Supporting Information

Improving the extraction and purification of immunoglobulin G by the use of ionic liquids as adjuvants in aqueous biphasic systems

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Determination of phase diagrams, tie-lines (TLs), tie-lines lengths (TLL) and critical points

The binodal curve of each ABS was determined through the cloud point titration method at 25 ˚C and atmospheric pressure, using aqueous solutions of salt at around 50 wt% and aqueous solutions of different PEGs (with concentrations ranging from 60 wt % to 90 wt %). Repetitive drop-wise addition of the aqueous salt solution to the polymer solution, or *vice-versa*, was carried out until the detection of a cloudy solution (biphasic region), followed by the drop-wise addition of ultra-pure water until the detection of a clear and limpid solution (monophasic region). This procedure was carried under constant stirring. Each mixture composition was determined by the weight quantification of all components (with an uncertainty of ± 10^{-4} g, and using an analytical balance, Mettler Toledo Excellence XS205 DualRange). Tie-lines (TLs) of each phase diagram were determined by a gravimetric method originally described by Merchuk et al. (1998). A mixture at the biphasic region was gravimetrically prepared (PEG + salt + water) within ± 10^{-4} g, vigorously stirred, and left for at least 12 h at 25 ˚C to reach the complete separation (and equilibrium) of the coexisting phases. After the separation step, both top and bottom phases were weighted. The experimental binodal curves were fitted using Eq. S1 (Merchuk et al; 1998):

$$[PEG] = A\exp[(B[salt]^{0.5}) \quad (C[salt]^3)] \tag{S1}$$

where, $[PEG]$ and $[salt]$ are, respectively, the PEG and salt weight percentages and A, B and C are constants obtained by the regression.

Each tie-line (TL) and respective tie-line length (TLL) was determined by the application of the lever-arm rule to the relationship between the top phase weight and the overall system composition. In general, for the determination of TLs it was solved the following system of four equations (Eqs. S2 to S5, with four unknown values, namely $[PEG]_{\mathrm{PEG}}$, $[PEG]_{\mathrm{salt}}$, $[salt]_{\mathrm{PEG}}$ and $[salt]_{\mathrm{salt}}$):

$$[PEG]_{\mathrm{PEG}} = A\exp\left[\left(B[salt]_{\mathrm{PEG}}^{0.5}\right) \quad (C[salt]_{\mathrm{PEG}}^{3})\right] \tag{S2}$$

$$[PEG]_{\mathrm{salt}} = A\exp\left[\left(B[salt]_{\mathrm{salt}}^{0.5}\right) \quad \left(\mathrm{C}[salt]_{\mathrm{salt}}^{3}\right)\right] \tag{S3}$$

$$[PEG]_{\mathrm{PEG}} = \frac{[PEG]_{\mathrm{M}}}{\alpha} \quad \left(\frac{1-\alpha}{\alpha}\right)[PEG]_{\mathrm{salt}} \tag{S4}$$

$$[salt]_{\mathrm{PEG}} = \frac{[salt]_{\mathrm{M}}}{\alpha} \quad \left(\frac{1-\alpha}{\alpha}\right)[salt]_{\mathrm{salt}} \qquad \text{(S5)}$$

The subscripts PEG, salt and M represent the top, bottom and the mixture phases, respectively. The parameter α is the ratio between the PEG-rich phase weight and the total weight of the two phases. The solution of the referred system gives the concentration (wt %) of PEG and salt in the top and bottom phases, and thus TLs can be easily represented.

For the calculation of the tie-line lengths (TLLs) it was applied Eq. (S6).

$$TLL = \sqrt{([salt]_{\mathrm{PEG}} \quad [salt]_{\mathrm{salt}})^2 + ([PEG]_{\mathrm{PEG}} \quad [PEG]_{\mathrm{salt}})^2} \qquad \text{(S6)}$$

The critical point of each ABS was determined by extrapolating the TLs' slopes of each phase diagram according to Eq. S7,

$$[\mathrm{Salt}] = f[\mathrm{PEG}] + g \qquad \text{(S7)}$$

where, f and g are fitting parameters.

Experimental data on ABS phase diagrams

Table S1. Experimental weight fraction data for the systems composed of PEG (1) + $C_6H_5K_3O_7$/$C_6H_8O_7$ (2) + H_2O (3) at 25˚C and atmospheric pressure.

PEG 400					
100 w_1	**100 w_2**	**100 w_1**	**100 w_2**	**100 w_1**	**100 w_2**
17.6667	0.1208	1.0534	1.0162	0.5310	1.4661
10.2530	0.1774	1.0237	1.0374	0.5184	1.4845
5.9874	0.2684	0.9930	1.0568	0.5110	1.4868
3.7506	0.3644	0.9370	1.1047	0.4969	1.5076
2.9612	0.4313	0.9028	1.1277	0.4854	1.5174
2.7227	0.4547	0.8759	1.1521	0.4719	1.5424
2.4975	0.4891	0.8329	1.1895	0.4527	1.5662
2.2364	0.5405	0.8172	1.1940	0.4132	1.6276
2.1893	0.5457	0.7986	1.2099	0.3927	1.6530
2.1194	0.5648	0.7877	1.2150	0.3657	1.6916
2.0110	0.5975	0.7761	1.2240	0.3497	1.7120
1.8958	0.6356	0.7696	1.2299	0.3416	1.7185
1.8250	0.6473	0.7485	1.2454	0.3078	1.7760
1.7728	0.6671	0.7324	1.2605	0.2841	1.8076
1.7073	0.6871	0.7221	1.2670	0.1526	2.8601
1.5861	0.7519	0.7021	1.2858	0.1897	1.9307
1.4683	0.7879	0.6973	1.2877	0.3059	1.6282
1.3953	0.8211	0.6906	1.2950	0.7904	1.0007
1.3523	0.8397	0.6636	1.3195		
1.3358	0.8470	0.6554	1.3273		
1.2931	0.8659	0.6446	1.3380		
1.2756	0.8792	0.6368	1.3435		
1.2198	0.9177	0.6206	1.3675		
1.2061	0.9251	0.6122	1.3721		
1.1685	0.9480	0.5886	1.3928		

