Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supplementary Information

Synthesis of Polymers and Nanoparticles Bearing Polystyrene Sulfonate Brushes for Chemokine Binding.

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Table of contents

Materials

Tetraethyl orthosilicate (TEOS, 95%) was purchased from Acros Organics. 1-Butanethiol, (3-aminopropyl)triethoxysilane (APTS), 4-(Dimethylamino)pyridine (DMAP), 1,1'-azobis(cyclohexane-1-carbonitrile) (V40, 98%) and sodium 4-vinylbenzenesulfonate (**1**) were purchased from Sigma Aldrich. 4,4'-azobis(4-cyanovaleric acid) was purchased from VWR. Ammonia solution (NH_3, 28%), absolute ethanol, silica gel 63 µm, dichloromethane (DCM), carbon disulfide (99.9%), tetrahydrofuran (THF, 99%), dimethylformamide (DMF), magnesium sulfate ($MgSO_4$) and potassium hydroxide (KOH) were obtained from AJAX Chemicals. Hexane (99%), acetone (99%), methanol (MeOH, 99%) and ethanol (EtOH, 95%) were supplied by Redox Chemicals. Ethyl acetate (99.5%), 1,2 Dimethoxyethane, Toluene (99.5%) and *p*-tosyl chloride (≥98%) were obtained from Merck. N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC•HCl) was obtained from GL Biochem.

Analyses

Transmission electron microscope (TEM) images were obtained on a JEOL 1400 120 kV TEM with a LaB6 filament running Gatan Digital Micrograph software. Size distribution of TEM images were also analysed using Image J software. Size distributions of particles in colloidal suspensions in Milli Q water and DMF solvents were analysed using Dynamic Light Scattering (DLS) conducted on a Malvern High Performance Particle Sizer running Dispersion Technology Software (Version 4.00). Mass of tethered RAFT molecules and polymers were analysed *via* Thermogravimetric Analysis (TGA) conducted on a TA Instruments Hi-res TGA 2950 instrument using Thermal Advantage v1.1A software. The instrument was continuously purged with N_2 gas. Samples were heated to 100 °C and equilibrated at 100 °C to remove residual water prior to analysis. Data was analysed using Universal Analysis 2000 software v4.2E by TA Instruments.

Analysis of compounds and polymerization reactions was carried out by ^1H-NMR spectroscopy acquired at 300 or 200 MHz. D_2O, DMSO-D_6 or $CDCl_3$ were used as the solvents as indicated.

Molecular weight distributions were recorded on a Shimadzu modular GPC system, comprising DGU-12A solvent degasser, an LC-10AT pump, a CTO-10A column oven, and a RID-10A refractive index detector. The analyses were carried out at 50 °C using a flow rate of 1mL/min with an aqueous solution of NaN_3 (0.5%w/v) as the eluent. The system was equipped with a PL5.0 mm guard column (50×7.8 mm^2) followed by four linear PL columns (10^5, 10^4, 10^3, and 500). Calibration was performed with PEG/PEO standards ranging from 500 to 10^6 g/mol.

Synthesis of silica nanoparticles via Stöber synthesis

A modification of the Stöber synthesis [1, 2] was used for the synthesis of the silica core. A mixture of 26.9 g of a 28% ammonia solution and 223 mg of absolute EtOH were mixed and stirred in a 70 °C oil bath under a closed system with a condenser at 0 °C and a nitrogen filled balloon. A second solution of 53.3 g of absolute EtOH and 12.5 g of TEOS were mixed before adding to the reaction at a rate of 1.6mL/min with constant stirring. Following addition (after ca. 25 min) the mixture became cloudy and was stirred for a further 1 h before cooling. The reaction was washed with absolute EtOH (4 x 20 mL) *via* centrifugation at 14000rpm for 20 minutes. Washing was deemed satisfactory once the supernatant of the centrifuged sample had a pH of 7.

