ELECTRONIC SUPPLEMENTARY INFORMATION (ESI)

Supramolecular biosolvents made up of self-assembled rhamnolipids: synthesis and characterization

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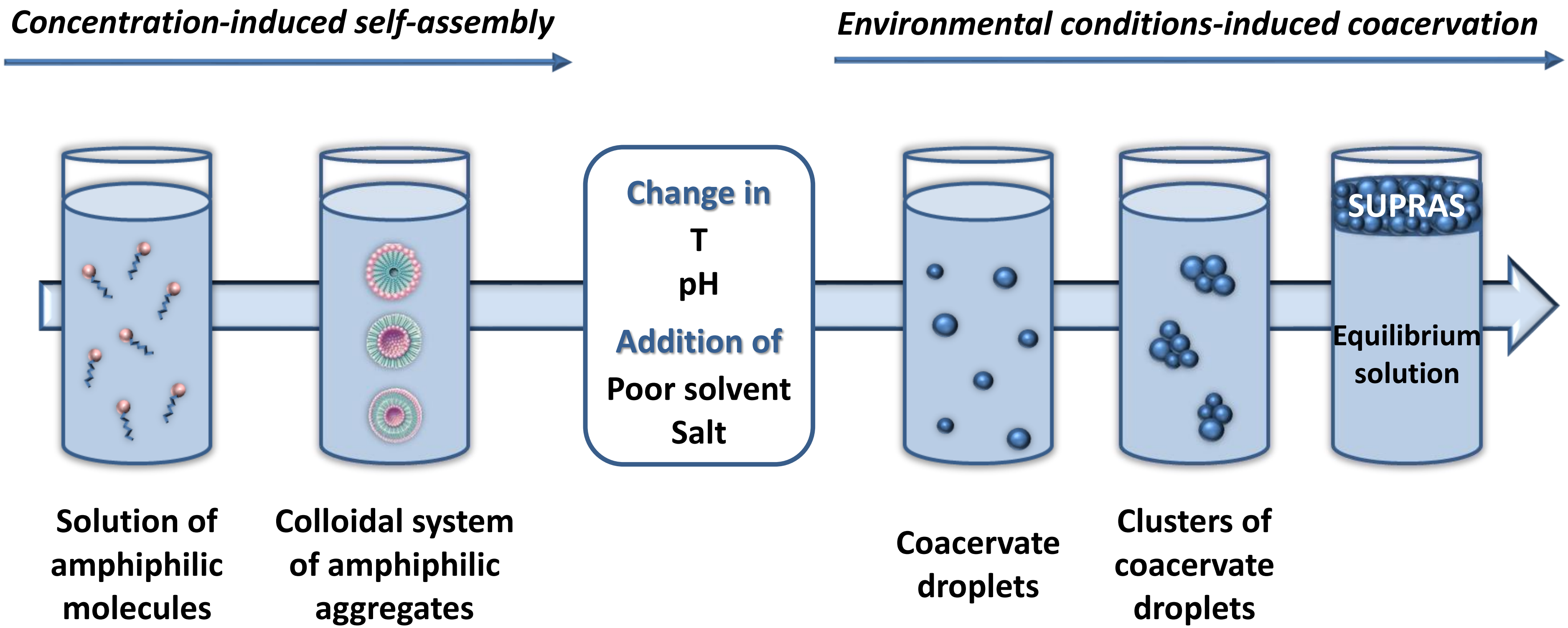


Figure ESI2. Chemical structures of Rha-C10-C10 (A) and Rha-Rha-C10-C10 (B)