Table S2. Experimental weight fraction data for the systems composed of PEG (1) + $C_6H_5K_3O_7/C_6H_8O_7$ (2) + H_2O (3) at 25˚C and atmospheric pressure.

PEG 600							
100 w_1	**100 w_2**	**100 w_1**	**100 w_2**	**100 w_1**	**100 w_2**	**100 w_1**	**100 w_2**
7.0842	0.0504	0.5065	0.7261	0.2511	1.0857	0.1570	1.2830
3.6416	0.1005	0.4980	0.7351	0.2462	1.0968	0.1517	1.2921
3.2769	0.1191	0.4896	0.7398	0.2438	1.1005	0.1456	1.3244
1.6936	0.2361	0.4769	0.7605	0.2409	1.1047	0.1393	1.3347
1.5467	0.2667	0.4640	0.7795	0.2391	1.1092	0.1364	1.3442
1.4595	0.2727	0.4526	0.7877	0.2356	1.1148	0.1303	1.3504
1.3641	0.2941	0.4394	0.8035	0.2323	1.1214	0.1250	1.3820
1.2798	0.3052	0.4272	0.8201	0.2288	1.1278	0.1218	1.3859
1.2184	0.3214	0.4165	0.8278	0.2249	1.1379	0.1188	1.3951
1.1758	0.3383	0.4076	0.8436	0.2215	1.1431	0.1099	1.4279
1.1393	0.3468	0.4023	0.8455	0.2171	1.1563	0.0963	1.4767
1.0986	0.3628	0.3983	0.8518	0.2124	1.1644	0.1450	1.8285
1.0668	0.3778	0.3936	0.8569	0.2087	1.1707	0.2073	1.6047
1.0319	0.3921	0.3894	0.8612	0.2061	1.1777	0.2786	1.4788
0.9945	0.3997	0.3836	0.8700	0.2034	1.1850	0.3238	1.3873
0.9615	0.4122	0.3728	0.8831	0.2012	1.1862	0.3769	1.3320
0.9423	0.4193	0.3656	0.8930	0.1982	1.1926	0.3988	1.2840
0.9232	0.4266	0.3595	0.902	0.1952	1.1980	0.4856	1.2068
0.8989	0.4389	0.3550	0.9078	0.1915	1.2088	0.5353	1.1413
0.8625	0.4542	0.3515	0.9128	0.1884	1.2111	0.5673	1.1112
0.8301	0.4768	0.3465	0.9226	0.1842	1.2228		
0.8010	0.4855	0.3426	0.9252	0.1810	1.2280		
0.7804	0.5001	0.3386	0.9311	0.1773	1.2384		
0.7644	0.5113	0.3344	0.9374	0.1720	1.2518		
0.7309	0.5270	0.3274	0.9476	0.1654	1.2683		

Table S3. Experimental weight fraction data for the systems composed of PEG (1) + $C_6H_5K_3O_7/C_6H_8O_7$ (2) + H_2O (3) at 25˚C and atmospheric pressure.

PEG 1000		**PEG 2000**		**PEG 4000**	
100 w_1	**100 w_2**	**100 w_1**	**100 w_2**	**101 w_1**	**101 w_2**
0.9761	0.3060	0.2650	0.2429	0.1034	0.2536
0.8536	0.3474	0.2562	0.2528	0.0902	0.2830
0.7166	0.3576	0.2470	0.2631	0.0798	0.3113
0.6533	0.3794	0.2388	0.2711	0.0694	0.3386
0.6049	0.3948	0.2316	0.2818	0.0503	0.3996
0.5622	0.4253	0.2218	0.2898	0.0436	0.4386
0.530	0.4358	0.1624	0.3737	0.0390	0.4690
0.0542	1.2678	0.1593	0.3782	0.0375	0.4752
0.0666	1.1863	0.1548	0.3821	0.0350	0.4969
0.0798	1.1314	0.1182	0.4583	0.0340	0.5059
0.0885	1.1064	0.1162	0.4625	0.0331	0.5082
0.1016	1.0688	0.1145	0.4681	0.0197	0.6183
0.1103	1.0384	0.1106	0.4821	0.0225	0.5952
0.1175	1.0149	0.1052	0.4976	0.0244	0.5789
0.1287	0.9863	0.0259	0.8948	0.0264	0.5623
0.1352	0.9708	0.0259	0.8948	0.0324	0.5245
0.1401	0.9574	0.0366	0.7975	0.0344	0.5059
0.1431	0.9479	0.0401	0.7755	0.0364	0.4904
		0.0712	0.6352		
		0.0932	0.5546		

Table S4. Experimental weight fraction data for the systems composed of PEG (1) + $C_6H_5K_3O_7/C_6H_8O_7$ (2) + H_2O (3) at 25˚C and atmospheric pressure.

