

Supplementary Information

Unravelling the nature of citric acid:L-arginine:water mixtures: The bifunctional role of water

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Table S1 - T_m variation (ΔT) predicted by PC-SAFT for citric acid and L-arginine in the mixture citric acid:L-arginine:H₂O. $\Delta T = 0$ correspond to the binary mixture without water.

$\chi(\text{water})$	$\chi(\text{citric acid})$	$\chi(\text{L-arginine})$	ΔT (citric acid)	ΔT (L-arginine)
0	0.5	0.5	0	0
0.1	0.45	0.45	-5	-7
0.2	0.4	0.4	-11	-16
0.3	0.35	0.35	-19	-26
0.4	0.3	0.3	-28	-39
0.5	0.25	0.25	-39	-55
0.6	0.2	0.2	-54	-76
0.67	0.165	0.165	-68	-95
0.71	0.145	0.145	-78	-108
0.75	0.125	0.125	-89	-123
0.78	0.11	0.11	-98	-137
0.8	0.1	0.1	-105	-146

Table S2 - Activity coefficients predicted by ePC-SAFT for citric acid (γ_{cit}) and [L-arginine]⁺ (γ_{arg}^+) in the mixture citric acid:L-arginine:H₂O.

$\chi(\text{water})$	$\chi(\text{citric acid})$	$\chi(\text{L-arginine})$	γ_{cit}	γ_{arg}^+
0	0.5	0.5	1.51	1.45
0.1	0.45	0.45	1.45	1.49
0.2	0.4	0.4	1.37	1.52
0.3	0.35	0.35	1.25	1.53
0.4	0.3	0.3	1.10	1.53
0.5	0.25	0.25	0.90	1.48
0.6	0.2	0.2	0.66	1.38

0.67	0.165	0.165	0.47	1.25
0.71	0.145	0.145	0.36	1.14
0.75	0.125	0.125	0.26	1.01
0.78	0.11	0.11	0.19	0.89
0.8	0.1	0.1	0.15	0.81

Table S3 - Activity coefficients predicted by ePC-SAFT for citric acid (γ_{cit}) in the mixture citric acid:L-arginine:H₂O, at a water molar ratio of 0.8 and different molar fractions of L-arginine and citric acid.

$\chi(\text{water})$	$\chi(\text{L-arginine})$	$\chi(\text{citric acid})$	$\chi(\text{L-arginine})$ in respect to citric acid	γ_{cit}
0.8	0.199	0.001	0.995	0.09
0.8	0.175	0.025	0.875	0.14
0.8	0.15	0.05	0.75	0.14
0.8	0.125	0.075	0.625	0.14
0.8	0.1	0.1	0.5	0.15
0.8	0.075	0.125	0.375	0.15
0.8	0.05	0.15	0.25	0.16
0.8	0.025	0.175	0.125	0.17
0.8	0.001	0.199	0.005	0.18

Table S4 - Activity coefficients predicted by ePC-SAFT for citric acid (γ_{cit}) in the mixture citric acid:L-arginine:H₂O, at a water molar ratio of 0.7 and different molar fractions of L-arginine and citric acid.

$\chi(\text{water})$	$\chi(\text{L-arginine})$	$\chi(\text{citric acid})$	$\chi(\text{L-arginine})$ in respect to citric acid	γ_{cit}
0.7	0.299	0.001	0.997	0.50
0.7	0.25	0.05	0.833	0.45
0.7	0.2	0.1	0.667	0.41
0.7	0.15	0.15	0.5	0.39
0.7	0.1	0.2	0.333	0.38
0.7	0.05	0.25	0.167	0.39
0.7	0.001	0.299	0.003	0.41

Table S5 - Activity coefficients predicted by ePC-SAFT for citric acid (γ_{cit}) in the mixture citric acid:L-arginine:H₂O, at a water molar ratio of 0.6 and different molar fractions of L-arginine and citric acid.

$\chi(\text{water})$	$\chi(\text{L-arginine})$	$\chi(\text{citric acid})$	$\chi(\text{L-arginine})$ in respect to citric acid	γ_{cit}
0.6	0.399	0.001	0.998	1.32
0.6	0.35	0.05	0.875	0.94
0.6	0.3	0.1	0.75	0.80
0.6	0.25	0.15	0.625	0.71
0.6	0.199	0.201	0.498	0.66
0.6	0.175	0.225	0.438	0.64
0.6	0.15	0.25	0.375	0.62
0.6	0.125	0.275	0.313	0.61
0.6	0.1	0.3	0.25	0.61

0.6	0.075	0.325	0.188	0.60
0.6	0.05	0.35	0.125	0.60
0.6	0.025	0.375	0.063	0.61
0.6	0.001	0.399	0.003	0.62

Table S6 - Activity coefficients predicted by ePC-SAFT for citric acid (γ_{cit}) in the mixture citric acid:L-arginine:H₂O, at a water molar ratio of 0.5 and different molar fractions of L-arginine and citric acid.