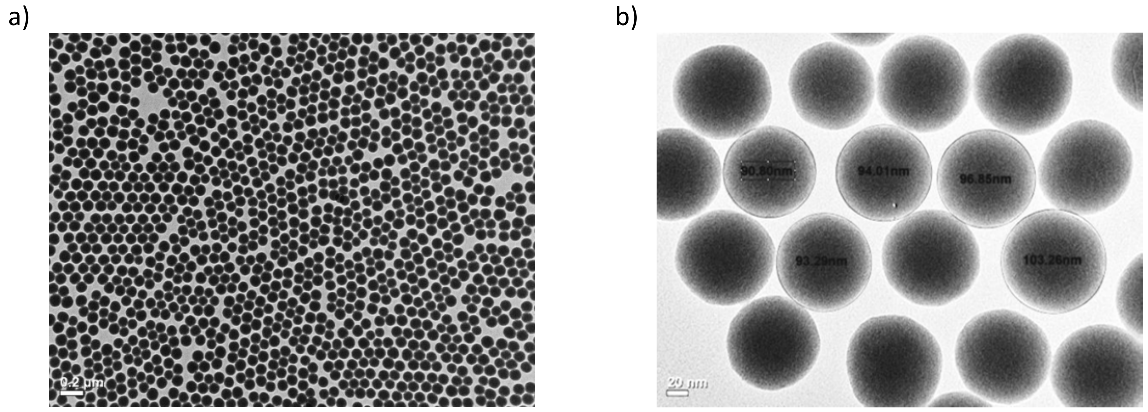

Figure 1: TEM images of silica particles synthesised by Stöber synthesis.

Mean	92.13757
Standard Error	0.776431
Median	91.69611
Mode	93.35091
Standard Deviation	6.259788
Minimum	78.50219
Maximum	108.7547

Table 1 : Results from Image J analysis of TEM images of synthesised silica particles

