

Supporting information

Separation of immunoglobulin G using aqueous biphasic systems composed of cholinium-based ionic liquids and poly(propylene glycol)

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Table S1. Experimental binodal weight fraction data for the systems composed of cholinium-based ILs (1) + PPG 400 (2) + H₂O (3) at 25°C.

[Chol][Caf]				[Chol][Gal]				[Chol][Van]				[Chol][Syr]	
100 w_1	100 w_2	100 w_1	100 w_2	100 w_1	100 w_2	100 w_1	100 w_2	100 w_1	100 w_2	100 w_1	100 w_2	100 w_1	100 w_2
2.083	86.82	47.23	19.47	0.7585	89.16	13.71	39.35	0.9459	89.42	15.78	43.76	0.6427	88.47
3.269	81.03	46.00	20.24	1.431	82.07	14.58	37.95	2.086	85.24	16.51	42.62	4.291	73.70
4.687	78.06	44.78	21.00	1.920	74.61	15.28	35.94	2.886	78.51	17.03	41.20	4.820	71.56
5.919	75.18	43.64	21.75	2.521	72.62	16.46	34.10	3.712	74.99	17.25	40.16	5.758	68.96
8.020	71.41	42.97	22.05	3.069	70.84	17.09	32.88	4.467	72.86	17.58	39.53	6.843	66.09
8.975	69.25	42.35	22.42	3.614	68.67	17.71	32.53	5.266	71.11	18.33	38.21	7.654	63.87
10.86	65.97	41.14	22.98	4.151	67.03	18.96	30.83	5.858	69.41	19.01	36.73	9.172	59.69
11.68	64.02	40.25	23.58	4.712	65.42	20.50	29.24	6.570	67.53	20.06	35.06	10.01	57.53
13.37	61.13	39.49	24.22	5.222	63.96	22.00	27.59	7.229	65.93	21.03	33.53	10.81	55.68
14.76	58.61	38.73	24.80	6.265	61.12			7.903	64.21	23.64	30.95	12.22	52.06
15.33	57.09	37.91	25.42	6.608	59.32			9.013	61.34	27.30	26.18	18.12	38.95
16.64	54.79	37.12	25.92	6.969	58.20			9.448	60.04	31.59	22.28	24.17	29.35
17.17	53.51	36.36	26.29	8.388	54.42			10.00	58.73	34.11	20.22		
17.86	52.02	35.47	27.15	8.735	53.27			11.02	56.33	38.55	17.01		
18.99	50.06	33.79	29.38	9.407	51.66			11.47	54.48	42.71	15.29		
20.48	47.28	32.94	30.15	9.887	48.89			11.96	53.36	45.19	12.99		
21.39	45.71	32.00	31.15	10.57	47.50			12.56	52.18	49.47	10.44		
22.82	43.64	31.08	32.08	11.03	46.12			12.94	51.17	51.53	8.60		
24.10	41.79	30.20	33.01	11.25	45.41			13.34	50.19	54.72	7.58		
24.86	40.55	28.99	34.54	11.50	44.66			13.69	49.19	57.43	6.40		
54.71	14.79	28.36	35.21	12.03	43.62			14.06	47.78	61.12	5.07		
51.79	16.38	27.42	36.33	12.53	42.24			14.84	46.40	65.12	3.50		
50.15	17.53	26.50	37.48	12.79	41.55			15.19	45.51	67.87	2.68		
48.70	18.46			13.48	39.99			15.45	44.50				

Table S2. Correlation parameters of Eq. (1) applied to describe the experimental binodal data and respective standard deviations (σ).

