

Electronic Supporting Information (ESI)

An integrated process combining the reaction and purification of PEGylated proteins

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Table S.1: Parameters obtained through the Merchuk equation (Eq. 1) with the respective standard deviations (std) and correlation factors (R^2) along with the weight fraction data (wt%) for the systems composed of polyethylene glycol polymers (1) + $C_6H_5K_3O_7/C_6H_8O_7$ pH 7 (2) + H_2O systems determined in the present work.

mPEG 2 kDa		PEG 2 kDa	
$A \pm \text{std} = 87.44 \pm 5.58$		$A \pm \text{std} = 83.18 \pm 5.50$	
$B \pm \text{std} = -0.354 \pm 0.029$		$B \pm \text{std} = -0.314 \pm 0.030$	
$C \pm \text{std} = 2.3 \times 10^{-4} \pm 2.4 \times 10^{-5}$		$C \pm \text{std} = 2.0 \times 10^{-4} \pm 2.1 \times 10^{-5}$	
$R^2 = 0.9888$		$R^2 = 0.9855$	
wt%(1)	wt%(2)	wt%(1)	wt%(2)
6.13	19.19	12.05	15.36
7.01	18.39	12.25	15.15
8.80	17.31	12.56	14.98
9.85	16.56	12.89	14.95
10.57	16.14	13.18	14.74
12.47	15.03	13.51	14.64
13.63	14.31	13.75	14.41
15.08	12.99	14.13	14.29
15.56	12.81	14.39	14.03
15.93	12.46	14.79	13.92
16.49	12.29	15.25	13.76
16.88	11.95	15.92	13.33
17.45	11.68	16.44	13.09
17.88	11.26	16.82	12.76
18.59	10.95	17.41	12.53
19.30	10.70	18.04	12.36
20.27	10.53	18.73	12.14
21.83	10.42	19.75	12.00
22.90	10.08	20.82	11.24
24.32	9.26	21.70	10.88
25.60	8.81	22.66	10.46
27.01	8.29	23.76	10.01
29.22	7.82	24.49	9.45
31.05	7.09	26.22	9.13
33.90	6.53	28.07	8.70
36.89	5.95	31.05	8.31
40.02	5.11	35.88	6.62
43.11	4.27	41.59	5.77
45.93	2.75	44.28	4.29
		47.05	2.69

Table

S.2:

Parameters

obtained through the Merchuk equation (Eq. 1) with the respective standard deviations (std) and correlation factors (R^2) along with the weight fraction data (wt%) for the systems composed of polyethylene glycol (1) + $C_6H_5K_3O_7/C_6H_8O_7$ pH 7 (2) + H_2O systems determined in the present work.

PEG 6 kDa		PEG 10 kDa		PEG 20 kDa	
$A \pm \text{std} = 118.9 \pm 6.2$ $B \pm \text{std} = -0.501 \pm 0.024$ $C \pm \text{std} = 3.0 \times 10^{-4} \pm 2.3 \times 10^{-5}$		$A \pm \text{std} = 171 \pm 21.8$ $B \pm \text{std} = -0.665 \pm 0.054$ $C \pm \text{std} = 4.0 \times 10^{-4} \pm 4.3 \times 10^{-5}$		$A \pm \text{std} = 74.20 \pm 2.62$ $B \pm \text{std} = -0.526 \pm 0.022$ $C \pm \text{std} = 5.8 \times 10^{-4} \pm 8.2 \times 10^{-5}$	
$R^2 = 0.9966$		$R^2 = 0.9937$		$R^2 = 0.9941$	
wt%(1)	wt%(2)	wt%(1)	wt%(2)	wt%(1)	wt%(2)
10.43	12.52	8.27	11.98	7.39	10.02
10.68	12.50	8.49	11.93	8.19	9.49
10.94	12.42	8.70	11.93	9.56	8.99
11.13	12.22	8.90	11.89	11.46	8.27
11.29	12.12	9.17	11.86	13.01	7.74
11.66	12.13	9.47	11.65	15.03	7.09
11.97	12.05	9.69	11.54	17.54	6.38
12.28	11.92	9.90	11.42	17.72	6.53
12.63	11.78	10.03	11.25	18.71	6.10
13.00	11.66	10.37	11.22	19.83	5.58
13.37	11.53	10.79	11.31	20.77	5.34
13.87	11.44	11.15	11.04	22.26	4.70
14.32	11.29	11.62	11.00	24.70	4.02
15.08	10.85	11.92	10.72	26.49	3.26
15.62	10.67	12.23	10.53	28.32	3.00
16.35	10.56	13.13	10.71	32.86	2.31
17.01	10.27	13.75	10.04	46.52	0.84
17.68	10.03	14.16	9.77		
18.69	9.94	14.79	9.59		
19.57	9.64	15.47	9.31		
20.50	9.30	16.35	9.27		
21.50	8.86	17.18	9.00		
23.42	8.00	17.94	8.65		
25.12	7.65	19.16	8.43		
27.11	7.21	20.30	8.09		
29.30	6.66	22.33	7.98		
32.24	6.00	24.14	7.43		
35.66	5.27	26.60	7.00		
41.46	4.57	35.64	5.11		
46.09	3.37				