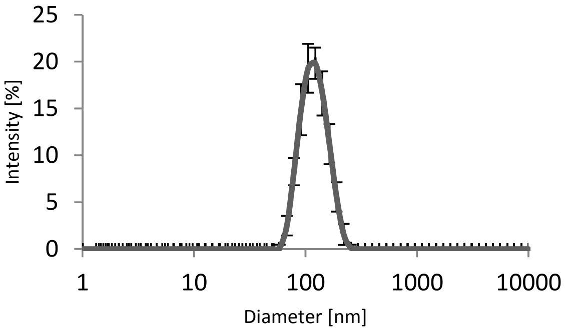

Figure 2: Size distribution of silica particles in EtOH measured by DLS

Characterisation of CBP-RAFT agent 2

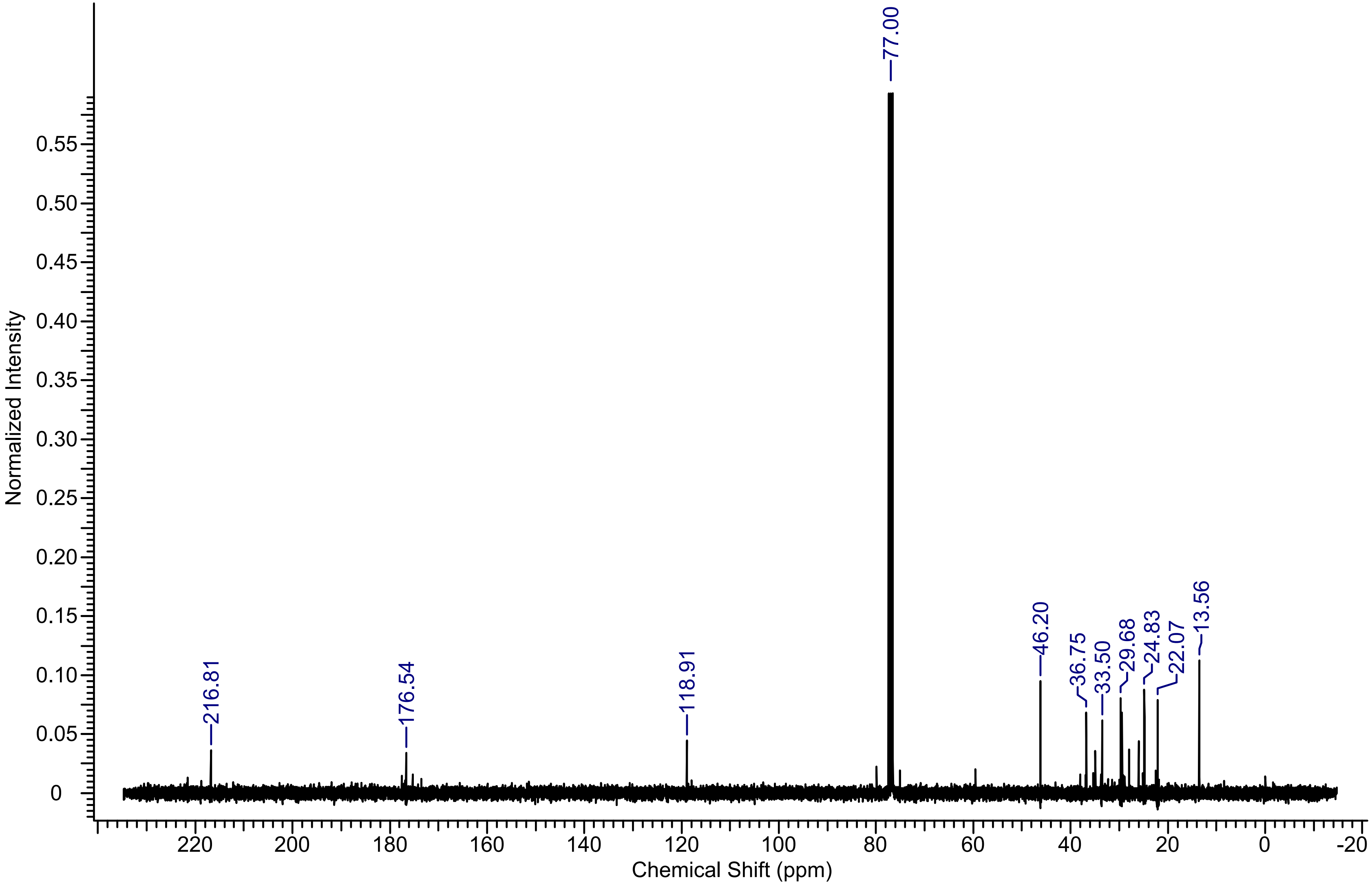

Figure 3: ^{13}C NMR spectrum of CBP-RAFT agent 2 in $CDCl_3$

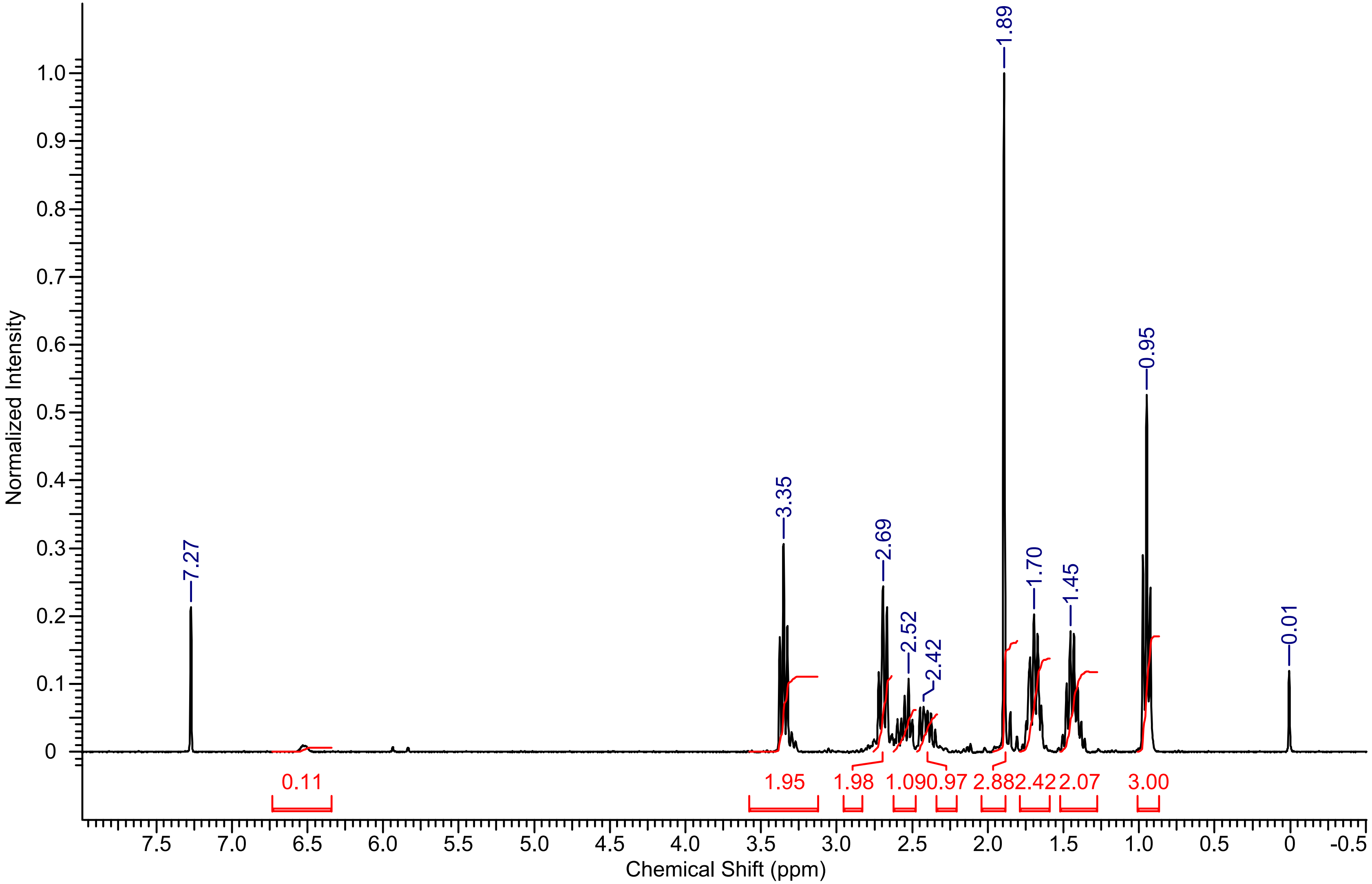

