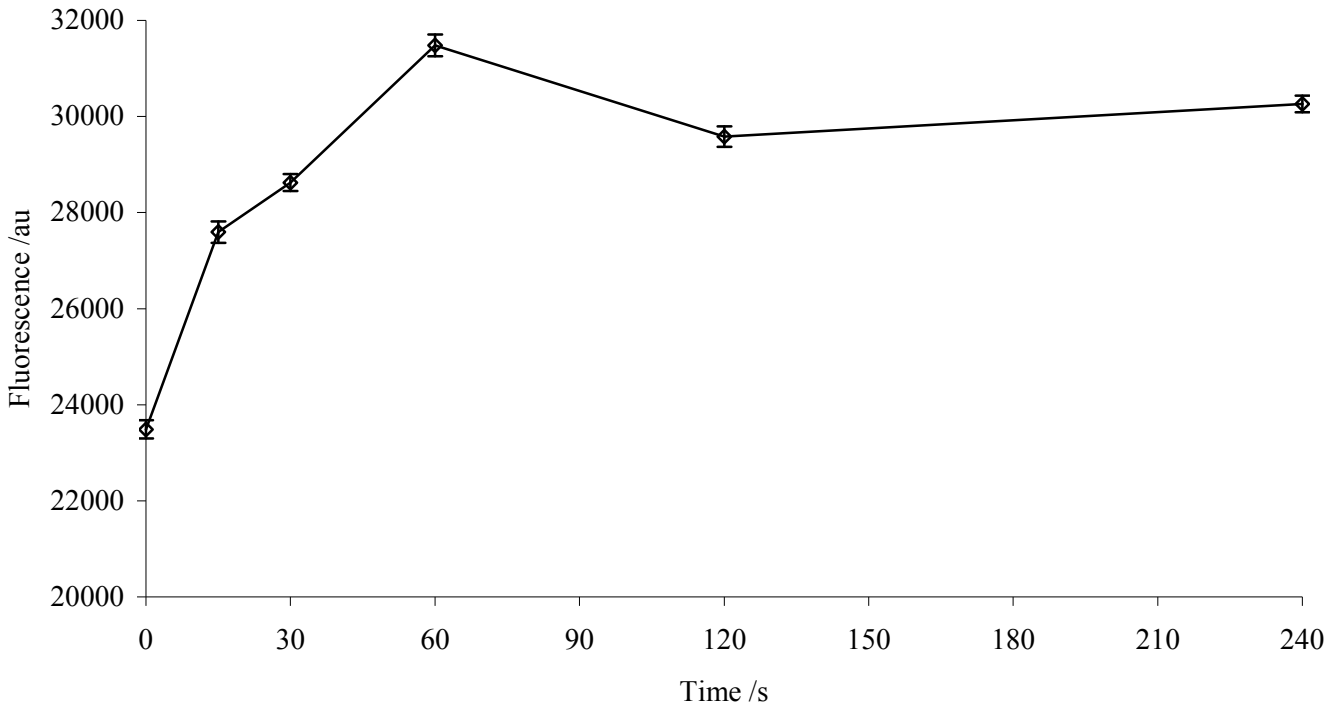


**Figure S4:** Change in fluorescence due to release of carboxyfluorescein on ultrasonic breakdown of emulsion containing microspheres with 50 mM 5,6-carboxyfluorescein as the internal aqueous phase.