Electronic Supporting Information

Synthesis of Hollow Polymeric Nanoparticles for Protein Delivery *via* Inverse Miniemulsion Periphery RAFT Polymerization

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1. Materials and methods:

1.1. Chemicals

N-(2- Hydroxypropyl) methacrylamide (HPMA, Polysciences), bovine serum albumin (BSA, >97% Sigma-Aldrich) and sodium chloride (NaCl, Univar), potassium chloride (KCl, Univar), di-sodium hydrogen orthophosphate (Na_2HPO_4, Univar), potassium di-hydrogen orthophosphate (KH_2PO_4, Univar), hydrochloric acid (HCl, Univar), 1,3,5-trioxane (Sigma Aldrich), *p*-nitrophenyl acetate (Sigma Aldrich) and trifluoroacetic acid (TFA, >99%, Sigma Aldrich) were used without further purification. Methyl methacrylate (MMA, 99% Sigma-Aldrich) and ethylene glycol dimethacrylate (EGDMA, 98%, Sigma-Aldrich) were de-inhibited by passing through a column of activated basic alumina. De-inhibited monomers were stored at below 4^{o}C and used within 7-days. 2,2'-azobisisobutyronitrile (AIBN) was re-crystallized twice from methanol. Deionized (DI) water was produced by a Milli-Q reverse osmosis system and had a resistivity of 19.6 mΩ cm^{-1}. Deuterated NMR solvents ($CDCl_3$ and D_2O) were purchased from Cambridge Isotope Laboratories. Toluene (99.5%, Univar), dimethylacetamide (DMAc, 99.9%, Sigma Aldrich), diethyl ether (Et_2O, 99%, Univar), methanol (Ajax Chemicals), acetonitrile (99.99%, Honeywell), 1,4-dioxane (99.5%, Sigma Aldrich) and n-hexane (95%, Univar) were used without further purification.

1.2. Analyses

Gel Permeation Chromatography (GPC) was performed using a Shimadzu modular system, comprised of an SIL-10AD auto-injector, LC-10AT pump, a DGU-12A degasser, CTO-10A column oven and an RID-10A differential refractive index detector. Column arrangement comprising of a Polymer Laboratories 5.0 µm bead size guard column (50 × 7.8 mm), followed by four linear PL column (300 × 7.8 mm, 500, 10^3, 10^4 and 10^5 Å, 5 µm pore size) was used for the analysis. *N*,*N*-dimethylacetamide (DMAc, 0.03% w/v LiBr, 0.05% w/v 2,6-di-butyl-4-methylphenol(BHT)) was used as the mobile phase at constant temperature 50^{o}C and a constant flow rate of 1mL.min^{-1}. The GPC system was calibrated using linear polystyrene standards, ranging from 500 to 10^6 g/mol (Polymer Laboratories). Chromatograms were processed using Cirrus 2.0 software (Polymer Laboratories).

Nuclear Magnetic Resonance (NMR) was utilized to analyze the structure of the synthesized compounds as well as to determine the polymerization rate. ^{1}H NMR spectroscopy was carried using Bruker Avance III 300 MHz for hydrogen nuclei, equipped with auto injector system. All measurements were run in either $CDCl_3$ or D_2O. Chemical shifts were reported in parts per million (PPM), in referenced to the solvent residual peak.

pH measurements were carried out using Mettler Toledo SevenCompact™ pH/Ion meter S220. The system was calibrated prior to every measurement using three pH buffer solutions at pH 4.01, 7 and 9.21 (Mettler Toledo).

Dynamic Light Scattering (DLS) analyses were run on a Zetasizer Nano ZS (Malvern), with a 4 mV He-Ne laser operating at λ =632 nm and non-invasive backscatter detection at 173^{o}, capable of measuring hydrodynamic diameters from 0.3 nm to 10 microns. Measurements were conducted in a Quartz cuvette, at 25^{o}C, with 60 seconds equilibration period prior to each measurement. A total of 3 measurements with a variable number of runs were carried on each sample, with the presented results representing the average of these three measurements. Number of runs, attenuator used and path length were automatically adjusted by the instrument, depending on the quality of the sample.

UV-Visible Spectroscopy (UV-Vis) spectra were recorded using a CARY 300 spectrophotometer (Bruker) equipped with CART temperature controller. Baseline correction was conducted prior to each measurement. The measurements were conducted in disposable plastic cuvettes, made from high quality polystyrene (suitable for assays between 340 nm – 900 nm).

Fluorescence Spectroscopy was carried out at 25^{o}C with Cary Eclipse Fluorescence Spectrophotometer. Both of the excitation and emission slits were set at 5 nm. The measurements were conducted in a four-sided glass cuvette at 200 nm/second scan rate.

High Performance Liquid Chromatography (HPLC) system comprised a LC-20AD pump, DGU-20A degasser and SPD-20A UV-Vis detector. Resteck C18 column (5.0 µm bead sizes, 250 × 10 mm) was used in all measurements. All samples were prepared in PBS buffer solution and filtered through a 0.45 µm syringe filter. The mobile phase was a mixture of acetonitrile (65% v/v) and water with 0.1%

trifluoroacetic acid (TFA) (35% v/v), operating at a flow rate of 1 ml min^{-1}. Analysis was carried out at 285 nm wavelength, with the maximum peak at 7.8 minutes retention time corresponding to BSA.

Transmission Electron Microscopy (TEM) JEOL1400 transmission electron microscope (TEM), operating at accelerating voltage of 120kV. Images were recorded via the Gatan CCD imaging software. All TEM samples were prepared by dropping a 1 mg ml^{-1} solution on the formvar supported copper grid, with excess solvent drained using filter paper after 1 minute.

2. Experimental

2.1. Synthesis of RAFT Agent

4-cyanopentanoic acid dithiobenzoate (CPADB) RAFT agent was synthesized according to the literature.[1]

2.2. Preparation of PBS-buffer solution

Phosphate buffer saline (PBS) solution was prepared by dissolving sodium chloride (8.01 g), potassium chloride (0.2 g), anhydrous di-sodium hydrogen orthophosphate (1.44 g) and potassium di-hydrogen orthophosphate (0.27 g) in deionized water (1 L). The pH of the solution was adjusted with HCl and measured to yield a pH 7 buffer solution.