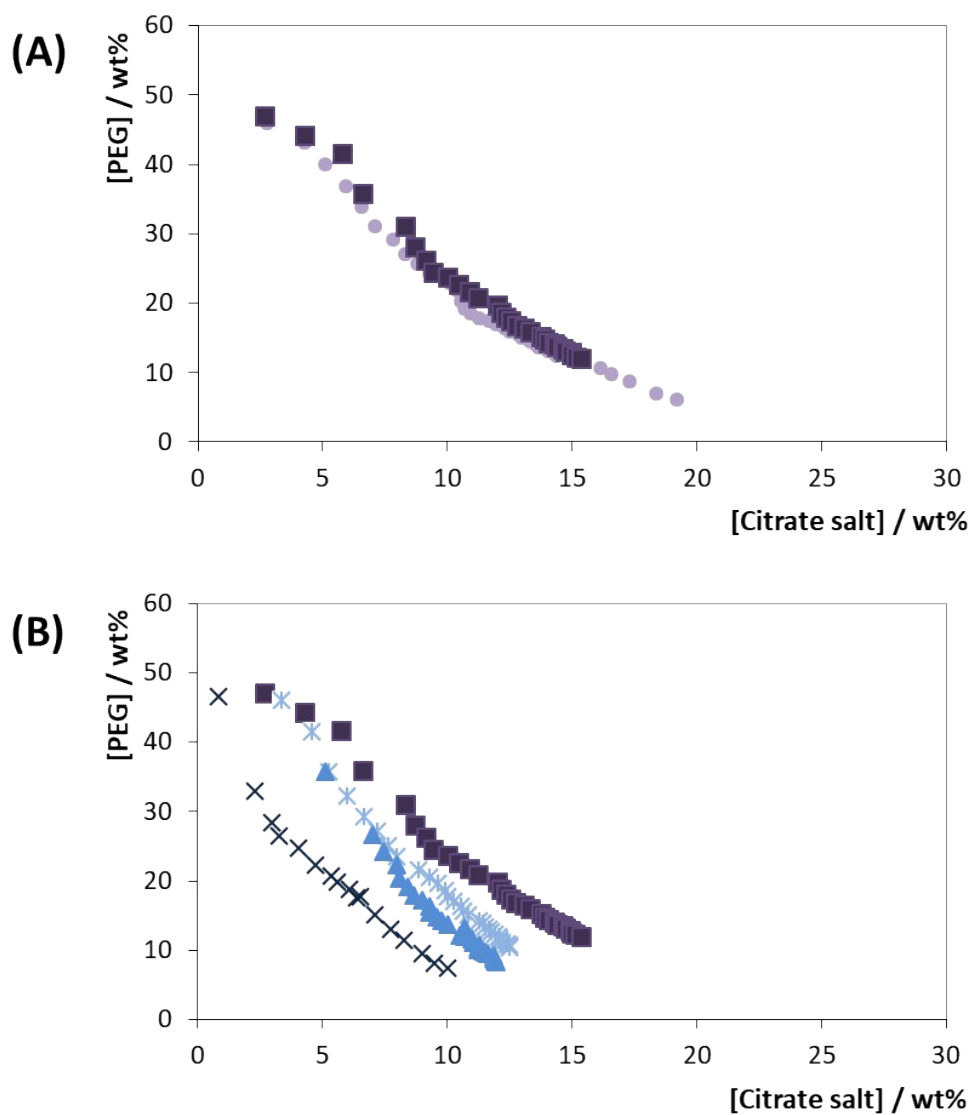
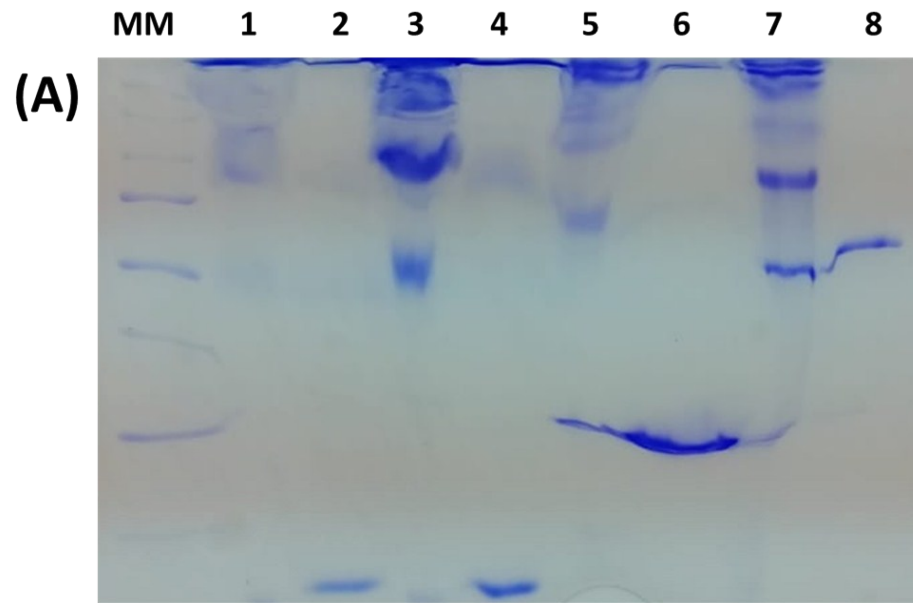
