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Supplementary Information (SI)

Entrapment and release kinetics study of dyes from BSA microspheres forming a matrix and a reservoir system

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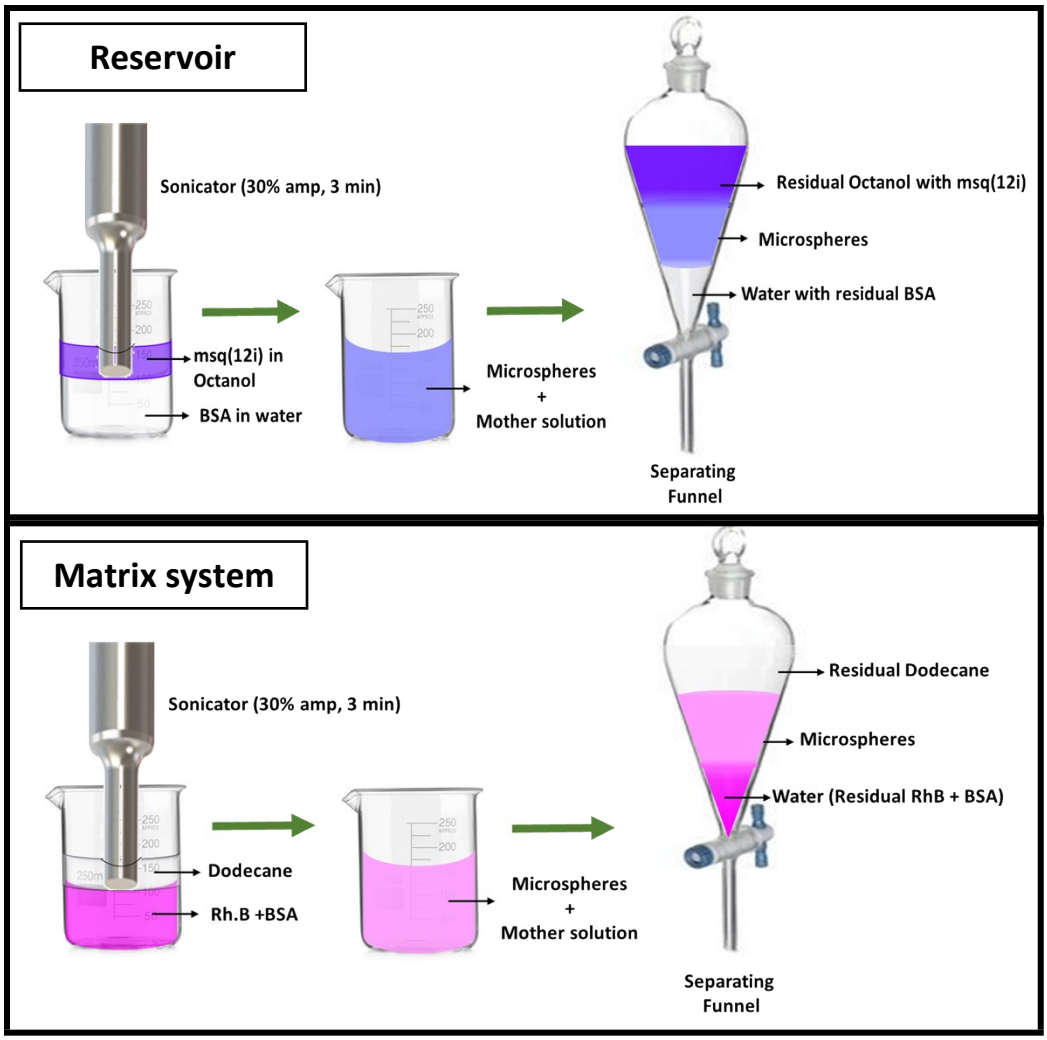

Fig. S1: Schematic presentation of the sonochemical synthesis and separation of the BSA microspheres containing Rhodamine B (matrix system) and MSQ (12i) (reservoir system)

1. Choosing the model systems

Several combinations of the types of dye, its initial concentrations and the types of organic phase were tried in order to find suitable matrix and reservoir systems for the leaching experiments. The requirements were: (1) Complete dissolution of the dye in water (matrix system) or in the organic solvent (reservoir system), (2) Good phase-separation after the sonication in a reasonable time. (3) Strong and well resolved absorption bands for the dyes. For the matrix system, Congo red, Brilliant blue, Evans blue, Coumarin, Rhodamine B were examined as dyes, and we selected Rhodamine B and Congo red. Three concentrations of Rhodamine B were tested (0.1, 0.25 and 0.5 mM) showing better phase-separation with the lower concentration. As for the organic phase, dodecane was found to be superior over kitchen oil (poor phase-separation) or octanol (poor dye solubility). For the reservoir system, Quinoline yellow dissolved in dichloromethane or Toluene and Curcumin dissolved in octanol were examined. However, the best combination was found to be a 0.1 mM solution of the dye MSQ (12i) in octanol and Nile red in octanol.

