SUPPORTING INFORMATION

Lysozyme-silica hybrid materials: from nanoparticles to capsules and double emulsion mineral capsules

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General

All reactants (TEOS, Lysozyme, Glycine buffer) were obtained from Merck and used without any further purification. The changes in the interfacial tension between the protein solution and the oil phase were determined at room temperature and monitored with a DSA100 pendant-drop tensiometer with a CCD-camera for drop-image processing, which allows rapid drop-image acquisition, edge detection and fitting of the Young-Laplace equation. Small blunt-end cannulas of 1.83 mm in diameter (NE44 Krüss) were used with 100 µl min^{-1} for the speed of drop-formation. A Bandelin Sonorex operating at 35 kHz at ambient temperature was used for the sonication treatment, an IKA Vortex Genius 3 operating speed 2500 rpm for vortex-mixing and a Grant-Bio Rocker PMR-3C operating at 5 oscill/min for mild shaking experiments. SEM analysis was performed on a HITACHI S-3000N or S-4800 scanning electron microscope and the EDX data were taken using an EDAX detecting unit.
TEM analysis was performed on a ZEISS LIBRA120 PLUS electron microscope, operating at 120 kV. Fluorescence and Optical Microscopy was done with a Keyence Biozero (BZ-8100E) microscope, with excitation-mode Texas Red for visualization of Nile Red. For TGA measurements a PerkinElmer STA 6000 was used operating at 10 °C/min with a gas flow of 20 ml/min (artificial air; 20% Oxygen, 80% Nitrogen).

S1

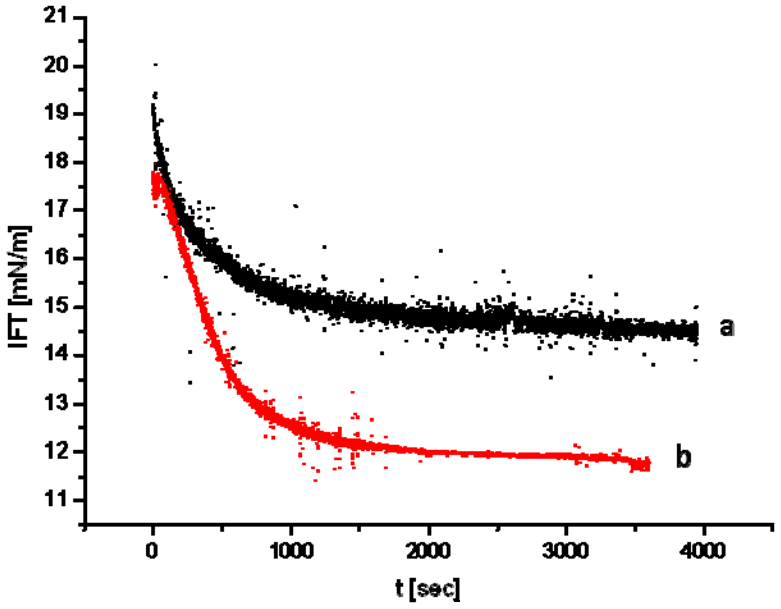


Figure S1: Interfacial tension measurements of interfaces (a) TEOS vs. Glycine buffer (pH 9) and (b) TEOS vs. lysozyme (5mg mL^{-1}) in Glycine buffer (pH 9).

