Supplementary Information for

Improved accuracy in pentraxin-3 quantification assisted by aqueous biphasic systems as serum pretreatment strategies

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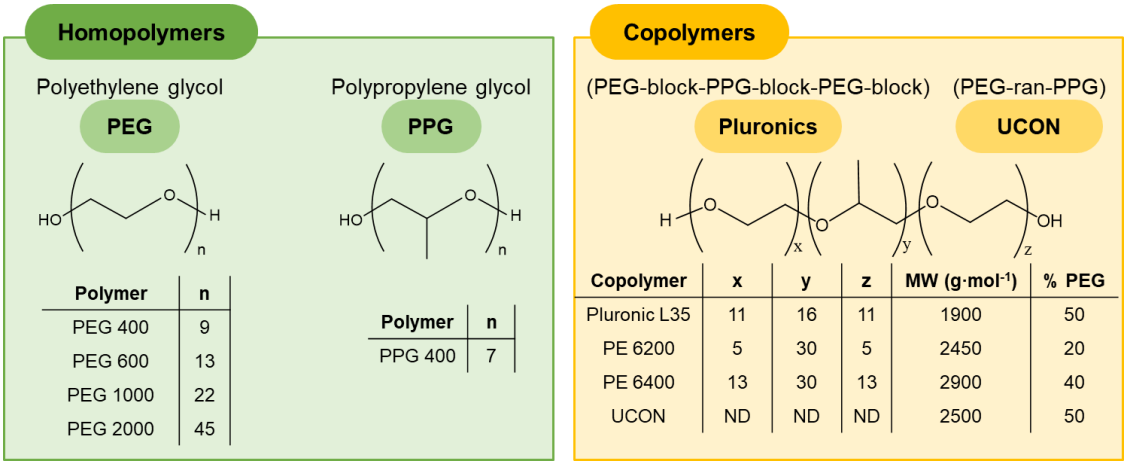
SI Figures

Materials



Polymer	n
PEG 400	9
PEG 600	13
PEG 1000	22
PEG 2000	45

Polymer	n
PPG 400	7

Copolymer	x	y	z	MW (g·mol^{-1})	% PEG
Pluronic L35	11	16	11	1900	50
PE 6200	5	30	5	2450	20
PE 6400	13	30	13	2900	40
UCON	ND	ND	ND	2500	50

Figure S1. Chemical structures, names, and abbreviations of polymers and copolymers studied.

SI Tables

Phase diagrams

Table S1. Experimental binodal data in weight percent for the ternary systems composed of homopolymers (1) + $K_3C_6H_5O_7/C_6H_8O_7$ (pH ≈ 7) (2) + water at 25 °C and atmospheric pressure.