2. Size measurements

Dynamic Light Scattering was used to determine the average size of the microspheres and their size distribution. Fig. S3 presents the DLS spectrum of pristine BSA microspheres. It is composed of seven batches that were prepared in a similar manner, two samples were taken from each batch and each of them was measured three times.

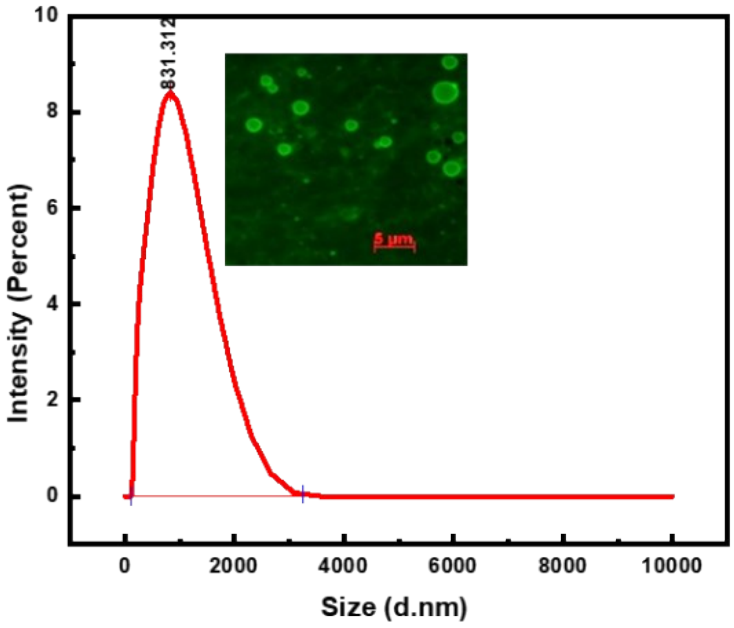

Fig. S2: Combined DLS spectra of seven batches of BSA microspheres prepared under the same conditions. Two samples from each batch were taken and each of them was measured three times.

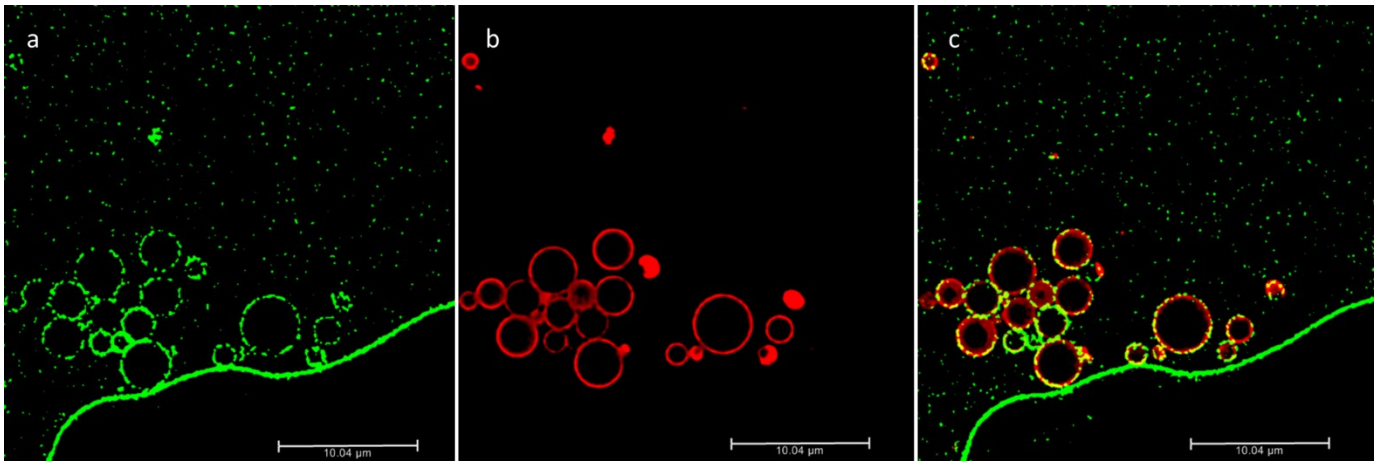

Fig. S3: Confocal images of BSA microspheres encapsulating Rhodamine B: **(a)** Excitation at 470 nm of BSA only, **(b)** excitation at 575 nm of Rhodamine B and **(c)** overlap image of (a) and (b) showing the respective positions of BSA and Rhodamine B in a microsphere.

