

Supplementary Materials

Fast Centrifugal Partition Chromatography for C- Phycocyanin Purification from *Limnospira platensis* using Eutectic Solvent-based Biphasic Systems

Bruno A. Pereira^{a,b,c}, Alexandra Conde^c, João Vasco Valente^c, Mara G. Freire^c, Sónia
P. M. Ventura^{c*}

^a LAQV-REQUIMTE, Department of Chemistry, NOVA School of Science and
Technology, NOVA University Lisbon, 2829-516 Caparica, Portugal

^b A4F – Algae for future, Estrada do Paço do Lumiar, Campus do Lumiar, Edif. E-R/C,
1649-038 Lisboa, Portugal

^c CICECO – Aveiro Institute of Materials, Department of Chemistry, University of
Aveiro, 3810-193 Aveiro, Portugal

*Corresponding author

E-mail address: spventura@ua.pt (Sónia Ventura)

Table S1 – Composition and stability assessment of hydrophobic ESs.

ESs				
Code	Molar ratio	Component A	Component B	Observation
OctA:DecA	1:2; 3:1	Octanoic acid	Decanoic acid	Instable
OctA:Ment	1:5	Octanoic acid	Menthol	Instable
DecA:Ment	2:1	Decanoic acid	Menthol	Instable
DodA:Ment	2:1	Dodecanoic acid	Menthol	Instable

Table S2 – Macroscopic evaluation of ES-Based **biphasic systems (BSs)** combined by hydrophilic and hydrophobic ESs.

Biphasic System								
Hydrophilic Phase		Hydrophobic ESs						
ESs	Co-solvent	AA:Thy (1:4)	LA:Thy (1:4)	LevA:Thy (2:1; 2:3)	DecA:Thy (2:1)	1,3-Prop:Thy (2:1)	1,3-Prop:OctA (2:1)	OctA:DecA:DodA (3:2:1)
Suc:ChCl:H₂O (2:5:5)	Water	Precipitated	Precipitated	Precipitated	-	Turbidity	Turbidity	-
Glu:Gly:H₂O (1:2:2.5)		Turbidity	Turbidity	Turbidity	-	Turbidity	Turbidity	-
Bet:Sor:H₂O (1.5:1:2)		Precipitated	Precipitated	Precipitated	-	-	-	-
Suc:ChCl:H₂O (2:5:5)	PBS (14 wt%)	Precipitated	Precipitated	Precipitated	Precipitated	Precipitated	Precipitated	Precipitated
Glu:Gly:H₂O (1:2:2.5)		Precipitated	Turbidity	Precipitated	Precipitated	Precipitated	Precipitated	-
Bet:Sor:H₂O (1.5:1:2)		Precipitated	Precipitated	Precipitated	-	Precipitated	Precipitated	-

Macroscopic Observation

Table S3 – Dynamic viscosity (μ) and density (ρ) of ESs used in this work at room temperature.

ESs	μ mPa · s	ρ g · mL ⁻¹
AA:Thy (1:4)	3.53 ± 0.05	1.15 ± 0.11
LevA:Thy (2:1; 2:3)	36.09 ± 1.20	1.27 ± 0.07
DecA:Thy (2:1)	11.46 ± 0.45	1.25 ± 0.13
DecA:Ment (2:1)	13.14 ± 0.31	1.26 ± 0.09
OctA:DecA:DodA (3:2:1)	7.71 ± 0.53	1.21 ± 0.10
Bet:Sor:H ₂ O (1.5:1:2)	3598.80 ± 30.02	1.42 ± 0.12
Glu:Gly:H ₂ O (1:2:2.5)	1716.80 ± 12.00	1.27 ± 0.04
Suc:ChCl:H ₂ O (2:5:5)	2971.47 ± 32.91	1.35 ± 0.14

Table S4 – Estimated cost *per liter* and *per kilogram* of individual and combined phases used in the hydrophilic and hydrophobic ES-based biphasic systems (systems (i) and (ii)) employed on FCPC to fractionate C-PC from total proteins and chlorophylls. Costs were calculated based on reagent prices (€ · L⁻¹ or € · kg⁻¹) and phase composition (*wt%*), considering laboratory- and industrial-scale preparation.