S2

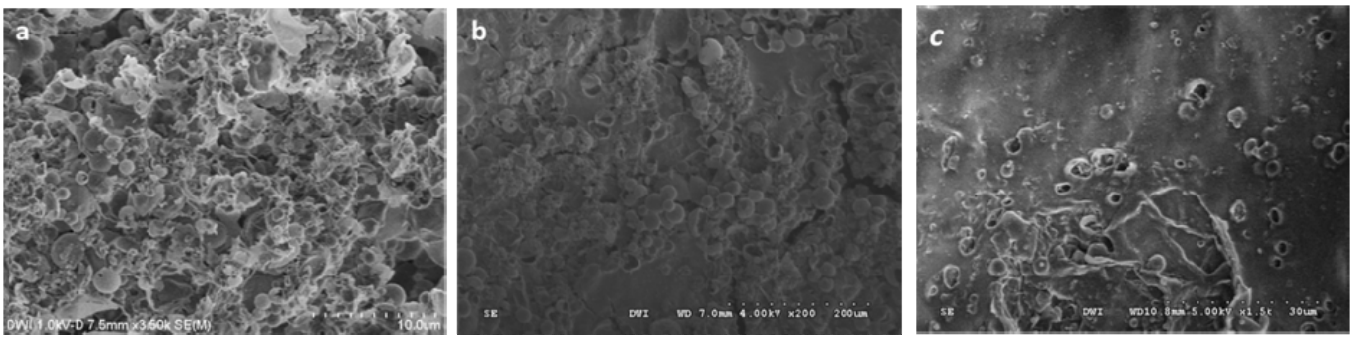


Figure S2: SEM images of hybrid lysozyme-silica capsules (**HS3**) prepared by addition of TEOS in one step to lysozyme solution (5mg mL^{-1}) followed by ultrasound treatment for 15min. Micrographs were taken after (a) 6 days (b) 10 days and (c) 1month of mineralization.

S3

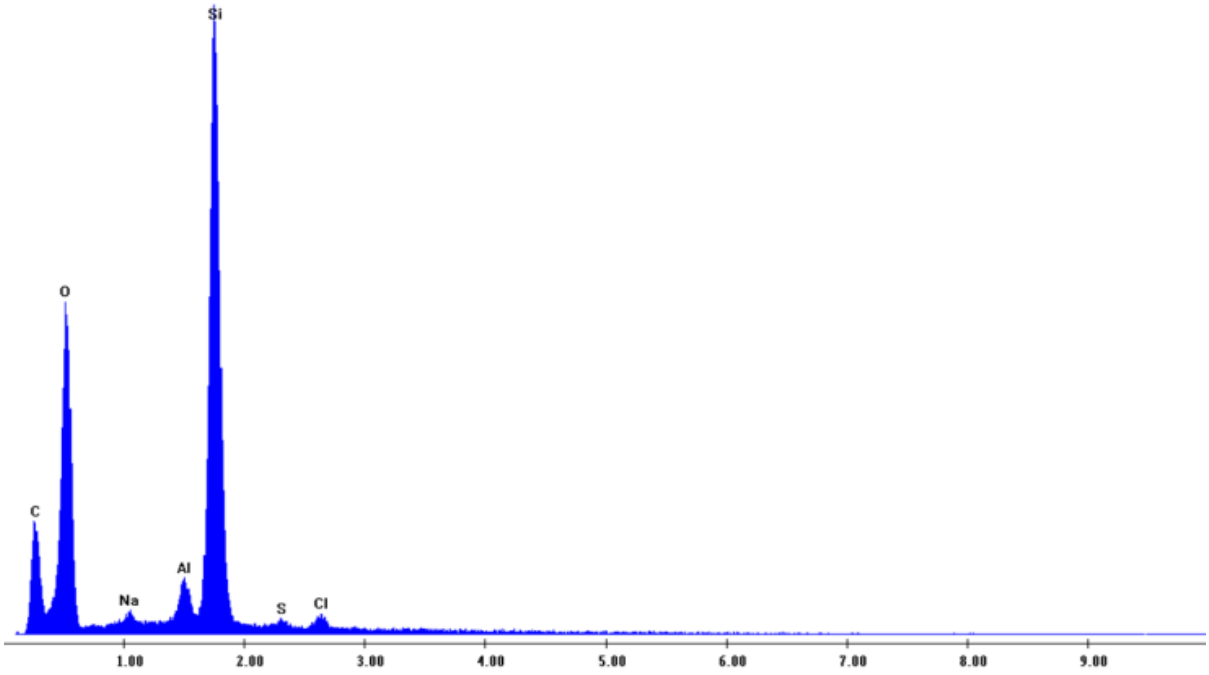


Figure S3: Typical EDX spectrum of solid hybrid nanoparticles obtained by addition of TEOS in one part to lysozyme solution (5mg mL^{-1}) after 24h mild stirring. The Na and Cl traces originate from the buffer and C and Al from the sample holder, energy in keV. The Si is from the mineralized shell and the presence of S is indicative for the protein-siliconoxide hybrid material.

