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USE OF ACCELL™ SEP-PAK® CARTRIDGES FOR PROTEIN BINDING CAPACITY AND MASS RECOVERY DETERMINATIONS

Accell™ media has recently become available in the form of SEP-PAK® cartridges for methods development and sample preparation. Two ways in which the cartridges can be used as part of a methods development process are in the evaluation of protein binding capacity and mass recovery. These two parameters are often investigated for maximum loading and efficiency, after the initial methods development stages of mobile phase optimization with respect to pH and ionic strength. Optimum chromatographic conditions in terms of resolution are not necessarily the optimum conditions for maximum binding and mass recovery and, therefore, these parameters should be investigated during the course of any methods development scheme. Sometimes re-optimization of the mobile phase is then necessary to achieve the best compromise.

The binding capacity of the packing is dependent on the ionic strength and pH of the moible phase, as well as the particular protein of interest and the matrix from which it is to be isolated. It is important to determine the maximum (100%) binding capacity as this will determine how much can be loaded onto the column in any one injection, for the desired recovery and purification. The cartridges provide a convenient means of evaluating binding capacity, as they can be used quickly and easily with small amounts of sample.

A specific example of how the SEP-PAK® cartridge can be used in an application for determining binding capacity was recently reported (1). Table I shows the maximum binding capacity, in terms of mg of protein per gram of QMA, as determined using a QMA cartridge for the particular sample matrix and mobile phase conditions and then a scaling of this figure from 1 to 10% of the value for optimum chromatographic efficiency. This particular method employed the "breakthrough" technique to determine the maximum binding capacity.

TABLE I

System	Column	Grams QMA	(ml) Column Volume	% of Total Binding Capacity	Loading,mg Protein/g QMA	(mg) Actual Loading
Model	SEP-PAK Cart.	0.35	0.70	100	49.0	17.2
Meth Dev.	9mm X 16cm glass	5.1	10	1	0.49	2.5
Prep	25mm X 16cm glass	39.3	80	8	3.9	153

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If the sample is totally retained it is a simple matter to load the cartridge in chromatographically-optimized low salt buffer until a "breakthrough" is seen as measured by absorbance, with the detector either on or off-line. The number of milligrams of protein retained by the packing is determined from the volume and concentration. In turn, the binding capacity, in terms of milligrams of protein per gram of packing (SEP-PAK® cartridge mass = 0.35g ± 10%) can also be calculated. If undesired components of the sample are unretained under the particular conditions employed, it becomes necessary to monitor the protein concentrations in the eluent versus the volume of sample loaded. The procedure would call for loading the sample onto the cartridge in the low salt buffer, and eluting the protein with a high salt buffer. A plot of the total number of milligrams loaded versus milligrams of protein bound per gram of packing (determined according to previously outlined calculations using volume, concentration, and cartridge mass) will plateau at maximum binding capacity.

Protein mass recovery is another important parameter which can be determined quite easily using a SEP-PAK® cartridge method. In this method, a known amount of a standard protein or sample matrix is loaded onto a cartridge in the low salt buffer and eluted from the cartridge with a high salt buffer. At this point, the concentration is redetermined, and, when compared with the starting concentrations, a figure for percent mass recovery is obtained.

Both protein binding capacity and mass recovery play an important role in the isolation and purification of the biopolymer of interest, and the ease of use of the new Accell™ SEP-PAK® cartridge has made the determination of these two parameters routine in the Accell™ methods development strategies used in our laboratory.

1. Phillips, D. J. and Tomany, M. J., "SEP-PAK® Cartridges as Model Systems for Preparative Chromatography of Proetins," Presentation #929 at The Fifth International Symposium on HPLC of Proteins, Peptides and Polynucleotides, November 4-6, Toronto, Ontario, Canada.