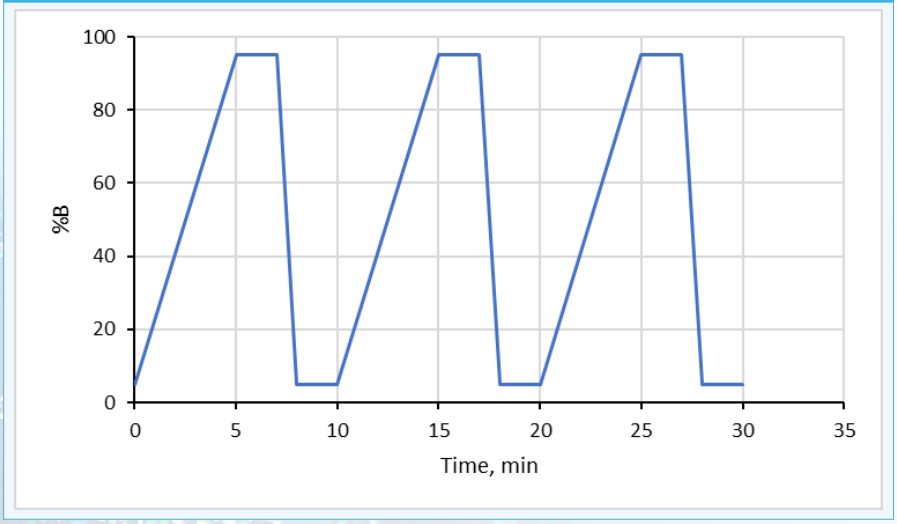
Column Conditioning for Protein & Peptide Analysis

For those new to working with peptides and proteins, it can be surprising to learn that these types of analytes are known to adsorb to metal surfaces under separation conditions that use low pH and low ionic strength mobile phase (0.1% formic acid). This sort of mobile phase is commonly used for LC-MS analyses. Charged sites on peptides and proteins may interact with metal surfaces in the column (frit/column body), instrument hardware, or in connecting materials. The resulting adsorption can cause low signal and/or reduced sample recovery when a new column is initially used. In extreme cases, no signal may be observed, even after multiple injections of the peptide or protein sample are completed.

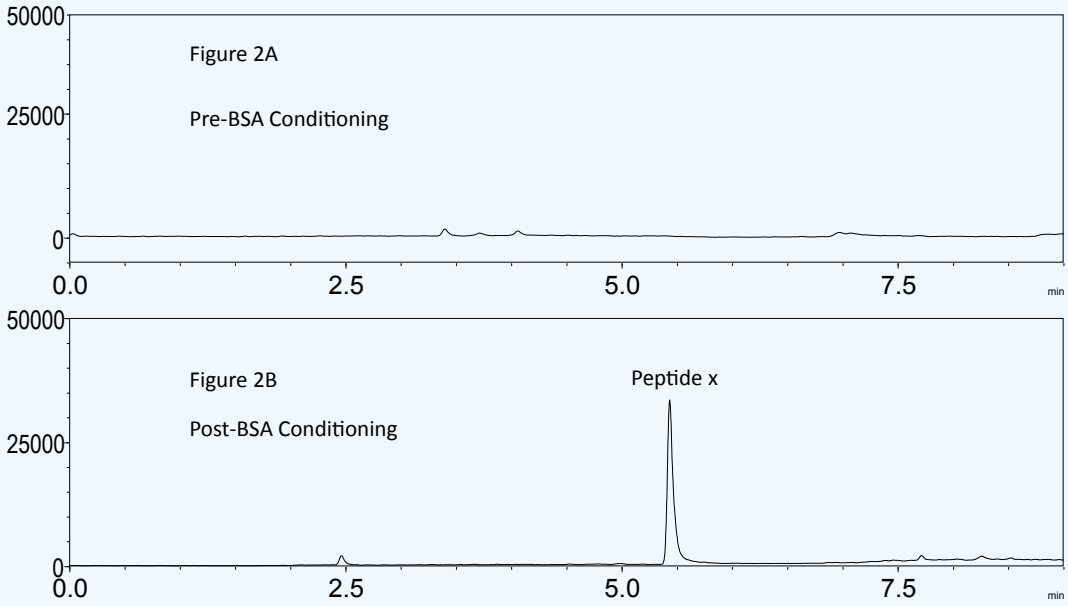
A common method to overcome this adsorption is to condition the new column with *either* your target peptide or protein or bovine serum albumin (BSA). According to Powell and Timperman (1), "conditioning improves column performance by irreversibly binding to sites of specific adsorption, and blocking these sites from future nonideal interactions." A conditioning protocol that has been successfully employed uses injection of 20 µL of a 20 mg/mL BSA solution. The solution of BSA can be prepared using lyophilized material purchased from Sigma-Aldrich (B6917) and dissolved in water/0.1% formic acid. Run the BSA sample using a sawtooth gradient (5-95% organic in 5 minutes repeated 3 times) as shown in Figure 1. Five replicate injections should be made to condition the column.