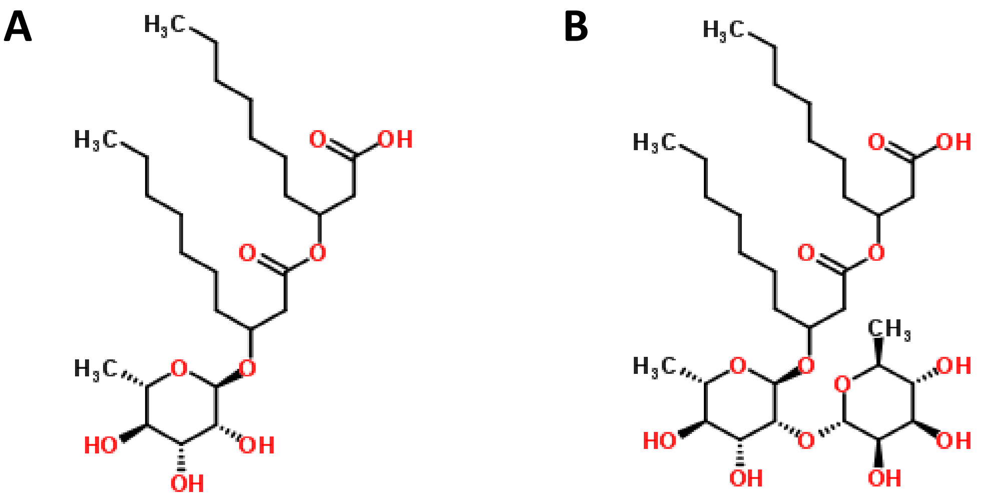


Figure ESI3. Variation of the slopes of the linear regression lines of SUPRAS volume (µL·mL^{-1} mixture) versus rhamnolipid percentage (w/v), as a function of salt concentration (M) in the synthesis mixture

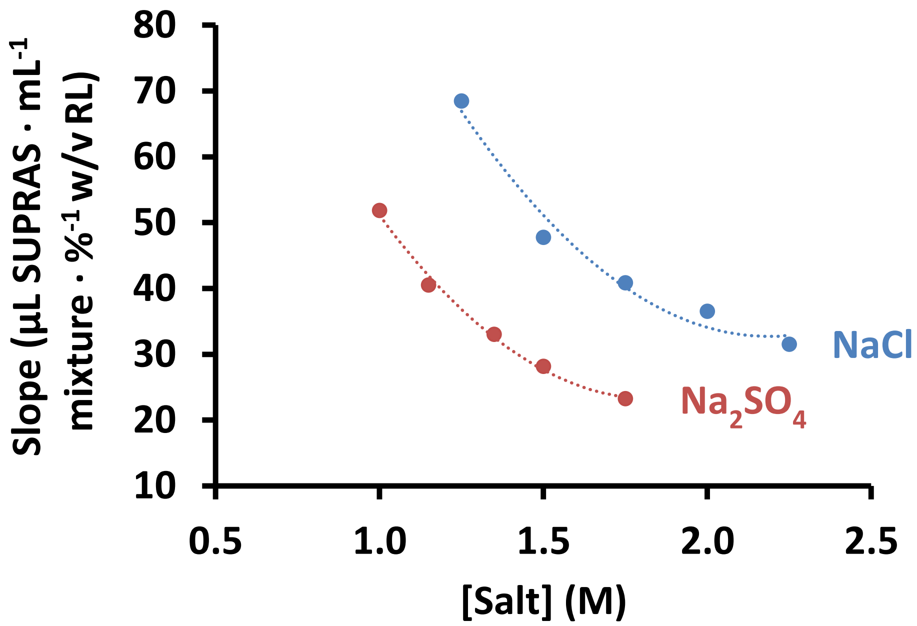


Figure ESI4. Volume of bioSUPRAS (µL·mL^{-1} mixture) versus initial concentration of rhamnolipid (%, w/v), at two concentrations of salt (A: 1.50 M; B: 1.75 M)

