HPLC DATA SHEET

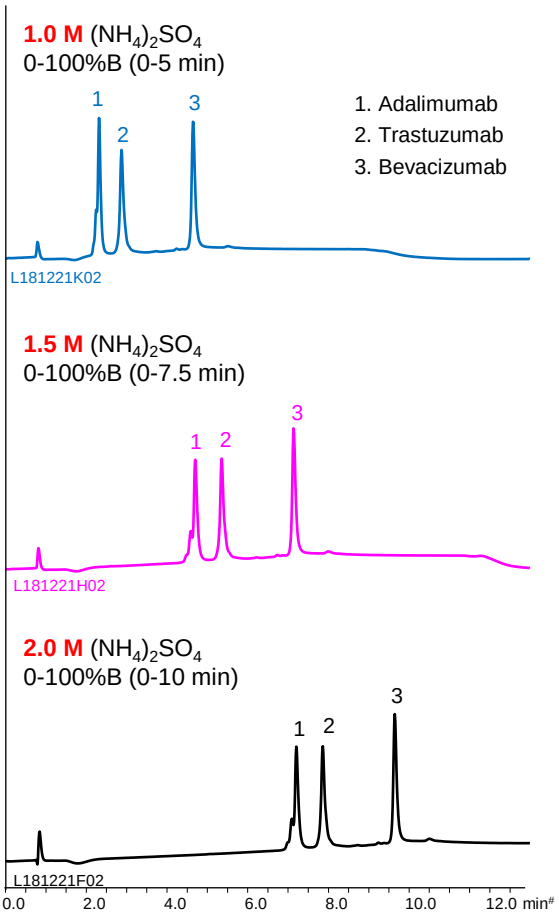
The factor affecting separation of hydrophobic interaction chromatography

U181127AE

Hydrophobic interaction chromatography (HIC) is a technique used to separate proteins such as antibodies by hydrophobic interactions between proteins and stationary phase. The mobile phase is typically an aqueous buffer with high concentration. Proteins are adsorbed to the stationary phase at high concentration of salt, and elute in the order of increasing hydrophobicity by decreasing the salt concentration. Unlike reversed-phase, proteins can be separated without any denaturation, thereby maintaining its activity.
In this report, we introduce the factor affecting separation of HIC.

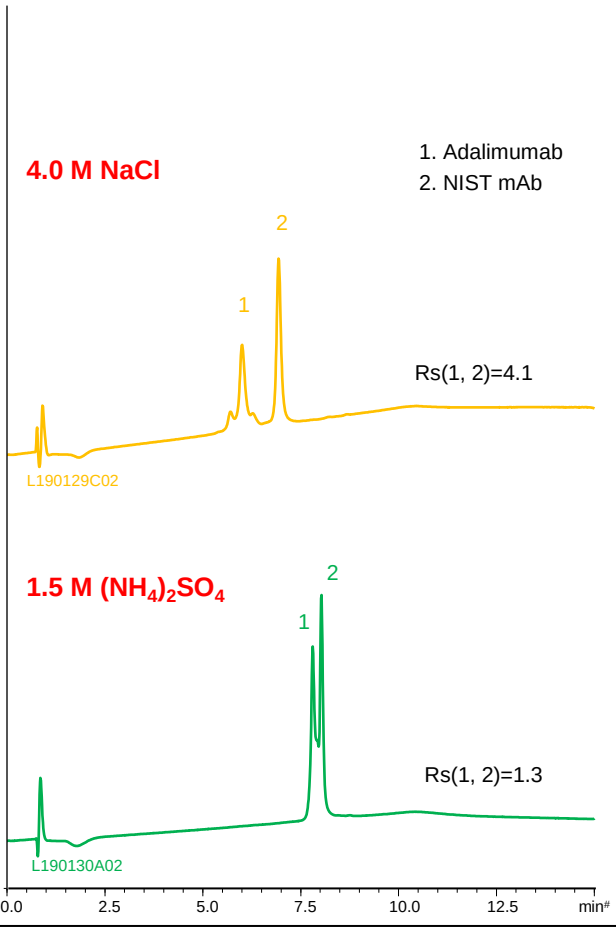
The effects of initial salt concentration and type of salt

≪The effects of initial salt concentration≫



Column	: BioPro HIC BF 4 µm, 100 X 4.6 mmI.D.
Eluent	: A) 100 mM NaH_2PO_4-Na_2HPO_4 (pH 7.0) containing salt B) 100 mM NaH_2PO_4-Na_2HPO_4 (pH 7.0) 0.2 M/min (The gradient slope is same.)
Flow rate	: 1.0 mL/min
Temperature	: 25℃
Detection	: UV at 280 nm
Injection	: 5 µL (0.5 mg/mL)

≪The effects of type of salt≫



Column	: BioPro HIC BF 4 µm, 100 X 4.6 mmI.D.
Eluent	: A) 100 mM NaH_2PO_4-Na_2HPO_4 (pH 7.0) containing salt B) 100 mM NaH_2PO_4-Na_2HPO_4 (pH 7.0) 0-100%B (0-10 min), 100%B (10-15 min)
Flow rate	: 1.0 mL/min
Temperature	: 25℃
Detection	: UV at 280 nm
Injection	: 10 µL (0.25 mg/mL)

The buffer containing $(NH_4)_2SO_4$ is often used as mobile phase of HIC because $(NH_4)_2SO_4$ has strong salting-out effect. The higher the concentration of initial $(NH_4)_2SO_4$, the stronger retention of proteins, so a buffer with high salt concentration is effective for separation of the low hydrophobic proteins with weak retention.