PEG 6000						PEG 8000			
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**
0.0691	0.2680	0.0113	0.5950	0.0070	0.6646	0.0054	0.6103	0.0065	0.5743
0.0348	0.3721	0.0111	0.6051	0.0067	0.6667	0.0065	0.5848	0.0072	0.5657
0.0297	0.3984	0.0108	0.6100	0.0066	0.6628	0.0083	0.5526	0.0078	0.5550
0.0250	0.4360	0.0105	0.6131	0.0064	0.6721	0.0098	0.5293	0.0084	0.5424
0.0245	0.4402	0.0103	0.6081	0.0063	0.6731	0.0123	0.4911		
0.0197	0.4931	0.0102	0.6210	0.0062	0.6765	0.0148	0.4558		
0.0186	0.4972	0.0100	0.6155	0.0060	0.6789	0.0169	0.4287		
0.0182	0.5072	0.0098	0.6219			0.0057	2.8291		
0.0178	0.5113	0.0096	0.6272			0.0029	0.6626		
0.0173	0.5137	0.0094	0.6216			0.0036	0.6423		
0.0170	0.5165	0.0093	0.6243			0.0044	0.6247		
0.0167	0.5228	0.0091	0.6281			0.0051	0.6056		
0.0163	0.5233	0.0090	0.6270			0.0058	0.5891		
0.0161	0.5288	0.0089	0.6337			0.0065	0.5743		
0.0158	0.5330	0.0087	0.6394			0.0072	0.5657		
0.0154	0.5460	0.0086	0.6329			0.0078	0.5550		
0.0149	0.5505	0.0085	0.6407			0.0084	0.5424		
0.0146	0.5443	0.0083	0.6478			0.0232	0.3923		
0.0143	0.5583	0.0080	0.6423			0.0225	0.3970		
0.0139	0.5560	0.0079	0.6472			0.0057	2.8291		
0.0135	0.5694	0.0078	0.6513			0.0029	0.6626		
0.0130	0.5783	0.0076	0.6474			0.0036	0.6423		

Table S5. Correlation parameters used to describe the experimental binodal data at pH 7 and 25˚C by Equation (1).

PEG	**$A \pm \sigma$**	**$B \pm \sigma$**	**$10^5 (C \pm \sigma)$**	**R^2**
400	167.4 ± 2.7	-0.360 ± 0.005	1.67 ± 0.67	0.9971
600	126.0 ± 0.9	-0.355 ± 0.003	3.36 ± 0.06	0.9986
1000	198.9 ± 7.3	-0.479 ± 0.012	5.06 ± 0.21	0.9981
2000	135.6 ± 6.0	-0.507 ± 0.016	9.83 ± 0.54	0.9991
4000	172.0 ± 17.7	-0.642 ± 0.039	14.6 ± 1.68	0.9983
6000	337.1 ± 38.2	-0.872 ± 0.041	16.3 ± 1.44	0.9953
8000	160.2 ± 138.4	-0.583 ± 0.292	37.6 ± 8.16	0.9947

Table S6. Experimental data of TLs and TLLs of ABS composed of PEG + $C_6H_5K_3O_7$/$C_6H_8O_7$ at pH 7 and 25˚C.

PEG	Weight fraction composition / wt %								TLL	α
	$[PEG]_{PEG}$	$[Salt]_{PEG}$	pH_{PEG}	$[PEG]_{M}$	$[Salt]_{M}$	$[PEG]_{Salt}$	$[Salt]_{Salt}$	pH_{Salt}		
400	74.83	4.97	7.10	34.54	25.18	6.35	39.31	6.64	76.60	0.41
	42.43	13.64	6.82	24.86	24.64	11.78	32.83	6.80	36.17	0.57
	69.47	5.94	6.54	32.40	24.40	8.71	36.20	7.09	67.88	0.61
	65.91	6.66	6.49	31.17	24.00	10.24	34.45	6.98	62.22	0.62
	41.33	14.09	6.92	34.72	18.29	11.73	32.88	6.37	35.06	0.78
600	39.94	9.88	6.93	33.04	15.00	2.29	37.86	6.29	46.91	0.82
	34.81	11.96	6.71	18.86	23.32	4.56	33.50	6.58	37.14	0.47
	39.94	9.88	6.93	33.04	15.00	2.29	37.86	6.29	46.91	0.82
1000	55.62	6.90	6.22	26.18	24.61	0.90	40.15	6.30	64.49	0.47
	50.90	7.81	5.99	31.31	19.52	0.68	37.81	6.22	58.50	0.61
	36.65	11.39	6.17	19.39	20.72	4.61	28.71	6.19	36.41	0.46
2000	36.88	16.06	6.82	24.86	24.64	8.73	36.15	6.80	34.59	0.57
	51.33	10.46	6.54	32.40	24.40	2.80	46.20	7.09	60.30	0.61
	47.87	11.62	6.49	31.17	24.00	3.47	44.54	6.98	55.26	0.62
	30.28	19.46	6.64	24.33	23.50	15.35	29.59	6.75	18.04	0.60
4000	51.49	3.49	6.83	24.90	19.99	0.01	35.44	6.51	60.59	0.52
	42.23	4.68	6.79	24.75	15.04	0.12	29.65	6.48	48.95	0.58
	31.33	6.68	6.63	17.52	14.18	1.34	22.98	6.50	34.13	0.54
6000	21.53	9.09	6.41	15.05	12.03	4.22	16.94	6.37	19.00	0.63
	24.97	8.29	6.45	15.07	12.81	2.94	18.35	6.37	24.22	0.55
	27.33	7.80	6.45	12.22	14.96	1.95	19.82	6.32	28.08	0.40
	31.21	7.09	6.51	17.42	13.86	1.02	21.92	6.30	33.64	0.54
8000	23.49	8.45	6.42	12.02	14.16	0.64	19.83	6.41	25.53	0.50
	21.31	8.98	6.31	6.04	16.03	1.40	18.18	6.30	21.94	0.23
	30.03	7.02	6.53	16.46	14.10	0.13	22.61	6.37	33.72	0.55
	30.70	6.89	6.51	15.54	14.77	0.12	22.78	6.34	34.47	0.50