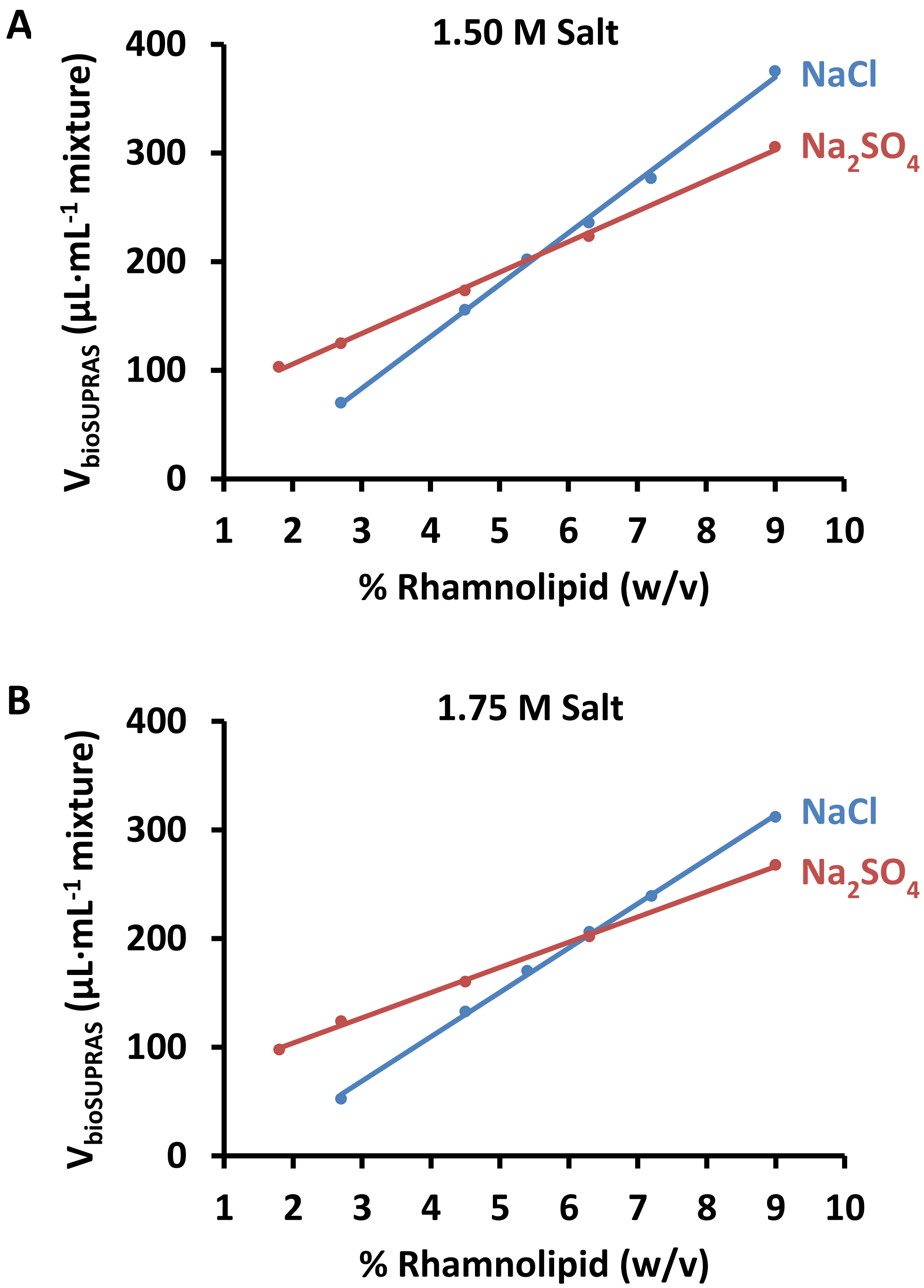


Figure ESI5. Agreement between the measured volumes of bioSUPRASs (µL·mL^{-1} mixture) and those calculated using the proposed equations (A: NaCl; B: Na_2SO_4)