Systems	Lab scale		Industrial scale		
	Cost € · L ⁻¹	Cost € · kg ⁻¹	Cost € · L ⁻¹	Cost € · kg ⁻¹	
Hydrophilic ES (Glu:Gly:H ₂ O 1:2:2.5)	101.62	80.02	0.62	0.79	
PBS 14 <i>wt%</i>	28.81	26.19	5.16	5.68	
System (i)	Hydrophilic ES (60% Glu:Gly:H ₂ O 1:2:2.5 + 40% H ₂ O)	56.25	48.41	0.86	0.74
	Hydrophobic ES (DecA:Ment 2:1)	117.97	93.63	12.17	9.66
System (ii)	Hydrophilic ES (60% Glu:Gly:H ₂ O 1:2:2.5 + 40 <i>wt%</i> PBS)	70.3	58.49	2.93	2.44
	Hydrophobic ES (OctA:DecA:DodA 3:2:1)	83.85	69.3	9.31	7.7

Laboratory-scale reagent prices were obtained from supplier catalogues (Sigma-Aldrich) corresponding to the exact materials used in this study. Bulk industrial prices were obtained from open-access market databases and B2B supplier listings (IMARC, ChemAnalyst, Made-in-China, Echemi) covering ton-scale purchases of glucose, glycerol, fatty acids (from C8 to C12), menthol and phosphate salts.

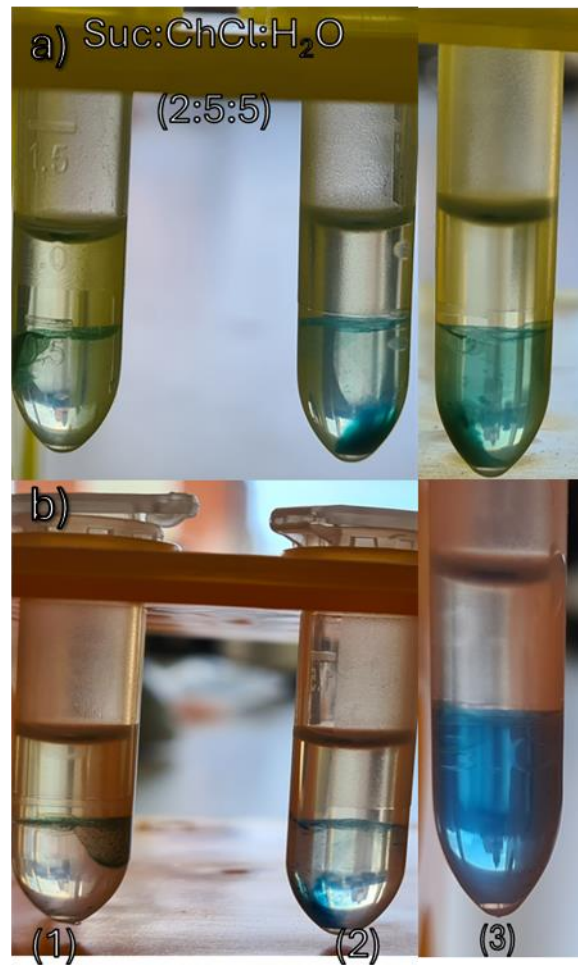


Figure S1 – ESs-based biphasic systems after contact composed by Suc:ChCl:H₂O a) diluted with water and b) diluted with PBS (14 wt%) combined with (1) – DecA:Thy (2:1); (2) – OctA:DecA:DodA (3:2:1); (3) – Dec:Ment (2:1).