2.3. Polymerization of *N*-(2-hydroxypropyl) methacrylamide

Polymerization of HPMA was carried out in DMAc, utilising AIBN as the initiator and CPADB as the RAFT agent. In a typical polymerization, HPMA (1 g, 6.984 x 10^{-3} mol), CPADB (0.04g, 1.4 x 10^{-4} mol) and AIBN (0.0115 g, 6.98 x 10^{-5} mol) were dissolved in DMAc (5.68 ml) to give a [monomer]:[RAFT]:[Initiator] ratio of 50:1:0.5. The solution was thoroughly purged with nitrogen gas for 30 minutes before being placed in oil bath at 70^{o}C for 7 hours. At the completion of the polymerization, the reaction was stopped by placing the solution into ice water and exposing it to air for 30 minutes. An aliquot (0.2ml) was then collected for ^{1}H NMR analysis. The conversion was calculated by comparing the integration of the signals at δ 5.35 ppm and δ 5.58 ppm to that of the characteristic CH signal in the repeat unit at δ 3.78 ppm. The polymer was isolated by precipitation in

diethyl ether to yield poly(HPMA) (38%) as a highly-viscous red liquid. The molecular weight of the polymer was then determined via DMAc GPC.

2.4. Chain extension of poly(HPMA) macroRAFT with methyl methacrylate

A solution of poly(HPMA) macroRAFT, AIBN, MMA in DMAc was initially prepared. A typical value for the copolymerization was as followed: Poly(HPMA) macroRAFT (0.195 g, 7.22 x 10^{-5} mol), MMA (1.45 g, 1.4 x 10^{-2} mol) and AIBN (0.0024 g, 1.44 x 10^{-5} mol) were dissolved in DMAc (14ml) to give a [monomer]:[RAFT]:[Initiator] ratio of 200:1:0.2. The solution was then purged with nitrogen for 30 minutes, before placing it in an oil bath at 70^{o}C for 6 hours. At the completion of the reaction, the polymerization was stopped by immersing the solution in ice water and exposing it to air. An aliquot (0.2 ml) was collected for ^{1}H NMR analysis. The conversion was calculated by comparing the signals at δ 4.85 ppm and δ 2.85 ppm. The polymer was isolated by precipitation in diethyl ether to yield PHPMA-b-PMMA (40%) as a brittle, red solid. The purified polymer was analyzed by DMAc GPC.

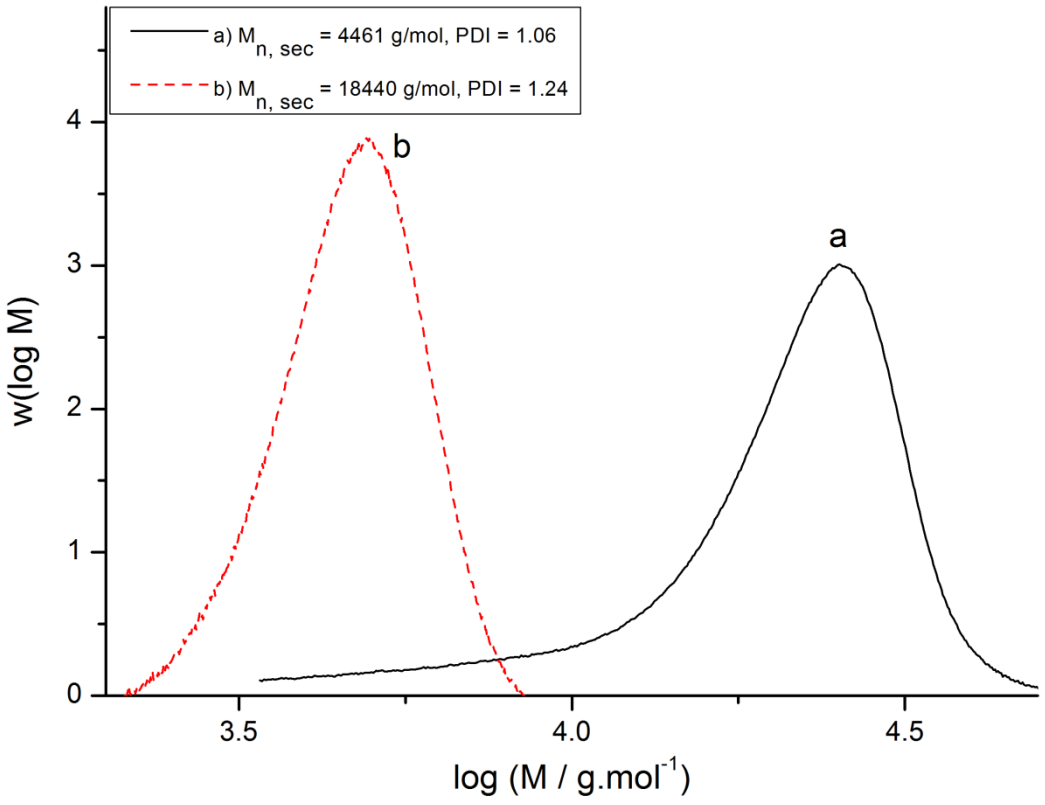

Figure S1. Molecular weight distributions of a) poly(HPMA); and b) PHPMA-b-PMMA, synthesized *via* RAFT polymerization

2.5. Preparation of inverse miniemulsion and inverse miniemulsion periphery RAFT polymerization

A representative preparation process is as follow; into a glass container, toluene (6.5 g) was mixed with PHPMA-b-PMMA (0.065 g) to create the continuous phase of the emulsion. Distilled water (0.65 g) and NaCl (0.013 g) were mixed together, to create the dispersed phase of the system, and added into the continuous phase. If BSA was to be incorporated, BSA (0.013 g) was dissolved in the dispersed phase. A third solution, consisting of MMA, EGDMA and AIBN was prepared. For this purpose, AIBN (0.485 mg, 2.96 x 10^{-6} mol) was dissolved in a solution of MMA (0.12 g, 1.18x 10^{-3} mol) and EGDMA (0.03 g, 1.48 x 10^{-4} mol) to give a [MMA]:[EGDMA]:[macroRAFT]:[AIBN] ratio of 200:25:1:0.5. This solution was subsequently added into the continuous-dispersed phase mixture. The resulting mixture was then ultrasonicated (Branson 450 sonifier, 70% amplitude, 5mm tip diameter) for 5 minutes while immersed in an ice bath. DLS analysis was carried out on the diluted miniemulsion solution to establish the initial diameter of the initial droplets. The resulting inverse miniemulsion was then transferred into a glass ampoule to undergo a series of nitrogen purging and vacuuming. The ampoule was finally flame sealed while under vacuum before placing it in an oil bath. The reaction was carried at 60^0C with constant shaking. At the completion of the reaction, 0.5 ml of the sample was collected for DLS, TEM and ^{1}H NMR analyses. The conversion was calculated by comparing the signals at δ 5.6 ppm and δ 6.2 ppm of the double bond to the signal of 1,3,5-trioxane, which has been added as internal standard, at δ 5.0 ppm. The conversion was determined gravimetrically by transferring 1 ml of the raw reaction solution into a pre-weighted aluminium pan and the solvents and unreacted monomers were left to evaporate. The mass of the leftover solid was determined and subtracted by the initial solid content of the inverse miniemulsion. The mass of polymer was compared to the initial monomer amount to calculate the conversion. Hollow nanoparticles were purified from the raw solution by firstly de-emulsifying the system using n-hexane, followed by drying of the wet solid under vacuum. Determination of the nano-shell integrity was carried by re-dispersing the resulting nanoparticles in 1,4-dioxane (1 mg/ml), followed by ultrasonication (Branson 450 sonifier, 15% amplitude, 5mm tip diameter) for 1 minute.