IL	$A \pm \sigma$	$B \pm \sigma$	$10^5 (C \pm \sigma)$	R^2
[Chol][Caf]	120.4 ± 5.6	-0.196 ± 0.014	0.55 ± 0.10	0.9896
[Chol][Van]	121.5 ± 5.0	-0.244 ± 0.014	0.73 ± 0.17	0.9909
[Chol][Gal]	109.7 ± 3.4	-0.246 ± 0.015	3.09 ± 0.87	0.9929
[Chol][Syr]	103.1 ± 4.6	-0.170 ± 0.019	3.36 ± 0.87	0.9942

Table S3. Tie-lines (TLs), tie-lines length (TLLs) and volume ratio (V_r) between phases. Initial mixture compositions are represented as $[\text{PPG}]_M$ and $[\text{IL}]_M$, whereas $[\text{IL}]_{\text{IL}}$ and $[\text{PPG}]_{\text{IL}}$ are the compositions of IL and PPG at the IL-rich phase, respectively, and vice-versa.

IL	Weight fraction composition / wt %						TLL	V_r
	$[\text{IL}]_{\text{IL}}$	$[\text{PPG}]_{\text{IL}}$	$[\text{IL}]_M$	$[\text{PPG}]_M$	$[\text{IL}]_{\text{PPG}}$	$[\text{PPG}]_{\text{PPG}}$		
[Chol][Caf]	60.81	7.62	24.92	44.68	15.75	54.14	64.76	3.9
	70.67	3.357	27.53	47.18	9.751	64.96	86.64	2.5
[Chol][Van]	70.86	4.876	24.98	45.07	5.534	55.69	82.76	1.5
	64.02	2.511	27.69	48.28	3.068	79.25	98.00	1.5
[Chol][Gal]	76.26	2.189	25.17	44.76	0.04319	57.75	94.32	1.4
	53.19	0.1753	19.85	41.07	7.278	55.87	72.18	2.8
[Chol][Syr]	77.12	2.879	24.78	44.78	0.1094	54.98	92.98	1.4
	60.15	0.0179	27.50	47.22	1.256	85.14	103.5	1.2
	60.77	0.0145	29.96	49.50	0.1081	97.46	114.8	1.0
[Chol][Bit] ^a	43.42	2.115	44.92	25.21	1.651	99.44	106.0	0.9
	43.27	2.210	31.09	30.39	1.700	98.39	104.8	n.a.
	31.72	6.170	24.88	24.64	2.430	85.19	84.28	n.a.
	23.60	11.540	19.98	20.28	5.320	55.69	47.79	n.a.
[Chol][Lac] ^a	45.86	0.9074	44.52	25.36	1.841	94.55	103.5	1.1
	44.05	1.120	30.45	30.05	2.030	90.51	98.78	n.a.
	34.69	2.920	16.22	39.68	3.700	64.59	68.81	n.a.

	26.77	5.980	12.19	34.90	5.610	47.95	47.40	n.a.
	41.68	6.283	45.50	24.84	4.489	92.89	94.25	1.0
[Chol][DHCit] ^a	39.98	7.410	29.71	29.62	4.740	89.47	88.92	n.a.
	34.28	9.180	23.56	34.81	5.828	77.36	73.87	n.a.
	27.47	13.800	19.63	29.38	9.700	50.21	66.57	n.a.
	47.424	1.124×10^{-5}	44.44	24.78	1.335	90.46	101.5	1.1
[Chol][Prop] ^a	46.58	2.420×10^{-5}	31.55	29.49	1.450	88.58	99.41	n.a.
	26.32	1.310	20.18	19.96	2.390	74.01	38.43	n.a.
	22.90	3.360	13.18	34.18	3.120	66.06	65.78	n.a.
	46.76	0.1139	44.77	25.16	1.415	93.87	104.1	1.1
[Chol][Gly] ^a	44.52	0.1700	29.99	30.00	1.567	88.98	98.65	n.a.
	33.21	0.9400	14.88	40.07	2.530	66.43	72.32	n.a.
	23.41	3.200	9.13	40.21	3.240	55.48	56.03	n.a.
	45.40	1.480	44.75	25.33	3.327	92.19	99.99	1.2
[Chol][But] ^a	43.31	1.790	30.19	30.07	3.870	86.83	94.52	n.a.
	24.56	6.090	19.91	20.25	5.370	64.58	70.83	n.a.
	36.47	2.830	30.19	30.07	5.800	59.94	61.56	n.a.
	44.44	0.4607	44.64	25.14	1.197	99.43	108.0	1.1
	42.89	0.5500	29.98	30.11	1.300	95.74	103.9	n.a.
[Chol][Ac] ^a	36.83	1.090	20.25	29.72	2.990	59.54	91.86	n.a.
	27.93	2.710	10.10	30.40	4.950	39.87	43.69	n.a.
	39.75	0.7900	29.75	20.09	2.160	73.38	81.75	n.a.
	34.78	1.350	29.15	10.69	3.490	53.21	60.57	n.a.
	42.39	3.650	44.83	24.84	1.297	100.1	104.8	1.2
[Chol]Cl ^a	41.99	3.725	30.00	29.98	1.622	92.14	97.19	n.a.
	31.94	6.223	25.05	25.05	1.811	88.15	87.29	n.a.
	26.22	8.638	20.20	25.05	2.757	72.63	84.57	n.a.
	49.96	0.1931	44.94	25.08	1.202	87.88	100.3	1.0
[Chol][DHPh] ^a	46.53	0.3000	29.98	30.06	1.420	81.42	92.82	n.a.
	39.54	0.6900	29.67	20.12	1.650	75.34	83.72	n.a.