Table S7. Critical points of the investigated systems PEG (400, 600, 1000, 2000, 4000, 6000 and 8000) + $C_6H_5K_3O_7/C_6H_8O_7$ at pH ≈ 7 + H_2O.

Critical Point / (wt %)		
Mw PEG / (g mol^{-1})	**[$C_6H_5K_3O_7/C_6H_8O_7$]**	**[PEG]**
400	23.86	22.98
600	22.96	15.29
1000	18.09	19.22
2000	15.53	12.74
4000	12.23	13.94
6000	12.00	12.42
8000	12.48	9.85

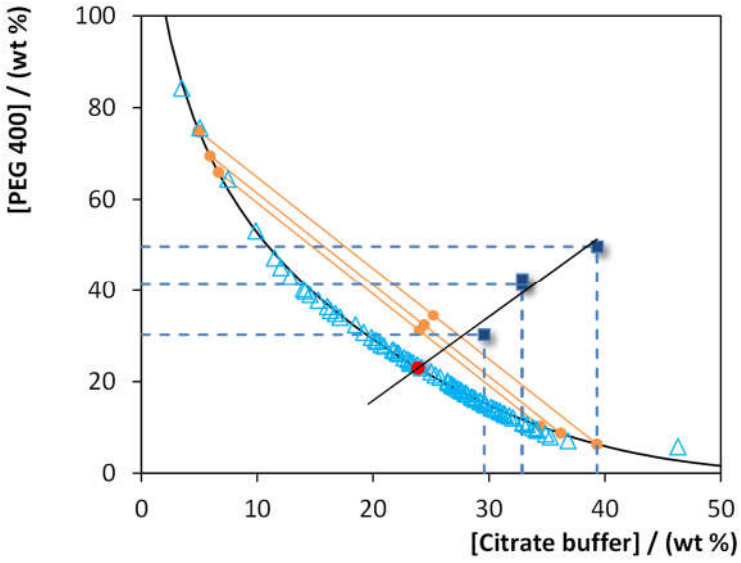

Figure S1. Phase diagram for the ternary system composed of PEG 400 + $C_6H_5K_3O_7/C_6H_8O_7$ + H_2O at pH 7 and 25˚C: binodal curve data (△,); TL data (●); adjusted binodal data through Equation 1 (-), critical point (●) and TL relation (■).

Table S8. Experimental weight fraction data for the systems composed of PEG 400 (1) + $C_6H_5K_3O_7/C_6H_8O_7$ (2) + H_2O (3) at different pH values, at 25°C and atmospheric pressure.

pH≈5		**pH≈6**			
100 w_1	**100 w_2**	**100 w_1**	**100 w_2**	**100 w_1**	**100 w_2**
97.6785	1.1474	83.1460	3.1973	26.1275	24.0347
59.4245	10.5023	80.2857	3.9381	27.1130	23.3590
54.8208	12.4952	74.0831	5.5981	27.8631	22.8964
51.8751	13.4461	70.2003	6.3838	28.4717	22.4577
43.1889	17.5656	67.5146	7.2412	29.0311	21.9991
37.4680	20.1883	62.7472	8.2251	30.0644	21.4199
29.6068	25.8032	60.1183	8.7867	30.7338	20.9344
79.5788	4.5648	52.5302	11.5347	31.3464	20.2928
75.1961	5.3173	42.9395	14.2884	32.5943	19.6152
42.5024	17.6484	41.0086	15.0556	33.0527	19.2490
37.5837	20.4752	39.3498	15.8188	33.9441	18.5133
43.8210	17.3961	37.4010	16.9016	34.8239	18.0871
38.0120	20.3881	8.3076	39.0424	35.6767	17.4873
35.6522	21.0005	10.2215	36.9386		
31.98544	24.1809	11.0343	36.0117		
33.04591	23.1484	12.6053	34.5915		
34.39002	22.2523	13.2279	33.8544		
35.73221	21.4667	15.3038	32.1482		
39.17455	19.1808	16.7716	31.1742		
24.76493	30.11694	17.2677	30.5668		
21.7746	32.0034	18.5333	29.6867		
20.3919	34.5012	19.1007	29.1624		
25.2329	28.9867	20.3021	28.3650		
16.3097	37.7884	21.5244	27.5649		
18.7841	35.7096	21.9908	27.1238		
17.5897	36.5537	22.9232	26.4286		
12.0404	41.5647	23.9513	25.7182		
		24.2902	25.2971		
		25.1437	24.6179		

