Electronic Supplementary Material (ESI) for Analyst.

Monodisperse spheres-on-sphere silica particles for fast HPLC separation of peptides and proteins

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Supplementary information

Experimental Details

Chemicals. All chemicals, peptides and proteins were obtained from Sigma Aldrich and used as received, with the exception of Gly-Tyr which was obtained from TCI. All solvents used were analytical grade, except acetonitrile (HPLC grade). Distilled water was used during particle preparation and Milli-Q water (18 MΩ) was used for HPLC testing. The Accucore 150-C4 100 x 2.1 mm 2.6 µm column was purchased from Crawford Scientific.

Reaction conditions to produce standard SOS particles. 0.25 g PVA (MW = 10k) and 0.1 g CTAB were dissolved in 5mL distilled water. 8 mL methanol was added with stirring, followed by 2 mL of 5.6% ammonium hydroxide solution. Reaction was stirred vigorously for 15 minutes before adding 500 µL MPTMS dropwise over 30 seconds. The reaction was allowed to stir at room temperature for at least 3 hours.

Reaction conditions to produce monodisperse SOS particles. 0.25 g PVP (MW = 10k) and 0.05 g CTAC (25% solution) were dissolved in 5 mL distilled water. 8 mL methanol was added with stirring, followed by 2 mL of 1.4% ammonium hydroxide solution. Reaction was stirred vigorously for 15 minutes before adding 400 µL MPTMS dropwise over 30 seconds. The reaction was allowed to stir at room temperature for at least 3 hours.

Microwave bonding. All microwave reactions were performed using a closed vessel method on a CEM Explorer reactor. Typically, 1.0 g of the calcined SOS particles were dispersed in 7.5 mL toluene, 0.5 g butyl(chloro)dimethyl silane (C4) and 0.1 g imidazole and stirrer bar were added, vessel sealed and placed into the reactor. The reaction was microwaved for 20 minutes at 110 °C, at a power setting of 200 W. Resultant particles were thoroughly washed with toluene, methanol, 50% aqueous methanol, then methanol. End-capping was performed using the same method with 0.5 g 1-(trimethylsilyl)imidazole in place of C4 and the omission of imidazole.

HPLC column packing method. 0.6 g of C4 bonded SOS silica was dispersed in 30 mL slurry solvent comprised of chloroform, methanol and isopropanol (72:8:20 ratio by volume). The slurry was sonicated for 3 minutes before packing into a 100 × 2.1 mm stainless steel HPLC column using a Knauer K-1900 packing pump. The push solvent was methanol pumped at a pressure of 600 bar until 50 mL had passed through the column.

Characterisation. Particle morphologies were observed with a Hitachi S4800 scanning electron microscope (SEM). Samples were coated in gold using an Emitech K550X sputter coater for 2 minutes at 25 mA. Particles were calcined using a Carbolite CWF1200 furnace by heating in air at 1

°C/min to the desired temperature, holding for 300 minutes and allowing to cool to room temperature. The surface area by nitrogen adsorption at 77 K was measured using a Quantachrome NOVA 4200e adsorption analyser. Samples were degassed overnight at 120 °C before analysis. Carbon content was analysed using a Thermo FlashEA 1112. Particle sizing and distributions were obtained using a Beckman Coulter Multisizer 3 at Thermo Scientific (Runcorn). Samples were prepared by dispersing in Isoton II electrolyte and analysed using a 50 µm diameter aperture and 800 µA current for 30000 counts. For HPLC separation, a Thermo Scientific Accela system was utilised. Data analysis was performed using ChromQuest 5.0 software, version 3.2.1 (2008, Thermo Fisher Scientific).

HPLC test mixtures. The peptide test mixture was composed of Gly-Tyr, Val-Tyr-Val, met-Enk, leu-Enk and Angiotensin II (all 25 µg/mL). The protein test mixture components were ribonuclease A (25 µg/mL), insulin (100 µg/mL), lysozyme (25 µg/mL), myoglobin (25 µg/mL), carbonic anhydrase (100 µg/mL) and ovalbumin (100 µg/mL). The large protein text mixture components were BSA (1 mg/mL), myoglobin (50 µg/mL) and thyroglobulin (1 mg/mL).