S4

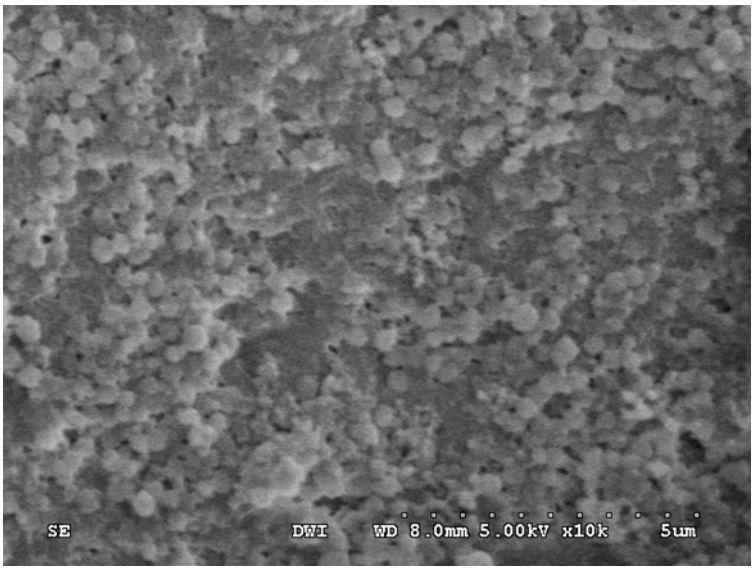


Figure S4: SEM image of the template product formed at intermediate stage (**I.M.**) in the two-step procedure after the first addition of 20μL TEOS and 30 min. of mild stirring.

S5

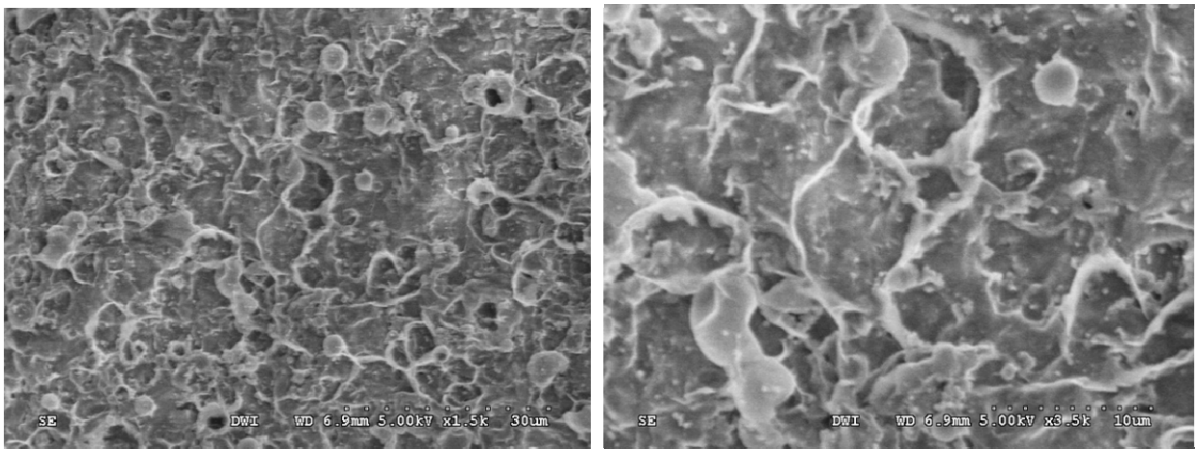


Figure S5: SEM images of hybrid lysozyme-silica capsules prepared by addition of TEOS in two steps to lysozyme solution (5mg mL^{-1}) followed by applying ultrasonic for 15min. directly after second TEOS addition after which sample was stirred mildly for 5h instead of 24h. It is observed that there is an incomplete mineralization and mostly broken and incomplete mineral shells result.