Fig. 1 Sawtooth gradient example showing 5-95% B in 5 minutes repeated three times.



Once this procedure is complete, the injections of the peptide/protein of interest will show the expected response and analysis can proceed as usual. Figure 2 shows an example comparing the results before and after BSA conditioning.

Figure 2A. TIC shows no signal for peptide x pre-BSA conditioning. Figure 2B. TIC shows signal for peptide x post-BSA conditioning.



Before BSA conditioning, there is no visible signal present for peptide x when it is run under standard conditions with a gradient of water/ACN/0.1% formic acid. After BSA conditioning following the gradient shown in Figure 1, the signal for peptide x is visible (Figure 2B).

Another example of the impact of BSA conditioning is shown in Figure 3. In this case, the response for trastuzumab was improved in terms of the peak area, including the minor components that elute after the main peak. For this conditioning, one 20 µL injection of a 20 mg/mL BSA solution in water/0.1% formic acid was employed.

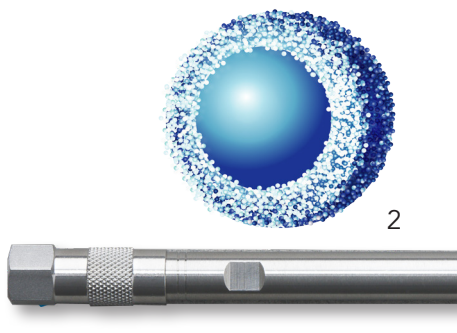
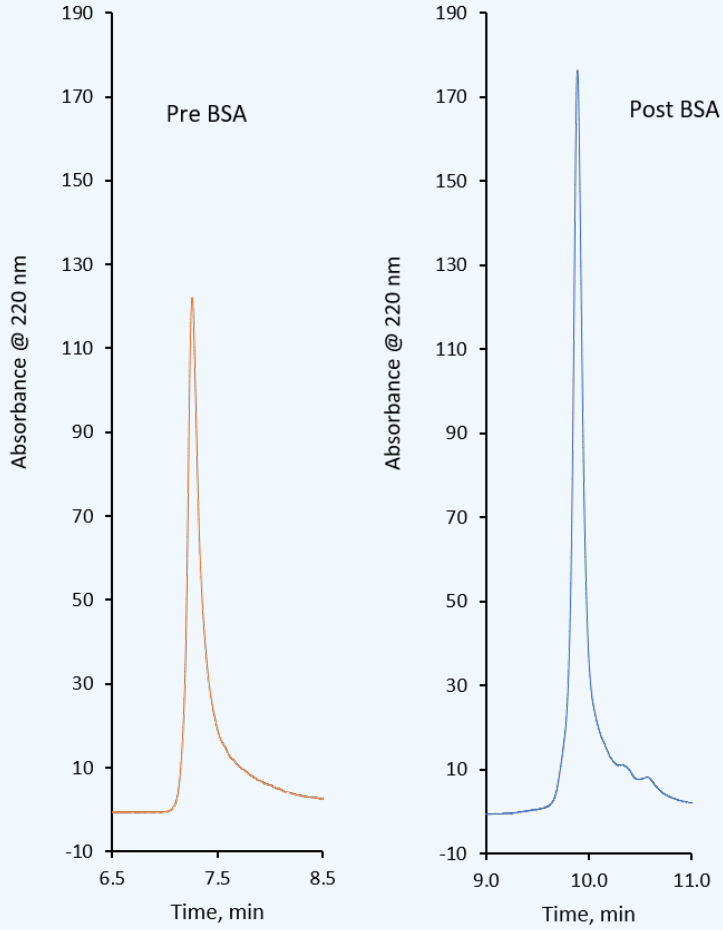


Figure 3. Left chromatogram shows reduced signal pre-BSA conditioning while right chromatogram shows increased signal and the presence of minor components post-BSA conditioning.



CONCLUSIONS

When working with peptides and proteins, it is good practice to be aware of the potential for these analytes to adsorb to metal surfaces and be prepared to condition the column if the expected response is not obtained. The conditioning steps are simple to perform and will yield the expected chromatographic results.

REFERENCE

1. Powell, M. J. and Timperman, A. T. (2004) Proteome Analysis. In Aguilar, M-I. (editor) *HPLC of Peptides and Proteins Methods and Protocols*. Humana Press, 387-400.