PPG 400		**PEG 400**		**PEG 600**		**PEG 1000**		**PEG 2000**	
$100w_1$	**$100w_2$**	**$100w_1$**	**$100w_2$**	**$100w_1$**	**$100w_2$**	**$100w_1$**	**$100w_2$**	**$100w_1$**	**$100w_2$**
47.19	2.24	40.95	13.60	49.04	6.63	40.68	7.51	40.83	6.03
43.34	3.03	38.03	14.82	47.04	7.71	35.00	9.43	37.39	6.96
39.59	3.81	35.87	16.14	44.48	8.92	31.91	10.41	34.92	7.66
36.40	5.10	34.18	16.85	41.58	10.65	29.50	11.08	31.97	8.13
33.19	5.49	31.21	18.68	40.22	11.39	27.44	12.16	30.23	8.81
31.25	5.88	28.89	19.99	38.03	12.73	25.61	13.23	28.69	9.38
29.84	6.36	26.30	21.86	36.33	13.61	24.18	13.93	27.13	9.99
28.80	6.84	24.22	23.29	35.00	14.53	22.92	14.63	25.48	10.41
27.39	7.37	22.12	24.91	33.33	15.48	22.07	15.25	24.63	10.93
25.81	7.51	21.61	25.24	31.75	16.46	21.04	15.72	23.55	11.06
24.82	7.83	19.98	26.45	30.62	17.11	19.96	16.34	23.01	11.36
24.22	7.91	18.88	27.30	30.02	17.39	18.93	17.10	22.24	11.59
23.63	8.01	17.26	28.46	29.33	17.79	18.05	17.60	21.78	11.86
23.23	8.20	16.28	29.13	28.73	18.14	17.21	18.12	21.33	12.10
22.61	8.89	15.17	30.03	27.70	18.72	16.61	18.51	20.69	12.16
21.56	8.77	14.33	30.69	26.01	19.68	15.97	18.85	20.27	12.43
21.28	8.88	13.57	31.22	25.14	20.13	15.57	19.24	19.82	12.63
20.81	8.91	12.93	31.73	24.34	20.64	15.10	19.74	19.42	12.79
20.10	9.48	12.39	32.13	23.40	21.29	14.45	20.26	18.92	13.19
19.27	9.51	11.85	32.54	22.58	21.72	13.80	20.42	18.43	13.63
18.72	9.53	11.11	33.25	22.05	21.98	13.44	20.46	17.87	13.75
18.01	10.01	10.68	33.53	21.40	22.29	12.99	20.73	17.57	13.91
17.37	10.10	10.19	33.91	20.79	22.70	12.52	21.14	17.27	14.15
16.84	10.20	9.76	34.36	20.58	22.68	12.17	21.30	17.00	14.30
16.37	10.30	9.39	34.70	20.24	22.81	11.80	21.52	16.71	14.40
16.07	10.40			19.82	23.13	11.41	21.73	16.46	14.57
15.66	10.56			19.41	23.46	11.11	21.90	16.18	14.77
15.41	10.74			19.11	23.62	10.78	22.11	15.83	15.22
14.54	10.75			18.54	23.96	10.51	22.51	15.35	15.32
14.22	11.00			18.13	24.15	10.08	22.75	15.01	15.31
13.77	11.12			17.78	24.41	9.84	22.83	14.64	15.67
13.30	11.05			17.34	24.67	9.59	22.98	14.37	15.62
12.92	11.33			17.05	24.78	9.31	23.22	14.18	15.73
12.51	11.45			16.55	25.12	9.01	23.57	14.00	15.81
12.05	11.67			16.14	25.36	8.83	23.64	13.79	15.98
11.60	11.74			15.86	25.49	8.62	23.80	13.65	16.12
11.19	11.98			15.60	25.68	8.38	23.86	13.40	16.24
10.87	12.31			15.29	25.79	8.13	24.15	13.20	16.37
10.45	12.30			15.14	25.91	7.87	24.31	12.84	16.48
10.11	12.49			14.78	26.16	7.63	24.47	12.51	16.72
9.83	12.73			14.31	26.58	7.38	24.71	12.25	16.90
9.37	13.06			13.90	26.81	7.17	24.84	12.03	16.90

Table S1 (cont.) Experimental binodal data in weight percent for the ternary systems composed of homopolymers (1) + $K_3C_6H_5O_7/C_6H_8O_7$ (pH ≈ 7) (2) + water at 25 °C and atmospheric pressure.

PPG 400		PEG 400		PEG 600		PEG 1000		PEG 2000	
$100w_1$	$100w_2$	$100w_1$	$100w_2$	$100w_1$	$100w_2$	$100w_1$	$100w_2$	$100w_1$	$100w_2$
9.09	13.02			13.66	26.94	6.96	24.97	11.61	17.34
8.87	13.10			13.36	27.06	6.79	25.21	11.29	17.54
8.62	13.28			13.08	27.27	6.61	25.35	11.01	17.61
8.46	13.41			12.75	27.40	6.43	25.47	10.78	17.78
8.24	13.45			12.59	27.48	6.24	25.69	10.57	17.94
8.07	13.64			12.35	27.62	6.07	25.83	10.33	18.10
7.78	13.75			12.04	27.82	5.96	25.87	10.10	18.33
7.61	13.89			11.81	27.95	5.82	26.12	9.82	18.56
7.47	14.02			11.54	28.17	5.70	26.15	9.53	18.66
7.27	14.11					5.57	26.28	9.15	19.02
7.06	14.25					5.42	26.45	8.93	18.89
6.89	14.29					5.25	26.60	8.69	19.12
6.75	14.33					5.09	26.76	8.46	19.11
6.59	14.52					4.99	26.81	8.23	19.34
6.41	14.70							7.91	19.53
6.26	14.68							7.78	19.60
6.16	14.91							7.61	19.74
5.96	15.03							7.43	19.91
5.73	15.17							7.23	19.99
5.58	15.33							7.05	19.89
5.46	15.41							6.87	20.21
5.28	15.53							6.71	20.19
5.18	15.61							6.55	20.48
5.05	15.63							6.38	20.46
								6.24	20.69
								6.09	20.69
								5.97	20.80
								5.84	20.93
								5.66	21.03
								5.47	21.43
								5.26	21.29