Figure 4: ^{1}H NMR spectrum of CBP-RAFT agent 2 in $CDCl_3$

Characterisation of triethoxysilane functionalised CBP-RAFT agent 6

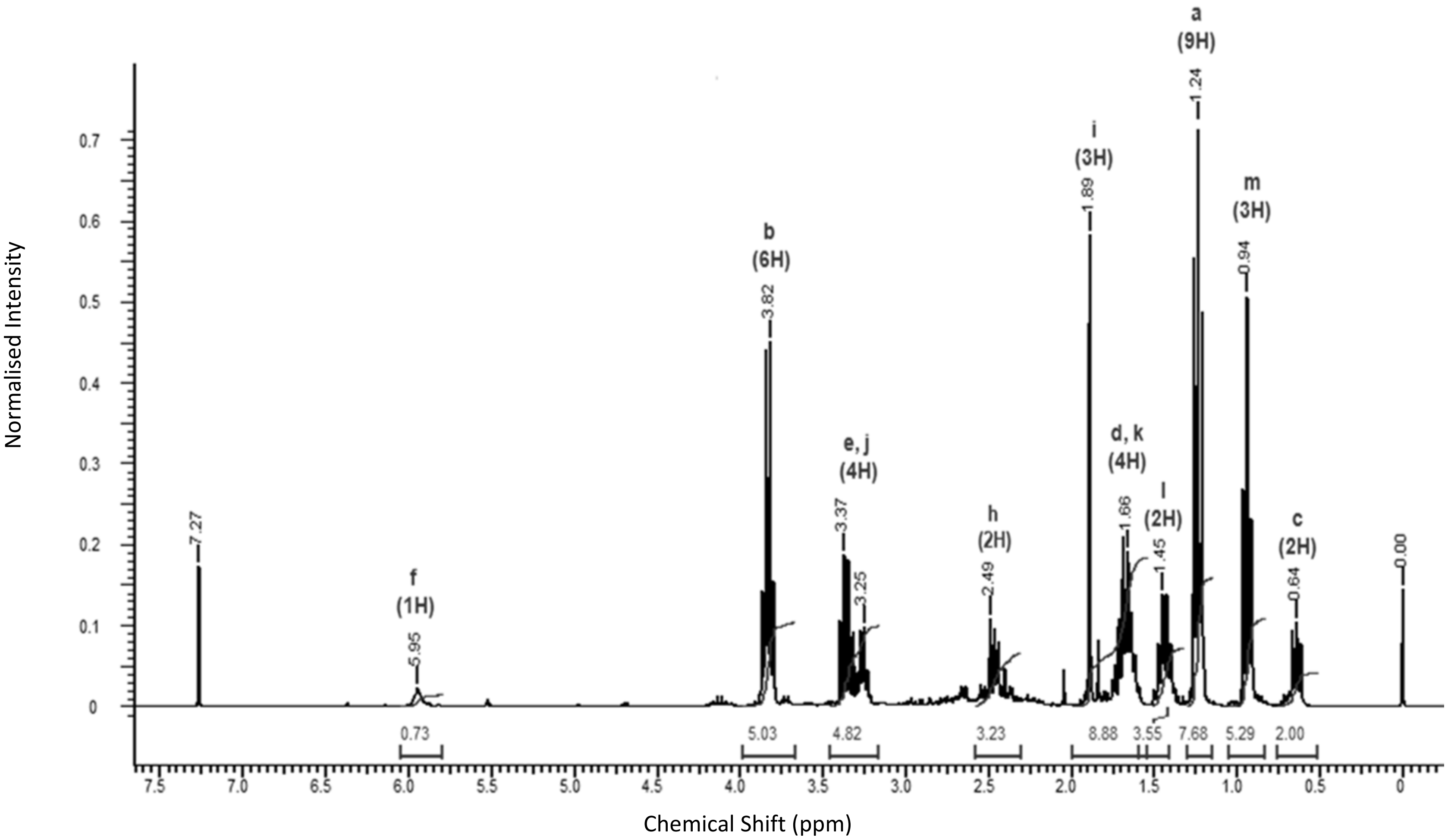

Figure 5: ^{1}H NMR spectrum of CBP-RAFT functionalised with triethoxysilane groups 6.