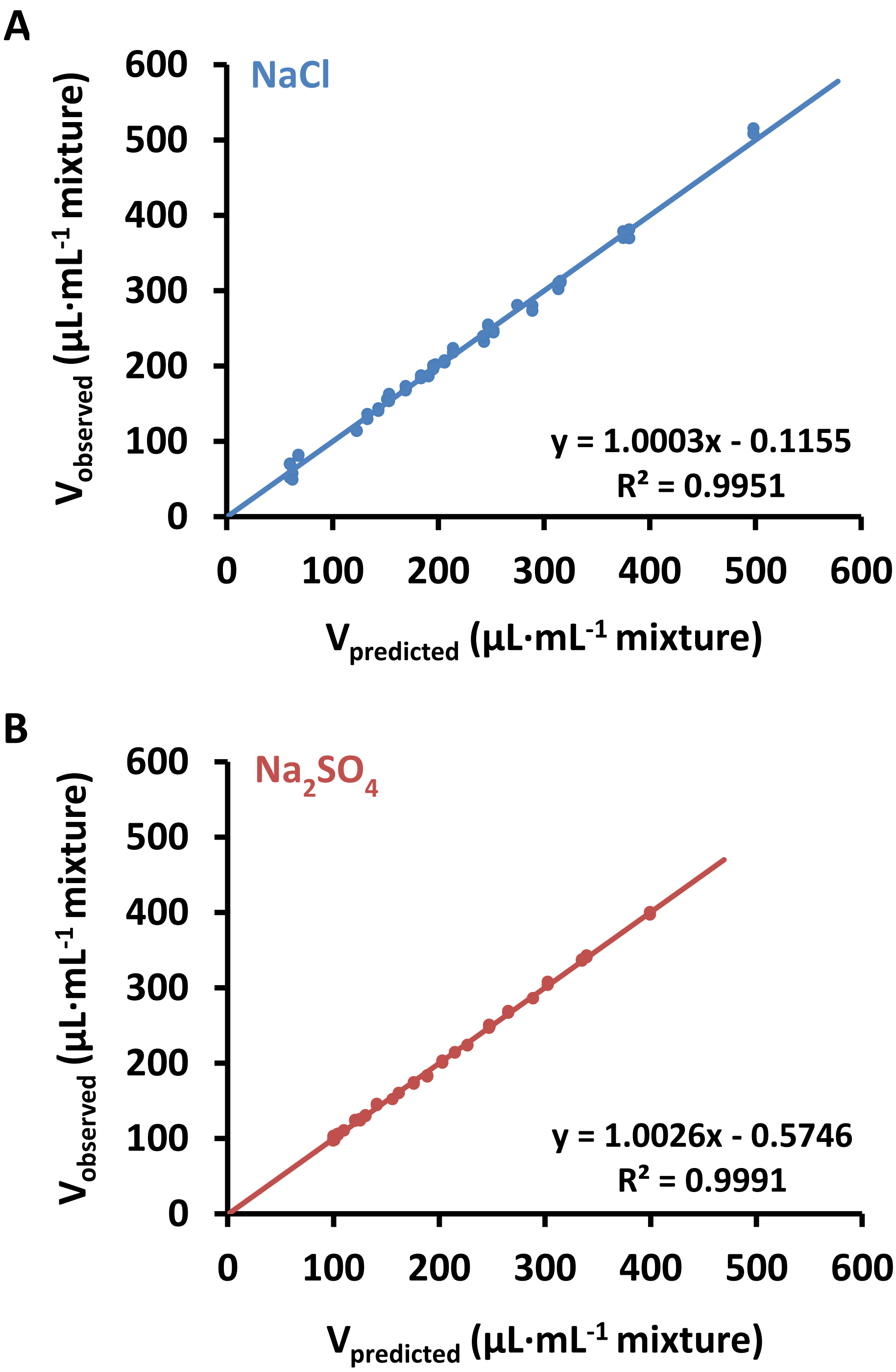


Figure ESI6. Water content in bioSUPRASs (%, w/w) formed using different initial concentrations of salts (M) and rhamnolipid (1.8-9%, w/v)

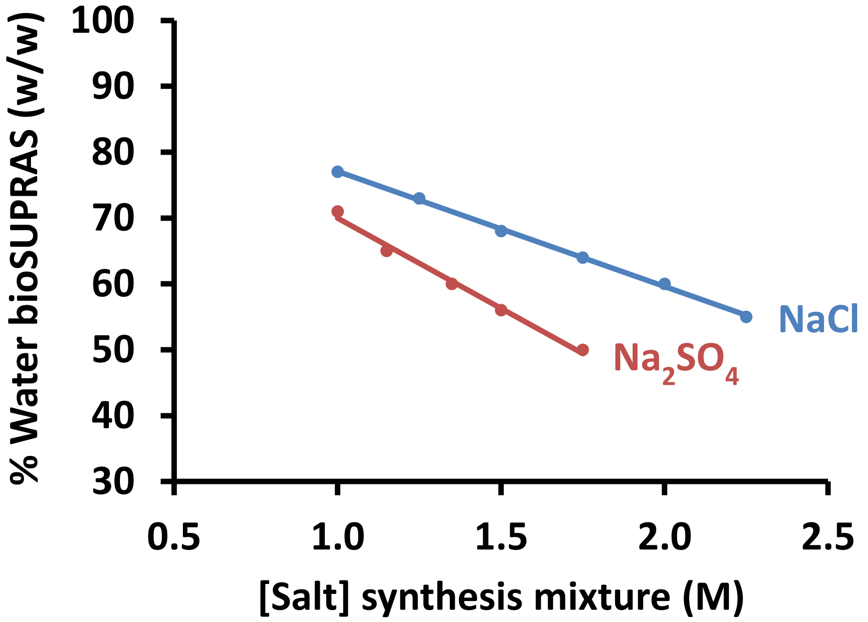


Figure ESI7. Optical micrograph of a bioSUPRAS produced from a mixture containing 4.5% of rhamnolipid (w/v) and 0.85 M of $NH_4CH_3CO_2$