	33.81	2.340	16.74	34.09	2.340	61.79	68.21	n.a.
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^a Taken and estimated using the TLs described in the literature¹; n.a. = not available.

Table S4. pH of the top and bottom phases of systems used to extract and purify IgG from rabbit serum.

IL	Phase pH	
	Top	Bottom
[Chol][DHPH]	4.57	4.42
[Chol][Lac]	7.45	7.69
[Chol][Van]	8.24	8.40
[Chol][Gly]	9.12	10.7

Table S5. Mixture compositions of the systems used to extract commercial IgG and corresponding $EE_{IgG}\%$ and $Y_{IgG}\%$.

IL	Mixture composition / (wt%)		$EE_{IgG}\%$	$Y_{IgG}\%$
	IL	PPG 400		
[Chol][Bit]	25.02 ± 0.27	45.05 ± 0.18	100	89.8 ± 9.8
[Chol][Lac]	25.35 ± 0.02	44.78 ± 0.37	100	20.4 ± 43.9
[Chol][DHCit]	25.44 ± 0.72	44.91 ± 0.31	93.3 ± 10.1	72.1 ± 9.8
[Chol][Prop]	24.71 ± 0.26	45.11 ± 0.35	100	100 ± 9.2
[Chol][Gly]	25.01 ± 0.22	45.00 ± 0.33	100	100 ± 5.5
[Chol][But]	25.17 ± 0.23	44.91 ± 0.22	100	67.2 ± 11.4
[Chol][Ac]	25.24 ± 0.13	44.65 ± 0.01	97.9 ± 3.1	87.6 ± 2.3
[Chol][DHPH]	25.05 ± 0.05	44.87 ± 0.10	96.2 ± 5.5	40.7 ± 25.9
[Chol]Cl	24.86 ± 0.04	44.90 ± 0.10	98.5 ± 2.2	94.5 ± 5.7
[Chol][Van]	24.96 ± 0.03	45.01 ± 0.09	100	95.9 ± 2.8
[Chol][Caf]	25.54 ± 0.37	44.80 ± 0.26	100	89.1 ± 7.5
[Chol][Syr]	24.95 ± 0.23	44.83 ± 0.07	100	73.5 ± 9.8
[Chol][Gal]	25.03 ± 0.43	44.46 ± 0.20	96.2 ± 5.6	59.4 ± 10.2

Table S6. Mixture compositions of the systems used to separate IgG from rabbit serum and corresponding $EE_{IgG}\%$, $Y_{IgG}\%$ and purity of IgG at the IL-rich phase.