Table S1. Summary of the hydrodynamic diameter by number, volume and intensity obtained by DLS of the droplet prior to the IMEPP in toluene and the nanoparticles after the IMEPP in toluene

Run Number	Sample Name	Z-Average	Number mean	Volume mean	Intensity mean	PDI
1	HollowNPs – Prior IMEPP	189	177	212	199	0.03
2	HollowNPs – Prior IMEPP	187	173	210	197	0.04
3	HollowNPs – Prior IMEPP	185	171	209	196	0.05
Run Number	**Sample Name**	**Z-Average**	**Number mean**	**Volume mean**	**Intensity mean**	**PDI**
1	HollowNPs – After IMEPP	241	224	293	263	0.08
2	HollowNPs – After IMEPP	244	228	291	264	0.06
3	HollowNPs – After IMEPP	239	219	291	260	0.10

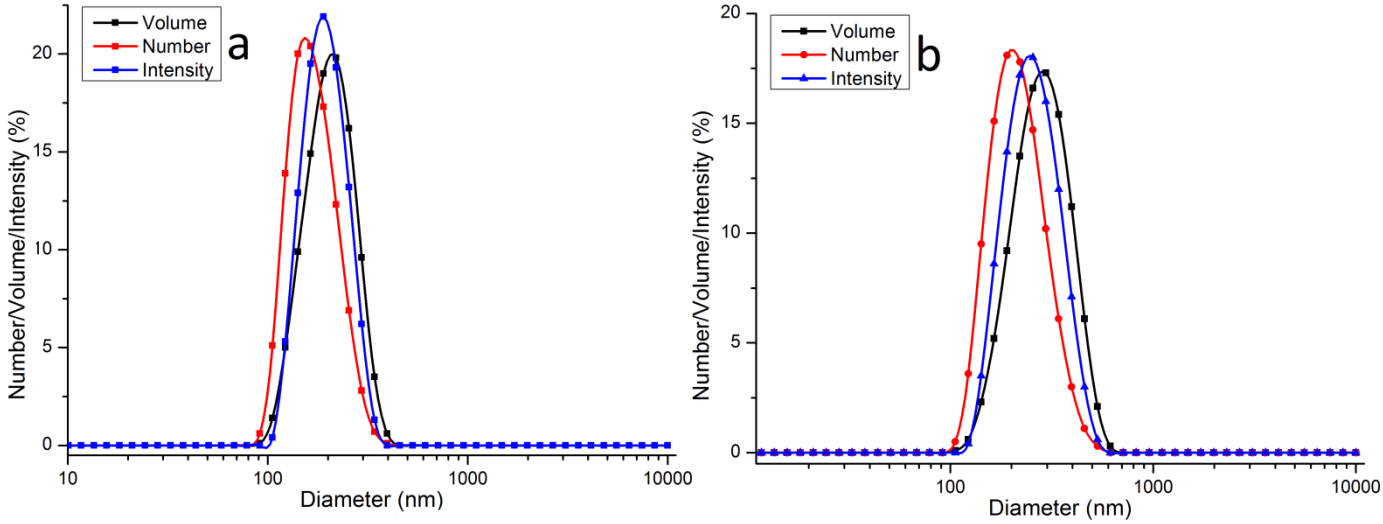

Figure S2. Droplet/particle size distributions obtained by DLS (a) before and (b) after inverse miniemulsion periphery polymerization based on number (red), intensity (blue) and number (black).

Table S2. Summary of the hydrodynamic diameter by number, volume and intensity of the droplet prior to the IMEPP in toluene, the nanoparticles after the IMEPP in toluene and of the re-dispersed nanoparticles in 1,4-dioxane.

Run Number	Sample Name	Z-Average	Number mean	Volume mean	Intensity mean	PDI
1	BSA-NPs – Prior IMEPP	188	176	210	198	0.04
2	BSA-NPs – Prior IMEPP	186	165	217	201	0.07
3	BSA-NPs – Prior IMEPP	187	170	212	199	0.05
Run Number	**Sample Name**	**Z-Average**	**Number mean**	**Volume mean**	**Intensity mean**	**PDI**
1	BSA-NPs – After IMEPP	214	181	267	234	0.15
2	BSA-NPs – After IMEPP	216	181	291	248	0.12
3	BSA-NPs – After IMEPP	215	188	261	233	0.13
Run Number	**Sample Name**	**Z-Average**	**Number mean**	**Volume mean**	**Intensity mean**	**PDI**
1	BSA-NPs – Re-dispersed	209	166	277	246	0.14
2	BSA-NPs – Re-dispersed	213	190	262	240	0.13
3	BSA-NPs – Re-dispersed	214	172	284	249	0.15