Table S2. Experimental binodal data in weight percent for the ternary systems composed of copolymers (1) + $K_3C_6H_5O_7/C_6H_8O_7$ (pH ≈ 7) (2) + water at 25 °C and atmospheric pressure.

Pluronic PE6200		**Pluronic PE6400**		**Pluronic L35**		**UCON**	
$100w_1$	**$100w_2$**	**$100w_1$**	**$100w_2$**	**$100w_1$**	**$100w_2$**	**$100w_1$**	**$100w_2$**
51.85	3.38	51.25	3.08	27.44	6.13	25.26	7.19
45.94	5.79	45.65	4.04	25.27	7.36	23.85	7.51
36.92	5.62	40.00	5.15	22.68	8.24	22.80	7.94
33.51	6.14	37.85	5.48	20.61	9.20	21.89	8.42
30.94	6.20	35.80	6.01	18.37	9.38	20.63	8.73
29.28	6.79	29.27	7.19	16.85	10.03	19.91	9.17
27.24	7.22	28.04	7.77	15.64	10.66	18.67	9.73
24.65	7.38	23.23	8.83	14.07	10.80	17.45	9.79
22.97	7.63	22.04	8.96	13.05	11.15	16.86	10.08
21.11	7.99	21.44	9.10	12.36	11.04	16.38	10.33
19.98	8.16	20.85	9.36	11.77	11.46	15.84	10.57
18.89	8.31	19.93	9.96	11.19	11.81	15.42	10.70
18.34	8.59	18.69	9.90	10.56	11.92	14.89	11.01
17.63	8.95	18.03	10.12	9.93	12.08	14.44	11.14
16.75	8.94	17.28	10.21	9.47	12.25	14.06	11.27
15.92	9.07	16.65	10.64	9.02	12.22	13.74	11.53
15.09	9.14	15.87	10.89	8.70	12.35	13.17	11.90
14.09	9.13	15.10	10.78	8.33	12.82	12.72	12.04
13.39	9.01	14.62	10.89	8.20	12.76	12.24	12.43
12.88	8.99	14.25	10.94	7.96	13.01	11.83	12.72
12.51	9.03	13.94	11.13	7.60	12.96	11.28	12.98
12.15	9.11	13.42	11.23	7.30	12.99	10.78	13.09
11.71	9.17	12.94	11.41	7.11	12.96	10.50	13.23
11.34	9.18	12.60	11.43	6.96	13.15	10.16	13.55
10.95	9.25	12.30	11.47	6.70	13.21	9.82	13.77
10.52	9.22	12.02	11.56	6.45	13.25	9.43	13.78
10.15	9.25	11.71	11.75	6.22	13.17	9.14	14.05
9.71	9.13	11.43	11.74	6.05	13.43	8.78	14.13
9.42	9.15	11.16	11.83	5.81	13.38	8.50	14.17
9.13	9.12	10.93	11.83	5.63	13.38	8.06	14.02
8.87	9.14	10.67	11.98	5.44	13.34	7.88	14.16
8.60	9.21	10.36	12.14	5.31	13.46	7.65	14.18
8.31	9.34	10.14	12.27	5.15	13.49	7.47	14.32
8.19	9.38	9.86	12.31	5.01	13.52	7.34	14.50
8.06	9.44	9.64	12.36	4.86	13.48	7.12	14.57
7.90	9.46	9.47	12.46	4.76	13.59	6.95	14.71
7.74	9.44	9.30	12.39	4.62	13.59	6.71	14.67
7.57	9.54	9.19	12.52	4.49	13.60	6.59	14.88
7.36	9.42	9.03	12.58	4.38	13.60	6.41	14.74
7.16	9.44	8.87	12.60	4.29	13.62	6.32	15.00
6.95	9.40	8.73	12.63	4.20	13.59	6.16	15.11
6.72	9.27	8.59	12.63	4.01	13.33	6.00	15.10
		8.42	12.81	3.90	13.29	5.87	15.14
		8.21	12.86			5.75	15.38
		8.00	12.89			5.62	15.36
		7.82	12.90			5.50	15.42
		7.72	12.84			5.38	15.46
		7.59	13.01			5.29	15.44