IL	Mixture composition / (wt%)		$EE_{IgG}\%$	$Y_{IgG}\%$	% purity
	IL	PPG 400			
[Chol][Lac]	25.12 ± 0.29	44.96 ± 0.08	100	88.0 ± 1.4	46.9 ± 0.1
[Chol][DHPH]	24.93 ± 0.28	44.83 ± 0.06	100	82.3 ± 3.7	49.2 ± 1.8
[Chol][Gly]	25.18 ± 0.30	45.09 ± 0.71	100	100 ± 0.8	21.5 ± 2.3
[Chol][Van]	24.90 ± 0.07	45.07 ± 0.11	100	100 ± 0.6	20.6 ± 0.8

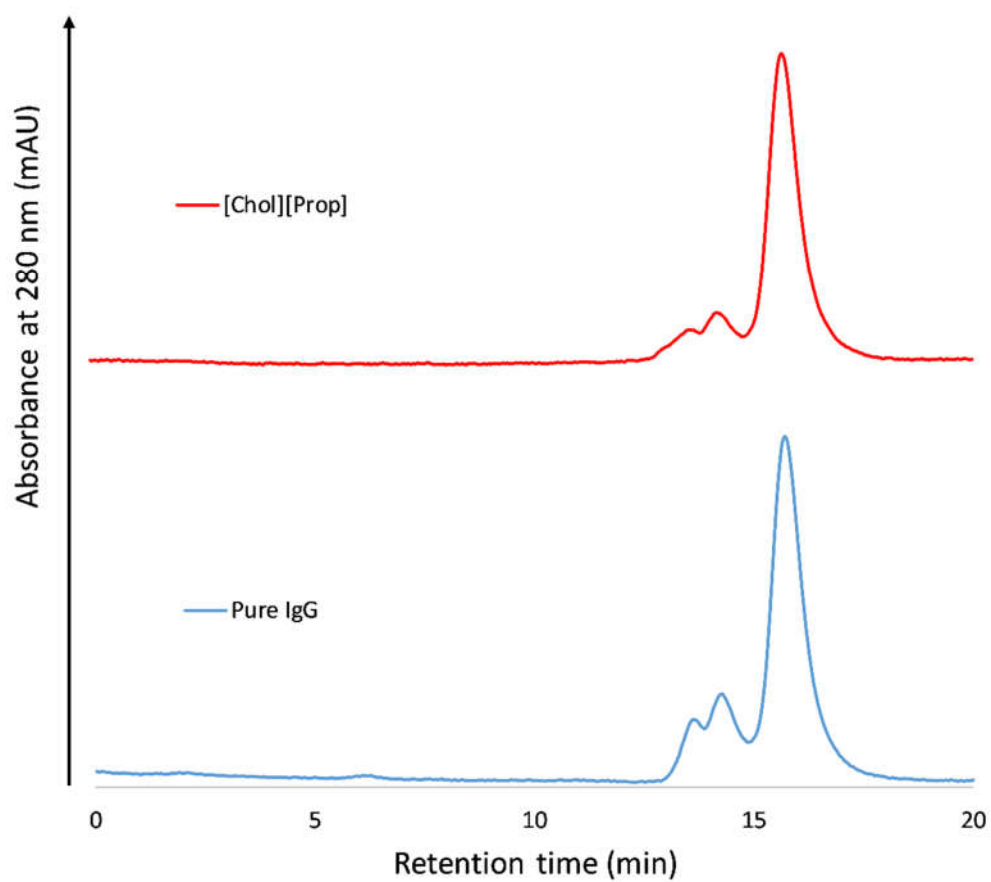


Figure S1. SE-HPLC chromatograms of commercial IgG ($0.1 \text{ g}\cdot\text{L}^{-1}$) and of the IL-rich phase of the ABS composed of [Chol][Prop].

