Supplementary Material

Effects of Miniemulsion Operation Conditions on the Immobilization of BSA onto PMMA Nanoparticles

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S.1 CMC Calculations

Formation of micelles in the reaction medium was calculated based on the surfactant coverage of monomer droplets at each particular experimental condition. Table S1 presents the main calculated values. For these calculations, the diameter used to evaluate the total available interfacial area (A_T) was the average droplet size obtained at each experimental condition and the number of droplets was estimated by dividing the volume of the organic phase by the average droplet volume. Therefore, the total interfacial area was obtained by multiplying the number of droplets by each droplet area. Surfactant coverage area (A_{TS}) was calculated by multiplying the number of surfactant molecules in each experimental condition by the superficial area of one surfactant molecule (a_s). The percentage of surfactant coverage (β) can be obtained with help of Eqs. 1, 2 and 3:[1]

$$\beta = \frac{2}{b_1 + \sqrt{b_1^2 - 4b_2}} \quad (1)$$

$$b_1 = 1 + b_2 + \left(\frac{1}{[S]_{aq} \cdot b'}\right) \quad (2)$$

$$b_2 = \frac{A_T}{(a_s \cdot [S]_{aq} \cdot V_{aq} \cdot Na)} \quad (3)$$

where β is the percentage of surfactant coverage, A_T is the total interfacial area (m^2), $[S]_{aq}$ is the surfactant concentration in the aqueous phase ($mol.m^{-3}$), V_{aq} is the aqueous phase volume (m^3), Na is the Avogadro number, b' is the constant of the adsorption isotherm for the analyzed surfactant (b' was considered equal to 0.750 $m^3.mol^{-1}$)[1], a_s is the superficial area of the surfactant molecule (for SDS surfactant, a_s = 57 $Å^2$ and for CTAB surfactant, a_s = 82 $Å^2$)[1,2].

Table S1. Values estimated for droplets surfactant coverage.

Test	**Organic phase volume (m^3)**	**Droplets number**	**Interfacial area (m^2) (A_T)**	**SDS molecules number**	**SDS coverage area (m^2) (A_{TS})**	**% coverage (β)**	**A_{TS}/A_T**
1	1.02E-05	2.02E+16	619	2.00E+20	114	18	0.18
2	1.70E-05	8.20E+15	644	3.34E+20	190	29	0.30
3	1.02E-05	1.62E+16	574	1.00E+21	571	96	1.00
4	1.70E-05	1.62E+16	807	1.67E+21	952	98	1.18
5	1.02E-05	2.33E+16	648	1.59E+20	130	20	0.20
6	1.70E-05	4.50E+16	1135	2.64E+20	217	19	0.19
7	1.02E-05	5.41E+16	859	7.93E+20	650	75	0.76
8	1.70E-05	5.93E+16	1244	1.32E+21	1084	85	0.87
9	1.70E-05	4.24E+16	1112	1.00E+21	571	51	0.51
10	1.70E-05	7.54E+16	1348	1.00E+21	571	42	0.42
11	1.70E-05	9.30E+16	1446	1.00E+21	571	39	0.40
12	1.70E-05	2.08E+16	878	7.93E+20	650	73	0.74
13	1.70E-05	4.32E+16	1119	7.93E+20	650	58	0.58
14	1.70E-05	3.44E+16	1037	7.93E+20	650	62	0.63

According to Table S1, it is observed that the total areas of droplets were always higher than the surfactant coverage areas. As a consequence, the percentages of surfactant coverage were always smaller than 100%. These values allow us to infer that the surfactant used for preparation of the miniemulsions was located mainly at the interface, indicating that the analyzed systems did not present free micelles, as expected for classical miniemulsion systems. Therefore, in polymerization runs performed with both SDS and CTAB, free surfactant concentrations in the aqueous media were below the respective CMC (CMC_{SDS} = 8 mM and CMC_{CTAB} = 1 mM) [3,4].

S.2 Comparisons between particle and droplet size distributions.