Table S2 (cont.). Experimental binodal data in weight percent for the ternary systems composed of copolymers (1) + $K_3C_6H_5O_7/C_6H_8O_7$ (pH ≈ 7) (2) + water at 25 °C and atmospheric pressure.

Pluronic PE6200		Pluronic PE6400		Pluronic L35		UCON	
$100w_1$	$100w_2$	$100w_1$	$100w_2$	$100w_1$	$100w_2$	$100w_1$	$100w_2$
		7.44	13.01			5.19	15.48
		7.32	13.00			5.08	15.57
		7.20	12.92			4.97	15.56
		7.09	13.09			4.88	15.82
		6.98	13.17			4.69	15.97
		6.86	13.10			4.55	16.03
		6.77	13.17			4.41	16.11
		6.64	13.18			4.29	16.15
		6.52	13.16			4.21	16.32
		6.43	13.25			4.07	16.39
		6.33	13.26			3.93	16.47
		6.24	13.32			3.83	16.67
		6.17	13.29			3.70	16.82
		6.10	13.32			3.60	16.92
		6.04	13.34				
		5.97	13.34				
		5.89	13.40				
		5.82	13.40				

Table S3. Correlation parameters *A*, *B* and *C* used to describe the experimental binodal data for the ternary systems composed of homopolymer/copolymers, $K_3C_6H_5O_7/C_6H_8O_7$ (pH ≈ 7) and water obtained by equation (1), respective standard deviations (σ) and correlation coefficients (R^2).

Homopolymer/Copolymer	$A \pm \sigma$	$B \pm \sigma$	$C \pm \sigma$	R^2
PPG 400	78 ± 6	-0.33 ± 0.04	0.0004 ± 0.0001	0.9766
PEG 400	107 ± 12	-0.249 ± 0.03	$2\times10^{-5} \pm 2\times10^{-6}$	0.9992
PEG 600	76 ± 1	-0.168 ± 0.006	$4\times10^{-5} \pm 8\times10^{-7}$	0.9997
PEG 1000	102 ± 9	-0.33 ± 0.03	$6\times10^{-5} \pm 4\times10^{-6}$	0.9977
PEG 2000	131 ± 9	-0.47 ± 0.02	$9\times10^{-5} \pm 6\times10^{-6}$	0.9977
Pluronic PE6200	100 ± 5	-0.27± 0.05	$2\times10^{-3} \pm 3\times10^{-4}$	0.9775
Pluronic PE6400	89 ± 6	-0.31 ± 0.03	$6\times10^{-4} \pm 3\times10^{-5}$	0.9972
Pluronic L35	100 ± 2	-0.42 ± 0.03	$5\times10^{-4} \pm 5\times10^{-5}$	0.9937
UCON	46 ± 10	-0.20 ± 0.08	$4.0\times10^{-4} \pm 0.0$	0.9904

Table S4. Experimental TLs in weight percent (wt%), TLLs and pH of the phases for ternary systems composed of homopolymer/copolymer, $K_3C_6H_5O_7/C_6H_8O_7$ (pH ≈ 7) and water at 25 °C and atmospheric pressure. $[Polymer]_M$ and $[Salt]_M$ indicate the initial mixture composition, while $[Polymer]_{Top}$, $[Salt]_{Top}$, $[Polymer]_{Bottom}$ and $[Salt]_{Bottom}$ stand for the top and bottom phases compositions.

