

# Single-Step Purification of Ovalbumin from Egg White Using Aqueous Biphasic Systems

Matheus M. Pereira<sup>1</sup>, Rafaela A. P. Cruz<sup>1</sup>, Mafalda R. Almeida<sup>1</sup>, Álvaro S. Lima<sup>2</sup>, João A. P. Coutinho<sup>1</sup> and Mara G. Freire<sup>1\*</sup>

<sup>1</sup>CICECO-Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal

<sup>2</sup>Programa de Pós-Graduação em Engenharia de Processos, Universidade Tiradentes, Farolândia, CEP 49032-490 Aracaju, SE, Brazil

\*Corresponding author

Tel: +351-234401422; Fax: +351-234-370084; E-mail address: maragfreire@ua.pt

## Supporting Information

**Table S1**

Experimental weight fraction data for the systems composed of PEG 400 (1) + C<sub>6</sub>H<sub>5</sub>K<sub>3</sub>O<sub>7</sub>/C<sub>6</sub>H<sub>8</sub>O<sub>7</sub> (2) + H<sub>2</sub>O (3) at 25°C.

pH 5		pH 6		pH 8	
100 w <sub>1</sub>	100 w <sub>2</sub>	100 w <sub>1</sub>	100 w <sub>2</sub>	100 w <sub>1</sub>	100 w <sub>2</sub>
13.8784	44.0714	12.7242	41.2876	68.0207	1.4982
15.8030	41.1839	13.5478	39.3030	60.6933	2.8980
17.1646	38.9245	14.7701	37.6565	57.5232	3.7124
19.3922	36.7353	15.7787	35.3873	54.2955	4.3635
21.0099	34.8396	16.2211	34.0678	51.6291	5.1016
23.5517	32.7621	17.5670	32.2227	48.3830	6.0044
25.2393	31.1759	18.7109	30.6367	44.8083	6.9456
26.8034	29.9046	19.9625	29.1688	40.2519	8.2738
28.2945	28.6035	21.6725	27.7436	39.5334	8.5822
29.5790	27.4259	23.4239	26.5159	38.7919	8.9058
31.3389	26.2354	23.8886	25.6818	38.0258	9.2458
32.3955	25.2020	25.2992	24.6300	37.2332	9.6035
32.6902	24.6885	26.6489	23.6464	36.3949	9.9819
34.1753	23.6390	27.8100	22.7248	35.5420	10.3786
35.7246	22.6037	28.9584	21.9689	34.6619	10.7976
36.4717	21.9259	29.8614	21.2308	33.7523	11.2408
37.6852	21.0508	30.7524	20.4976	32.8105	11.7106
39.0763	20.1973	32.3364	19.6229	31.8337	12.2092
41.3708	19.1531	33.2482	18.9739	30.7015	12.7517
42.1115	18.5689	34.3542	18.2869	29.8047	13.3088
44.3205	17.4301			28.9110	13.8516
				27.2947	14.6005

26.3131	15.2395
25.7023	15.7740
25.1869	16.2964
23.8576	17.0889
22.5121	17.9341
21.8801	18.5595
20.4643	19.4835
19.2419	20.3042
18.4269	21.1170
16.6452	22.2122
15.7167	23.1358
13.7114	24.4315
11.3638	25.9401
10.3368	27.0210
7.8421	28.7068
6.4377	31.3587

**Table S2**

Experimental weight fraction data for the systems composed of PEG (1) + C<sub>6</sub>H<sub>5</sub>K<sub>3</sub>O<sub>7</sub>/C<sub>6</sub>H<sub>8</sub>O<sub>7</sub> (2) + H<sub>2</sub>O (3) at 25°C and pH 7.

PEG 400		PEG 600		PEG 1000	
100 w <sub>1</sub>	100 w <sub>2</sub>	100 w <sub>1</sub>	100 w <sub>2</sub>	100 w <sub>1</sub>	100 w <sub>2</sub>
82.6364	2.7688	85.9890	1.7175	57.8766	5.9077
75.1384	3.7415	75.6691	3.2334	52.8615	6.5404
69.3176	4.8225	66.0885	4.1373	47.5771	7.0310
64.5254	5.9201	61.2011	5.2283	43.2552	8.0531
60.4327	6.9732	57.0016	6.2553	40.5045	9.0397
56.8465	7.0909	53.3512	7.1548	38.5181	9.3272
53.6422	8.1477	48.9937	7.7503	37.3214	9.7977
50.7347	9.1522	45.9932	8.3937	36.3024	10.2048
50.3763	9.2678	43.8617	9.0435	34.8850	10.7059
44.9721	10.9316	41.6540	9.5154	33.3928	11.0325
42.1959	12.4068	40.2461	10.0757	32.4179	11.4377
40.9067	12.9465	38.8544	10.6452	31.5184	11.7518
39.3859	13.5980	37.2160	11.0289	30.6611	12.1286
37.4982	14.6429	36.0051	11.5597	29.8253	12.4888
36.3936	15.1547	34.9330	12.0149	29.0518	12.8247
35.2881	15.5759	34.0072	12.4572	28.2799	13.2131
33.4970	16.6700	33.1104	12.8165	27.6278	13.4697
32.0660	17.4791	32.2384	13.1799	26.9766	13.6716
30.7214	18.2968	31.3807	13.4793	26.3515	13.9313
29.4833	19.0725	30.5355	13.8412	24.6438	14.6031
28.5894	19.5448	29.4578	14.5832	24.1790	14.9130
27.4451	20.3323	27.6537	15.0870	23.3543	15.3366