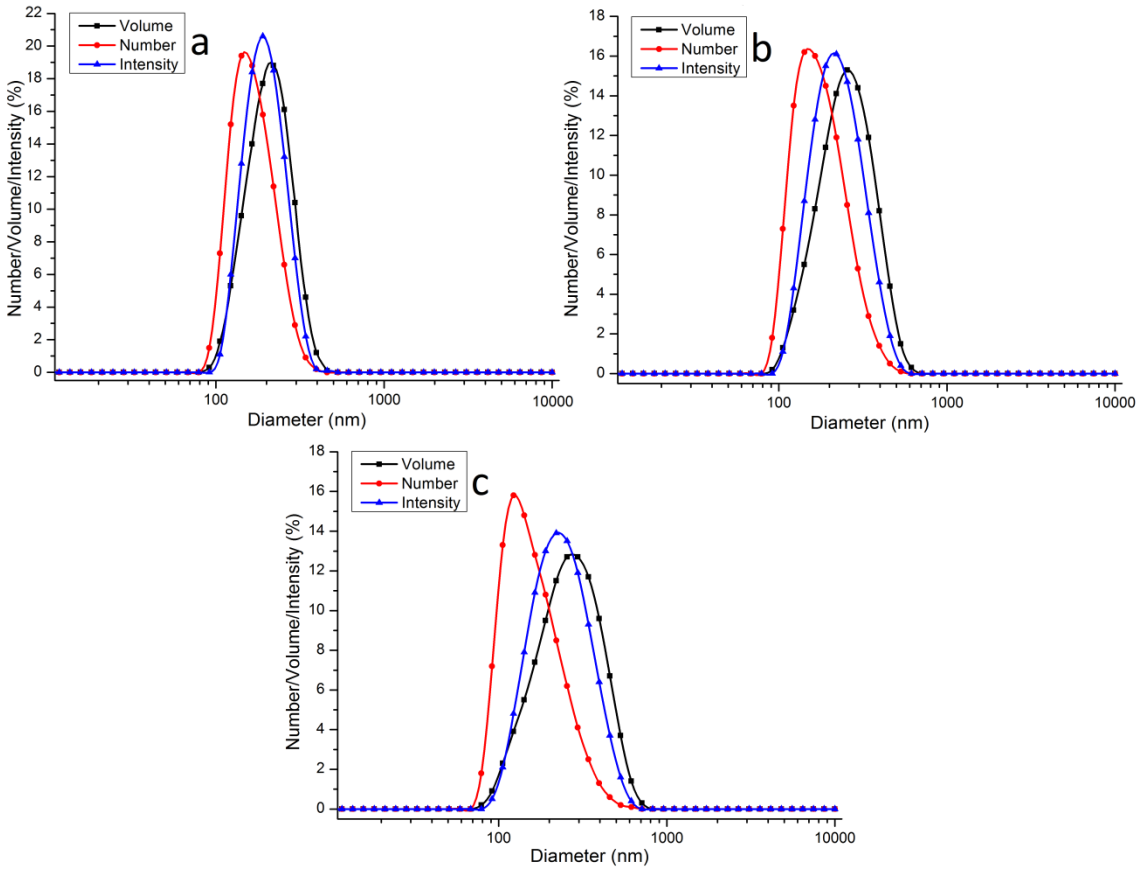

Figure S3. Droplet/particle size distributions obtained by DLS by number (red), intensity (blue) and number (black) of (a) the initial miniemulsion droplets, (b) nanoparticles after IMEPP, and (c) re-dispersed BSA-loaded particles.