Homopolymer/Copolymer	pH_{Top}	Weight fraction composition (wt%)						pH_{Bottom}	TLL
		$[Polymer]_{Top}$	$[Salt]_{Top}$	$[Polymer]_M$	$[Salt]_M$	$[Polymer]_{Bottom}$	$[Salt]_{Bottom}$		
PPG 400	7.73 ± 0.06	94.59	0.00	30.39	28.96	0.00	42.86	6.73 ± 0.01	103.95
PEG 400	7.25 ± 0.09	56.83	6.34	30.64	28.80	0.44	54.69	7.18 ± 0.04	74.29
PEG 600	7.22 ± 0.09	63.12	1.23	31.11	28.87	0.01	55.72	7.08 ± 0.00	83.37
PEG 1000	7.69 ± 0.08	72.96	0.98	29.91	29.21	0.01	48.82	7.06 ± 0.07	87.24
PEG 2000	7.18 ± 0.05	63.82	1.00	29.66	29.39	0.00	54.04	6.9 ± 0.2	82.99
Pluronic PE6200	6.51 ± 0.02	78.54	0.80	31.19	28.70	0.00	47.08	6.78 ± 0.08	91.16
Pluronic PE6400	6.53 ± 0.01	73.45	0.37	29.93	29.26	0.00	49.12	6.92 ± 0.05	88.16
Pluronic L35	7.62 ± 0.05	78.39	0.33	29.83	29.43	0.00	47.30	6.76 ± 0.03	91.38
UCON	8.48 ± 0.06	77.93	0.00	30.08	28.97	0.00	47.68	6.98 ± 0.09	91.66

Depletion efficiencies of human serum albumin and immunoglobulin G

Table S5. Depletion efficiencies of IgG (DE_{IgG}, %) and HSA (DE_{HSA}, %) obtained with ABS-TPP composed of 30 wt% of homopolymer/copolymer + 30 wt% of $K_3C_6H_5O_7/C_6H_8O_7$ (pH ≈ 7) + 5 wt% of human serum + 35 wt% of water.

Homopolymer/Copolymer	DE_{IgG} (%)	DE_{HSA} (%)
PPG 400	84.0 ± 0.3	91 ± 1
PEG 400	23.16 ± 0.09	9 ± 2
PEG 600	57 ± 1	24 ± 3
PEG 1000	93.4 ± 0.1	95 ± 1
PEG 2000	87 ± 3	83 ± 4
Pluronic PE6200	73 ± 4	41 ± 4
Pluronic PE6400	59 ± 7	39.9 ± 0.6
UCON	84 ± 2	92 ± 3
Pluronic L35	74 ± 3	85 ± 5

Extraction efficiencies and recovery yields of pentraxin 3

Table S6. Extraction efficiency of PTX-3 (EE_{PTX-3}, %) obtained with ABS composed of 30 wt% of homopolymer/copolymer + 30 wt% of $K_3C_6H_5O_7/C_6H_8O_7$ + 5 wt% of aqueous solution of PTX-3 (5 ng·mL^{-1}) + 35 wt% of water.

Homopolymer/Copolymer	EE_{PTX-3} (%)
PPG 400	100
PEG 1000	100
PEG 2000	100
UCON	100
Pluronic L35	100

Table S7. Recovery yield of PTX-3 (RY_{PTX-3}, %) to the top, inter, and bottom phase in the ABS-TPP composed of 30 wt% of homopolymer/copolymer + 30 wt% of $K_3C_6H_5O_7/C_6H_8O_7$ + 5 wt% human serum spiked with PTX-3 (5 ng·mL^{-1}) + 35 wt% of water.

Homopolymer/Copolymer	RY_{PTX-3} (%)		
	Top phase	Interphase	Bottom phase
PPG 400	0.2 ± 0.3	94 ± 2	5 ± 1
PEG 1000	99.8 ± 0.4	0.2 ± 0.4	0 ± 0
PEG 2000	0 ± 0	94 ± 5	6 ± 5
UCON	54 ± 12	46 ± 12	0 ± 0
Pluronic L35	48 ± 14	52 ± 14	0 ± 0