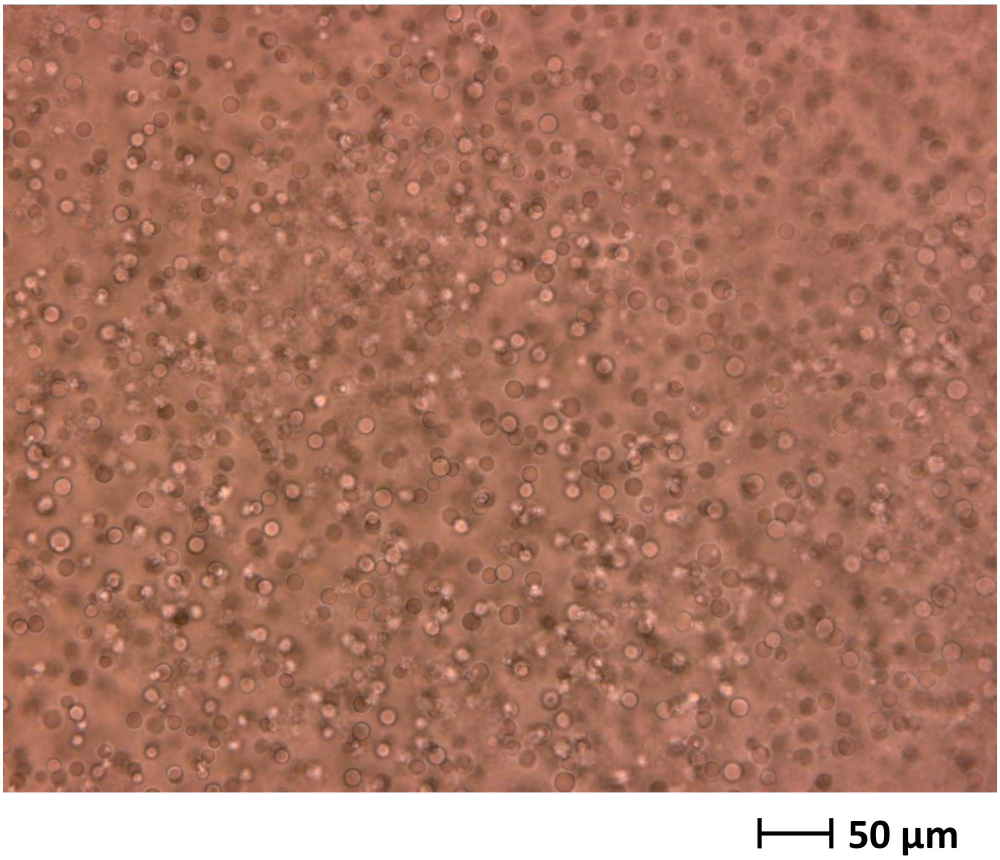
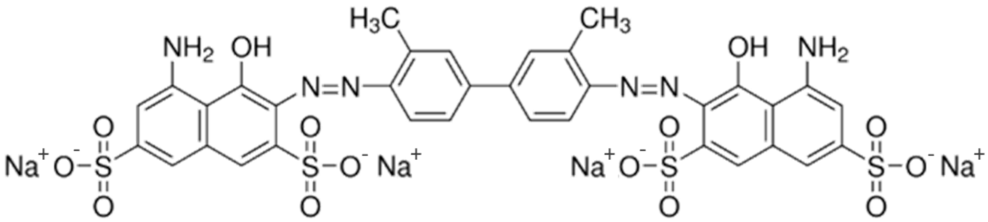


Figure ESI8. Chemical structures of trypan blue (A) and malachite green oxalate salt (B)

A



B

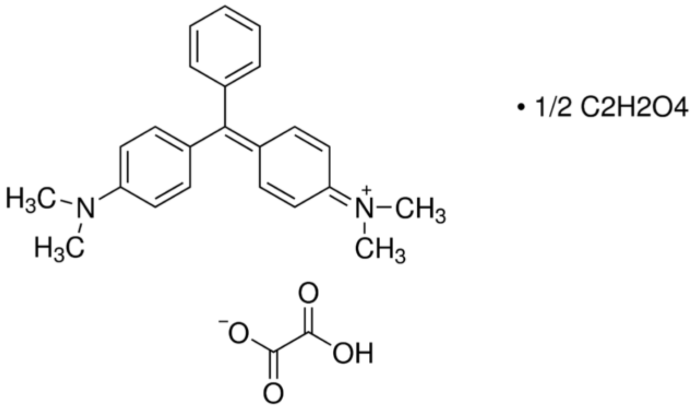


Table ESI1. Critical aggregation concentration (mM) reported for colloidal systems produced from pure and mixed rhamnolipids under different experimental conditions

