Optimizing SEC

SAMPLE LOAD

In SEC, sample load on the column is limited due to the absense of a staionary phase that participates in the retention process. High sample loads distort peak shapes and cause an overall decrease in efficiency due to column overload.

Optimal sample load highly depends on the sample properties (sample matrix) and the separation task. For analytical columns, sample concentrations of 1-20 mg/mL are recommended. Proteins can be loaded at higher concentrations and higher total loads than synthetic macromolecules. For preparative purposes for example, 100 mg of BSA can be loaded on two 21.5 mmID x 60 cm L TSKgel G3000SW columns, but only 20 mg of PEG 7500.

Sample volume depends very much on the type of column. On TSKgel SuperSW columns for example, a 5 µL injection volume ensures optimal results. Standard injection volumes for 7.5 and 7.8 mmID columns are 20-100 µL, whereas for preparative purposes on 21.5mmID columns, injection volumes may be raised up to 2 mL.

MOBILE PHASE

Proper selection of the mobile phase is necessary to maximize molecular sieving mechanism and to minimize secondary effects such as ionic and hydrophobic interaction between the sample and the column packing material. For each sample there will be an optimum buffer type and concentration that results in the highest resolution and recovery.

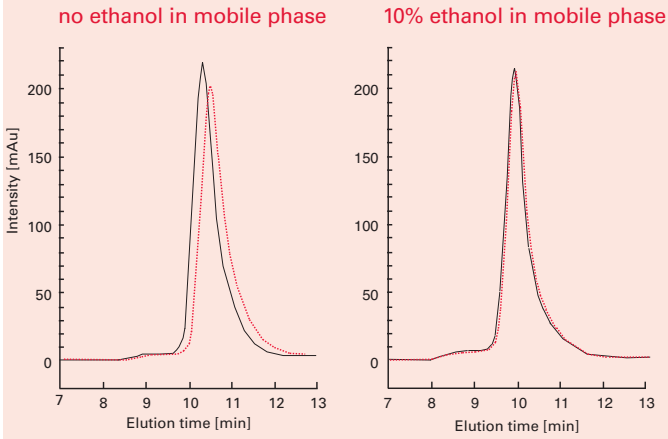
For TSKgel SW columns' mobile phases a buffer concentration between 0.1 M and 0.5 M is recommended. Under low ionic strength (< 0.1 M), ionic interactions between the sample molecules and the silica surface may occur. Under conditions of high ionic strength (>1.0 M), hydrophobic interactions are more likely to occur. A neutral salt, such as sodium sulphate may be added to the buffer to increase buffer ionic strength. Also the ionic species of the buffer has an effect on the separation. As a good starting point, a 0.1 M sodium phosphate buffer together with 0.1 M sodium sulphate has proved to be of value.

As the polymeric TSKgel PW and Alpha-type resins carry less residual charged groups on the surface than silica gels, salt concentration of the mobile phase can be lower. Non-ionic, non-polar compounds such as polyethylene glycols can simply be analysed with distilled water. For ionic polymeric compounds, a neutral salt such as sodium nitrate is added to the aqueous eluent. Generally, a concentration of 0.1 M to 0.2 M is sufficient to overcome undesirable ionic interactions.

If hydrophobic interaction occurs between the sample and the column matrix, a water soluble organic solvent can be added to the mobile phase. The addition of acetonitrile, acetone, ethanol or methanol up to a concentration of 20% may also prevent columns from fouling by suppressing interaction of hydrophobic impurities of the sample. An example is shown in Figure 20 with the analysis of a pegylated protein on a TSKgel G3000SW$_{XL}$ column. As pegylated products are more hydrophobic, they tend to interact with the column matrix. Over time enough of the pegylated product can foul the column, which is indicated by shifts of retention time and decreasing separation performance. By adding 10% of ethanol to the elution buffer, this problem is overcome. Figure 20 shows no differences in performance at the first and the 150th injection. (courtesy of J.J. Ratto et al. Amgen Inc., 1996)

Figure 20

SEC of pegylated proteins



Column:	G3000SW$_{XL}$, 5 µm, 7.8 mm ID x 30 cm L
Sample:	10 mL of PEG-r-HuMGDF
	——— initial Injection
	····· after 150 injections
Mobile phase:	0.1 M sodium phosphate, pH 6.9, with 0.5 M NaCl
Flow rate:	0.7 mL/min
Detection:	UV @ 215 nm

COLUMN PROTECTION

To protect the column and increase its lifetime, the use of a guard column is strongly recommended. An example for the influence of the guard column on column lifetime is depicted on page 6 in Figure 7.

Sample purity, sample load and the composition of the mobile phase have an influence on column lifetime as already demonstrated in Figure 20.

For additional information on TSKgel Size Exclusion columns for GFC, please consult the Tosoh Bioscience Laboratory Products Catalog.