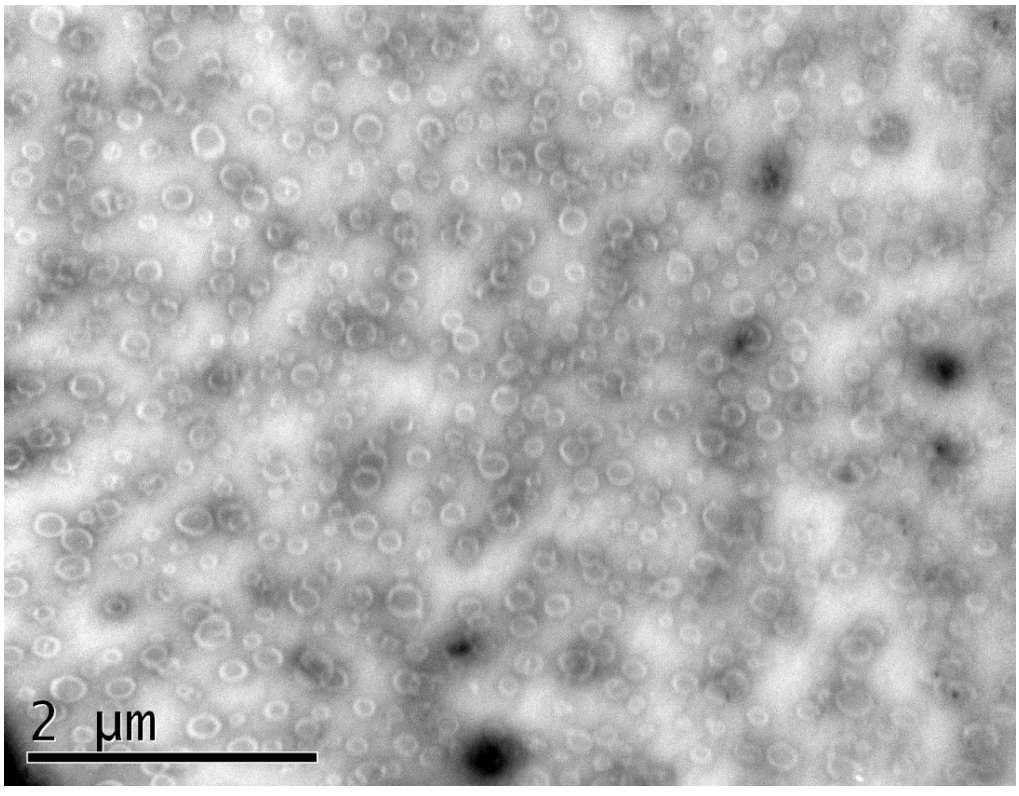

Figure S4. Low magnification TEM image of the synthesized hollow nanoparticles

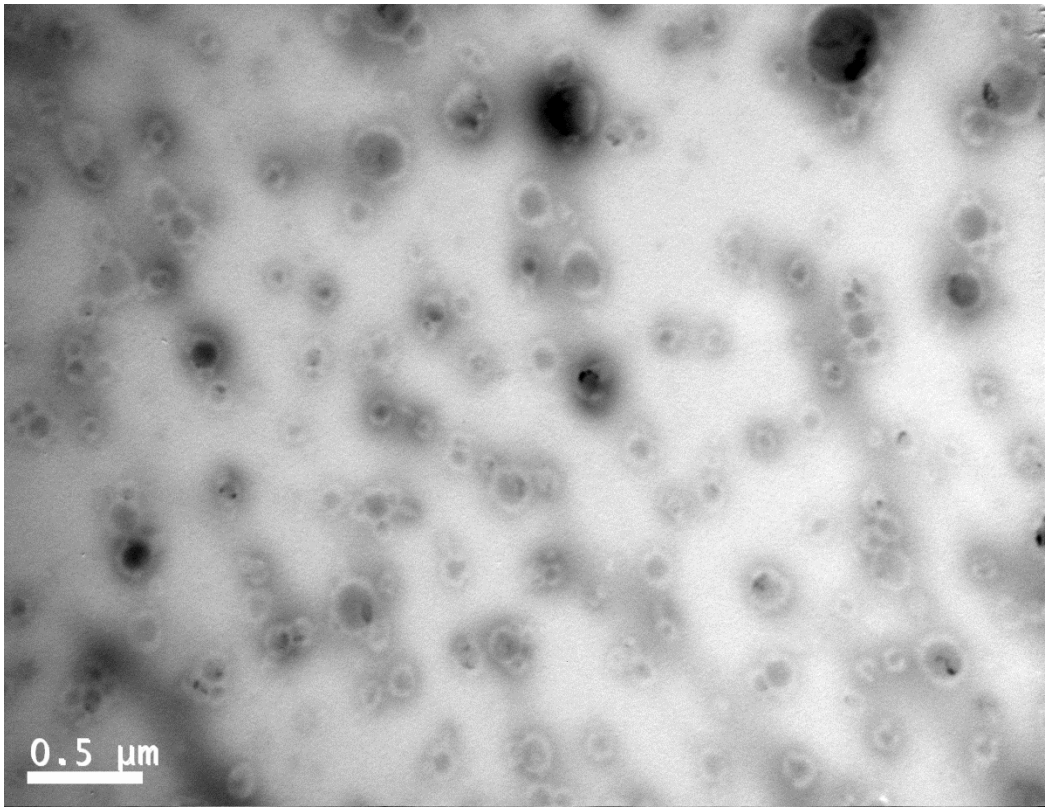

Figure S5. Low magnification TEM image of the synthesized BSA-loaded nanoparticles, with the dark spots in the core representing the BSA

2.6. Study of catalytic activity of ultrasonicated BSA towards *p*-nitrophenyl acetate

The effect of sonication on the properties of BSA was investigated by monitoring its catalytic activity towards the hydrolysis of *p*-nitrophenyl acetate. *p*-Nitrophenyl acetate (63 mg, 3.47×10^{-4} mol) was dissolved in methanol (1 ml). This solution was then transferred to PBS buffer solution (1 L) to yield a solution with an overall concentration of 3.48×10^{-4} M. Another solution comprising of BSA (1.564 g, 2.6×10^{-5} mol) in PBS buffer solution (15 ml) was prepared, yielding a final BSA concentration of 1.74×10^{-3} M or 5-fold of the concentration of *p*-nitrophenyl acetate. In the case with the ultrasonicated BSA, aforementioned BSA solution was exposed to an ultrasonication process (Branson 450 sonifier, 70% amplitude, 5 mm tip diameter) for 5 minutes. Three samples were then prepared, each sample containing 1 ml of the *p*-nitrophenyl acetate solution and either 1 ml of PBS buffer solution, 1 ml of the PBS buffer solution with BSA or 1 ml of PBS buffer solution with ultrasonicated BSA, each to be referred as control, non-ultrasonication and ultrasonication respectively. Reaction was carried at room temperature with constant stirring. UV-Vis measurements

were conducted at specific time intervals of 0 hour, 2 hours and 17 hours to investigate the production of the nitrophenol moiety, which is UV-Vis active at 405 nm.

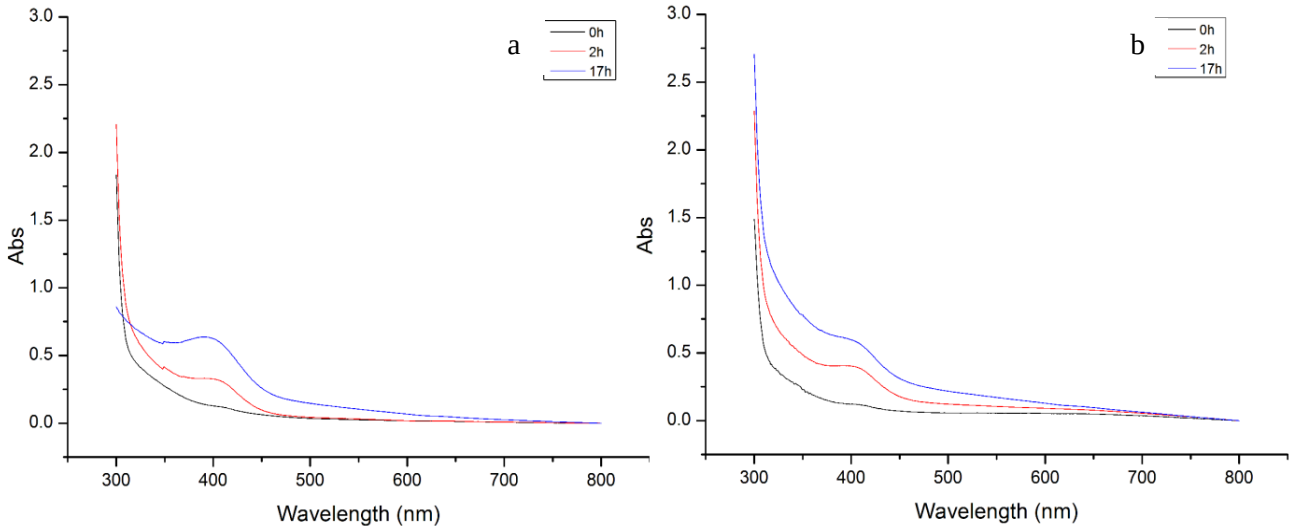

Figure S6. UV-Vis spectra at time = 0 h (black), 2 h (red) and 17 h (blue) of the hydrolysis reaction, catalyzed by pure BSA (a); and ultrasonicated BSA (b)

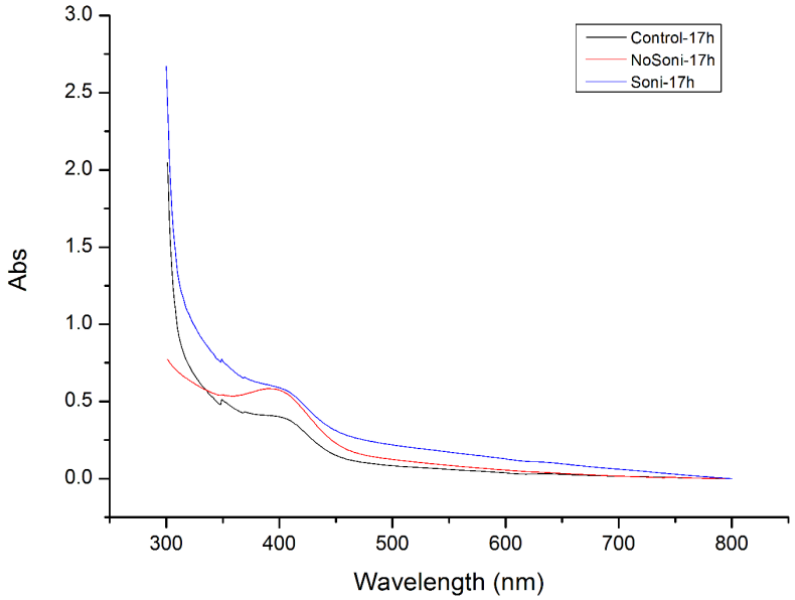

Figure S7. UV-Vis spectra of the control (black), ultrasonication (blue) and non-ultrasonication (red) solution, showing the production of nitrophenol after 17 hours of reaction time.