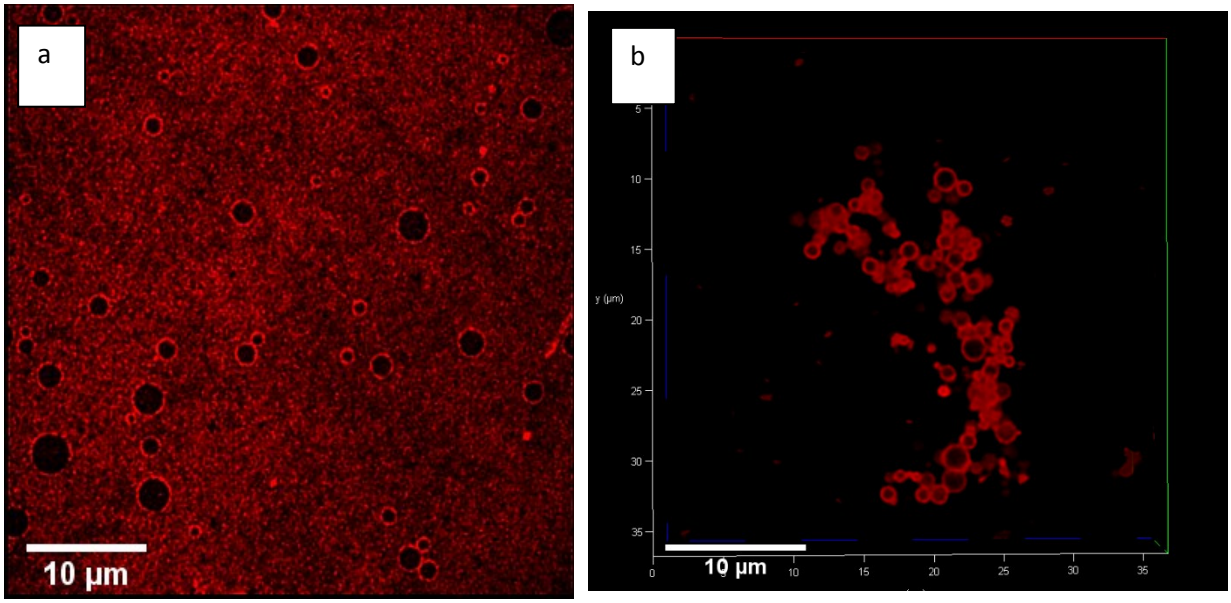

Fig S4: Confocal microscope image of BSA microspheres. **(a)** after reaching a plateau in the leaching experiment. **(b)** Plain microspheres that have been immersed for 24 h in a 0.1 mM aqueous solution of Rhodamine B.