Table S9. Experimental weight fraction data for the systems composed of PEG 400 (1) + $C_6H_5K_3O_7/C_6H_8O_7$ (2) + H_2O (3) at different pH values, at 25˚C and atmospheric pressure.

pH≈9				pH≈8			
100 w_1	**100 w_2**	**100 w_1**	**100 w_2**	**100 w_1**	**100 w_2**	**100 w_1**	**100 w_2**
98.7614	0.6114	19.6993	25.3901	97.9816	1.0131	6.0589	36.1366
68.2447	3.1421	18.8710	26.0018	72.0986	2.9437	7.0630	35.2505
65.9899	3.8872	18.3604	26.2245	65.5590	4.2215	9.0933	33.1761
63.3480	4.3566	17.8041	26.6026	61.8489	5.2348	9.9558	32.3053
61.1248	5.2798	17.4656	26.8562	58.1287	6.1471	10.9065	31.6091
57.1765	6.8965	16.6855	27.3949	53.9687	7.3423	11.7741	30.9423
54.4698	7.5474	16.3474	27.6280	51.7503	8.0110	12.5534	30.3797
53.3535	7.8675	16.1222	27.7605	46.5439	10.5737	13.3199	29.8972
50.7736	8.8696	15.7539	28.0582	44.9058	11.0891	13.9490	29.2169
49.7684	9.1850	15.5798	28.1564	43.1049	12.0416	15.2438	28.3406
46.7201	10.6741	15.2734	28.3902	41.6065	12.7035	15.8502	27.7407
44.9089	11.6286	15.0137	28.5631	40.3051	13.2035	17.2340	26.9222
43.2403	12.1418	14.8760	28.5919	38.3276	14.2975	17.8247	26.4605
42.1962	12.5506	14.5414	28.8819	36.8786	14.7359	18.8281	25.7318
40.4663	13.1536	2.4456	48.1525	33.2908	16.7460		
39.4923	13.5933	3.7524	44.0195	32.3498	17.2076		
38.1205	14.3601	4.6473	40.0228	31.8512	17.4923		
37.4959	14.5110	5.5669	38.1322	31.2476	17.8160		
35.8609	15.6126	6.6937	36.3331	30.1857	18.6230		
34.5416	16.0606	7.4991	34.8308	29.5314	18.9394		
33.8126	16.3824	8.9759	33.0886	28.9074	19.1716		
32.9396	16.9120	10.7990	31.8381	28.0889	19.6419		
32.0742	17.4605	11.5234	31.1063	27.5907	19.9676		
30.7456	18.0696	12.3085	30.3982	26.8083	20.5204		
29.1959	19.0198	13.9001	29.3979	26.3774	20.8019		
27.6533	20.0223	14.9116	28.6416	25.9860	21.0619		
26.5636	20.6568	16.4486	27.7227	25.2994	21.4896		
24.8188	21.8903	17.0294	27.1178	24.8228	21.8130		
24.2601	22.2001	19.6993	25.3901	24.4753	22.0550		
23.7156	22.5333	18.8710	26.0018	23.9810	22.3626		
22.8205	23.2478	18.3604	26.2245	23.5928	22.6428		
22.4224	23.4550	17.8041	26.6026	23.2114	22.9108		
21.8741	23.8240	17.4656	26.8562	22.8346	23.1516		
98.7614	0.6114	16.6855	27.3949	22.3768	23.4472		
68.2447	3.1421			21.8416	23.7413		
63.3480	4.3566			21.0831	24.2745		
61.1248	5.2798			20.7018	24.5273		
57.1765	6.8965			20.2810	24.8188		

Table S10. Correlation parameters used to describe the experimental binodal data by Equation 1 for the systems composed of PEG 400 + $C_6H_5K_3O_7$/$C_6H_8O_7$ + H_2O at different pH values.

PEG	pH	$A \pm \sigma$	$B \pm \sigma$	10^5 ($C \pm \sigma$)	R^2
	5	131.9 ± 2.1	-0.253 ± 0.006	1.10 ± 0.09	0.9979
	6	153.8 ± 3.0	-0.320 ± 0.007	1.51 ± 0.10	0.9967
400	7	167.4 ± 2.7	-0.360 ± 0.005	1.67 ± 0.67	0.9971
	8	127.2 ± 1.6	-0.305 ± 0.005	2.07 ± 0.10	0.9971
	9	119.4 ± 1.2	-0.285 ± 0.004	2.34 ± 0.08	0.9979

Table S11. Experimental data of TLs and TLLs of ABS composed of PEG 400 + $C_6H_5K_3O_7$/$C_6H_8O_7$ at different pH values and 25˚C.