2.7. Investigation of the BSA structural preservation by fluorescence analysis of tryptophan residues

The effect (or lack thereof) of sonication on the structure of BSA was investigated by analysing the fluorescence emission profiles of the tryptophan residues. To mimic the conditions used in the IMEPP reaction, solutions containing 26 mg of BSA in 15 ml of PBS (mimicking 2wt% reaction, [BSA]

=0.0226 mM) and 65 mg of BSA in 15 ml of PBS buffer (mimicking 5wt% reaction, [BSA] = 0.065 mM) were sonicated (Branson 450 sonifier, 70% amplitude, 5 mm tip diameter) for 5 minutes. As a comparison, a control solution was prepared (26 mg BSA in 15 ml of PBS, no sonication). Each solution was then diluted to a concentration of 4.722 µM. Fluorescence measurement was then carried at an excitation wavelength of 300 nm and with the emission spectra recorded in the wavelength region of 250-550 nm.

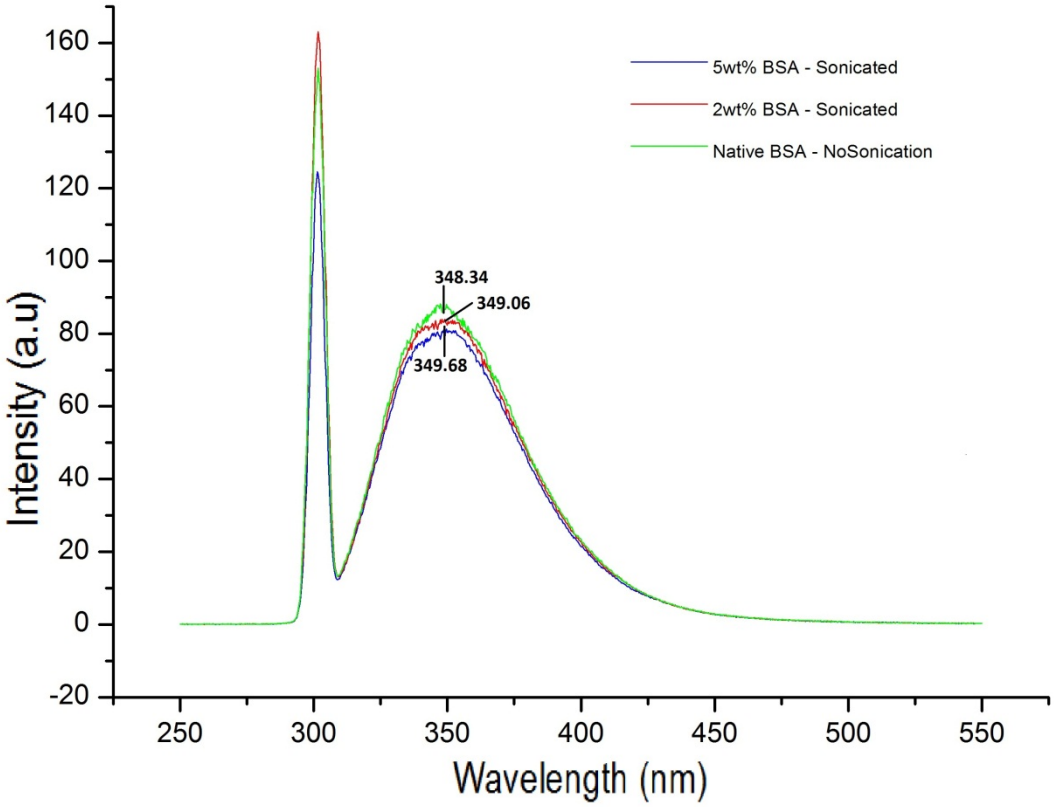

Figure S8. Fluorescence emission spectra of the control (green), 2wt%-ultrasonicated (red) and 5wt%-ultrasonicated (blue) solution, with the peak at 300 nm corresponding to the scattering of the excited beam and the peak at 349 nm corresponding to the tryptophan residues.

2.8. Release study of encapsulated BSA and its activity towards *p*-nitrophenyl acetate

Analysis over the leaching rate of the encapsulated BSA in aqueous environment was carried using HPLC. Initially, 6 standards comprising various concentrations of BSA in PBS buffer was prepared and analyzed. To promote the release of BSA, the synthesized nanoparticles (0.78 g) were transferred into PBS buffer solution (2 ml). The mixture was stirred continuously for 3 days at room temperature. At the completion of the stirring process, the mixture was filtered through 0.45 µm syringe filter to

remove any un-dissolved constituents. The filtrate (1 ml) was then analyzed through HPLC to determine the quantity the amount of BSA within the filtrate. To study the catalytic activity of the encapsulated BSA, the filtrate from the previous test (1 ml) was mixed with a solution of *p*-nitrophenyl acetate in PBS buffer (1 ml, 5.5×10^{-6} M). Two solutions comprising of pure BSA in PBS buffer (1 ml, 2.75×10^{-6} M) and DI water (1 ml) in *p*-nitrophenyl acetate solution (1 ml, 5.5×10^{-6} M) were prepared for comparison and referred to as pure BSA and control respectively. The reaction was carried at room temperature with constant stirring over the period of 24 hours. The quantity of nitrophenol produced was measured using UV-Vis spectroscopy.

Table S3. BSA standard solutions used to establish the calibration curve

BSA mass (mg)	PBS Buffer Volume (ml)	[BSA] (mg/ml)	Response (mV)
0.2	2	0.1	4.929
0.98	2	0.49	14.089
2.01	2	1.005	22.983
2.52	2	1.26	31.383
3	2	1.5	34.998
4.02	2	2.01	48.612
6.03	2	3.015	71.413