Reproducibility and stability of the SOS particles: All SOS particles produced by the modified method are monodisperse with d90/10 ranging from 1.36 to 1.50 and a mean particle size of 2.6 to 3.0µm. Morphology is consistent between separate reactions, with all particles having a complete shell of nanospheres of similar sizes.

The stability of the SOS particles has been assessed by dispersing un-bonded particles in water using a sonic bath for up to 60 minutes. These particles were then imaged using SEM to determine if any nanoparticles had been displaced from the surface. No free nanoparticles were observed in the SEM image. Bonded SOS particles were packed into a stainless steel HPLC column and tested with the peptide and protein test mixtures under the conditions described in this article. Following testing, the column was then unpacked and the particles imaged by SEM to see if any nanoparticles had been displaced from the surface or if any particles had been crushed or broken by the high-pressure conditions. Again no free nanoparticles were observed and all particles were intact indicating that these SOS materials are robust enough to withstand the operating pressures of a HPLC system.

Supporting Figures

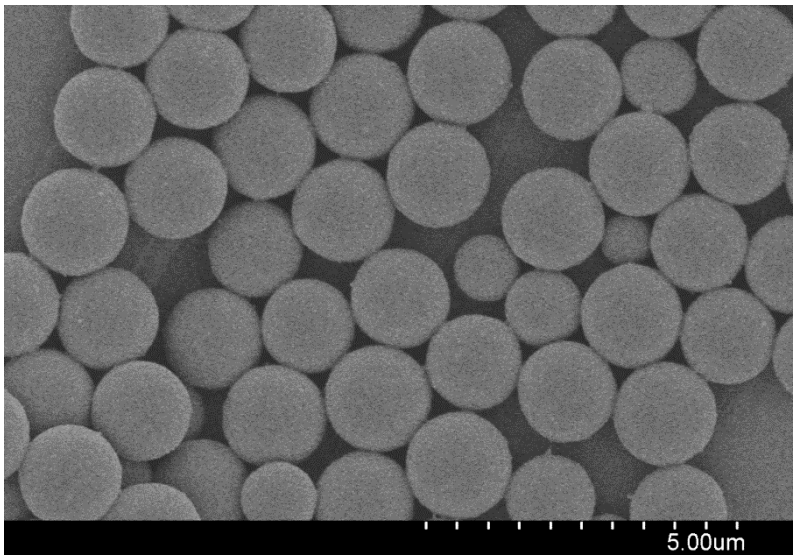

Fig. S1 SEM image of smooth spherical particles. Standard SOS reaction conditions modified by replacing PVA with PVP (MW = 10k). All other conditions remained the same.

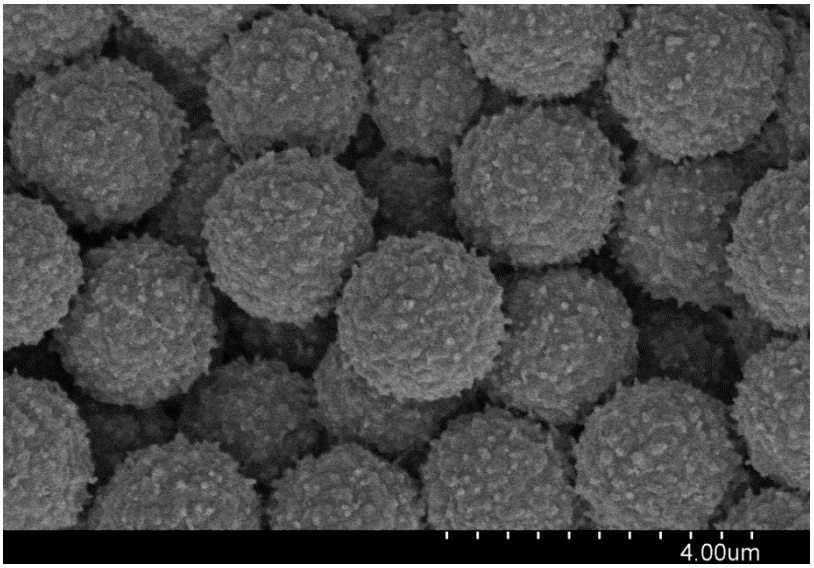

Fig. S2 SEM image of bumpy particles. Standard SOS reaction conditions modified by replacing PVA with PVP (MW = 10k) and omitting CTAB. All other conditions remained the same.