pH	Weight fraction composition / wt %								TLL	α
	$[PEG]_{PEG}$	$[Salt]_{PEG}$	pH_{PEG}	$[PEG]_{M}$	$[Salt]_{M}$	$[PEG]_{Salt}$	$[Salt]_{Salt}$	pH_{Salt}		
5	63.58	8.20	5.66	28.64	35.35	3.19	55.13	5.51	76.48	0.58
	36.67	21.48	5.41	20.50	35.53	7.60	46.74	5.38	38.51	0.44
	32.90	23.97	5.43	19.88	35.40	8.71	45.21	5.36	32.19	0.46
6	44.88	13.87	6.27	30.26	24.96	5.03	44.09	6.02	50.01	0.63
	50.57	11.59	6.27	32.24	26.13	2.53	49.69	6.11	61.32	0.62
	37.87	17.16	5.93	21.92	29.37	7.31	40.57	5.93	38.49	0.48
	74.83	4.97	7.10	34.54	25.18	6.35	39.31	6.64	76.60	0.41
	42.43	13.64	6.82	24.86	24.64	11.78	32.83	6.80	36.17	0.57
8	47.16	10.13	7.76	26.99	25.38	3.12	43.44	7.70	55.22	0.54
	49.13	9.38	7.77	29.91	24.52	2.15	45.97	7.78	59.55	0.59
	40.01	13.21	7.64	24.24	24.46	7.38	36.49	7.63	40.09	0.52
	36.67	10.13	7.76	21.42	25.68	9.19	34.36	7.61	33.69	0.45
9	48.19	9.68	7.86	27.01	25.02	3.30	55.55	8.04	55.55	0.53
	49.93	9.01	7.91	30.01	24.03	2.06	59.96	8.11	59.96	0.58
	40.69	2.97	7.84	24.25	24.25	8.11	35.01	7.90	39.33	0.50
	35.80	15.43	7.72	22.76	24.81	7.33	35.05	8.11	35.05	0.54

Table S12. Critical points of the investigated systems (PEG 400 + $C_6H_5K_3O_7/C_6H_8O_7$ + H_2O) at different pH values.

	Critical Points / (wt %)	
pH	**PEG 400**	**$C_6H_5K_3O_7/C_6H_8O_7$**
5	13.87	39.29
6	20.98	27.51
7	22.24	24.35
8	24.23	22.11
9	26.40	20.80

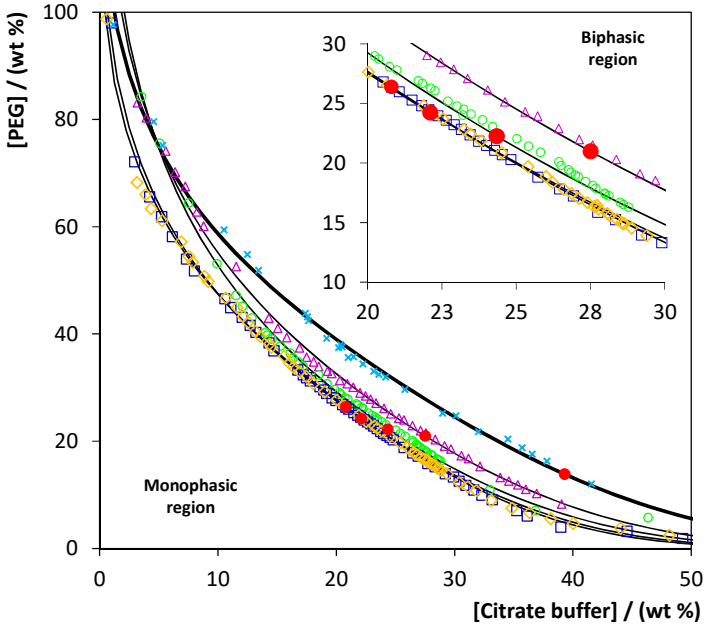

Figure S2. Ternary phase diagrams at 25ºC and atmospheric pressure. Systems composed of PEG 400 + $C_6H_5K_3O_7/C_6H_8O_7$ + H_2O at different pH values: pH 5 (×), pH 6 (△), pH 7 (○), pH 8 (□) and pH 9 (◇). Critical point of each system (●).

Effect of equilibration time on the extraction of IgG

To optimize the equilibrium conditions, the polymers with the highest (PEG 8000) and the lowest (PEG 400) molecular weight were used. The mixtures were prepared at pH ≈ 7. Some of them were submitted to centrifugation at 1000 rpm for 10 min (VWR, Micro Star 17), followed by different times of equilibrium, namely 10, 30, 60, 90 and 120 min of rest, while others were left to phase separate (with no centrifugation) for 300 and 720 min at 25°C. The following mixture compositions at pH ≈ 7 were used: 25 wt% of PEG 400 + 25 wt% of $C_6H_5K_3O_7/C_6H_8O_7$, and 16 wt% of PEG 8000 + 15 wt % of $C_6H_5K_3O_7/C_6H_8O_7$ (which correspond to a TLL of *circa* 35). This study was carried out in order to understand the best time conditions for the extraction and purification of IgG (aiming at avoiding precipitation and denaturation effects while guarantying the equilibrium). It is important to refer that in the systems composed of PEG 400 the protein precipitation was not observed, while all samples composed of PEG 8000 demonstrated the presence of precipitated protein. The results shown in Figure S1 confirm the higher yield of IgG (Y_{IgG}) obtained with the systems composed of PEG 400. In particular, the best results, both for extraction efficiency and recovery yield, were obtained with the phases' separation promoted by centrifugation at 1000 rpm for 10 min followed by 120 min of equilibrium time, and with the 300 and 720 min without the use of centrifugation.