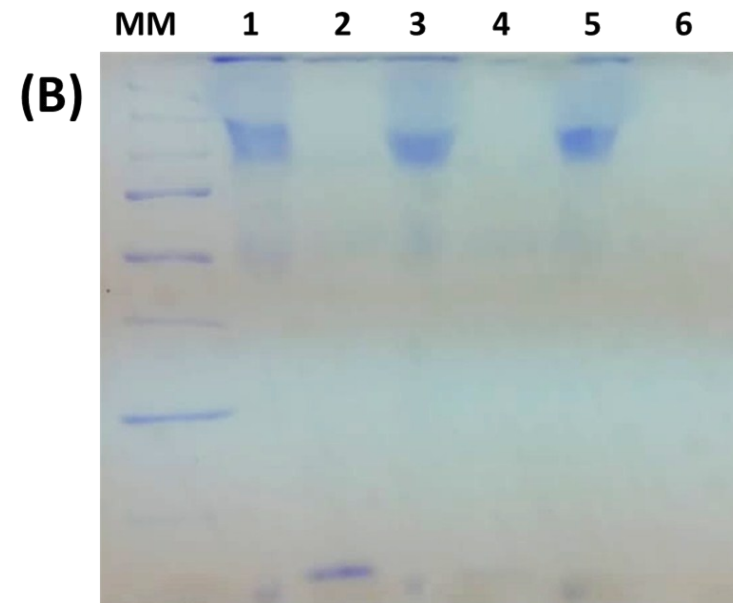