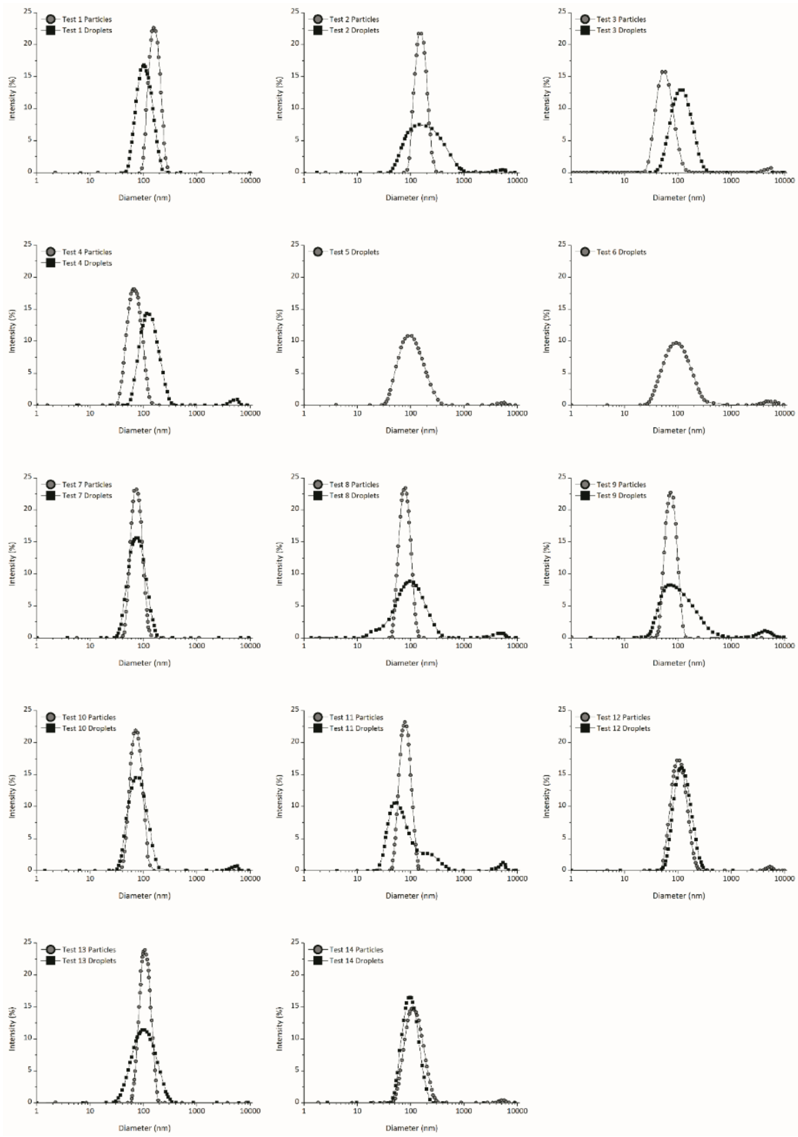

Figure S1. Particle and droplet size distributions.

S.3 Statistical analyses

Figure S2 illustrates the good quality of the proposed model fit for the average sizes of PMMA NPs (Equation 5 of the main manuscript). The absence of responses in tests 5 and 6, due to massive particle coalescence, caused some of the parameter correlations to be different from zero. Despite this, the correlations among parameters estimated in Equation 5 were low, as shown in Table S2, and are not expected to affect the proposed quantitative analyses.

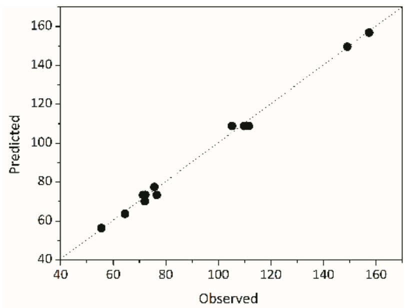

Figure S2. Representation of the quality of the model fit for the PMMA NPs average sizes.

Table S2. Correlation matrix for the estimated parameters in Equation 5.

Correlation Matrix						
	a_0	a_1	a_2	b_{12}	b_{23}	c
a_0	1.00	0.16	-0.10	-0.10	0.00	-0.49
a_1	0.16	1,00	-0.63	-0.63	0.00	-0.49
a_2	-0.10	-0.63	1.000	0.75	0.00	-0.54
b_{12}	-0.10	-0.63	0.75	1.00	0.00	-0.54
b_{23}	0.00	0.00	0.00	0.00	1.00	0.00
c	-0.49	0.49	-0.54	-0.54	0.00	1.00

Figure S3 illustrates the good quality of the proposed model fit for zeta potential of PMMA NPs (Equation 6 of the manuscript). Again, the absence of responses in tests 5 and 6 caused some of the parameter correlations to be different from zero. Despite this, the correlations among parameters estimated in Equation 6 were low, as shown in Table S3, and are not expected to affect the proposed quantitative analyses.

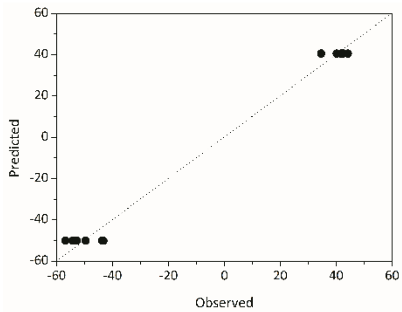

Figure S3. Representation of the quality of the model fit for the PMMA NPs zeta potential.

Table S3. Correlation matrix for the estimated parameters in Equation 6.

Matrix Correlation		
	a_0	a_1
a_0	1.00	-0.41
a_1	-0.41	1.00

Figure S4 illustrates the good quality of the proposed model fit for BSA adsorption (Equation 7 of the main manuscript). Again, the absence of responses in tests 5 and 6 caused some of the parameter correlations to be different from zero. Despite this, the correlations among parameters estimated in Equation 7 were usually low (with exception of the pair a_0-a_1), as shown in Table S4, and are not expected to affect the proposed quantitative analyses.