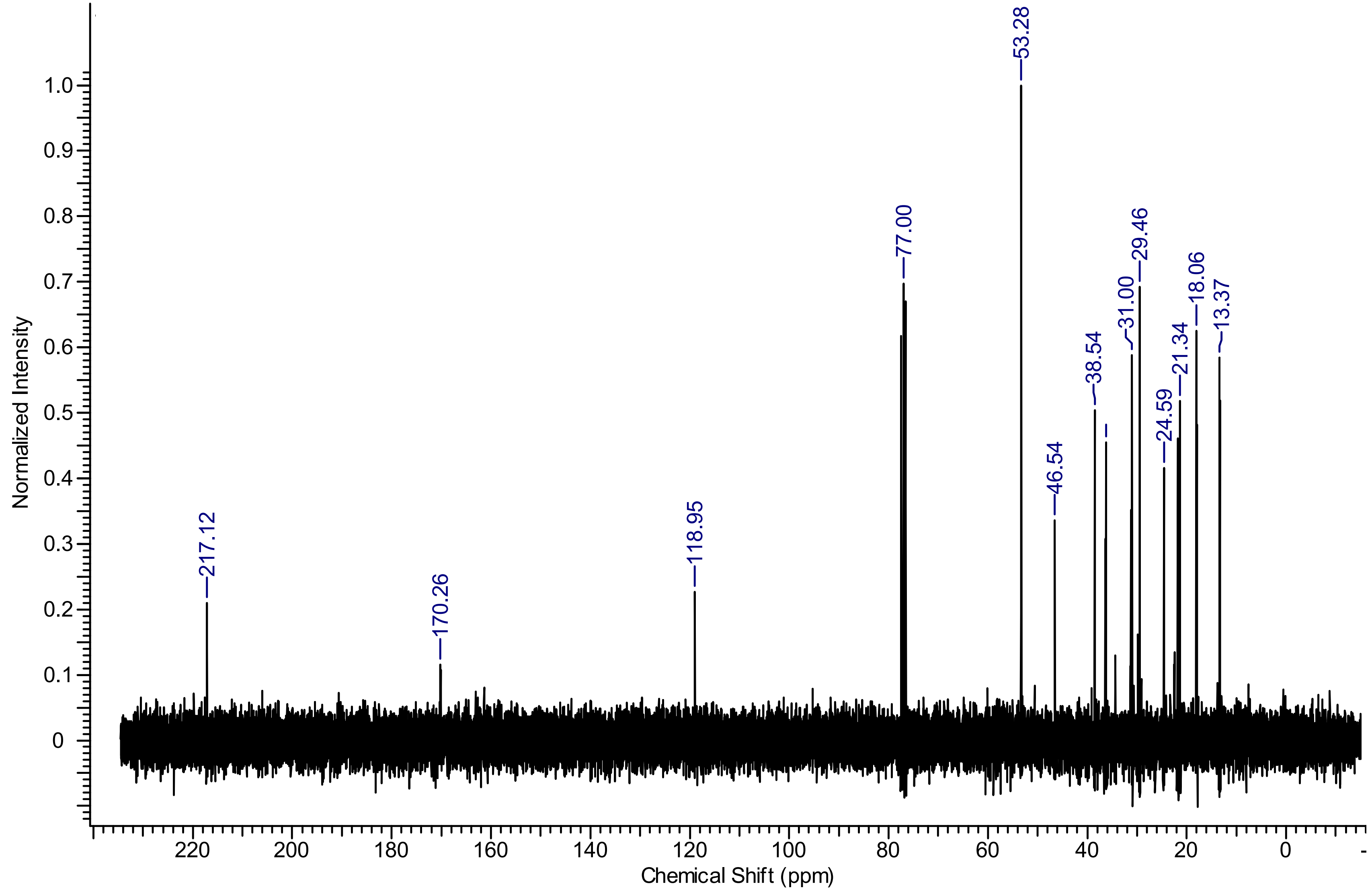

Figure 6: ^{13}C NMR spectrum of CBP-RAFT functionalised with triethoxysilane groups 6.

Characterisation of functionalised particles 9-11

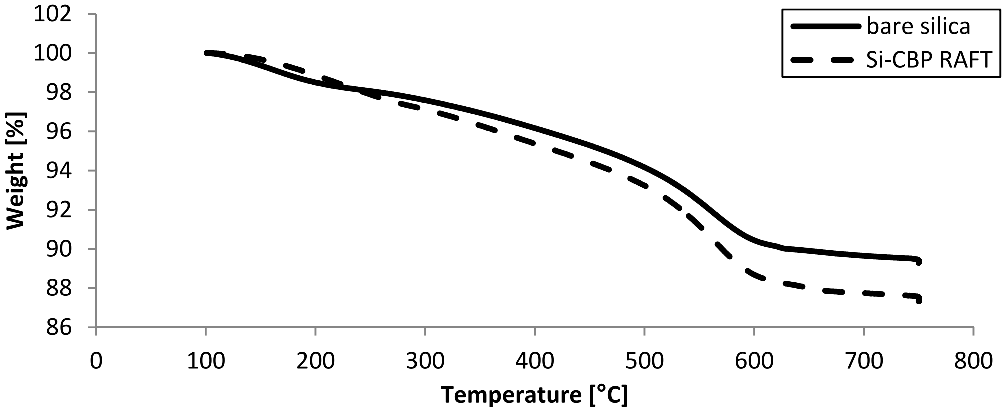

Figure 7: TGA of silica particles before and after functionalisation with triethoxysilane CBP-RAFT agent.