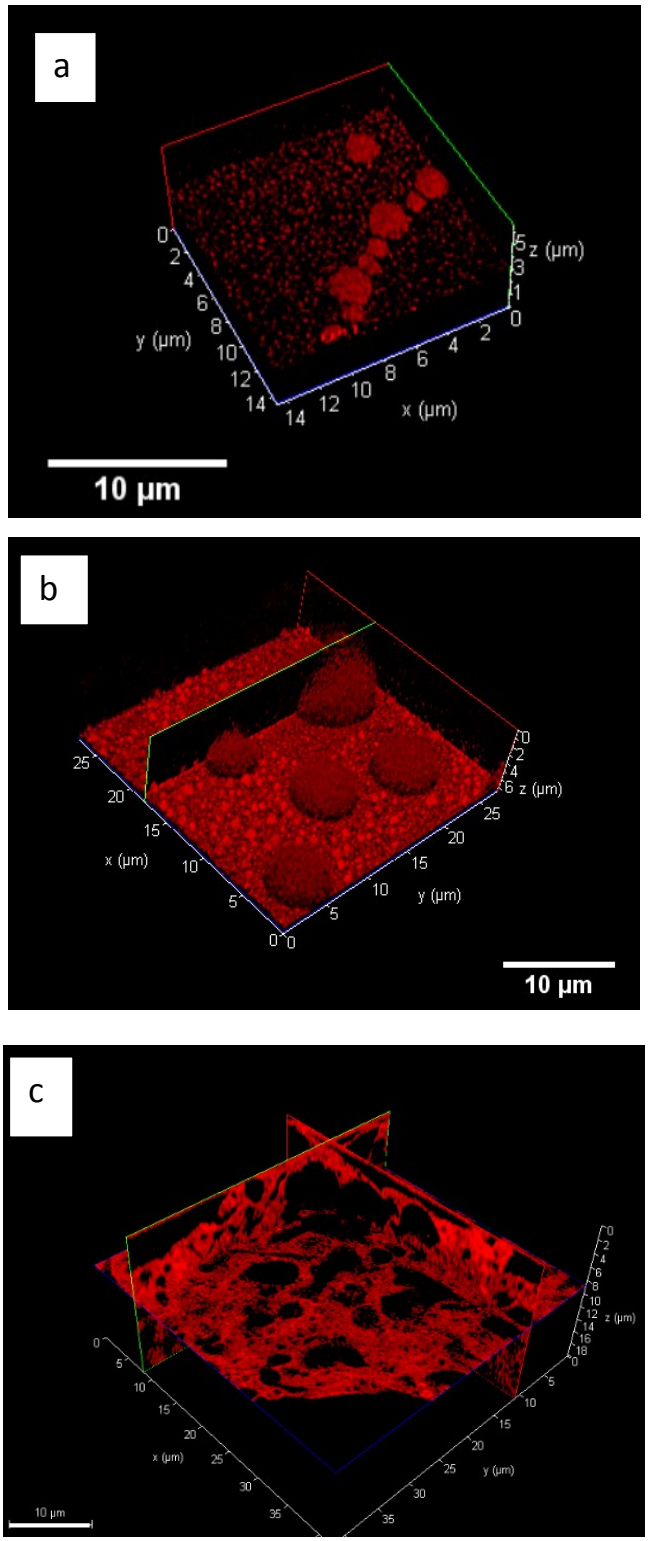

Fig. S5: 3D Confocal microscope images of the system BSA/octanol/MSQ (12i) **(a)** Right after immersion in octanol. **(b)** After 60 mins immersion in octanol **(c)** after total leaching (around 5 hours) of the dye.

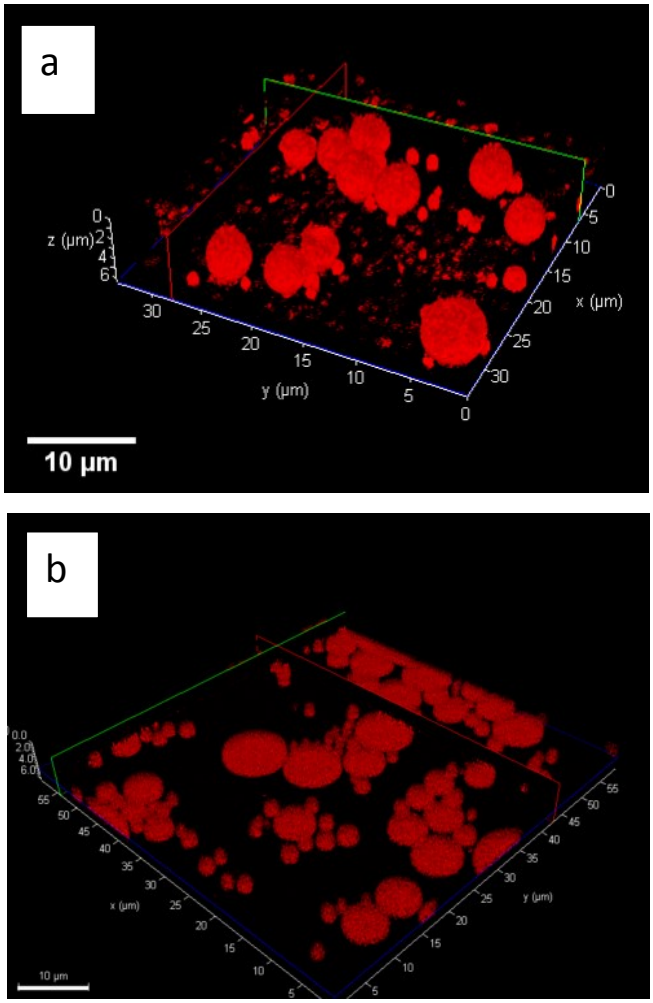

Fig. S6: 3D Confocal microscope images of samples of the system BSA/octanol/Nile red at various stages of the leaching experiment. **(a)** before leaching, **(b)** after 3 hours leaching.