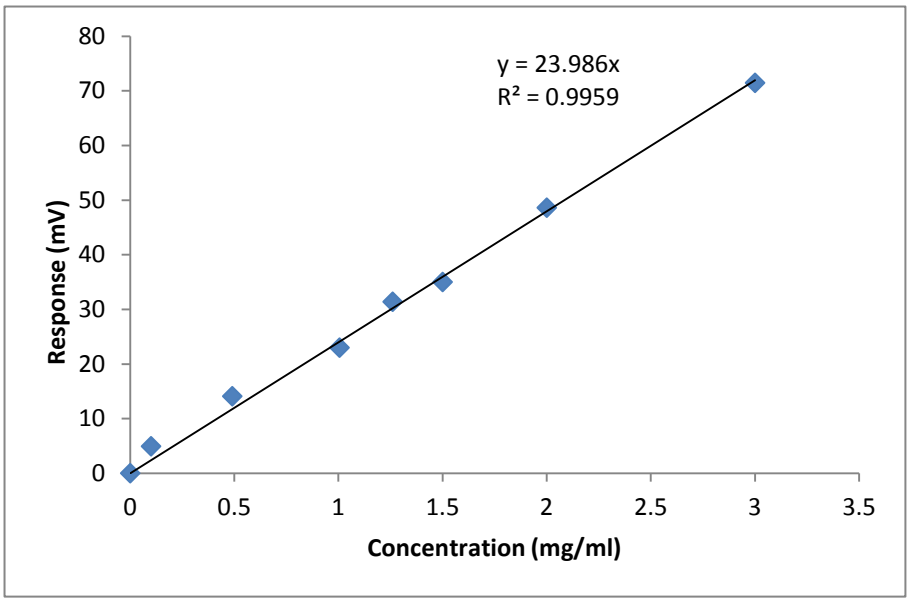

Figure S9. Calibration curve used to determine the BSA concentration within a given solution, correlating the concentration of the solution with the HPLC response.

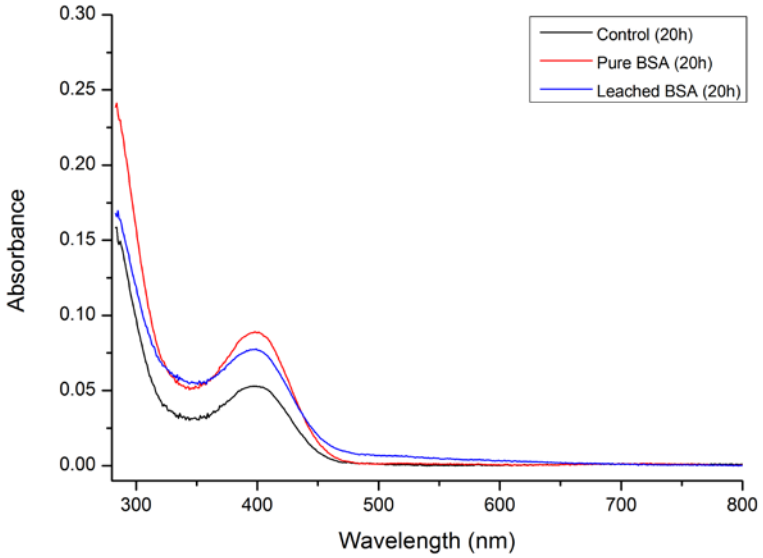

Figure S10. UV-Vis spectra showing the presence of nitrophenol evidenced by the absorbance at 405 nm, of the control (black), pure BSA (blue) and leached BSA (red) solutions.

2.9. Fluorescence analysis of the tryptophan residues on the encapsulated BSA after release

Fluorescence spectroscopy was carried out using the same procedure as described in 2.7 to confirm the structural integrity of the encapsulated BSA after release. The emission profile of the encapsulated BSA recovered after release from the carrier (Section 2.8) was compared with that of the pure BSA (control) and pure BSA in urea solution (positive control). The BSA concentration of each solution was adjusted to the same value to allow comparison of the three samples. Measurements were carried out at an excitation wavelength of 300 nm and the emission spectrum was recorded from 250 – 550 nm. The shift observed of the emission maximum from 355 nm (of the native or the encapsulated BSA) to 361 nm (of the native BSA in urea) indicates the unfolding of BSA in urea as a positive control for denatured BSA. Comparison of the emission spectra of native BSA and encapsulated BSA indicates that the denaturation of the BSA during the process is barely noticeable.

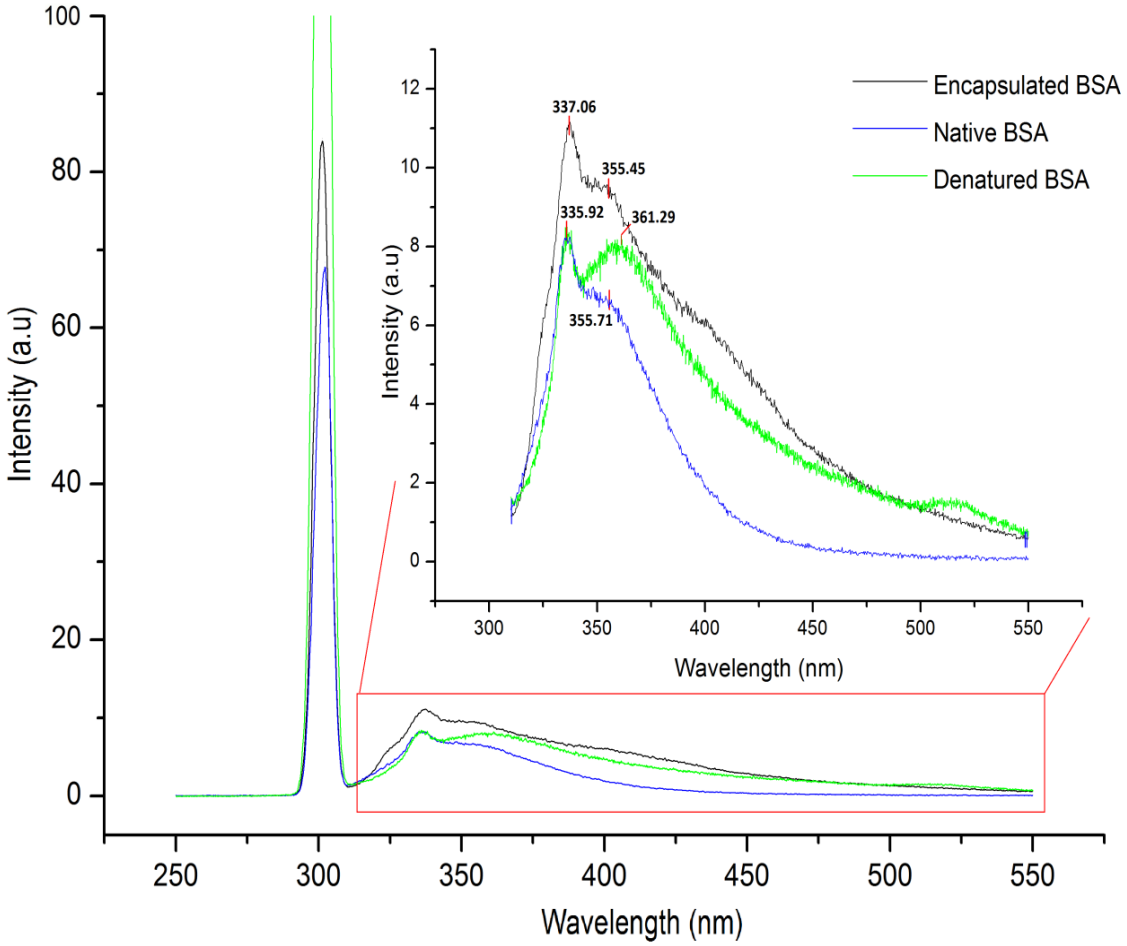

Figure S11. Fluorescence emission spectra of the native BSA (blue), encapsulated BSA (black) and denatured BSA (green), with an exploded version of spectra between 310 nm to 550 nm (insert) included to show the shift in the tryptophan peak from 355 nm (in folded BSA) to 361 nm (unfolded BSA).