Composition of rhamnolipid product	Production of colloidal systems		Reference
	Experimental conditions	Critical aggregation concentration (mM)	
Rha-Rha-C_{10}-C_{10} (50%) Rha-Rha- C_{10}-C_{12} (29%) Impurities (21%)	pH 4.0 pH 7.4 pH 4.0, 0.15 M NaCl pH 7.4, 0.15 M NaCl pH 7.4, 0.50 M NaCl	0.01 0.50 0.01 0.11 0.10	[52]
Rha-C_{10}-C_{10} Rha- C_{10}-$C_{12:1}$ Rha- C_{10}-C_{12}	pH 4.0 pH 7.4 pH 4.0, 0.1 M NaCl pH 7.4, 0.1 M NaCl	0.05 0.25 0.05 0.04	[53]
Rha-C_{10}-C_{10}	UHQ water pH 7.0 pH 9.0 0.5 M NaCl	0.04 0.18 0.36 0.03	[49]
Rha-Rha-C_{10}-C_{10}	UHQ water pH 7.0 pH 9.0 0.5 M NaCl	0.07 0.11 0.18 0.08	
Rha-C_{10}-C_{10} (30%) Rha-Rha-C_{10}-C_{10} (70%)	pH 9	0.26	
Rha-C_{10}-C_{10} (50%) Rha-Rha-C_{10}-C_{10} (50%)	pH 9	0.34	
Rha-C_{10}-C_{10} (70%) Rha-Rha-C_{10}-C_{10} (30%)	pH 9	0.34	
Rha-C_{10}-C_{10}	UHQ water 0.05 M NaCl 0.50 M NaCl 1 M NaCl	0.1 0.1 0.05 0.04	[54]
Rha-Rha-C_{10}-C_{10}	UHQ water 0.05 M NaCl 0.50 M NaCl 1 M NaCl	0.15 0.08 0.08 0.04	
Rha-C_{10}-C_{10}	UHQ water 0.375 M NaCl 0.875 M NaCl	0.10 0.05 0.05	[48]
Rha-Rha-C_{10}-C_{10}	UHQ water 0.375 M NaCl 0.875 M NaCl	0.02 0.02 0.02	

Table ESI2. Slopes and correlation coefficients calculated from the linear regression lines of SUPRAS volume (µL·mL^{-1} mixture) as a function of rhamnolipid percentage (w/v) in the colloidal system, at different concentrations of salts (M)

Synthesis conditions	**Slope (µL SUPRAS · mL^{-1} mixture · $\%^{-1}$ w/v RL)**	**Correlation coefficient**
1.25 M NaCl	64.49	0.9958
1.50 M NaCl	47.75	0.9977
1.75 M NaCl	40.85	0.9988
2.00 M NaCl	36.53	0.9975
2.25 M NaCl	31.08	0.9999
1.00 M Na_2SO_4	51.81	0.9995
1.15 M Na_2SO_4	40.49	0.9998
1.35 M Na_2SO_4	33.03	0.9992
1.50 M Na_2SO_4	28.15	0.9987
1.75 M Na_2SO_4	23.23	0.9987

Table ESI3. Density values (g·mL^{-1}) of bioSUPRASs formed from different NaCl (M) and rhamnolipid (%, w/v) concentrations

Synthesis conditions		*bioSUPRAS*
% Rhamnolipid (w/v)	**[NaCl] (M)**	**ρ±SD (g·mL^{-1})**
1.8	1.00	1.07±0.01
1.8	1.25	1.05±0.02
1.8	1.50	1.08±0.01
1.8	1.75	1.06±0.02
2.7	1.00	1.063±0.001
2.7	1.25	1.07±0.02
2.7	1.50	1.06±0.02
2.7	1.75	1.081±0.008
2.7	2.00	1.067±0.007
4.5	1.25	1.088±0.008
4.5	1.50	1.073±0.009
4.5	1.75	1.071±0.008
4.5	2.00	1.09±0.01
5.4	1.25	1.07±0.01
5.4	1.50	1.083±0.003
5.4	1.75	1.075±0.001
5.4	2.00	1.08±0.02
5.4	2.25	1.092±0.001
6.3	1.25	1.08±0.03
6.3	1.50	1.09±0.01
6.3	1.75	1.09±0.01
6.3	2.00	1.08±0.01
6.3	2.25	1.10±0.02
9.0	1.25	1.081±0.005
9.0	1.50	1.05±0.01
9.0	1.75	1.11±0.02
9.0	2.00	1.078±0.004
9.0	2.25	1.08±0.02

Table ESI4. Density values ($g \cdot mL^{-1}$) of bioSUPRASs formed from different Na_2SO_4 (M) and rhamnolipid (%, w/v) concentrations

Synthesis conditions		*bioSUPRAS*
% Rhamnolipid (w/v)	**[Na_2SO_4] (M)**	**ρ±SD ($g \cdot mL^{-1}$)**
1.8	1.00	1.07±0.04
1.8	1.15	1.10±0.02
1.8	1.35	1.04±0.01
1.8	1.50	1.11±0.02
1.8	1.75	1.12±0.01
2.7	1.00	1.12±0.04
2.7	1.15	1.087±0.007
2.7	1.35	1.117±0.003
2.7	1.50	1.10±0.02
2.7	1.75	1.10±0.05
4.5	1.00	1.11±0.03
4.5	1.15	1.142±0.009
4.5	1.35	1.13±0.02
4.5	1.50	1.035±0.005
4.5	1.75	1.10±0.06
6.3	1.00	1.13±0.02
6.3	1.15	1.13±0.02
6.3	1.35	1.12±0.04
6.3	1.50	1.136±0.008
6.3	1.75	1.11±0.003
9.0	1.00	1.10±0.06
9.0	1.15	1.07±0.06
9.0	1.35	1.16±0.01
9.0	1.50	1.149±0.001
9.0	1.75	1.13±0.02