S6

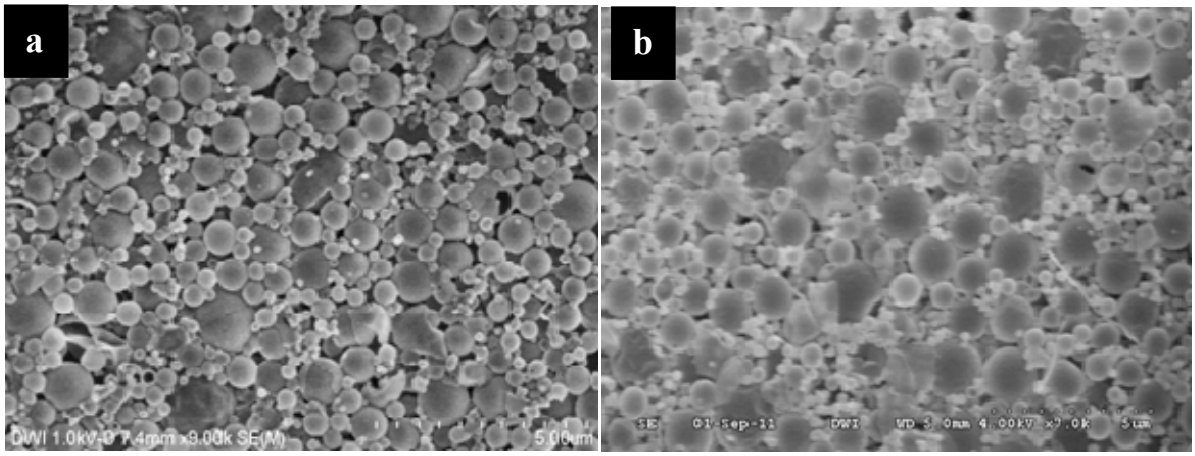


Figure S6: SEM images of hybrid lysozyme-silica capsules (**HS8**) prepared by adding TEOS in two parts to lysozyme solution (5mg mL^{-1}) followed by applying ultrasonic for 15min. directly after second TEOS addition after which samples were stirred mildly for 24 h. with applying second sonication again after stirring. Images are taken after (a) 10 days and (b) 1month mineralization.

S7

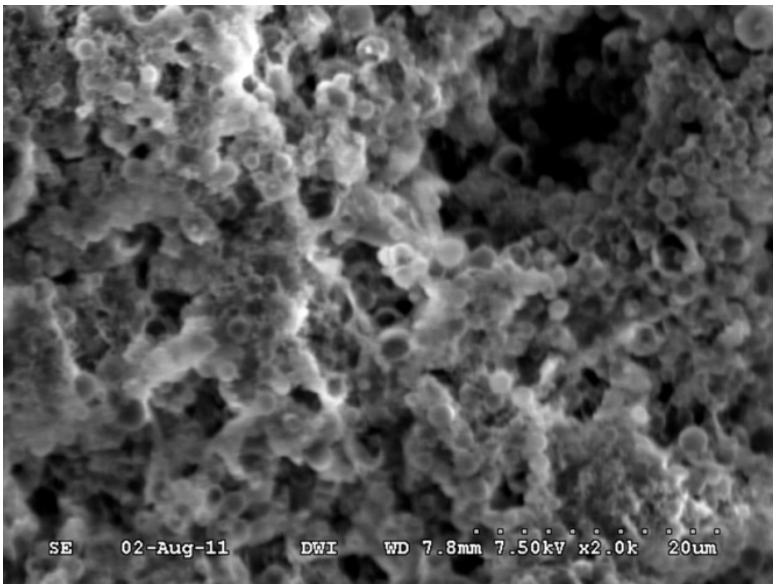
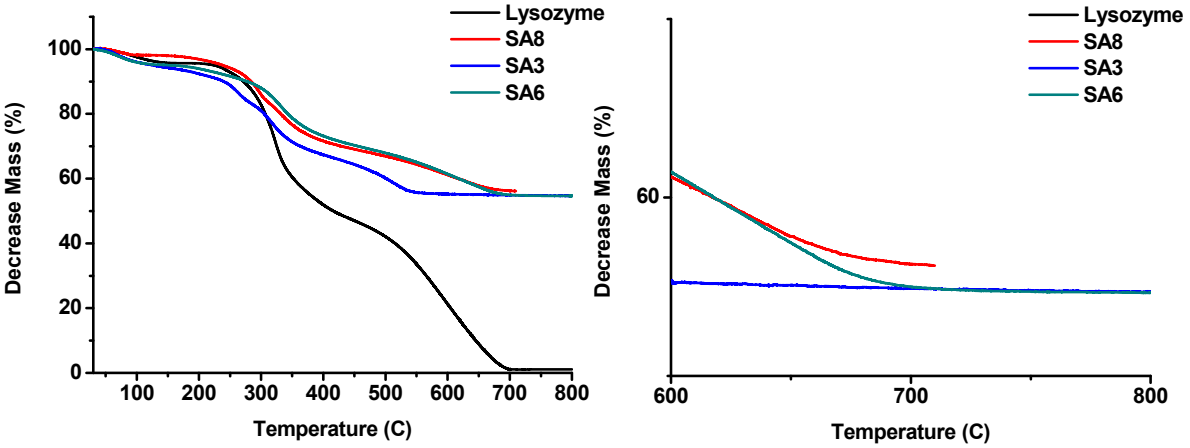


Figure S7: SEM image of hybrid lysozyme-silica hollow capsules prepared by adding TEOS in two parts to lysozyme solution (5mg mL^{-1}) followed by applying first 15min. ultrasonic directly after 30min. mild stirring after which second part of TEOS was added and samples were stirred mildly for 24 h.

