Figure 26. PAM affinity sorbent binding phenomena and impact on swine IgG binding efficiency. PAM ligands immobilized via EDAC-mediated chemistry. Comparison data from Table 7 (Fassina et al., 1996).
Figure 27. PAM affinity sorbent swine IgG static binding efficiency (mol IgG/mol PAM ligand).
Figure 28. PAM affinity sorbent swine IgG static binding efficiency (mol IgG/mol PAM recognition sequence).
Figure 29. Effect of PAM ligand orientation and local density on swine IgG binding efficiency (data from Table 8). PAM affinity sorbents were prepared using GAM-based, low-solids content (2.0 wt. %), large-particle diameter (average particle diameter: 500 µm) cellulose supports using the specified level of epichlorohydrin. PAM ligand (tetrameric structural form) recognition sequence: YTR. Classical one-step, bulk coupling of PAM ligand was performed through either amine-reactive, epoxy (random or nonoriented) coupling, or carboxyl-reactive, EDAC (oriented) coupling onto amine-terminated support. Volume-averaged PAM density for each sorbent is approximately 1.0 ± 0.15 mg/ml support. % static binding efficiencies (\(\eta_{eff}\)) are calculated assuming a 1:1, swine IgG:PAM ligand stoichiometric ratio.
Figure 30. Purification process of 7D7B10 mAb by PAM chromatography. PAM ligand density: 1.10 ± 0.15 mg PAM/ml support. Octameric branched-chain form was immobilized via the epoxy-GAM/TSC method using EDAC-mediated coupling chemistry onto low-solids content (3.0 wt. %), large-particle diameter (500 m) cellulose support. 8 – 12 % Silver-stained SDS-PAGE, nonreducing conditions (See Figure 31 for corresponding Western blot). Lane 1: 7D7B10 mAb standard reference (100 µg/ml); Lane 2: Hybridoma cell culture supernatant starting material; Lane 3: Column flowthrough fraction; Lane 4: 7D7B10 mAb product fraction (0.1 M Glycine/2 (v/v) % acetic acid, pH 2.0).
Figure 31. Purification process of 7D7B10 mAb by PAM chromatography. PAM ligand density: 1.10 ± 0.15 mg PAM/ml support. Octameric branched-chain form was immobilized via the epoxy-GAM/TSC method using EDAC-mediated coupling chemistry onto low-solids content (3.0 wt. %), large-particle diameter (500 m) cellulose support. Corresponding Western blot of 8 – 12 % SDS-PAGE (Figure 30), nonreducing conditions. Lane 1: 7D7B10 mAb standard reference (100 µg/ml); Lane 2: Hybridoma cell culture supernatant starting material; Lane 3: Column flowthrough fraction; Lane 4: 7D7B10 mAb product fraction (0.1 M Glycine/2 (v/v) % acetic acid, pH 2.0).