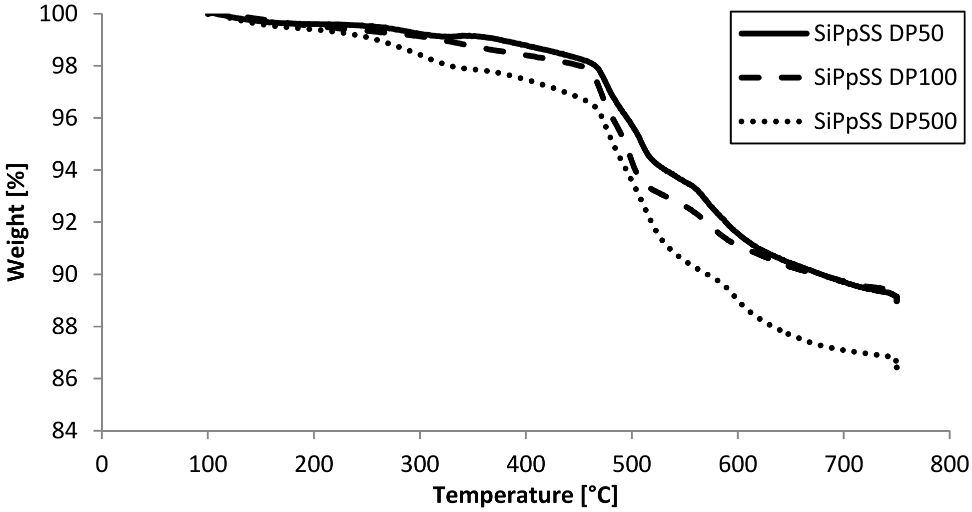

Figure 8: TGA of particles modified with pSS brushes

A grafting density of 0.967 chains/nm^2 of polymer was calculated from the values obtained from TGA using equation 1. This unusually high grafting density is due to the functionalisation of silica particles with a triethoxysilane funtionalised RAFT agent forming a possible multi-layer.

$$\sigma = \frac{\rho D N_A m_{(organic)}}{6 M_{CTA} m_{(Si)}} \tag{1}$$

σ is the grafting density in groups/nm^2, ρ is the density of silica nanoparticles (1.9 x 10^{-21} g/nm^3), D is the average diameter of core particles, $m_{(organic)}$ and $m_{(Si)}$ are the mass for RAFT agents and bare silica nanoparticles acquired from TGA.

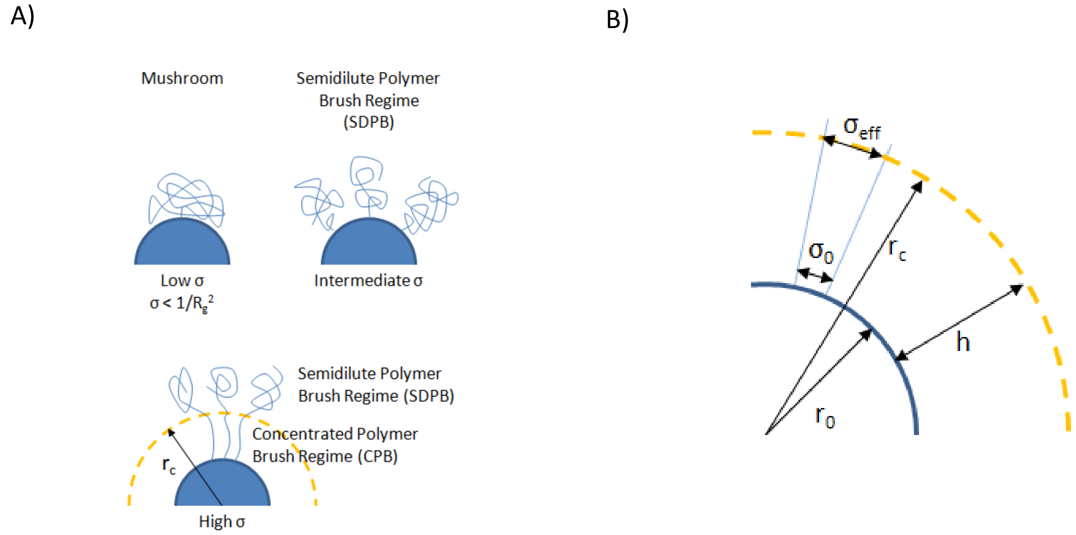

Figure 9: Polymer brush configurations on curved surfaces

The effective grafting density of the polymer brush, $\sigma_{eff,}$, decreases with increasing radial distance (shown in Figure 9b) and can be calculated accordingly using equation (2). Here, σ_0, the graft density on the core surface is 0.967 groups/nm^2, and r is the distance from the core center. When the σ_{eff} is high enough (greater than 0.03), the polymer brushes are said to fall within the concentrated polymer brush (CPB) regime, where the excluded-volume effect is screened out [3, 4].