Fig. S.1: Ternary phase diagrams of the ABS composed of **(A)** mPEG 2kDa (●) and PEG 2 kDa (■) + $C_6H_5K_3O_7/C_6H_8O_7$ (pH = 7) **(B)** PEG 2 (■), 6 (▼), 10 (▲), and 20 (⊗) kDa + $C_6H_5K_3O_7/C_6H_8O_7$ (pH = 7) at $T = (298 \pm 1)$ K and atmospheric pressure.



MM – Molecular Marker
 1 - Top Cyt-c
 2 - Bot Cyt-c
 3 - Top LYS
 4 - Bot LYS
 5 - Top ASNase
 6 - Bot ASNase
 7 - Top CAT
 8 - Bot CAT



MM – Molecular Marker
 1 - Top Cyt-c, time 7.5
 2 - Bot Cyt-c, time 7.5
 3 - Top Cyt-c, time 15
 4 - Bot Cyt-c, time 15
 5 - Top Cyt-c, time 30
 6 - Bot Cyt-c, time 30

Fig. S.2: SDS-PAGE gels of the bottom and top aqueous phases of the integrated ABS composed of 1.0 wt% Prot + 12.5 wt% Prot-PEG/PEG + 12.5 wt% potassium citrate buffer at pH 7.0, in terms of the protein type **(A)** and time assay **(B)**.

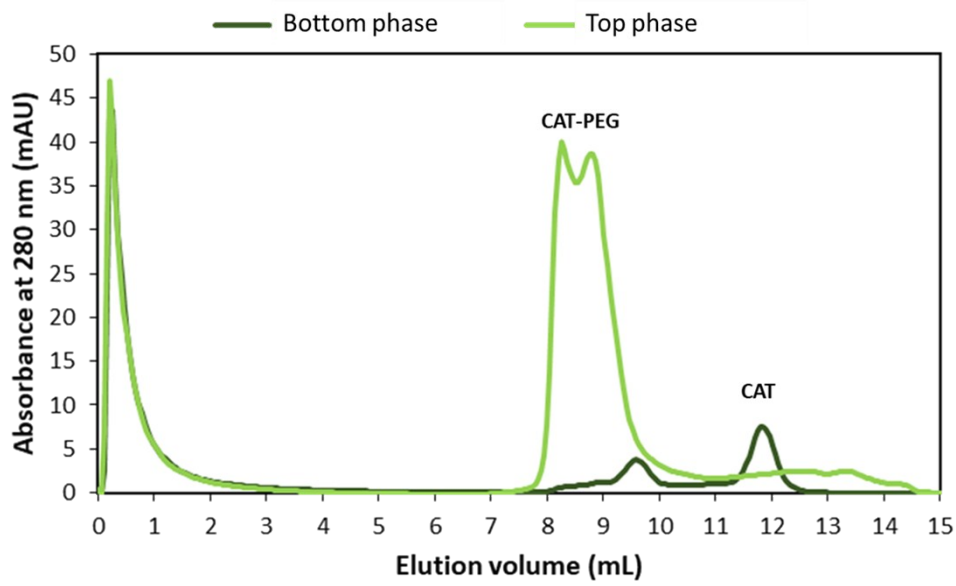


Fig. S.3: FPLC chromatogram of the top and bottom phases of integrated ABS composed of 1.0 wt% CAT + 12.5 wt% CAT-PEG/PEG + 12.5 wt% potassium citrate buffer, performed with mPEG-NHS of 20 kDa for a reaction time of 7.5 min.