$\chi(\text{water})$	$\chi(\text{L-arginine})$	$\chi(\text{citric acid})$	$\chi(\text{L-arginine})$ in respect to citric acid	γ_{cit}
0.5	0.499	0.001	0.998	2.44
0.5	0.45	0.05	0.9	1.53
0.5	0.4	0.1	0.8	1.27
0.5	0.35	0.15	0.7	1.10
0.5	0.3	0.2	0.6	0.99
0.5	0.25	0.25	0.5	0.90
0.5	0.2	0.3	0.4	0.84
0.5	0.15	0.35	0.3	0.80
0.5	0.1	0.4	0.2	0.77
0.5	0.05	0.45	0.1	0.76
0.5	0.001	0.499	0.002	0.77

Table S7 - Activity coefficients predicted by ePC-SAFT for citric acid (γ_{cit}) in the mixture citric acid:L-arginine:H₂O, at a water molar ratio of 0.2 and different molar fractions of L-arginine and citric acid.

$\chi(\text{water})$	$\chi(\text{L-arginine})$	$\chi(\text{citric acid})$	$\chi(\text{L-arginine})$ in respect to citric acid	γ_{cit}
0.2	0.799	0.001	0.999	6.27
0.2	0.75	0.05	0.938	3.31
0.2	0.7	0.1	0.875	2.75
0.2	0.65	0.15	0.813	2.36
0.2	0.6	0.2	0.750	2.07
0.2	0.5	0.3	0.625	1.65
0.2	0.4	0.4	0.500	1.37
0.2	0.3	0.5	0.375	1.18
0.2	0.2	0.6	0.250	1.06
0.2	0.15	0.65	0.188	1.02
0.2	0.1	0.7	0.125	0.99
0.2	0.05	0.75	0.063	0.98
0.2	0.001	0.799	0.001	0.98

Table S8 - Activity coefficients predicted by ePC-SAFT for citric acid (γ_{cit}) in the mixture citric acid:L-arginine:H₂O, at a water molar ratio of 0.001 and different molar fractions of L-arginine and citric acid.

$\chi(\text{water})$	$\chi(\text{L-arginine})$	$\chi(\text{citric acid})$	$\chi(\text{L-arginine})$ in respect to citric acid	γ_{cit}
0.001	0.998	0.001	0.999	8.52
0.001	0.95	0.049	0.951	4.30
0.001	0.9	0.099	0.901	3.59

0.001	0.8	0.199	0.801	2.73
0.001	0.7	0.299	0.701	2.17
0.001	0.6	0.399	0.601	1.79
0.001	0.5	0.499	0.501	1.51
0.001	0.4	0.599	0.400	1.31
0.001	0.3	0.699	0.300	1.17
0.001	0.2	0.799	0.200	1.08
0.001	0.1	0.899	0.100	1.02
0.001	0.05	0.949	0.050	1.00
0.001	0.001	0.998	0.001	1.00

Table S9 - Activity coefficients predicted by ePC-SAFT for [L-arginine]⁺ (γ_{arg}^+) in the mixture citric acid:L-arginine:H₂O, at a water molar ratio of 0.8 and different molar fractions of L-arginine and citric acid.

$\chi(\text{water})$	$\chi(\text{citric acid})$	$\chi(\text{L-arginine})$	$\chi(\text{L-arginine})$ in respect to citric acid	γ_{arg}^+
0.8	0.001	0.199	0.995	0.41
0.8	0.025	0.175	0.875	0.48
0.8	0.05	0.15	0.75	0.56
0.8	0.1	0.1	0.5	0.81
0.8	0.15	0.05	0.25	1.24
0.8	0.175	0.025	0.125	1.54
0.8	0.199	0.001	0.005	1.20