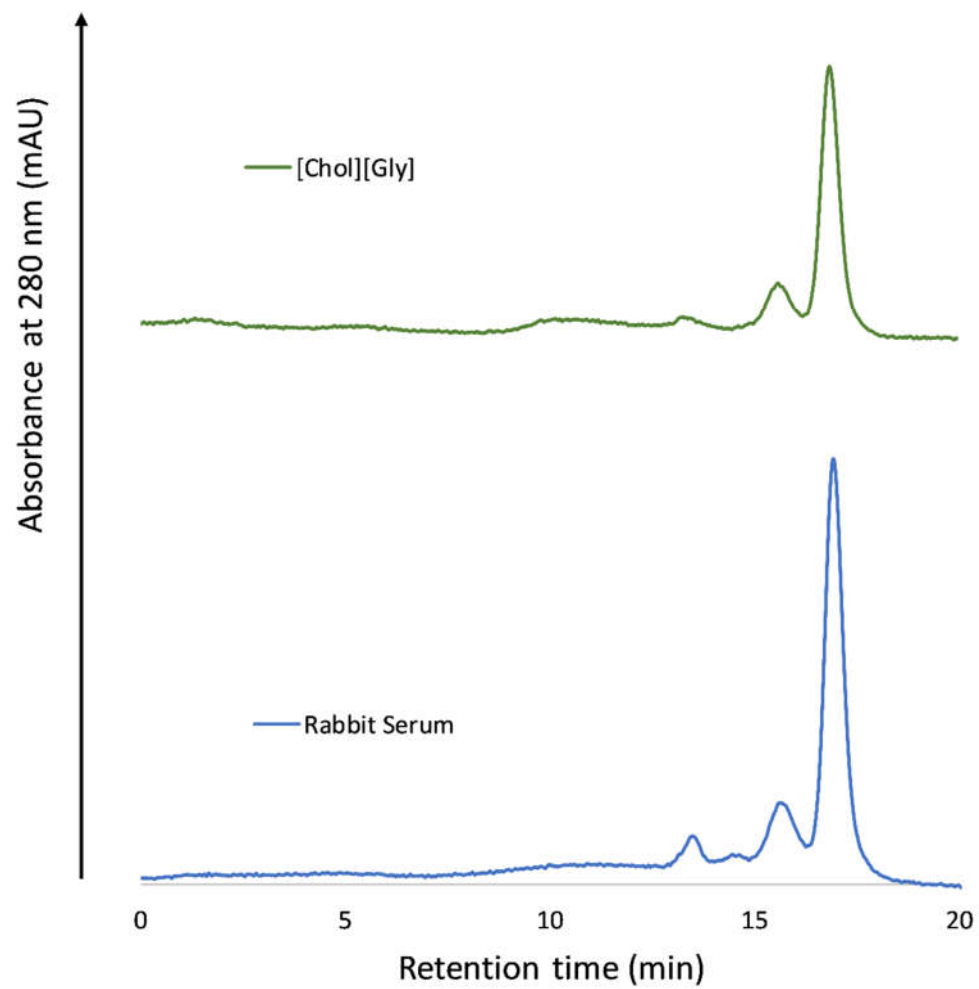


Figure S2. SE-HPLC chromatograms of commercial rabbit serum (dilution of 1:500 (v:v)) and of the IL-rich phase of the ABS composed of [Chol][Gly].