2.10. Theoretical calculation of shell thickness

The shell thickness would be the sum of the contribution by the PMMA chain of the initial block-copolymer and the resulting polymeric layer formed during the IMEPP reaction. This value is calculated using two different methods, thereby providing a minimum and a maximum value (the true value being somewhere between these limits). The first method (minimum value) considers the volume of the polymerized monomers while the other method (maximum value) is based on the total contour length of the polymerized monomers.

2.10.1. Shell thickness based on the contour length of the polymerized monomers

The contour length of a given chain is established using the following formula:

$$L_{cont} = N \times b \times \sin(0.5\,\Theta)$$

where, N is the number of repeating units, b is the C-C bond length (0.154 nm),[2] and Θ is the C-C bond angle within the polymer backbone (109°).

From previous analysis (Section 2.4), it was found that the repeating unit of the PMMA chain was 79 and therefore the contour length of this segment is:

$$L_{cont} = 79 \times 0.154 \times \sin(0.5 \times 109)$$

$$L_{cont} = 9.9\ nm$$

Using the overall monomer conversion of 51% and the initial molar ratio of the RAFT agent and monomers ([RAFT]:[MMA]:[EGDMA] = 1:200:25), the number of repeating units (N) of MMA and EGDMA within the chain was calculated:

$$N_{MMA} = \frac{[MMA]}{[RAFT]} \times Conversion$$

$$N_{MMA} = 200 \times 0.51 = 102$$

$$N_{EGDMA} = 25 \times 0.51 = 12.75$$

From the results obtained and with the number of EGDMA repeating unit rounded up to the closest integer, the total contour length of the polymerized shell is:

$$L_{cont} = (102 + 13) \times 0.154 \times \sin(0.5 \times 109)$$

$$L_{cont} = 14.4\ nm$$

Hence the total thickness of the shell calculated using this method (maximum thickness) is 14.4 + 9.9 = 24.3 nm.

2.10.2. Shell thickness based on the volume of the polymerized monomers

Using the result obtained from DLS, the hydrodynamic diameter was determined to be 170 nm. However, the actual diameter of the droplet would be the difference between this value and the contour length of the PMMA hairs (2 * 10 nm), i.e. 150 nm. The volume of an individual droplet is then:

$$V = \frac{4}{3} \times \pi \times r^3$$
$$V = 1.77 \times 10^{-15}\ cm^3$$

Correlating the volume of the individual droplet with the total volume of the droplet (or the volume of the dispersed phase), the total number of droplet is:

$$Total\ \#\ of\ droplet = 0.65 \div (1.77 \times 10^{-15})$$
$$Total\ \#\ of\ droplet = 3.68 \times 10^{14}$$

The overall thickness of the shell would be the sum of the IMEPP shell and the PMMA hairs. As for the IMEPP segment, initially, using the conversion of 51% and the initial volume of MMA and EGDMA used being 0.087 cm^3 and 0.0193 cm^3, respectively, the volume of the polymerized monomers is:

$$V_{polymerized\ monomers} = (0.51 \times 0.087) + (0.51 \times 0.0193)$$
$$V_{polymerized\ monomers} = 0.0542\ cm^3$$

Therefore, after polymerization, the volume of the nanoparticles is the sum of the volume of the dispersed phase and the volume of the polymerized monomers:

$$Total\ V = 0.65 + 0.0542$$
$$Total\ V = 0.7042\ cm^3$$

Dividing this value with the total number of droplet present,

$$V\ of\ NPs = 0.7042 \div (3.68 \times 10^{14})$$
$$V\ of\ NPs = 1.914 \times 10^{-15}\ cm^3$$

Hence, the diameter of each individual nanoparticle is:

$$d_{NPs} = 2 \times \sqrt[3]{1.914 \times 10^{-15} \times \frac{3}{4\pi}}$$
$$d_{NPs} = 1.54 \times 10^{-5}\ cm\ or\ 154\ nm$$

This results in a shell thickness of:

$$Thickness_{IMEPP} = \frac{154 - 150}{2}$$

$$Thickness_{IMEPP} = 2\ nm$$

The contribution of the PMMA hairs is calculated by first considering the moles of block-copolymer used ($5.9 \times 10^{-6} mol$) and the molecular weight of the PMMA block (7,909 g mol^{-1}) to obtain the mass of the PMMA hairs, and subsequently calculating the corresponding volume based on the density of MMA (0.94 g ml^{-1}):

$$Mass = 5.9 \times 10^{-6} \times 7909$$

$$Mass = 0.0467\ g$$

$$Volume = 0.0496\ ml$$

Based on the total number of droplets calculated above, the volume of the individual nanoparticles, comprising the liquid core and the PMMA hairs, is calculated as follows:

$$V\ of\ NPs = (0.65 + 0.0496) \div (3.68 \times 10^{14})$$
$$V\ of\ NPs = 1.902 \times 10^{-15}\ cm^3\ or\ 1.902 \times 10^{6}\ nm^3$$

Hence the thickness of the PMMA hair segment is:

$$Thickness_{PMMA} = \frac{\left(\left(2\sqrt[3]{1.902 \times 10^{6} \times \frac{3}{4\pi}}\right) - 150\right)}{2}$$

$$Thickness_{PMMA} = 1.86\ nm$$

Finally, the minimum thickness of the shell is the sum of the synthesized shell segment and the PMMA hair segment, i.e. 2 + 1.86 = 3.86 nm

3. References

1. Y. Mitsukami, M. S. Donovan, A. B. Lowe and C. L. McCormick, *Macromolecules*, 2001, **34**, 2248-2256.
2. D. R. Lide Jr, *Tetrahedron*, 1962, **17**, 125-134.