

Supporting Information

Enhancing Biomarker Detection in Human Serum for Lung Cancer Diagnosis: Aqueous Biphasic Systems for Simultaneous Depletion of High-Abundance Proteins and Efficient Extraction of CYFRA 21-1

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IgG and HSA depletion efficiencies

Table S1. Recovery yield of IgG (RY_{IgG} , %) and HSA (RY_{HSA} , %) in the top and bottom phases and depletion efficiencies obtained for IgG (DE_{IgG} , %) and HSA (DE_{HSA} , %) at the interphase using ABS-TPP composed of 30 wt% of PEG + 12 wt% $\text{K}_2\text{HPO}_4/\text{KH}_2\text{PO}_4$ (pH \approx 7) buffer + 10 wt% human serum + 48 wt% water (mean \pm SD, n=3). Data for the ABS-TPP composed of PEG 1000 is taken from the literature[1].

ABS-TPP	RY_{IgG} (%)		DE_{IgG} (%)	RY_{HSA} (%)		DE_{HSA} (%)
	Top phase	Bottom phase	Interphase	Top phase	Bottom phase	Interphase
PEG 400	77 \pm 6	0.2 \pm 0.1	23 \pm 6	81 \pm 3	1.2 \pm 0.4	18 \pm 3
PEG 1000	4 \pm 2	0.4 \pm 0.4	95 \pm 1	76 \pm 1	1.2 \pm 0.2	23 \pm 1
PEG 1500	1 \pm 1	0.1 \pm 0.2	99.2 \pm 0.8	26 \pm 1	3.6 \pm 0.1	70.1 \pm 0.7
PEG 2000	3 \pm 2	0.4 \pm 0.1	97 \pm 2	6 \pm 2	31 \pm 4	63 \pm 2
PEG 4000	16 \pm 1	0	84 \pm 1	48 \pm 1	0	52 \pm 1
PEG 6000	15 \pm 2	0	85 \pm 2	45 \pm 1	0	55.4 \pm 0.1

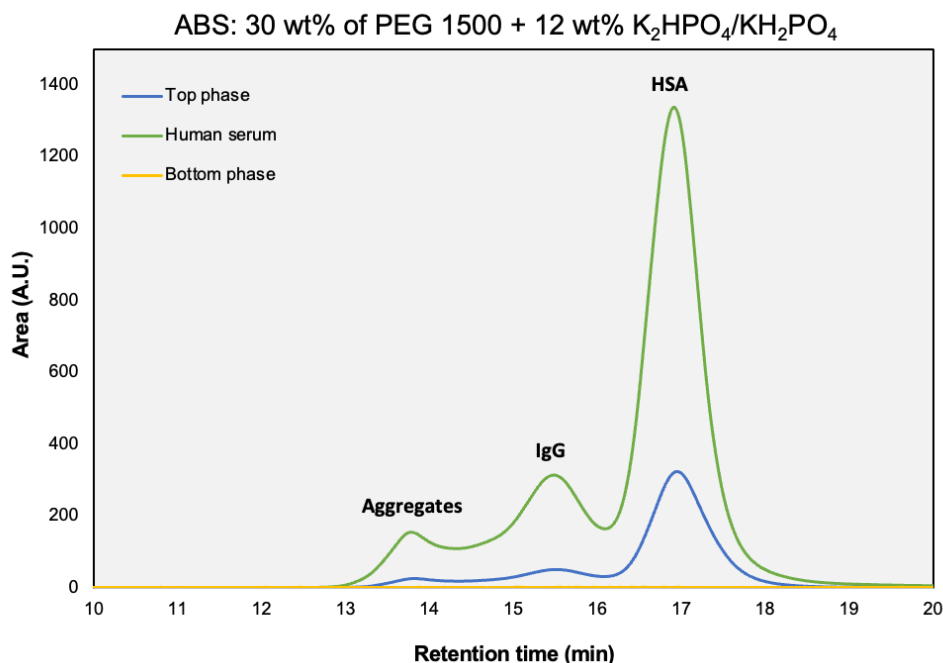


Figure S2. SE-HPLC chromatograms of human serum (green line) and top (blue line) and bottom (yellow line) phases of ABS composed of 30 wt% of PEG 1500 + 12 wt% $\text{K}_2\text{HPO}_4/\text{KH}_2\text{PO}_4$ + 48 wt% water + 10 wt% human serum.

Extraction efficiencies and recovery yields of CYFRA 21-1

Table S3. Extraction efficiency of CYFRA 21-1 ($EE_{\text{CYFRA 21-1}}$, %) obtained with ABS composed of 30 wt% of PEG + 12 wt% of $\text{K}_2\text{HPO}_4/\text{KH}_2\text{PO}_4$ (pH \approx 7) + 48 wt% water + 10 wt% CYFRA 21-1 aqueous solutions (1000 ng·mL⁻¹) (mean \pm SD, n=2).

ABS	$EE_{\text{CYFRA 21-1}}$ (%)
PEG 400	98 \pm 1
PEG 1000	100
PEG 1500	99.7 \pm 0.2
PEG 2000	99.1 \pm 0.8
PEG 4000	95.7 \pm 0.8
PEG 6000	93.0 \pm 0.5

Table S4. Recovery yield of CYFRA 21-1 ($RY_{\text{CYFRA 21-1}}$, %) in the top, inter and bottom phases in the ABS-TPP composed of 30 wt% of PEG + 12 wt% of $\text{K}_2\text{HPO}_4/\text{KH}_2\text{PO}_4$ (pH \approx 7) + 48 wt% water + 10 wt% CYFRA 21-1 spiked human serum (1000 ng·mL⁻¹) (mean \pm SD, n=3).

ABS-TPP	$RY_{\text{CYFRA 21-1}}$ (%)		
	Top phase	Interphase	Bottom phase
PEG 1000	85 \pm 6	11 \pm 1	4 \pm 4
PEG 1500	91.0 \pm 0.3	9.0 \pm 0.3	0
PEG 2000	74 \pm 5	10 \pm 6	16 \pm 1
PEG 4000	18 \pm 8	81 \pm 8	1 \pm 2
PEG 6000	100	0	0

References

- [1] M.E. Rosa, M.S.M. Mendes, E. Carmo, J.P. Conde, J.A.P. Coutinho, M.G. Freire, F.A. e Silva, Tailored pretreatment of serum samples and biomarker extraction afforded by ionic liquids as constituents of aqueous biphasic systems, *Sep. Purif. Technol.* 322 (2023) 124248. <https://doi.org/10.1016/j.seppur.2023.124248>.