S8

Thermogravimetric Analysis (TGA)



The decrease in mass and the resulting leftover mass indicates the percentage of protein and inorganic component (siliconoxide), respectively. As shown, the lysozyme in the pure form completely vanishes upon heat treatment in the presence of an air-flow (artificial air, O_2/N_2 20-80). The same transitions are observed in the hybrid structures as for the pure lysozyme, indicating that indeed it is the lysozyme that is being removed. The final composition remains relatively constant irrespectively of the method used for the preparation. The right graph shows details of the left graph in the temperature range from 600 to 800 °C.

S9

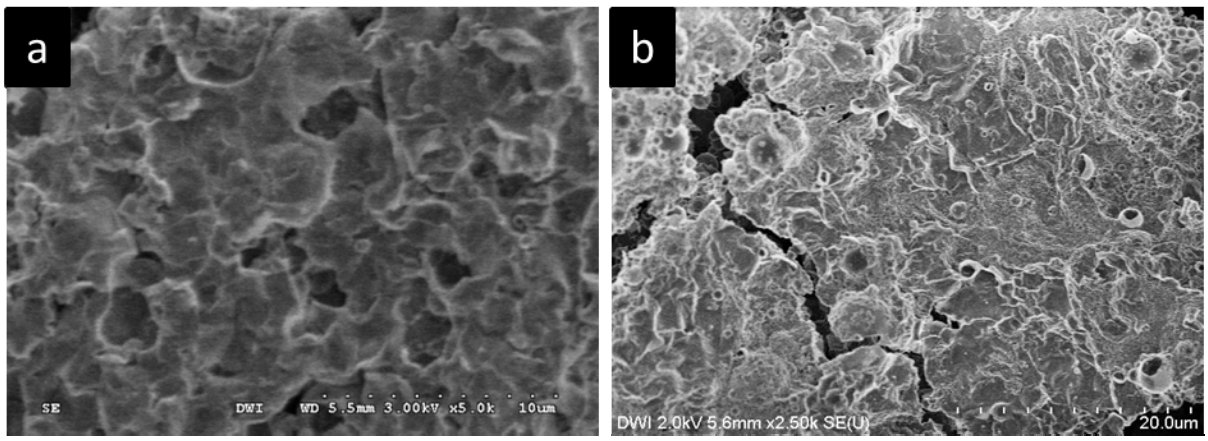


Figure S9: SEM image of particles prepared by adding TEOS to lysozyme solution (2.5mg mL^{-1}) (a) in two parts followed by applying sonication for 15min. directly after second TEOS addition after which the sample was stirred mildly for 5 h in a similar fashion as for **HS9** but with less reaction time (normally 24h) resulting in again an incomplete mineralization and (b) in one batch followed by applying ultrasonic for 15min which is also not suitable to produce fully mineralized capsules. It depicts that the exact treatment of the samples is crucial for structure formation.