26.1742	21.1703	25.0088	16.1531	22.7360	15.7350
25.2695	21.6338	22.7269	17.7926	21.5921	16.2943
24.0984	22.5744	22.2983	18.1895	20.3070	16.8851
23.0210	23.3475	21.4583	18.7162	19.3895	17.4345
22.9418	24.0731	20.7102	19.2075	17.9807	18.1916
22.1722	24.6153	19.8200	19.7896	16.8277	18.8238
21.4621	25.0926	18.8294	20.4667	15.9315	19.3771
20.9712	25.4513	18.6367	21.3598	14.7080	20.0678
20.8230	25.7781	16.6079	22.1350	13.0007	20.8642
20.3333	26.0970	16.0212	22.5600	11.7033	21.7123
20.4662	26.1916	14.8776	23.3935	10.9790	22.2316
19.9953	26.5007	13.5874	24.3492	9.4577	23.0413
19.4360	26.9010	12.1340	25.3038	8.8034	23.6258
17.7018	29.0287	10.4744	26.5086	7.1315	24.5652
16.9222	30.0241	9.6662	27.1884	6.3816	25.3675
15.8030	32.5812	8.6476	28.1055	5.3984	26.0209
15.3813	32.7344	7.5462	29.1223	3.4627	28.1891
14.1924	33.5291	6.5676	30.2392		
13.3104	35.7602	5.6147	31.3011		
12.4501	37.8980	3.6603	33.7064		
11.8434	39.2537				

---

**Table S3**

Partition coefficients ( $K_{Ova}$ ) and extraction efficiencies ( $EE\%_{Ova}$ ) of ovalbumin and weight fraction compositions of the initial mixtures at 25°C.

PEG	pH	Weight fraction composition / (wt %)		$K_{Ova} \pm \sigma$	$EE\%_{Ova} \pm \sigma$
		PEG	C <sub>6</sub> H <sub>5</sub> K <sub>3</sub> O <sub>7</sub> /C <sub>6</sub> H <sub>8</sub> O <sub>7</sub>		
400	5	30.01	29.99	12.6 ± 0.3	94.8 ± 0.7
	6	29.99	29.85	32.8 ± 0.3	96.1 ± 0.1
	7	29.98	29.97	54.7 ± 0.1	97.7 ± 0.7
	8	30.13	29.84	52.4 ± 0.1	96.5 ± 0.5

**Table S4**

Partition coefficients ( $K_{Ova}$ ) and extraction efficiencies ( $EE\%_{Ova}$ ) of ovalbumin and weight fraction compositions of the initial mixtures at 25°C and pH 7.

PEG	Weight fraction composition / (wt %)		$K_{Ova} \pm \sigma$	$EE\%_{Ova} \pm \sigma$
	PEG	C <sub>6</sub> H <sub>5</sub> K <sub>3</sub> O <sub>7</sub> /C <sub>6</sub> H <sub>8</sub> O <sub>7</sub>		
400	24.75	25.28	76.4 ± 0.4	98.8 ± 0.7
	29.98	19.97	89.5 ± 0.2	98.9 ± 0.1
	30.05	24.97	76.4 ± 0.6	98.8 ± 0.8
	30.01	29.84	54.7 ± 1.0	97.6 ± 0.5
	35.72	24.97	74.4 ± 0.4	98.4 ± 0.7
600	25.05	24.98	54.5 ± 0.1	97.1 ± 0.4
	30.13	20.38	55.7 ± 0.4	96.1 ± 0.3
	30.21	25.44	54.7 ± 0.5	97.1 ± 0.3
	29.79	30.01	52.7 ± 0.9	97.8 ± 0.1
	35.06	24.98	34.7 ± 0.6	97.7 ± 0.5
1000	24.93	25.42	35.5 ± 0.6	95.6 ± 0.4
	29.94	20.01	12.7 ± 0.2	93.6 ± 0.8
	29.87	25.06	35.5 ± 0.3	95.6 ± 0.7
	30.10	29.93	51.6 ± 0.9	97.8 ± 0.4
	34.95	25.15	22.1 ± 0.2	95.2 ± 0.9

**Table S5**

Partition coefficients ( $K_{Ova}$ ) and extraction efficiencies ( $EE\%_{Ova}$ ) of ovalbumin from egg white and weight fraction compositions of the initial mixtures at 25°C and pH 7.

PEG	Weight fraction composition / (wt %)		$K_{Ova} \pm \sigma$	$EE\%_{Ova} \pm \sigma$
	PEG	C <sub>6</sub> H <sub>5</sub> K <sub>3</sub> O <sub>7</sub> /C <sub>6</sub> H <sub>8</sub> O <sub>7</sub>		
400	25.43	25.28	74.4 ± 0.6	98.4 ± 0.3
600	24.65	25.05	34.7 ± 0.6	97.7 ± 0.1
1000	25.16	25.35	22.1 ± 0.1	95.2 ± 0.9