Table ESI5. Morphology and size distribution of anionic rhamnolipids at different concentrations of biosurfactant ($g\cdot L^{-1}$) and salt (M)

Composition of rhamnolipid product	Experimental conditions			Hydrodynamic diameter range or mean (nm)	Aggregate morphology (electron microscopy)	Reference
	Rhamnolipid	pH	NaCl			
Rha-Rha-C_{10}-C_{10} (50%) Rha-Rha- C_{10}-C_{12} (29%) Impurities (21%)	<0.7 $g\cdot L^{-1}$	7.4	0.15 M	Bimodal distribution (43-66; 350-550)	Spherical vesicles (150-200 nm); elongated vesicles with lengths >1000 nm and aggregated structures of larger size	[52]
	> 1.8 $g\cdot L^{-1}$	7.4	0.15 M	> 1500		
Rha-C_{10}-C_{10}	0.07-0.7 $g\cdot L^{-1}$	6.8	0.05-1 M	Not reported	Morphology transition from spherical micelles to bilayer structures and to hexagonal (rod shaped) micelles, for both monoRha and diRha, with increasing NaCl concentration.	[54]
Rha-Rha-C_{10}-C_{10}	0.07-0.7 $g\cdot L^{-1}$	6.8	0.05-1 M			
Rha-C_{10}-C_{10}	1 $g\cdot L^{-1}$		No salt 0.375 M 0.875 M	140.3±2.0 (PDI 0.263) 2212±444.1 (PDI 0.890) 4674±359.8 (PDI 1.0)	NaCl 0.875 M: Giant vesicle-like structures (in the µm range) by self-assembling of the crude RL mixture and spherical structures for mono-RLs and di-RLs.	[48]
Rha-Rha-C_{10}-C_{10}	0.5 $g\cdot L^{-1}$		No salt 0.375 M 0.875 M	133.1±4.9 (PDI 0.373) >10000 >10000		
Rha-C_{10}-C_{10} (10%) Rha-Rha-C_{10}-C_{10} (30%) Impurities (60%)	1.5 $g\cdot L^{-1}$		No salt 0.375 M 0.875 M	302.8±7.4 (PDI 0.549) 456.6±42.2 (PDI 0.596) 2343±154.1 (PDI 0.753)		

PDI: polydispersity index

Table ESI5. Morphology and size distribution of anionic rhamnolipids at different concentrations of biosurfactant ($g{\cdot}L^{-1}$) and salt (M)

Composition of rhamnolipid product	Experimental conditions			Hydrodynamic diameter range or mean (nm)	Aggregate morphology (electron microscopy)	Reference
	Rhamnolipid	pH	NaCl			
Mixture 1 (monoRha) Rha-$C_{14:2}$ (3.1%) Rha-$C_{12:2}$ (24 %)	1.8 $g{\cdot}L^{-1}$	6.8	0.01 M	Unimodal distribution (40-350)	Not reported	[62]
Rha-C_{12}-C_{10} (14.1%) Rha-C_{10}-$C_{12:1}$ (5.0%) Rha-C_{10}-C_{10} (43.4%) Rha-$C_{10:1}$–C8 (4.5%) Rha-Rha-C_{10}-C_{10} (5.9%)	3.6 $g{\cdot}L^{-1}$	6.8	0.01M	Multimodal distribution (50-300; 300-1000; 2000-3000)		
Mixture 2: (DiRha) Rha-Rha-C_{10}-$C_{14:1}$ (4.1%) Rha-Rha-C_{10}-C_{12} (2.2%)	1.8 $g{\cdot}L^{-1}$	6.8	0.01 M	Multimodal distribution (4-10; 40-100; 200-500)		
Rha-Rha-C_{10}-$C_{12:1}$(37.5%) Rha-Rha-C_{10}-C_{10} (45.1%) Rha-Rha-C_{10}-C_8 and Rha-Rha-C_8-C_{10} (6.9%) Rha-Rha-C_{10} (4.12%)	3.6 $g{\cdot}L^{-1}$	6.8	0.01 M	Multimodal distribution (10-40; 150-1000; 2000-4000)		
Crude extract containing mixture 1 (64%) and mixture 2 (36%)	1.8 $g{\cdot}L^{-1}$	6.8	0.01 M	Bimodal distribution (70-200; 400-1000)		
	3.6 $g{\cdot}L^{-1}$	6.8	0.01 M	Multimodal distribution (80-170; 500-2000; 2000-5000)		

