Zirconia Publication Abstracts – Antibody Purification

47. A.M. Clausen and P.W. Carr, "Chromatographic Characterization of Phosphonate Analog EDTA-Modified Zirconia Support for Biochromatographic Applications," *Anal. Chem.* **70,** 378-85 (1998).

Abstract: Zirconium dioxide (zirconia) has a great affinity for inorganic and organic phosphate. Previous work from is laboratory demonstrated the utility of phosphate-modified microparticulate zirconia as a support for protein separations. We have extended this investigation to include the study of ethylenediamine-N,N'-tetramethylphosphonic acid (EDTPA), a phosphonate analog of EDTA, as a surface modifier for zirconia. Our work explores the use of EDTPA-modified zirconia (PEZ) for its potential use as a high-performance inorganic cation-exchange support for the separation of proteins. The phosphate groups in EDTPA very effectively block the sites responsible for strong interactions of hard Lewis bases with zirconia's surface. Modification of zirconia with EDTPA provides a "biocompatible" stationary phase, resulting in high mass recoveries of proteins. We compare PEZ with inorganic phosphate-modified zirconia to show increased efficiency, as well as unique selectivities for chromatography of proteins on the chelator-modified surface. Finally, the selectivity, efficiency, and separation mechanism are reported. The studies show the PEZ is a useful high-performance ion-exchange support for the separation of cationic proteins and for modulating the sites responsible for the high affinity of zirconia toward certain classes of anions.

52. A.M. Clausen, A. Subramanian, and P.W. Carr, "Purification of Monoclonal Antibodies from Cell Culture Supernatants Using a Modified Zirconia Based Cation-Exchange Support," *J. Chromatogr. A* **831,** 63-72 (1999).

Abstract: A method suitable for the isolation of monoclonal antibodies (Mabs) is described. The protocol utilizes a zirconia based column modified with ethylenediamine-*N,N'*-tetra(methylenephosphonic) acid to create a novel cation-exchange chromatographic support. Initial experiments using a linear salt gradient demonstrate the ability of this support to efficiently separate Mab from transferrin and bovine serum albumin in a model matrix. Results of the purification of Mab from an actual cell culture supernatant over a range in protein concentrations are also shown. Analyses by enzyme-linked immunosorbent assay and gel electrophoresis demonstrate that Mabs can be recovered from a cell culture supernatant at high yield (92-98%) and high purity (>95%) in a single chromatographic step.

57. A. Subramanian, P.W. Carr, C.V. McNeff, "Use of Spray-Dried Zirconia Microspheres in the Separation of Immunoglobulins from Cell Culture Supernatant*," Journal of Chromatography*, **890(1)**, 15-23, 2000.

Abstract: A method suitable for the isolation of monoclonal antibodies (Mabs) on novel zirconia microspheres (20-30 micron) is described. Zirconia microspheres were generated by spray drying colloidal zirconia. Spray-dried zirconia microspheres were further classified and characterized by X-ray diffraction, BET porosimetry and scanning electron microscopy. Spray-dried zirconia microspheres were modified with ethylenediamine-N,N'-tetra(methylenephosphonic) acid (EDTPA) to create a cation-exchange chromatographic support. The chromatographic behavior of a semi-preparative column packed with EDTPA-modified zirconia microspheres was evaluated and implications for scale-up are provided. EDTPA-modified zirconia microspheres were further used to purify Mabs from cell culture supernatant. Analysis by enzyme linked immunosorbent assay and gel electrophoresis demonstrate that Mabs can be: recovered from a cell culture supernatant at high yield (92-98%) and high purity (>95%) in a single chromatographic step.

73. Anuradha Subramanian and Sabyaschi Sarkar "Use of a Modified Zirconia Support in the Separation of Immunoproteins." *Journal of Chromatography A*, **944**, 179-187 (2002).

Abstract: Zirconia beads (25–38 μm in diameter) were modified with *N,N,N',N'*-ethylenediaminetetramethylenephosphonic acid to generate a zirconia based pseudoaffinity support, further

referred to as r_PEZ. The influence of pH, salt concentration and temperature on the binding of human immunoglobulin G (hIgG) to r_PEZ was studied. Temperature had no significant impact on the maximum binding capacity (Q_{max}), and the equilibrium-binding constant (K_d), whereas pH and the salt concentration had a noticeable impact on both Q_{max} and K_d. The Q_{max} value of 55 mg hIgG/ml of bead was obtained at a pH of 5.5 and found to decrease with an increase of pH. The modified zirconia support allowed the separation of immunoglobulins (IgG, IgA and IgM) from untreated human serum. Elution was possible under mild conditions with a step salt gradient. Overall protein recoveries in the range of 109–125% were obtained with human serum. Human IgG, human IgA, and human IgM yields of 29.50±6.3, 3.22±0.7, and 6.84±0.7%, respectively, were obtained at a linear velocity of 4.32 cm/min. Purity of products, obtained from a single chromatographic step was estimated to be greater than 89.0±2.6%. The utility of r_PEZ in the selective removal of immunoglobulins, as in immunoadsorption was discussed.