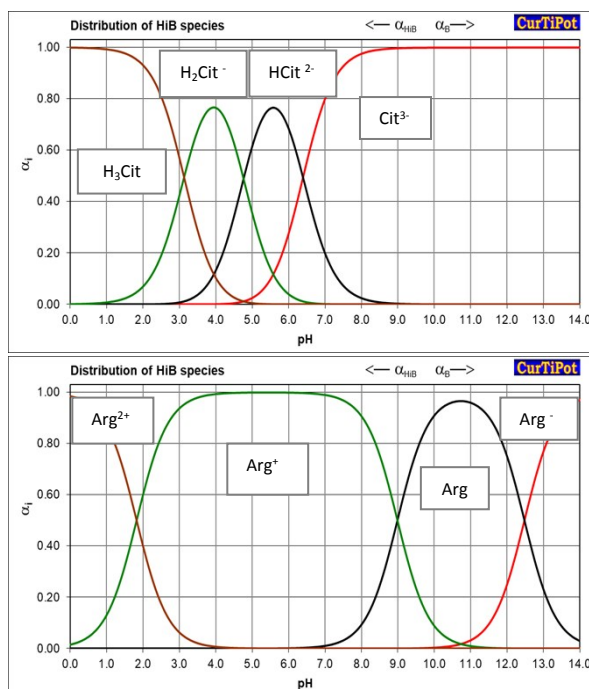
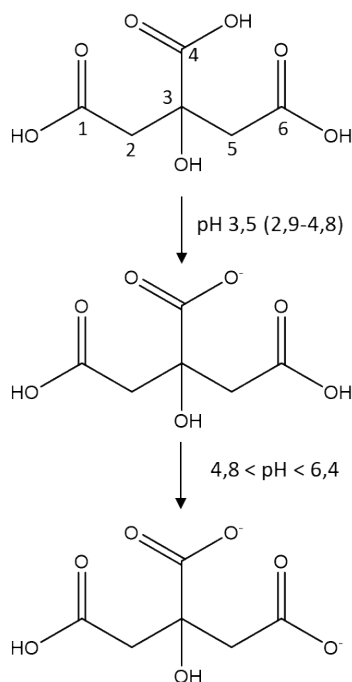


Fig. S1 - Diagram of the relative distribution of species (α_i) depending on pH, for citric acid (up) and L-arginine (down). Cit: citric acid; Arg: L-arginine. Calculated by CurTiPot⁴⁹ using pKa's from Table S10.

Table S10 – pKa's of citric acid and L-arginine. Functional group numbers' according to Fig. S2 b).

	citric acid	L-arginine
pKa	2.9 (COOH 4)	2.5 (OH 1)
	4.8 (COOH 6)	9 (NH ₂ 4)
	6.4 (COOH 1)	12.5 (guanidine 8, 10, 11)

Citric acid pH < 2,9



L-arginine pH > 12,5

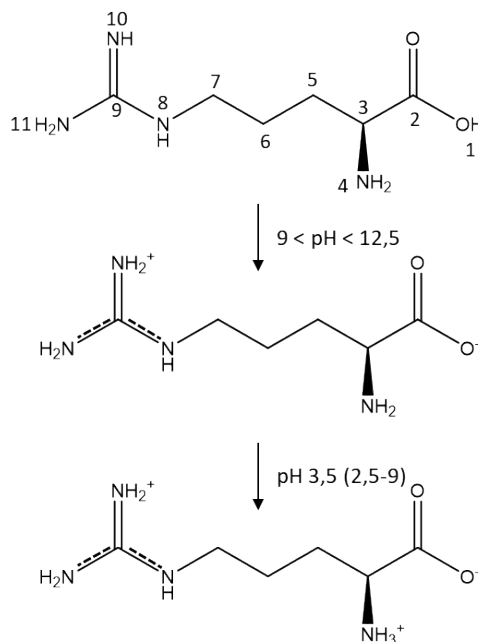


Fig. S2 - Chemical structure of citric acid (left) and L-arginine (right) at different pH's, according to pKa's from Table S10.

Table S11 – Assignment of L-arginine functional groups to the respective FTIR wavenumber. Functional group numbers' according to Figure S2 b), right.

Wavenumber (cm ⁻¹)	Assignment	Reference (in main article)
3358	s N-H 4	50-53
3302	s N-H 6 (guanidine)	50-53
3057	s OH (COOH) 1	50
2946	s CH 5,6,7	50.53
2863		
1722	-	-
1676	s C=O (COOH) 1	50, 52-54
1612	s C=N 9	50,51
1553	b NH	50,51,53
1472	b CH +/- b OH	50,53,54
1422		
1374		

1330		
1279	-	-
1180	s C-N 3	
1137	s C-N 9 (guanidine) +/- b NH	50-53
979	b OH	53
766	b NH	51
701	-	-
607		
551	b COO- 1	51,53
495		

Table S12 - Assignment of citric acid functional groups to the respective FTIR wavenumber. Functional group numbers' according to Figure S2 b), left.

