Figure 41. Adsorption number analysis: Effect of column length (L) on dynamic binding capacity (DBC) using 3.5 wt. % CL-DEAE cellulose supports. BSA (1 mg/ml) was loaded onto 9 × 5 cm (Ο) and 90 × 1.6 cm ( ) column beds (180 ml) in 15 mM sodium phosphate, pH 7.8 at room temperature (25 °C) to 20 – 25 % BT at 1, 2, 5 and 10 cm/min. After washing the column to baseline (absorbance 280 nm) with 1 – 2 CV of running buffer, the bound protein was eluted with 1 M NaCl. Column beds were reconditioned with 4 M NaCl (2 CV), binding buffer (1 CV), 0.5 N NaOH (1 CV) and binding buffer (6 CV) at 10 cm/min.
Figure 42. Dynamic binding capacities (DBC) of 2 wt. % and 3.5 wt. % CL-DEAE cellulose supports for BSA at varying conductivities (ionic strengths). The DBC of BSA (1 mg/ml) for 2 wt. % and 3.5 wt. % CL-DEAE cellulose supports (column dimensions: 90 × 1.5 cm I.D.) were determined at a linear velocity of 10 cm/min. Operating backpressures ranged from 2 – 3 PSI for all of the columns and all of the supports washed to a baseline within 2 – 3 CV following loading (except for the 3.5 wt. % CL-DEAE cellulose support using 50 mM Tris/50 mM NaCl). (a) 15 mM sodium phosphate, pH 7.8 (monobasic + dibasic); 2 wt. % CL-DEAE (0, 20, 50 and 100 mM NaCl), 3.5 wt. % CL-DEAE (0, 50 and 100 mM NaCl); (b) 50 mM Tris-HCl, pH 8.6; 2 wt. % CL-DEAE (0, 20, 50 and 100 mM NaCl), 3.5 wt. % CL-DEAE (0, 20, 35 and 50 mM NaCl). *Note: When loaded and washed using 50 mM NaCl, the 3.5 wt. % cellulose support would not wash below the 20 % BT level (the BSA just slowly desorbed in a trail for 10 – 20 CV). However, when loaded in 50 mM NaCl and washed in Tris buffer with no NaCl present, the 3.5 wt. % cellulose support washed to baseline in 2 – 3 CV and an accurate DBC could be obtained. The DBC with 100 mM NaCl could not be determined accurately on the 3.5 wt. % cellulose support due to minimal binding and extensive trailing.
Figure 43. Effect of column length (L) on the dynamic binding capacity (DBC) of 3.5 wt. % CL-DEAE cellulose support. BSA (1 mg/ml) was loaded onto 9 × 5 cm (O) and 90 × 1.6 cm ( ) column beds (180 ml) in 15 mM sodium phosphate, pH 7.8 buffer at room temperature to 20 – 25 % breakthrough. After washing to baseline (absorbance 280 nm) with 1 – 2 CV of running buffer, the bound protein was eluted with 1 M NaCl. Column beds were reconditioned with 4 M NaCl (2 CV), binding buffer (1 CV), 0.5 N NaOH (1 CV) and binding buffer (6 CV) at 10 cm/min.
Figure 44. Dynamic binding of BSA onto cellulose supports under optimal binding conditions at 10 cm/min. BSA (1 mg/ml) was loaded onto columns of 2.0 wt.%, 6.0 wt. % and 10 wt.% CL-DEAE cellulose supports at 10 cm/min to ~ 20 % BT according to standard procedures. The loading and binding buffer for all columns was 39 mM tris-phosphate, pH 8.6. Protein concentrations in the pools of the FT, wash and 1 M NaCl elution fractions were determined by absorbance at 280 nm. In all cases, the recovery of BSA was greater than 90 %.
Figure 45. Breakthrough loading of BSA onto 3.5 wt. % CL-DEAE cellulose and DEAE FF-Sepharose supports at 10 cm/min.
Figure 46. Breakthrough loading of BSA on 3.5 wt. % CL-Q cellulose and Q fast-flow sepharose supports at 10 cm/min.
Figure 47. hFib/BSA binding study on DEAE FF-Sepharose supports. hFib: BSA; 1:1 at 1.7 cm/min loading. Column: 15 × 5 cm; 9 cm/min. Pressure drop: 2.7 PSI. Binding buffer: 39 mM tris-phosphate, pH 8.6. BSA: ~ 1 mg/ml. hFib: ~ 1 mg/ml.
Figure 48. hFib/BSA binding study on 2 wt. % CL-DEAE cellulose supports. hFib: BSA; 1:1 at 1.7 cm/min loading. Column: 15 cm × 5 cm, 9 min/CV. Pressure drop: < 0.2 PSI. Binding buffer 39 mM tris-phosphate, pH 8.6. BSA: ~ 1 mg/ml. hFib: ~ 1 mg/ml.
Figure 49. hFib/BSA binding study on 2 wt. % CL-DEAE cellulose beads. hFib: BSA; 1:1 at 5 cm/min loading. Column: 90 cm × 1.6 cm. Binding buffer: 39 mM tris-phosphate, pH 8.6. BSA: ~ 1 mg/ml. hFib: ~ 1 mg/ml.
Figure 50. Analysis of 1:5 and 1:10 hFib/BSA chromatographic runs by SDS-PAGE. BSA standards and the eluents of the 1:5 and 1:10 hFib/BSA (FIB/ALB) runs on 2 wt. % CL-DEAE cellulose supports were analyzed by SDS-PAGE (4 – 12 % gradient gel). Lanes: (a) ALB (0.2 mg/ml); (b) ALB (0.5 mg/ml); (c) 1:5 feed; (d) 1:5 flowthrough/wash; (e) 1:5 NaCl eluent; (f) 1:10 feed; (g) 1:10 flowthrough/wash; (h) 1:10 NaCl eluent; (i) BSA (1 mg/ml); (j) BSA (2 mg/ml). Concentrations of hFib in the mixtures were determined by ELISA. Concentrations of BSA in the mixtures were determined by laser densitometry of the BSA bands versus BSA standards (e.g., lanes a, b, i and j). Note: the impurities in the central portion of the gels originate from the BSA.
Figure 51. Dynamic binding of hFib onto 2 wt. % CL-DEAE cellulose supports (0.5 cm/min loading).
Figure 52. Dynamic binding of hFib onto 2 wt. % CL-DEAE cellulose supports (10 cm/min wash and elution).