The high-resolution, high-efficiency ProPac™ ion-exchange columns are ideally suited to the characterization and quality control assessment of closely related protein variants. The rigid, nonporous pellicular resin provides exceptionally high resolving power, permitting the separation of proteins that differ by as little as one charged residue. In this brief application, we will examine the chromatography of a humanized monoclonal antibody.

High-resolution ion-exchange chromatography can be used to separate and identify protein variants that include glycosylated (1), phosphorylated (2), deamidated (3) and oxidized (4) forms of proteins (5). Processing of C-terminal lysine and arginine residues of proteins isolated from mammalian cell cultures was described by Harris in 1995 (6). It has been observed that C-terminal lysine or arginine residues, which could have been expected based on gene sequence information, are often absent in proteins isolated from mammalian cell culture as a result of processing. If this processing is incomplete, charge heterogeneity can result; the different charge variant forms can be resolved using cation-exchange chromatography.

In this study, the ProPac WCX-10 column was used to resolve the variants of a humanized monoclonal antibody, IgG1, having lysine residue truncations at the C-terminal of the heavy chains. The same MAb was digested by immobilized papain in the presence of cysteine and the Fab and Fc fragments separated using a protein A column. These fragments were then analyzed using the ProPac WCX-10 column. This cation-exchange column also resolves the components in the Fab and Fc fractions.

**Experimental Conditions**

Dionex BioLC™ consisting of a GS50 gradient pump, an AS50 autosampler, and a PD A-100 photodiode array detector, controlled via the Dionex PeakNet® 6 chromatography workstation.

**Results**

Figure 1 shows the resolution of three distinct peaks during the analysis of the MAb using a 2 mm × 250 mm ProPac WCX-10 column. This profile is typical of that observed as a result of truncations of the C-terminal lysine residues due to partial processing in the cell culture. Figure 2 shows the analysis of the protein A purified fractions of the digested MAb. It is seen that the three principal peaks observed for the MAb are also associated with the Fc fragment. Treatment of either the intact MAb or the Fc fragment with the aminopeptidase carboxypeptidase B will collapse the three peaks down to a single peak. Additional peaks are also seen on both the Fab and Fc. On the Fc separation there is an additional pair of peaks, which appear to be associated with the three principal components. Additional work is required to further identify these additional peaks.

**Conclusions**

The ProPac WCX-10 permits the high-resolution separation of MAb charge variants. These columns provide a simple, convenient, and rapid means to preparatively isolate fractions for further analysis. These columns are available in dimensions from 4 mm × 50 mm, which enable fast chromatography at high flow rates, to 4 mm × 250 mm for routine high-resolution analytical separations, to 9 mm × 250 mm and 22 mm × 250 mm columns for direct scale-up to tens of milligram amounts of protein loaded.

**References**