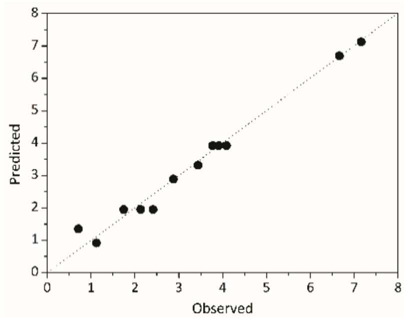

Figure S4. Representation of the quality of the model fit for BSA adsorption onto the PMMA NPs surfaces.

Table S4. Correlation matrix for the estimated parameters in Equation 7.

Correlation Matrix						
	a_0	a_1	a_2	b_{12}	b_{23}	c
a_0	1.00	0.84	-0.16	0.00	-0.33	1.00
a_1	0.84	1.00	-0.19	0.00	-0.07	0.84
a_2	-0.16	-0.19	1.000	0.00	-0.07	-0.16
b_{12}	0.00	0.00	0.00	1.00	0.00	0.00
b_{23}	-0.33	0.07	-0.07	0.00	1.00	-0.33
c	1.00	0.84	-0.16	0.00	-0.33	1.00

Figures S5 and S6 illustrate the good quality of the proposed model fits for NPs diameters and BSA adsorption using SDS as the surfactant agent (Equations 8 and 9 of the main manuscript).

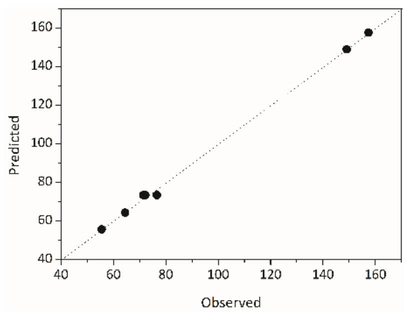

Figure S5. Representation of the quality of the model fit for average diameters for PMMA NPs considering only SDS surfactant tests.

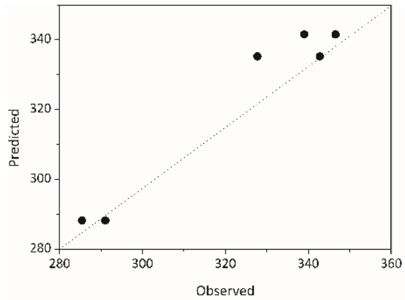

Figure S6. Representation of the quality of the model fit for BSA adsorption onto the PMMA NPs surfaces, considering only SDS surfactant tests.

Table S5. Correlation matrix of SDS surfactant model parameters for average NPs diameters (Equation 8).

Correlation Matrix				
	a_0	a_2	b_{23}	c
a_0	1.00	0.00	0.00	-0.76
a_2	0.00	1.00	0.00	0.00
b_{23}	0.00	0.00	1.00	0.00
c	-0.76	0.00	0.00	1.00

Table S6. Correlation matrix of SDS surfactant model parameters for BSA adsorption onto PMMA NPs surfaces (Equation 9).

Correlation Matrix				
	a_0	a_2	b_{23}	c
a_0	1.00	0.00	0.00	-0.76
a_2	0.00	1.00	0.00	0.00
b_{23}	0.00	0.00	1.00	0.00
c	-0.76	0.00	0.00	1.00

In the case of CTAB, the absence of responses in tests 5 and 6 caused the occurrence of some undesired parameter correlations, as shown in Table S7 and S8 (Equation 10 and 11 of the manuscript, respectively). Despite this, the correlation does not affect the proposed quantitative analyses. Figures S7 and S8 illustrate the proposed model fits for NPs diameters and BSA adsorption using CTAB as the surfactant agent.

Table S7. Correlation matrix of Size model parameters for CTAB surfactant (Equation 10).

Matrix Correlation		
	a_0	a_2
a_0	1.00	-0.63
a_2	-0.63	1.00

Table S8. Correlation matrix of BSA adsorption model parameters for CTAB surfactant (Equation 11).

Matrix Correlation		
	a_0	a_2
a_0	1.00	-0.63
a_2	-0.63	1.00

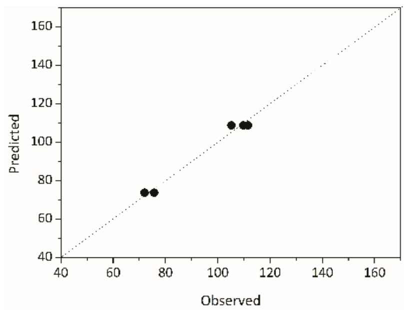

Figure S7. Representation of the quality of the model fit for average diameters for PMMA NPs considering only CTAB surfactant tests.

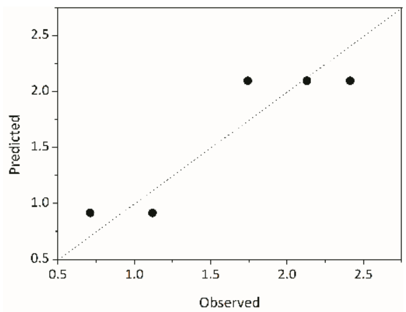

Figure S8. Representation of the quality of the model fit for BSA adsorption onto the PMMA NPs surfaces, considering only CTAB surfactant tests.

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