Waters
Lab Highlights
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PROTEIN LOADING CAPACITY STUDIES ON DEAE (ANION EXCHANGE) LC COLUMNS

Protein loading capacity of chromatographic supports was established by two methods. In the first technique, increasing loads (0.66 - 42.4 mg) of ovalbumin (OVA) were chromatographed and the resolution between the various components of OVA noted. The second technique consisted of injecting mixtures of OVA and bovine serum albumin (BSA) at different concentrations and calculating the resolution factor (R_S) between the major components of these proteins. OVA and BSA were chosen as models because of their stability and low cost. In addition, "the distribution of components throughout the elution gradient made them a sensitive indicator of both resolution and column loading capacity." (1)

The results of the first loading studies employing OVA are shown in Figure 1. Although the resolution between all components of OVA was lost at a load of 42.4 mg, almost complete resolution of a minor peak ($R_t \approx 10$ min) of OVA which eluted just before the major OVA component could be achieved at loads as high as 21.2 mg protein. Furthermore, a second component of OVA ($R_t \approx 14$ min) could also be partially separated at a load of 21.2 mg.

The results of the second protein loading studies are shown in Table 1. These data suggest that an R_S value of 0.9 between OVA and BSA could be achieved at a load of 2-3 mg protein.

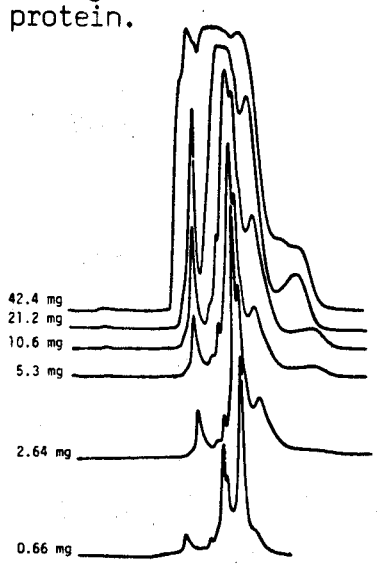

FIGURE 1: Loading capacity effect on the resolution of ovalbumin. Column: Protein-PAK DEAE-5PW 7.5 cm X 7.5 mm; id; Flow rate: 1 ml/min; Gradient: Solvent A: 0.02M Tris acetate, pH 8.0; Solvent B: 0.02M Tris acetate/0.5M NaCl, pH 8.0; 0% - 100%, Curve 6, 25 min; Detector: 441, Absorbance 280 nm.

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

TABLE 1

R_S VALUES OF OVA-BSA CHROMATOGRAPHED ON DEAE COLUMN WITH INCREASING LOADS

MILLIGRAM AMOUNTS	R_S BSA/OVA
0.66	1.9
1.32	1.6
2.30	0.9
4.60	0.5

See Figure 1 for chromatographic conditions.

1. G. Vanecek and F. E. Regnier, Variables in High Performance Anion Exchange Chromatography of Proteins, Anal. Biochem., 109 (1980) 345-353.