523b Functionalized Nanoporous Molecular Sieves for Chromatographic Separations of Proteins

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Protein, monoclonal antibody (MAb), and p-DNA purifications are of great importance in the evolving biopharmaceutical industry. The commercial production of these compounds as therapeutic drugs continues to be challenged by limitations in downstream purification techniques. Chromatography is an indispensable separation method for process-scale purifications of biomolecules. Overloaded chromatography is therefore the subject of intense basic research. Two issues of importance are the availability of highly selective adsorbents for the large variety of separations that are of commercial significance, and an incomplete understanding of the process of biomolecular adsorption/desorption.

We have investigated the potential of Mesoporous Molecular Sieve Silicates (MMSS) as chromatographic supports for size-selective and dual-mode biomolecular separations. The MCM class of MMSS materials was investigated in detail using lysozyme (LYS) and bovine serum albumin (BSA) as probe proteins. It has been shown that while very high adsorption capacities are observed (in the range of 500 mg protein/mg), the attainable pore-size range is limiting for protein separations, and the observed high capacity is due to significant specific external area on the adsorbent (Katiyar et al., 2005).

More recently, we have investigated the molecular sieve capability of the SBA class of MMSS materials in the globular protein size range. SBA adsorbents are distinguished by large mesopores, sharp pore-size distributions (molecular sieves), and high accessible surface areas (≈1000 m^2/g). SBA-15 adsorbents with pore sizes of 40 Å, 80Å, and 240Å were synthesized and used for equilibrium isotherm measurements. The procedures used to synthesize spherical supports will be described, and the importance of controlling both pore-size distribution and particle morphology will be illustrated. Our experiments show that LYS (14.3kDa; 19Å*25Å*43Å) adsorbs with very high capacity (≈275 mg/g) on the 80Å material, but was effectively excluded from the internal surface area of the 40Å material. The larger protein BSA (69kDa, 40Å*40Å*140Å) was also effectively excluded from the internal surface of the 40Å and adsorbed with high capacity on the 240Å material (≈400mg/g). These results illustrate the promising molecular sieve capability of the SBA-15 for protein separations.

The rate of adsorption of proteins on SBA-15 is of significant practical importance in protein chromatography. Rate studies with LYS and BSA will be presented to demonstrate very efficient protein uptake rates. TEM images of the synthesized materials reveal a very dense hexagonally close packed structure of uniform pores with minimal tortuosity. The very high protein capacities and rates of adsorption are attributed to this unique nanostructure. Confocal microscopy with fluorescently labeled proteins has been used to image the mass transfer of proteins in the supports.

Functionalization methods have been developed to activate SBA-15 molecular sieve adsorbents for Hydrophobic Interaction Chromatography (HIC). The effects of salts on protein capacity and rates of adsorption have been investigated on these novel size-selective HIC supports. The effects of solution conditions and surface ligands on protein binding have been determined with flow microcalorimetry, to better understand protein-adsorbent interactions.

References: Katiyar, A., L. Ji, P.G. Smirniotis and N.G. Pinto (2005), "Adsorption of Bovine Serum Albumin and Lysozyme on Siliceous MCM-41," Microporous & Mesoporous Matls., 80, 311-320.