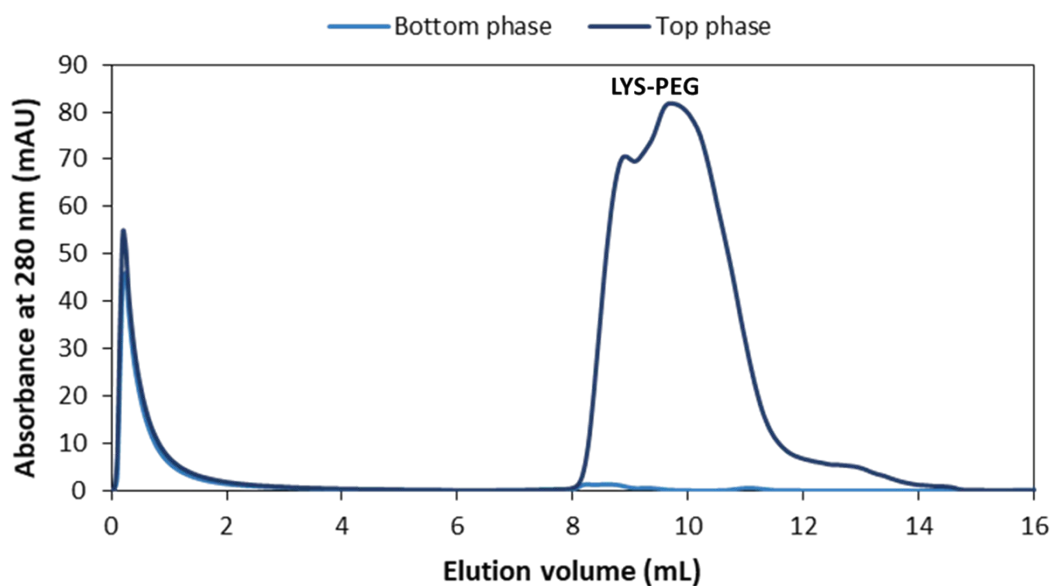


Fig. S.4: FPLC chromatogram of the top and bottom phases of integrated ABS composed of 1.0 wt% LYS + 12.5 wt% LYS-PEG/PEG + 12.5 wt% potassium citrate buffer, performed with mPEG-NHS of 20 kDa for a reaction time of 7.5 min.

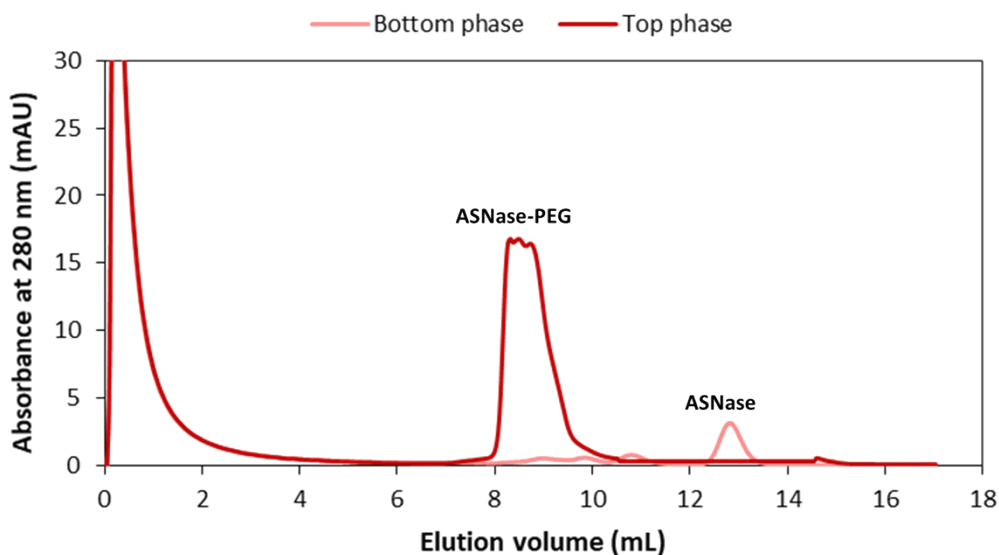


Fig. S.5: FPLC chromatogram of the top and bottom phases of integrated ABS composed of 1.0 wt% ASNase + 12.5 wt% ASNase-PEG/PEG + 12.5 wt% potassium citrate buffer, performed with mPEG-NHS of 20 kDa for a reaction time of 7.5 min.