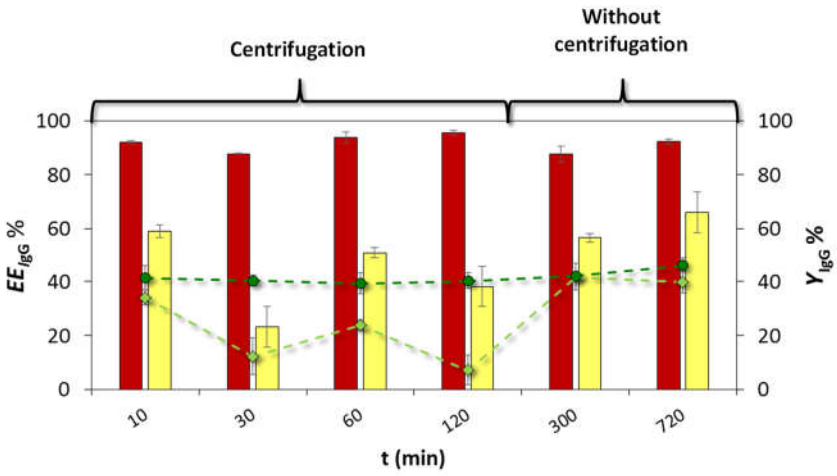

Figure S3. Extraction efficiencies (EE_{IgG} %) and recovery yields (Y_{IgG} %) of IgG using ABS composed of PEG 400 and PEG 8000 at pH 7 and at 25˚C: EE_{IgG} % PEG 400 (■) and Y_{IgG} % (——); EE_{IgG} % PEG 8000 (■) and Y_{IgG} % (——).

Results on the extraction and purification of IgG

Table S13. Extraction efficiency (EE_{IgG} %) and recovery yield (Y_{IgG}%) of rabbit IgG in the systems composed of PEG + $C_6H_5K_3O_7/C_6H_8O_7$ + H_2O at pH 7, using systems formed by PEG 400, 600, 1000, 2000, 4000, 6000 and 8000, and respective standard deviations given within brackets.

PEG	EE_{IgG}%	Y_{IgG}%
400	95.6 (± 0.9)	40.3 (± 2.9)
600	97.8 (± 3.1)	44.4 (± 0.7)
1000	89.5 (± 1.4)	43.8 (± 7.7)
2000	54.5 (± 9.9)	43.6 (± .9)
4000	64.9 (± 6.0)	33.0 (± 7.1)
6000	82.6 (± 9.1)	41.0 (± 3.5)
8000	38.2 (± 7.4)	7.1 (± 5.5)

Table S14. Extraction efficiency (EE_{IgG} %) and recovery yield (Y_{IgG} %) of rabbit IgG in the systems composed of PEG 400 + $C_6H_5K_3O_7/C_6H_8O_7$ + H_2O at different pH values at 25 C, and respective standard deviations given within brackets.

pH	EE_{Ig} %	Y_{IgG}%
5	94.7 (± 0.7)	53.7 (± 3.7)
6	96.7 (± 3.4)	45.7 (± 3.3)
7	95.6 (± 0.9)	40.3 (± 2.9)
8	97.3 (± 1.7)	49.4 (± 1.7)
9	97.1 (± 2.6)	43.3 (± 2.3)

Table S15. Extraction efficiency (EE_{IgG}%), and recovery yield (Y_{IgG}%) of rabbit IgG in the systems composed of PEG 400 + $C_6H_5K_3O_7$/$C_6H_8O_7$ + H_2O + 5 wt% of [C_4mim]-based ILs at pH 7, and respective standard deviations given within brackets.

IL	EE_{IgG}%	Y_{IgG}%
No IL	95.6 (± 0.9)	40.3 (± 2.9)
[C_4mim]Cl	100	36.9 (± 9.3)
[C_4mim][TOS]	100	36.8 (± 6.7)
[C_4mim]Br	89.7 (± 10.3)	55.1 (± 10.0)
[C_4mim]Ac	100	26.8 (± 5.9)
[C_4mim][$N(CN)_2$]	93.1 (± 8.0)	31.1 (± 1.4)

Table S16. Extraction efficiency (EE_{IgG}%) and recovery yield (Y_{IgG}%) of rabbit IgG in the systems composed of PEG 400 + $C_6H_5K_3O_7$/$C_6H_8O_7$ + H_2O + 5 wt% of Br-based ILs at pH 7.

IL	EE_{IgG}%	Y_{IgG}%
No IL	95.6 (± 0.9)	40.3 (± 2.3)
[C_2mim]Br	95.8 (± 4.2)	49.9 (± 2.5)
[C_4mim]Br	89.7 (± 10.3)	55.1 (± 10.0)
[N_{1111}]Br	100	45.8 (± 10.1)
[N_{2222}]Br	100	46.7 (± 1.8)
[N_{3333}]Br	100	34.2 (± 3.9)
[N_{4444}]Br	100	34.3 (± 4.4)
[P_{4444}]Br	100	45.6 (± 4.0)