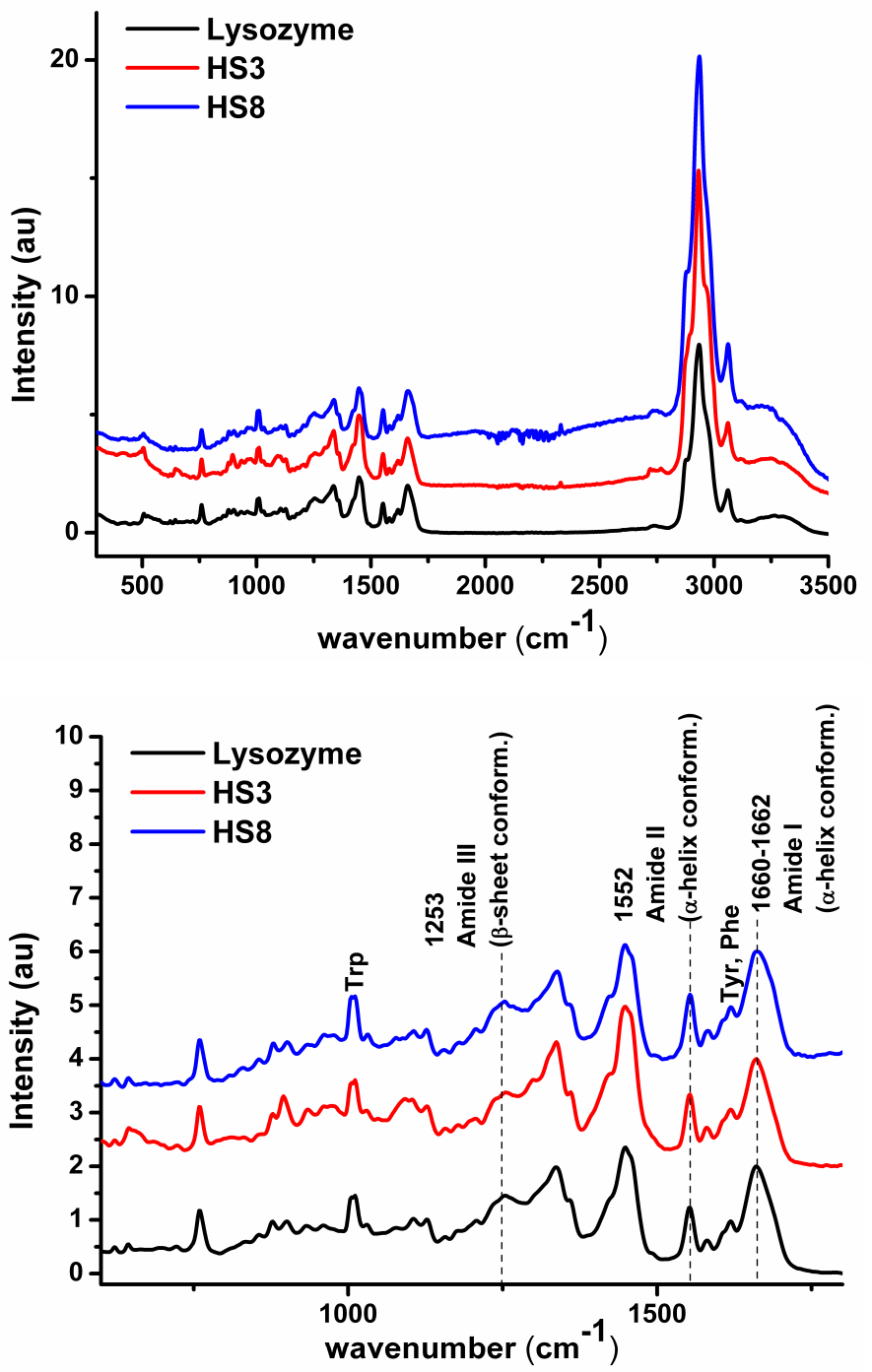


Figure S10: A comparative raman-study on the secondary structural features of pure lysozyme compared to the lysozyme embedded inside the silicon dioxide in the one-step approach (HS3) and two-step approach (HS8). All structural features like the the β-sheet as well as the α-helix motifs can be identified (assignment based on: A. Hedoux, Y. Guinet, and L. Paccou, *J. Phys. Chem. B*, 2011, **115**, 6740–6748/ Z. Chi, X. G. Chen, J. S. W. Holtz, and S. A. Asher, *Biochemistry*, 1998**, 37***,* 2854-2864). The relative intensities deviate showing higher intensities for pure lysozyme indicating that there is some loss of secondary structure upon mineralization.

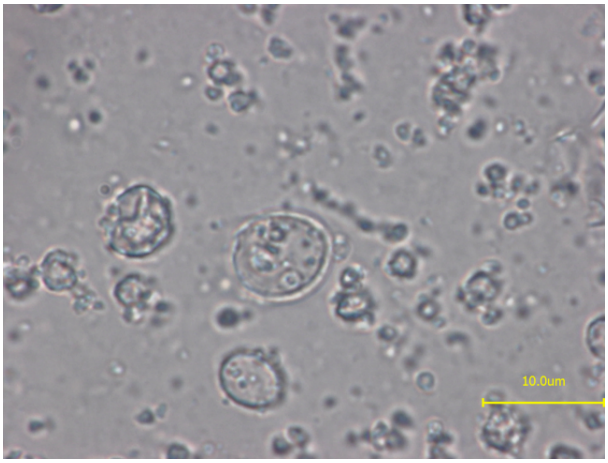


Figure S11: Optical microscope image of double emulsion (sample **HS10**).