Wavenumber (cm ⁻¹)	Assignment	Reference (in main article)
3499	s OH 4	-
3370		
3290	s OH(COOH) 1,4,6	50, 55-57
3242		
3000	s CH 2,5	55,56
2993		
2640		
2531	OH(COOH) 4	-
1750		
1719	s C=O 1,4,6	50,55,56
1681		
1421	b CH	56
1394	b OH	50,56
1351	b CH	56
1314	b COH + b CH	56
1292	b CH	56
1207	b CH + s COH	56
1171	b C-OH	56
1103	s CC	56
1055	s CC(COOH)	56
1025	-	-
944	s C-O	56
904	s CC	56
775	b CH/s CC	50, 55/56
725	-	-
687	-	-
635	-	-
599	b OH	56
505	b COOH	56
441	-	-
404	-	-

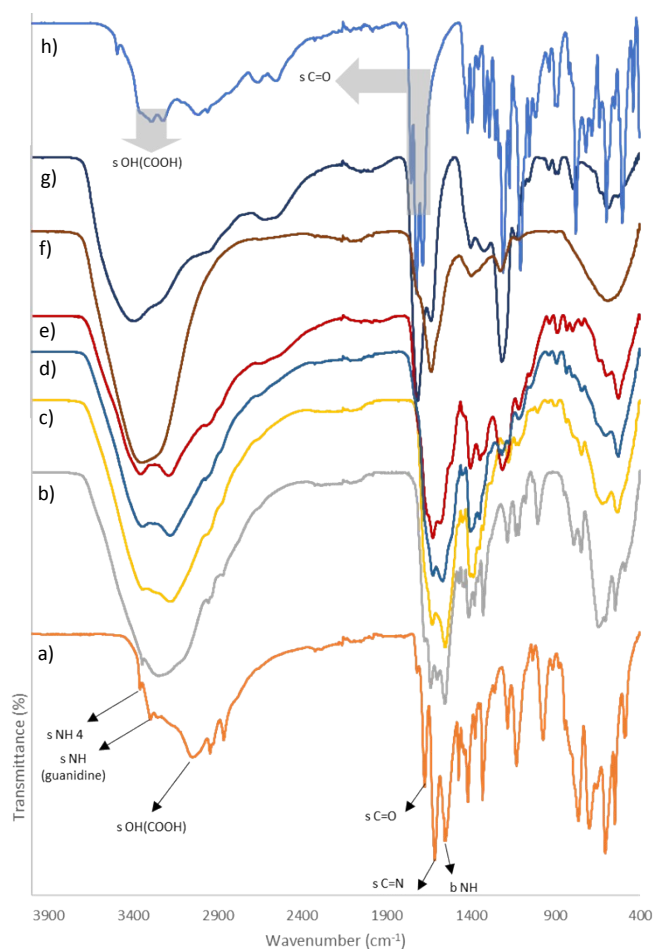


Fig. S3 - FTIR spectra of a) pure L-arginine (orange line); b) citric acid:L-arginine:H₂O 0.1:1:7 mol (grey line); c) citric acid:L-arginine:H₂O 0.2:1:7 mol (yellow line); d) citric acid:L-arginine:H₂O 0.5:1:7 mol (blue line); e) citric acid:L-arginine:H₂O 1:1:7 mol (red line); f) citric acid:H₂O pH 3.5 (brown line); g) citric acid:H₂O 1:7 mol (dark blue line); h) pure citric acid (light blue line). s and b refer to stretching and bending vibrations, respectively. Numbers attributed according to the chemical structures from Figure S1 b).

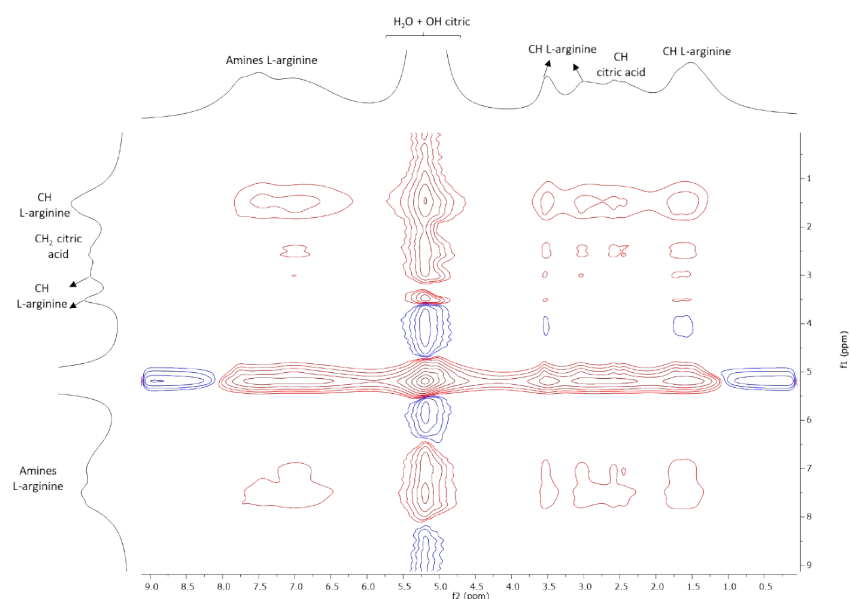


Fig. S4 - 2D NOESY spectra of the mixture citric acid:L-arginine:H₂O 0.5:1:7 mol. The functional groups of the protons identified are aligned with the respective peaks.