Waters
Lab Highlights

An Internal Communication
of Applications and Techniques

LAH 0291 1/86
SP/LS/ED/0T/0T

COLUMN PACKING CONSIDERATIONS USING ACCELL™ ION EXCHANGE MEDIA

Accell™ QMA and CM preparative ion exchange packings are available as bulk packings, allowing the flexibility of packing various columns of assorted hardware and dimensions. Applications involving the use of this packing in both glass and stainless steel columns, open columns, and standard HPLC instrument configurations, as well as SEP-PAK® Cartridges, have been reported in-house since the introduction of this material in the Spring of 1985. The large 40µ particle size and rigid silica base of the material are significant contributions to the packing's versatility that people familiar with conventional gels may not recognize.

Accell™ media can be dry or slurry packed, either at atmospheric pressure, or under elevated pressures using a standard HPLC packing apparatus. Columns can be packed quickly and efficiently since many considerations such as allowing the material to swell according to the ionic strength of the eluents, removal of fines, settling time, etc. , are not an issue with this packing.

Column size is usually dictated by the particular instrument application, whether open column or HPLC, and by the amount of sample, in terms of total protein concentration, that is to be loaded onto the column in any given injection. Depending on the resolution, purity, etc., desired, it is advised to maintain the loading of the column anywhere from one to ten percent of maximum binding capacity. Maximum binding capacity is in the area of 70 and 120 mg of protein per gram of packing material for QMA and CM, respectively, so that the amount of packing for a particular application can be easily determined. Some applications, however, may range in loading up to 100%, depending on the goals of the particular separation. However, what usually occurs is the reverse; that is, once a column is packed, the amount of packing material is determined, and hence the load. The amount of packing material in grams necessary to pack a column is equal to one half its volume in milliliters (D = .5g/ml). Therefore, once the column dimensions are selected, it is an easy matter to calculate how much material to use regardless of packing technique, and hence the appropriate load. As with any HPLC technique, one can always perform multiple injections in order to purify even more sample, as fast run times and column equilibration are characteristic of Accell™ separations.

While columns of Accell™ media can be packed dry, we have had more success in our laboratory with slurry packing in terms of column efficiency, time, and ease of use in low- to medium-pressure applications. Typical procedure calls for preparing a slurry in a small Erlenmeyer flask by swirling the calculated amount of material (plus a small excess) in two to three volumes of the low salt eluent. The resulting slurry can be degasssed at this point, by applying a vacuum if necessary, and simply poured into the column. Although the packing will settle quite rapidly, it can be accellerated (no pun intended) by pulling a vacuum at the column outlet with a 10cc priming syringe. Once the slurry has settled to the desired bed height, the syringe is disconnected, and the column inlet connections, such as a flow adapter, plunger, or other type of end frit arrangement, are made and the column is ready for equilibration. At this point, 5-10 column volumes of high salt buffer is introduced to condition the column, and once the column is re-equilibrated back to the low salt buffer, it is ready to use.

One of the major advantages of the packing material is its ease of use, and if you keep in mind a few simple parameters, such as density and binding capacity among others, you can indeed accellerate your methods development.

MILLIPORE
Waters Chromatography Division 34 Maple St. / Milford, MA 01757 / 617-478-2000

Michael Swartz