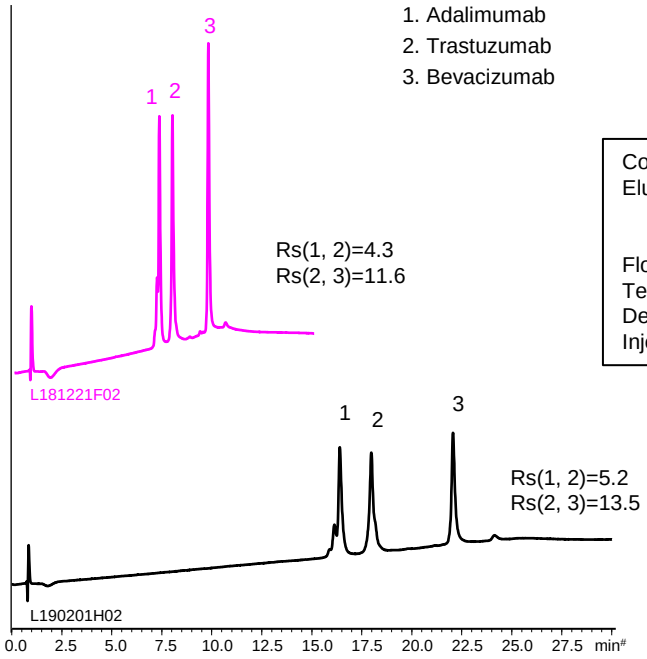
NaCl and CH_3COONH_4 are also used as salts. The separation selectivity vary with the type of salt in some cases (see chromatograms above), so changing the type of salt is also effective when the separation is poor. However, these salts are used very high concentration to gain retention comparable to $(NH_4)_2SO_4$. It is need attention that precipitation of salts in the buffer and damage of LC system.

YMC's column for hydrophobic interaction chromatography, BioPro HIC BF, is designed to high hydrophobicity of stationary phase. Therefore, it enables to analyze low hydrophobic proteins that can't be retained using other commercial columns even in lower salt concentration buffer or low salting-out effect salts buffer.

The effects of gradient slope

0.2 M/min

0-100%B (0-10 min),
100%B (10-15 min)



0.08 M/min

0-100%B (0-25 min),
100%B (25-37.5 min)

Column	: BioPro HIC BF 4 μm, 100 X 4.6 mmI.D.
Eluent	: A) 100 mM NaH_2PO_4-Na_2HPO_4 (pH 7.0) containing 2.0 M $(NH_4)_2SO_4$
	B) 100 mM NaH_2PO_4-Na_2HPO_4 (pH 7.0)
Flow rate	: 1.0 mL/min
Temperature	: 25℃
Detection	: UV at 280 nm
Injection	: 10 μL (0.5 mg/mL)

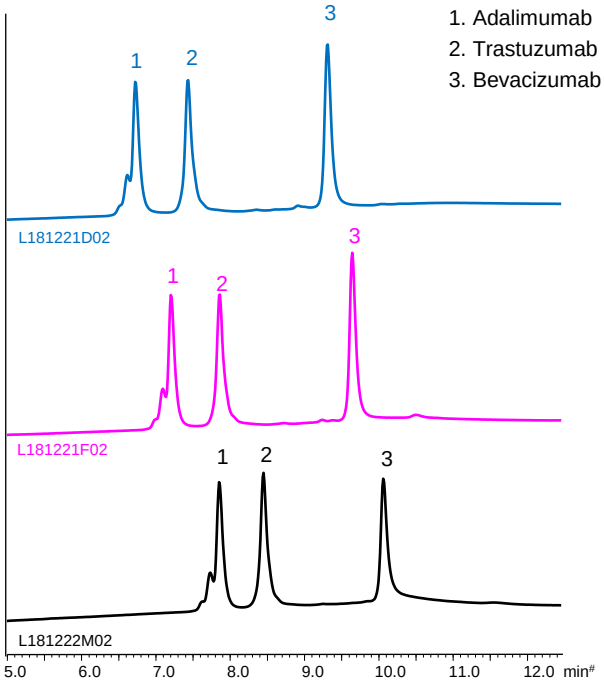
Using more shallow gradient make improve separation.

The effects of temperature

15℃

25℃

40℃



Column	: BioPro HIC BF 4 μm, 100 X 4.6 mmI.D.
Eluent	: A) 100 mM NaH_2PO_4-Na_2HPO_4 (pH 7.0) containing 2.0 M $(NH_4)_2SO_4$
	B) 100 mM NaH_2PO_4-Na_2HPO_4 (pH 7.0)
	0-100%B (0-10 min), 100%B (10-15 min)
Flow rate	: 1.0 mL/min
Detection	: UV at 280 nm
Injection	: 5 μL (0.5 mg/mL)

In HIC mode, the higher temperature, the longer retention time of proteins. It assume that the hydrophobic area interacted with stationary phase become large by changing the structure of proteins with temperature increased, so the hydrophobic interaction become strong.