

Supplementary Material

Ionic liquid-based sample pretreatment platforms: comparing solid supports and aqueous biphasic systems for enhanced bioanalytical performance

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Figure S1. PSA calibration curve obtained by ELISA with the corresponding linear regression equation, coefficient of determination (*R*²), limit of detection (LOD), and limit of quantification (LOQ). The LOD was calculated from the calibration curve analysed in duplicate, using the ratio between the standard error of the regression (*sy/x*) and the slope of the calibration curve, multiplied by a factor of 3.3. The LOQ was subsequently estimated as three times the corresponding LOD value.

Figure S2. CD spectra of PSA at 4 ng·mL⁻¹ in PBS (purple dashed line) and in the liquid phase of a representative SPE system composed of [Si][N₃₁₁₄]Cl and K₂HPO₄/KH₂PO₄ buffer after recovery (green line).

Characterization of SILs

Table S1. Elemental analysis results showing the weight percent of carbon (%C), hydrogen (%H), and nitrogen (%N) for activated silica, [Si][C₃]Cl, and all SIL materials ([Si][C₃mim]Cl, [Si][N₃₁₁₄]Cl, and [Si][N₃₈₈₈]Cl).

Material	C (%)	H (%)	N (%)
Activated silica	-	-	-
[Si][C ₃]Cl	5.61	1.64	-
[Si][C ₃ mim]Cl	9.91	2.32	2.22
[Si][N ₃₁₁₄]Cl	9.68	2.17	1.01
[Si][N ₃₈₈₈]Cl	6.43	1.35	0.13

Depletion of immunoglobulin G and human serum albumin using SILs

Table S2. Depletion efficiencies of IgG and HSA using SPE with activated silica and SILs in the presence of human serum diluted in PBS, and K₂HPO₄/KH₂PO₄ buffer, using a S:L of 100 mg·mL⁻¹ and a contact time of 60 minutes. Control experiments were performed in the absence of silica and SILs.

Buffer	SILs	DE _{IgG} (%)	DE _{HSA} (%)
PBS (0.15M)	Control	18 ± 1	0
	Activated Silica	17 ± 2	0
	[Si][C ₃ mim]Cl	14 ± 1	0
	[Si][N ₃₁₁₄]Cl	30 ± 1	0
	[Si][N ₃₈₈₈]Cl	42 ± 5	0
K ₂ HPO ₄ /KH ₂ PO ₄ buffer (4.68M)	Control	64.8 ± 4.3	46.8 ± 2.0
	Activated Silica	63 ± 2	48 ± 2
	[Si][C ₃ mim]Cl	98 ± 3	97 ± 3
	[Si][N ₃₁₁₄]Cl	95 ± 1	92 ± 2
	[Si][N ₃₈₈₈]Cl	99 ± 1	99 ± 1

Binodal curves of ABS

Table S3. Experimental binodal data in mass percent for the ternary systems composed of IL (1) + K₂HPO₄/KH₂PO₄ (pH $\approx$ 7) (2) + water at 25°C and atmospheric pressure.

[N ₁₄₄₄] ⁺ Cl ⁻		[C ₃ mim] ⁺ Cl ⁻	
100w ₁	100w ₂	100w ₁	100w ₂
30.85	9.27	36.00	4.36
27.89	10.64	34.13	6.41
26.36	11.59	32.27	8.45
24.81	12.35	29.53	10.04
22.56	13.87	28.34	10.89
21.37	14.66	27.09	11.96
19.99	15.63	25.87	13.07
18.88	16.47	24.52	14.71
17.85	17.28	23.14	16.63
16.28	18.61	21.31	17.97
15.32	19.44	20.52	18.40
14.52	20.14	19.47	19.54
13.90	20.62	18.30	20.37
13.21	21.20	17.49	22.54
12.22	22.14	16.71	23.20
11.25	23.01	15.06	25.61
10.33	23.86	13.39	27.71
9.68	24.58	11.96	29.07
9.03	25.22	10.09	31.90
8.24	26.08	8.83	34.29
7.60	26.77	8.03	35.79
6.88	27.61	7.40	36.95
6.32	28.25	6.79	38.35

Depletion of immunoglobulin G and human serum albumin using ABS

Table S4. Depletion efficiencies of IgG and HSA obtained using ABS composed of different 0.3 g of IL, 0.5 g of K₂HPO₄/KH₂PO₄ and 0.1 g of water in the presence of 0.1 g of human serum.

Ionic liquid	DE _{IgG} (%)	DE _{HSA} (%)
[C ₃ mim] ⁺ Cl ⁻	19 $\pm$ 1	12 $\pm$ 2
[N ₂₂₂₂] ⁺ Cl ⁻	0	0
[N ₃₃₃₃] ⁺ Cl ⁻	0	0
[N ₄₄₄₄] ⁺ Cl ⁻	56 $\pm$ 3	44 $\pm$ 1
[N ₁₄₄₄] ⁺ Cl ⁻	19 $\pm$ 1	31 $\pm$ 1