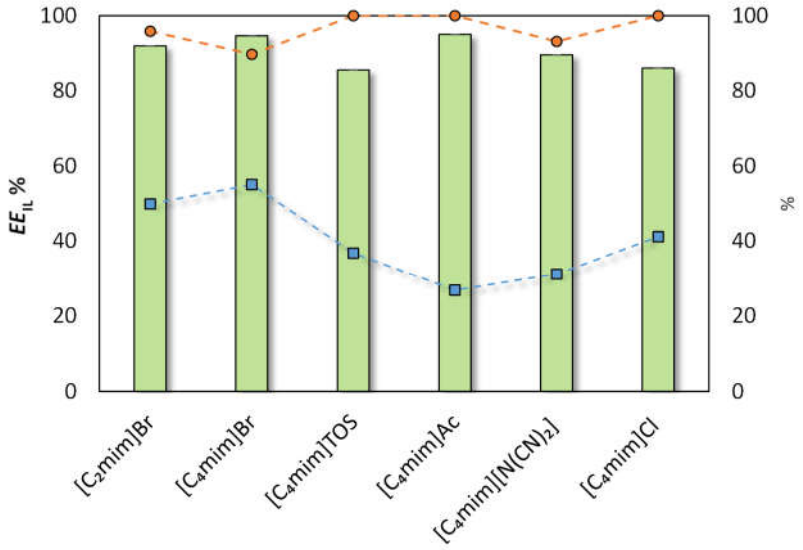

Fig. S4. Extraction efficiencies (EE_{IL}%) of ILs (bars) in ABS composed of PEG 400 + $C_6H_5K_3O_7/C_6H_8O_7$ + H_2O + ILs at 5 wt%, at pH ≈ 7 and 25˚C; and (●) extraction efficiency and (■) recovery yield of IgG.

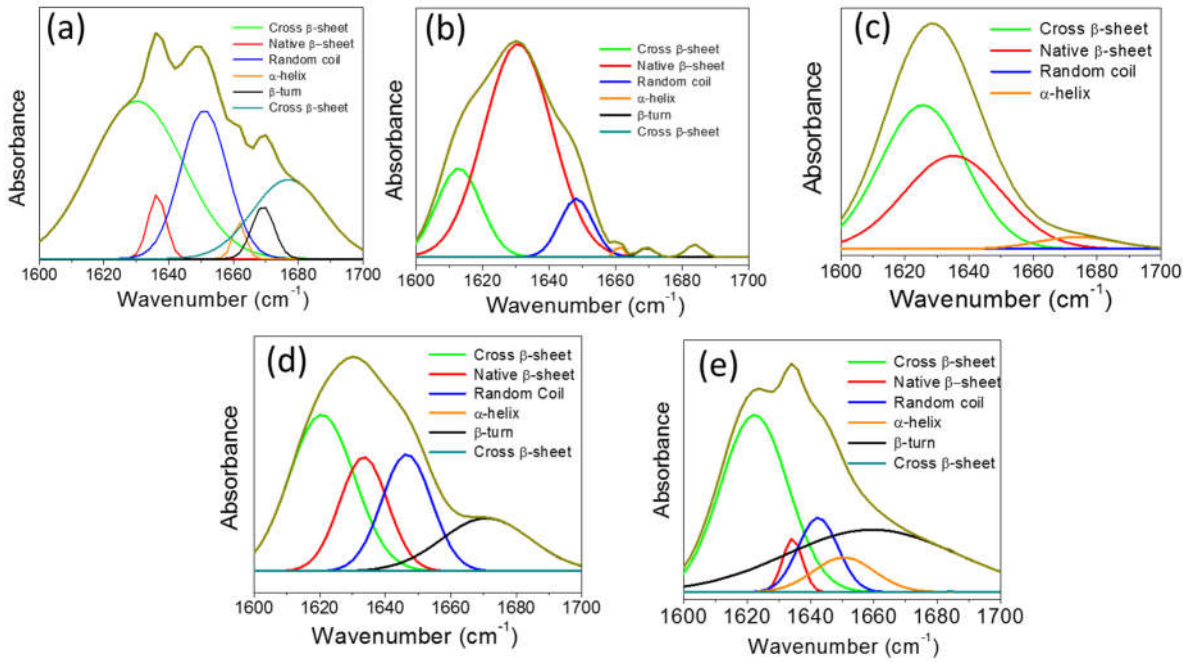

Figure S5. FT-IR spectra of IgG (amide I region) (a) in PBS, 10 mM, pH 7.4, (b) in presence of PEG 400 + $[N_{4444}]Br$, (c) in presence of PEG 400 + $[C_4mim]Cl$, (d) in presence of PEG 400 + $[C_4mim]Br$ and (e) in presence of PEG 400 + $[C_4mim][N(CN)_2]$.

Table S17. Extraction efficiency of IgG reported in the literature using different aqueous biphasic systems.

Aqueous biphasic system	**Extraction efficiency of IgG (%)**	**Reference**
8% PEG 3350 + 10% potassium phosphate + 15% NaCl , pH 6	76	1
12% PEG 6000 + 10% potassium phosphate + 15% NaCl, pH 6	88	2
8% PEG 3350 + 10% potassium phosphate + 10% NaCl, pH 6, 5 steps	89	3
8% UCON 2000 + 6% Dextran 500 000 + 20% TEG-AG, 2 stages	85	4
7% PEG 3350 + 5% + 1.3% Dextran 500000 TEG-AG, pH 4, 5 stages	95	5
8% UCON 50HB + 5% Dextran 3520-500000, pH 5, 2 stages	82	6
8% PEG 3350 + 5% Dextran 500000 + 200 mM NaCl particles coated with modified aminophenyl boronic acid gum arabic	92	7
PEG 3350 + potassium phosphate + NaCl , pH 6, 3 stages	100	8
7% PEG 6000 + 5% Dextran 500000 + 150 mM NaCl, pH 3	84	9
PEG 400 + $C_6H_5K_3O_7$/$C_6H_8O_7$ + ILs at 5 wt%, single-step	100	Present study

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