Waters
Lab Highlights

An Internal Communication
of Applications and Techniques

LAH 0297 2/86
AN,SP/LS/MD/PR/EZ

NO. 172 EVALUATION OF A NEW CATION EXCHANGE MEDIA FOR THE ISOLATION AND PURIFICATION OF PROTEINS, ENZYMES AND IMMUNOGLOBULINS

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Over the past several years, ion exchange chromatography has played an important role in the isolation and purification of biological molecules (1,2). Previously, much of the work in this area involved slow, laborious fractionation and electrophoretic methods, or traditional soft-gel chromatography (3,4). Recently, however, Waters has introduced an ion exchange media, called ACCELL™ that addresses the requirements of the biochemist in terms of capacity, recovery, speed, resolution, and ease of use. ACCELL™ is a unique, rigid, preparative ion exchange media available in both bulk and pre-packed cartridges. ACCELL™ media does not shrink or swell like conventional gels and provides high resolution with improved reproducibility. Its rigid structure permits linear scale-up at all reasonable flow rates and pressures, from analytical to all preparative scales. Available chemistries are QMA (quaternary methylamine) anion, and CM (carboxymethyl) cation exchangers, both having a 40μ particle size and 500Å pore size. The porous silica base gives the media high available capacity for large biopolymers while the rigid structure allows high flow rates without the compression typically associated with conventional soft-gel media.

We will present detailed information on the physical and chromatographic characteristics of the cation form of this new ion exchange media, ACCELL™ CM. This will include mass recovery, recovery of biological activity, flow properties, chemical compatibility, and methods development, including optimization of buffer type, pH, and ionic strength.

In addition, we will report applications that have been developed in our laboratories utilizing this media to accomplish the isolation and purification of several enzymes and proteins of current biological interest.

In one such application, the enzyme aldolase (molecular weight 150,000, PI=6.1) was isolated from rabbit skeletal muscle using ACCELL™ medium. In a single pass through the ACCELL™ medium, a ten-fold increase in purification was obtained, with recovery of biological activity greater than 95 percent. The crude protein extract was prepared by homogenization of rabbit muscle tissue in an equal volume of cold Buffer A mobile phase (50 mM NaH_2PO_4, pH 6.0), centrifugation, and filtration of the supernatant through a Millex® HV filter. Purity of the fractions collected were evaluated using such additional methods as electrophoresis, gel filtration chromatography, and re-chromatography on analytical cation exchange and reverse phase material. Peak homogeneity was also evaluated by determining the amino acid composition of each fraction collected across the peak using a PICO·TAG™ system. Total time for the sample workup through final purification using the ACCELL™ medium is less than two hours, as compared to several hours or even days by previously reported techniques using ammonium sulphate precipitation and/or soft-gel chromatographic techniques (5,6). This data will be presented, in its entirety, along with additional applications and specific details of methods development and scale-up strategies for isolation of larger quantities of enzymes and proteins of this type.

Use of this media in open columns, existing analytical liquid chromatographic (LC) systems, and preparative LC systems will also be demonstrated.

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