ELISA calibration curve for PSA

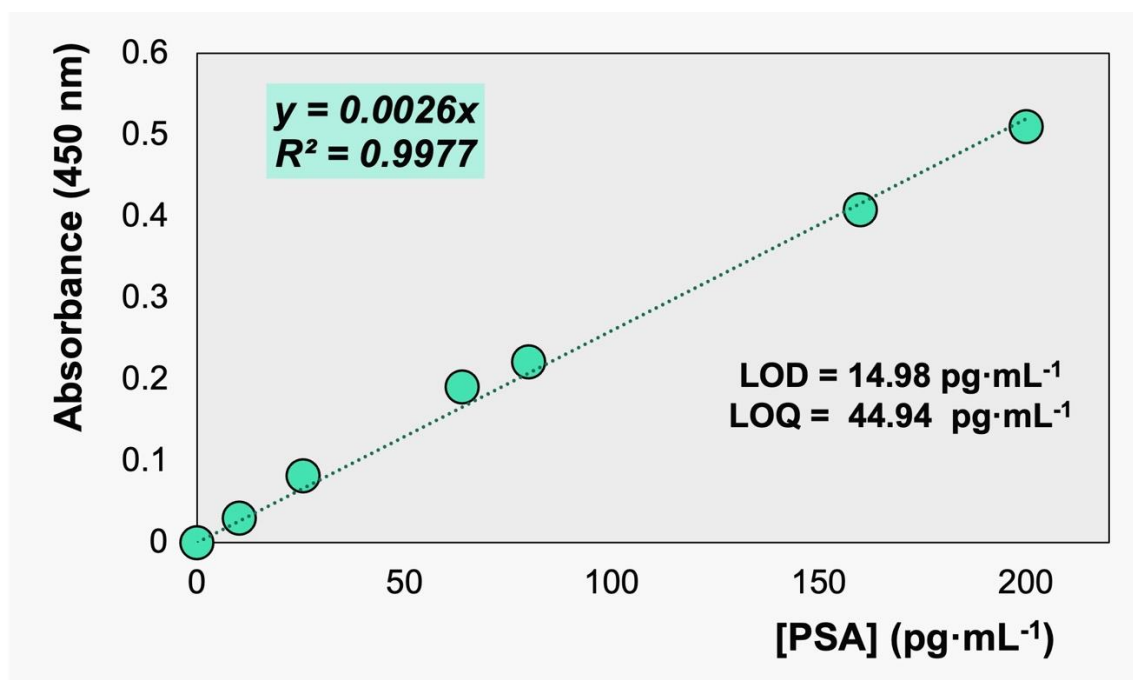


Figure S1. PSA calibration curve obtained by ELISA with the corresponding linear regression equation, coefficient of determination (R^2), limit of detection (LOD), and limit of quantification (LOQ). The LOD was calculated from the calibration curve analysed in duplicate, using the ratio between the standard error of the regression (sy/x) and the slope of the calibration curve, multiplied by a factor of 3.3. The LOQ was subsequently estimated as three times the corresponding LOD value.

Isolation of PSA

Table S5. Recovery yield of PSA in the liquid phase, along with the corresponding relative error of analysis, using SPE with activated silica and SILs in the presence of human serum diluted in K_2HPO_4/KH_2PO_4 buffer, using a S:L of $100 \text{ mg} \cdot \text{mL}^{-1}$ and a contact time of 60 minutes. Control experiments were performed in the absence of silica and SILs.

Buffer	SIL	Liquid phase (%)	RE (%)
K_2HPO_4/KH_2PO_4 buffer (4.68M)	Control	92 ± 2	8 ± 2
	Activated Silica	67 ± 6	33 ± 6
	[Si][C ₃ mim]Cl	26 ± 2	74 ± 2
	[Si][N ₃₁₁₄]Cl	89 ± 5	11 ± 5
	[Si][N ₃₈₈₈]Cl	47 ± 3	53 ± 3

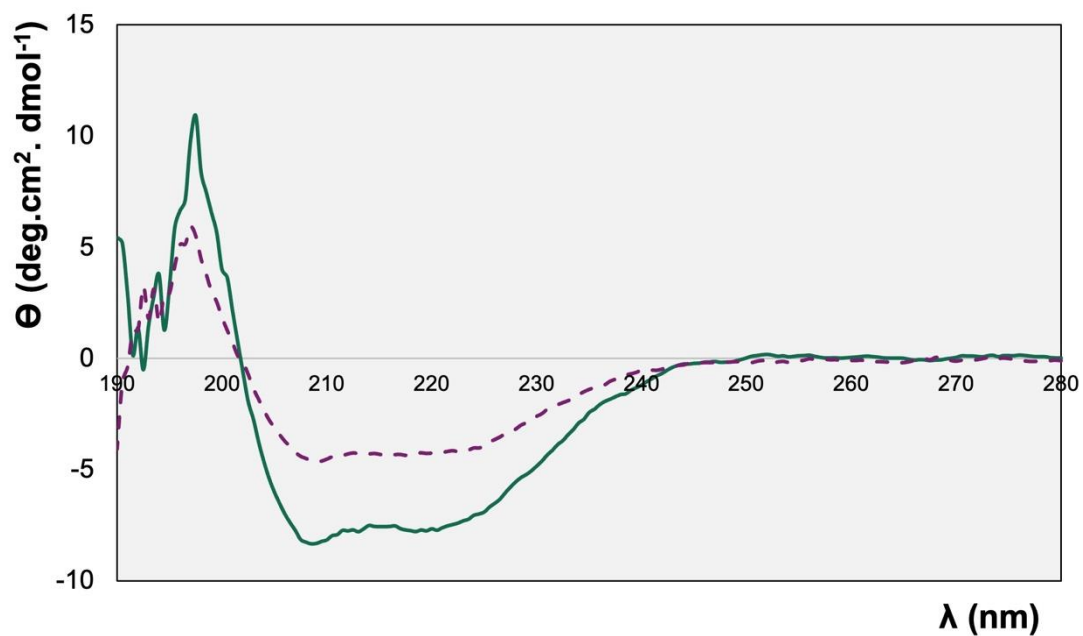


Figure S2. CD spectra of PSA at $4 \text{ ng} \cdot \text{mL}^{-1}$ in PBS (purple dashed line) and in the liquid phase of a representative SPE system composed of $[\text{Si}][\text{N}_{3114}]\text{Cl}$ and $\text{K}_2\text{HPO}_4/\text{KH}_2\text{PO}_4$ buffer after recovery (green line).