Table ESI6. Comparison of environmental/health and sustainability concerns and market prices for different SUPRAS developed in extraction processes

Extraction of compounds/ samples	Surfactant(s) and solvents (percentage[a])	Coacervation-inducing agent[b]	Environmental/health concerns and specific regulations[c]	Sustainability concerns	Surfactant market price	Reference
Bixin and norbixin in food	Octanoic acid (2%, v/v) THF (35%, v/v)	Poor solvent (type II water, 3.207 mL; H_2O/THF volume ratio 1.80; pH 3)	Decanoic acid: harmful to aquatic life with long lasting effects. THF is **suspected of causing cancer**, highly flammable. Included in the Community rolling action plan (CoRAP). Other: acid conditions needed.	Synthetic surfactant and organic solvent. Mild acidic synthesis conditions	Carboxylic acids: $ 0.5-2/kg	[64]
Xanthohumol in beer	Triton X-114 (2.5%, v/v) Water content in sample, pH 5	Temperature (70 ºC) and salt (NaCl, 15% w/v)	Triton X-114 is an **endocrine disruptor**, very toxic to aquatic life, with long lasting effects, harmful if swallowed and suspected of damaging fertility or the unborn child. It is classified as substance of very high concern (SVHC)	Synthetic surfactant. High temperature synthesis conditions	Triton series: $ 2-10/kg	[65]
Vitamin E in water	Sodium dodecane sulphonic acid, SDSA (1%, w/v) Water sample	Acid (HCl, 4.2 M)	-	Synthetic surfactant. Strong acidic synthesis conditions	SDSA: $ 1-1.5/kg	[66]
Erythrosine and tartrazine in food	Triton X-100 (~2%, w/v) Cetyl trimethyl ammonium bromide, CTAB (~$6\cdot10^{-4}$%, w/v), type II water, pH 2.5	Temperature (70 ºC) and salt (KCl, 0.1% w/v)	Triton X-100 is an **endocrine disruptor** very toxic to aquatic life with long lasting effects, causes serious eye damage, harmful if swallowed. It is classified as substance of very high concern (SVHC)	Synthetic surfactant. High temperature synthesis conditions	Triton series: $ 2-10/kg Cetyl trimethyl ammonium bromide: $ 1-10/kg	[67]

[a] Percentage of each compound in the synthesis mixture.
[b] In the case of water-induced coacervation, water volume per sample and H_2O/solvent volume ratio are indicated.
[c] EU ECHA database (hazards regarding eye or skin or respiratory irritation not included).

Table ESI6. Comparison of environmental/health and sustainability concerns and market prices for different SUPRAS developed in extraction processes

Extraction of compounds/ samples	Surfactant(s) and solvents (percentage[a])	Coacervation-inducing agent[b]	Environmental/health concerns and specific regulations[c]	Sustainability concerns	Surfactant market price	Reference
Perfluoroalkyl compounds in blood serum	Hexanoic acid (6.5%, v/v), THF (40%, v/v)	Poor solvent (water content in sample;~765 µL/volume ratio H_2O/THF 1.28, acid pH	THF: see first table row	Synthetic surfactant and organic solvent. Mild acidic synthesis conditions	Carboxylic acids: $ 0.5-2/kg	[68]
Chlorophenols in river water	Cetrimide (0.5%, w/v) 1-octanol (0.08%, v/v) Water sample	Salt (NaCl, 37.8% w/v)	Cetrimide: substance very toxic to aquatic life, harmful if swallowed, causes serious eye damage, may cause damage to organs through prolonged or repeated exposure, causes skin irritation and may cause respiratory irritation. 1-octanol: very toxic to aquatic life with long lasting effects, causes serious eye damage, harmful if swallowed and in contact with skin, highly flammable, may cause respiratory irritation and may cause drowsiness or dizziness	Synthetic surfactant and co-surfactant	Cetrimide: $ 1-10/kg	[69]
Dyes in water	Rhamnolipids	Salt (NaCl at ~1-2 M, Na_2SO_4, ~0.5-1.5M or $NH_4CH_3CO_2$ ~0.5-1.5M	-	-	$ 20-25/kg	This study

[a] Percentage of each compound in the synthesis mixture.
[b] In the case of water-induced coacervation, water volume per sample and H_2O/solvent volume ratio are indicated.
[c]EU ECHA database (hazards regarding eye or skin or respiratory irritation not included).