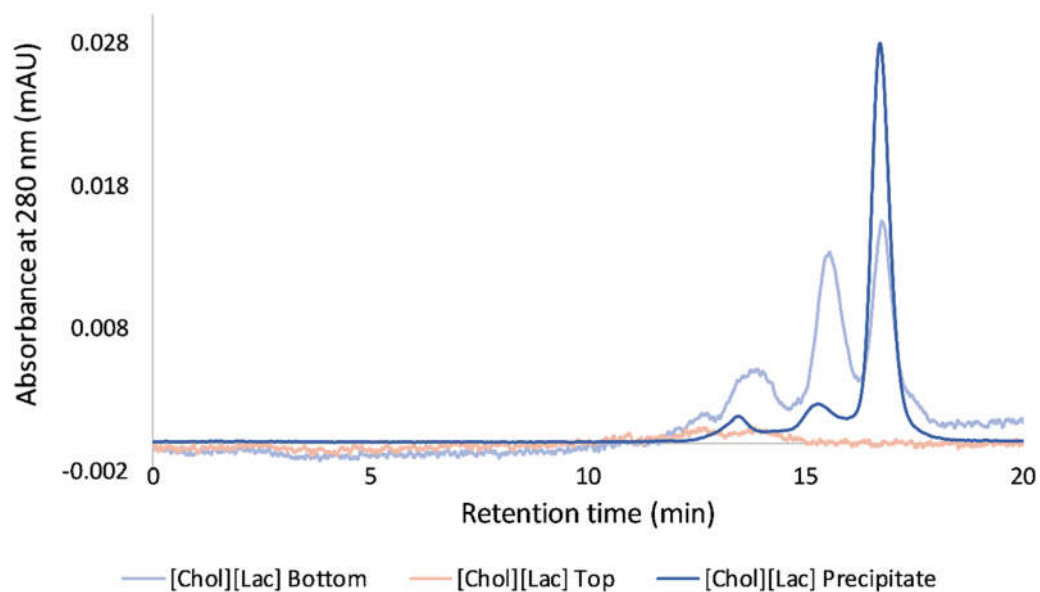


Figure S3. SE-HPLC chromatograms for the several fractions resultant from the ABS composed of [Chol][Lac] used in the separation studies with rabbit serum.

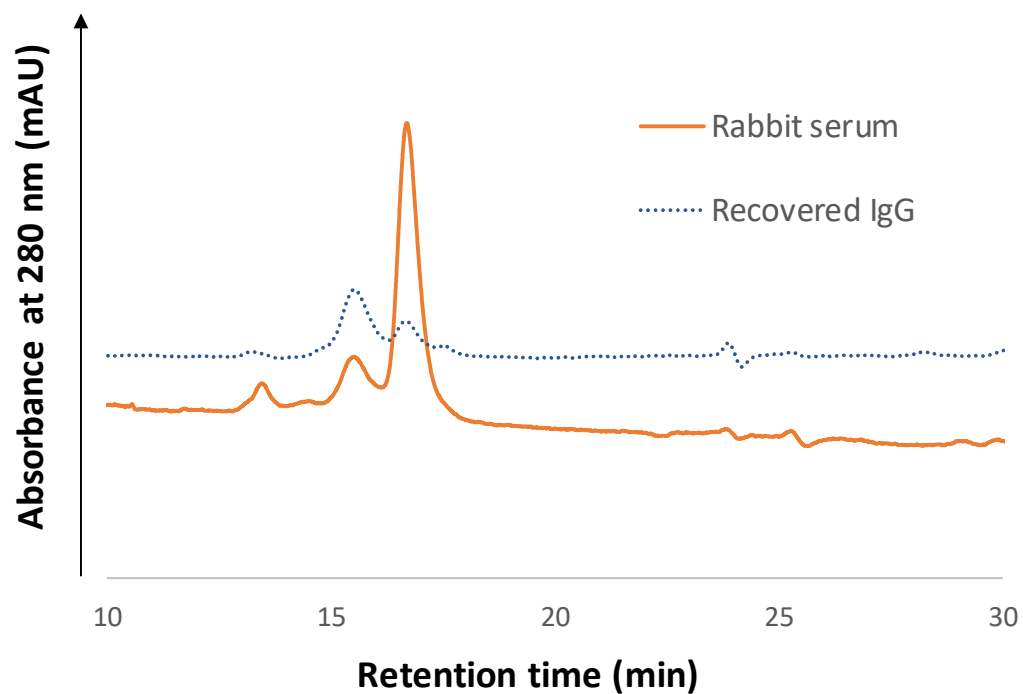


Figure S4. SE-HPLC chromatograms of rabbit serum (1:500 (v:v)), and of the recovered IgG after the ultrafiltration step from the IL-rich phase of the ABS formed by [Chol][DHPH].

References

1. Quental MV, Caban M, Pereira MM, Stepnowski P, Coutinho JAP and Freire MG, Enhanced extraction of proteins using cholinium-based ionic liquids as phase-forming components of aqueous biphasic systems. *Biotechnol. J.* **10**: 1457–1466 (2015).