

Electronic Supplementary Information

Hybrid alginate-protein cryogel beads: efficient and sustainable bio-based materials to purify immunoglobulin G antibodies

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Adsorption isotherms

The equilibrium behaviour of the IgG adsorption was evaluated by the Langmuir and Freundlich isotherms.^{1,2} The Langmuir model parameters were estimated by a non-linearized fitting of equation (S1) to the equilibrium experimental data:

$$q_e = \frac{q_m \times k_d \times C}{1 + k_d \times C} \quad \text{Eq. (S1)}$$

where C ($\text{mg}\cdot\text{mL}^{-1}$) is the IgG concentration in the serum sample, q_e ($\text{mg}\cdot\text{g}^{-1}$) is the equilibrium concentration of adsorbed IgG per mass of beads, q_m ($\text{mg}\cdot\text{g}^{-1}$) is the maximum adsorption capacity of IgG per unit mass of beads to form a complete monolayer on the surface (saturation capacity) and k_d ($\text{mL}\cdot\text{mg}^{-1}$) is the Langmuir equilibrium constant related to the strength of affinity between the protein and the surface.¹

The Freundlich model parameters were estimated by non-linearized fitting by equation (S2) to the equilibrium experimental data:

$$q_e = k_F \times C^n \quad \text{Eq. (S2)}$$

where k_F ($\text{mg}\cdot\text{g}^{-1}$) is the Freundlich binding constant related with the adsorption of IgG per weight of materials and n is an empirical parameter that is a measure of the intensity of adsorption in Freundlich adsorption isotherms.^{1,2} IgG concentrations in human serum from 0.06 to 1.8 $\text{mg}\cdot\text{mL}^{-1}$ were applied for both models. The equilibrium time was fixed at 90 min and pH 5.0.

References:

1. B. Liu, S. Cao, X. Deng, S. Li and R. Luo, *Appl. Surf. Sci.*, 2006, **252**, 7830.
2. J. D. Andrade and R. Vanwagenen, *Abstr. Pap. Am. Chem. Soc.*, 1983, **185**, 189.

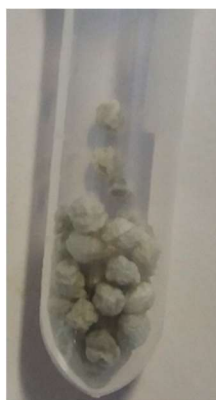


Figure S1. Optical image of restructured Alg-SSF hybrid cryogel beads (R-Alg-SSF).

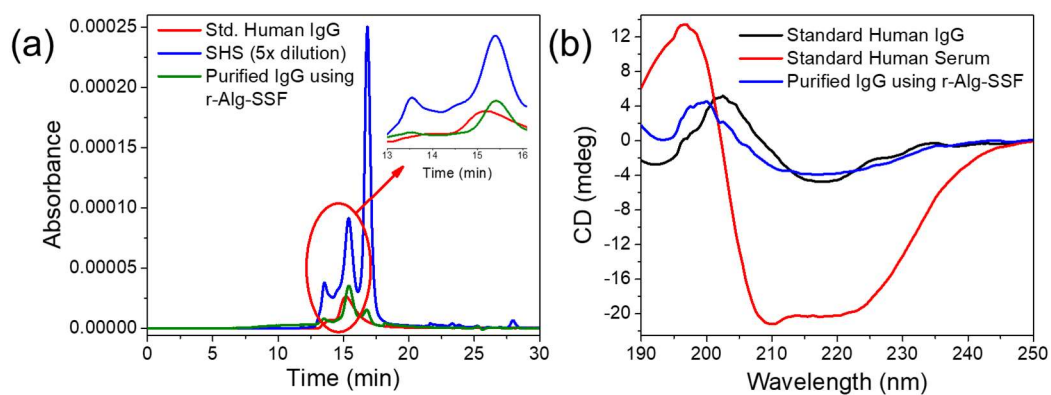


Figure S2. (a) SE-HPLC chromatograms for standard human IgG (Std Human IgG), human serum aqueous solution (SHS) diluted 5 times and purified IgG using recovered r-Alg-SSF hybrid cryogel bead materials after the desorption steps. (b) CD spectra of standard human IgG, human serum and purified IgG from human serum using recovered r-Alg-SSF hybrid cryogel beads after the desorption steps.

Table S1. Langmuir and Freundlich isotherm parameters for the adsorption of IgG from human serum onto Alg-SSF composite-based cryogel beads.

Parameters			
Langmuir isotherm	q_m (mg g ⁻¹)	k_d (L mg ⁻¹)	R ²
	175	3.3	0.994
Freundlich isotherm	k_F (mg g ⁻¹)	n	R ²
	122	2.6	0.954

Table S2. Optimization of the human serum concentration and contact time for the IgG adsorption onto alginate-protein hybrid cryogel beads.

Contact time (min) / Alg-SSF (pH 5.0)	Purity (%)	Yield (%)
30	55.3±3.1	62.5±2.1
60	60.0±2.5	68.7±1.6
90	67.6±1.3	71.4±2.4
120	66.6±0.7	70.6±5.6
150	63.9±0.4	68.6±3.4
180	64.7±0.6	72.5±2.9
Human serum concentration (dilution times) / Alg-SSF (pH 5.0)		
5	91.0±1.2	31.0±1.0
10	86.6±2.3	68.7±1.1
15	79.0±0.9	71.7±0.1
20	69.8±1.1	73.8±0.4
25	58.1±2.6	75.6±0.2
50	53.0±3.6	65.6±2.3