$$\sigma_{eff} = \sigma_0 \left(\frac{r_0}{r}\right)^2 \qquad (2)$$

Using the following equation, the σ_{eff} for particles decorated with p(SS) brush of DP 50, 100 and 500; the σ_{eff} of these particles are 0.310 chains/nm^2, 0.240 chains/nm^2 and 0.208 chains/nm^2, respectively. With a σ_{eff} falling well above 0.03, it suggests that the polymer brushes on these particles exhibit a CPB regime even if the charges of the polymer brushes were neutralized by counterions [4], thus removing the possible problems of changes in polymer brush conformations due to its radius of gyration (R_g) affecting access to sulfonate groups within the brush layers.

Characterisation of functionalised particles 9-11 in 50 mM MOPS buffer vs water

1 wt% of particles were measured in 50 mM MOPS buffer and water to provide a direct comparison of brush conformations in the presence of buffering salts used in the fluorescence anisotropy chemokine binding assay.

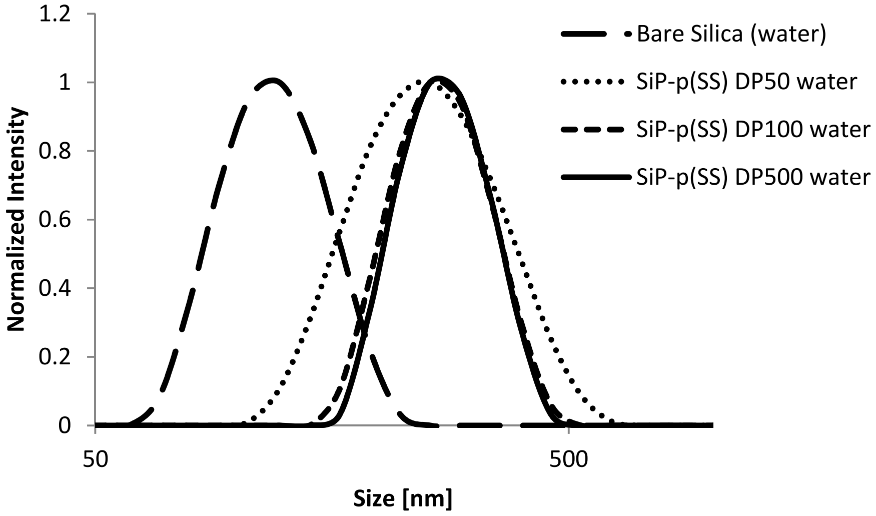

Figure 10: Size distribution of 1 wt% of hybrid nanoparticles in water.

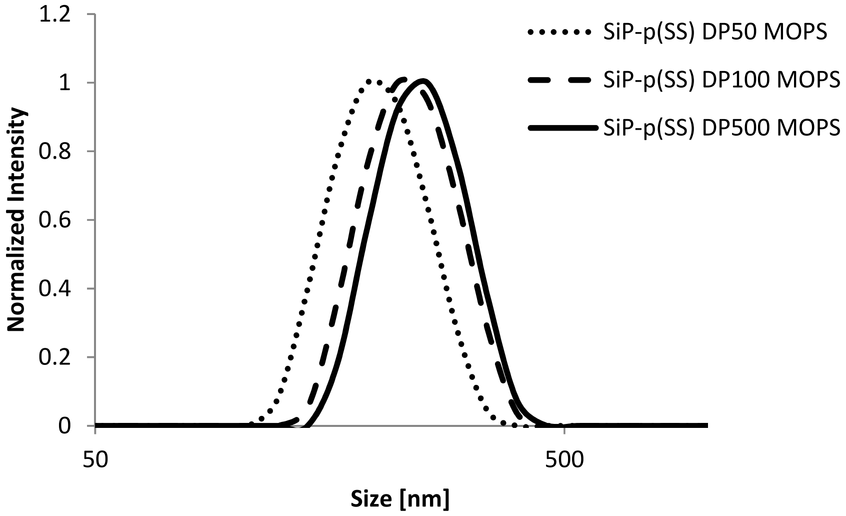

Figure 11: Size distribution of 1 wt% of hybrid nanoparticles in 50 mM MOPS buffer.