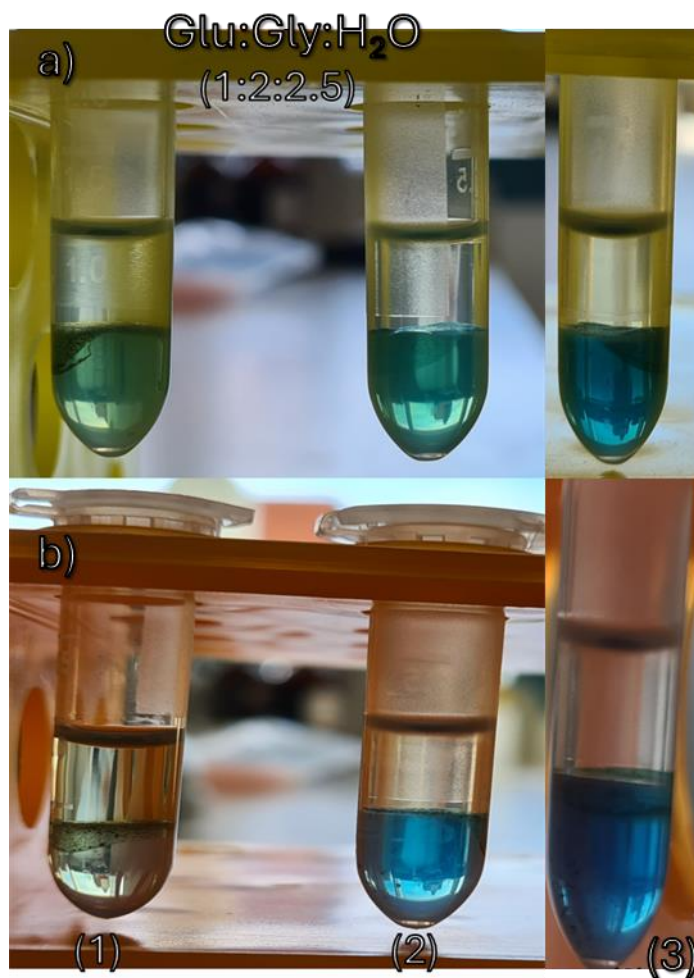


Figure S2 – ESs-based biphasic systems after contact composed by Glu:Gly:H₂O a) diluted with water and b) diluted with PBS (14 wt%) combined with (1) – DecA:Thy (2:1); (2) – OctA:DecA:DodA (3:2:1); (3) – Dec:Ment (2:1).

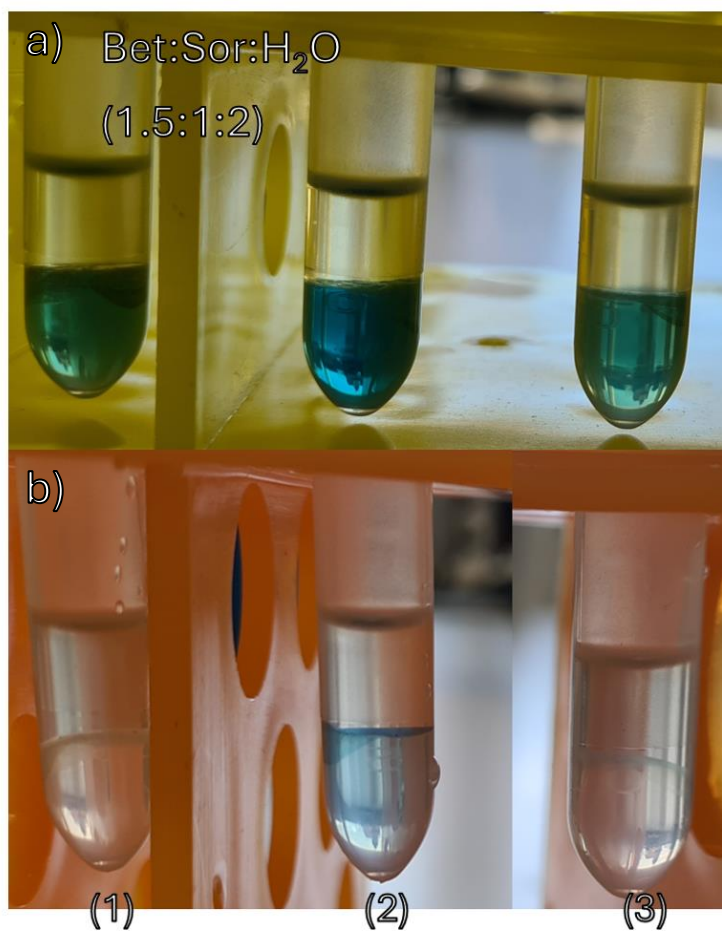


Figure S3 – ESs-based biphasic systems after contact composed by Bet: Sor: H₂O a) diluted with water and b) diluted with PBS (14 wt%) combined with (1) – DecA: Thy (2:1); (2) – OctA: DecA: DodA (3:2:1); (3) – Dec: Ment (2:1).