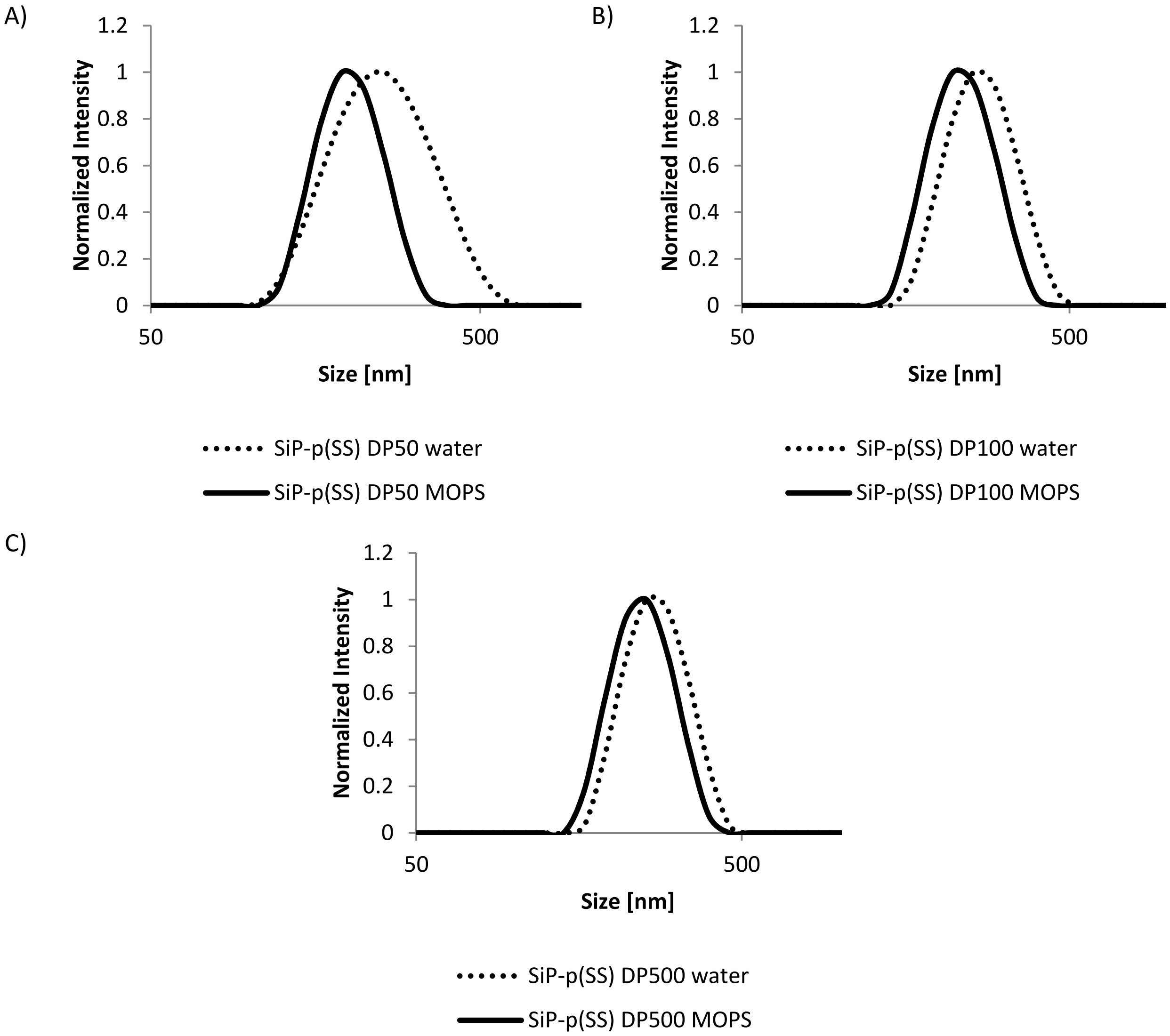

Figure 12: Size distribution of hybrid nanoparticles indicating change in hydrodynamic volume due to increased ionic strength in 50 mM MOPS buffer. A) SiP-p(SS)$_{50}$, B)SiP-p(SS)$_{100}$ and C)SiP-p(SS)$_{500}$.

References

1. C.A.R. Costa,, C.A.P. Leite, and F. Galembeck, J. Phys. Chem. B, 2003. **107**, 4747-4755.
2. W. Stöber, A. Fink, and E. Bohn, *J. Coll. Inter. Sci*. 1968. **26**, 62-69.
3. D. Dukes, L. Yu, S. Lewis, B. Benicewicz, L. Schadler, S. K. Kumar, *Macromolecules*, 2010. **43**, 1564-1570.
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