

Aspen Chromatography

Hydrophobic Interaction Chromatography (HIC)

Bioprocess Laboratory

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Introduction (HIC experimental data)

Column: HiTrap Phenyl HP, 1 ml

Samples: 1) Monoclonal antibody anti-lac cl 507, 8.8 mg/ml, 100 μ l
 2) Monoclonal antibody anti-trn cl 739, 1.0 mg/ml, 500 μ l
 3) Monoclonal antibody anti-TSH cl 79, 6.7 mg/ml, 100 μ l
 (All three monoclonal antibodies are pure and a kind gift from Pharmacia Diagnostics AB, Uppsala, Sweden)

Sample pretreatment: Dilution 1:1 (v/v) with start buffer

Start buffer: 50 mM NaH₂PO₄, 1.0 M (NH₄)₂SO₄, pH 7.0

Elution buffer: 50 mM NaH₂PO₄, pH 7.0

Gradient: Linear from 100% start buffer to 0% start buffer in 15 ml (15 x V_T)

Flow rate: 1.0 ml/min (156 cm/h) at room temperature

Table B2.Specify

	Value	Spec	Units	Description
Flowrate	1.0	Fixed	ml/min	Feed flowrate
Component_Concentration(*)				
Component_Concentration("A")	8.8e-005	Fixed	eq/l	Component concentration
Component_Concentration("B")	5.e-005	Fixed	eq/l	Component concentration
Component_Concentration("C")	6.5e-005	Fixed	eq/l	Component concentration
Component_Concentration("K")	1.0	Fixed	M	Component concentration
Pressure	3.0	Fixed	bar	Feed pressure

Simulation conditions

	Value	Spec	Units	Description
Hb	2.5	Fixed	cm	Height of adsorbent layer
Db	0.7	Fixed	cm	Internal diameter of adsor
Ei	0.35	Fixed	m ³ void/m ³ bed	Inter-particle voidage
Ep	0.0	Fixed	m ³ void/m ³ bed	Intra-particle voidage

Column dimensions 0.7 x 2.5 cm (1 ml), 1.6 x 2.5 cm (5 ml)

Column volumes 1 ml and 5 ml

Rec. flow rate 1.0 ml/min (1 ml), 5.0 ml/min (5 ml)

Max. flow rate* 4.0 ml/min (1 ml), 20.0 ml/min (5 ml)

Max. back pressure 0.3 MPa, 3 bar, 43 psi

	Value	Spec	Units	Description
* Flowrate	1.0	Fixed	ml/min	Feed flowrate
Component_Concentration(*)				
Component_Concentration("A")	8.8e-005	Fixed	eq/l	Component concentration
Component_Concentration("B")	5.e-005	Fixed	eq/l	Component concentration
Component_Concentration("C")	6.5e-005	Fixed	eq/l	Component concentration
Component_Concentration("K")	1.0	Fixed	M	Component concentration
Pressure	3.0	Fixed	bar	Feed pressure

Aspen chromatography simulation

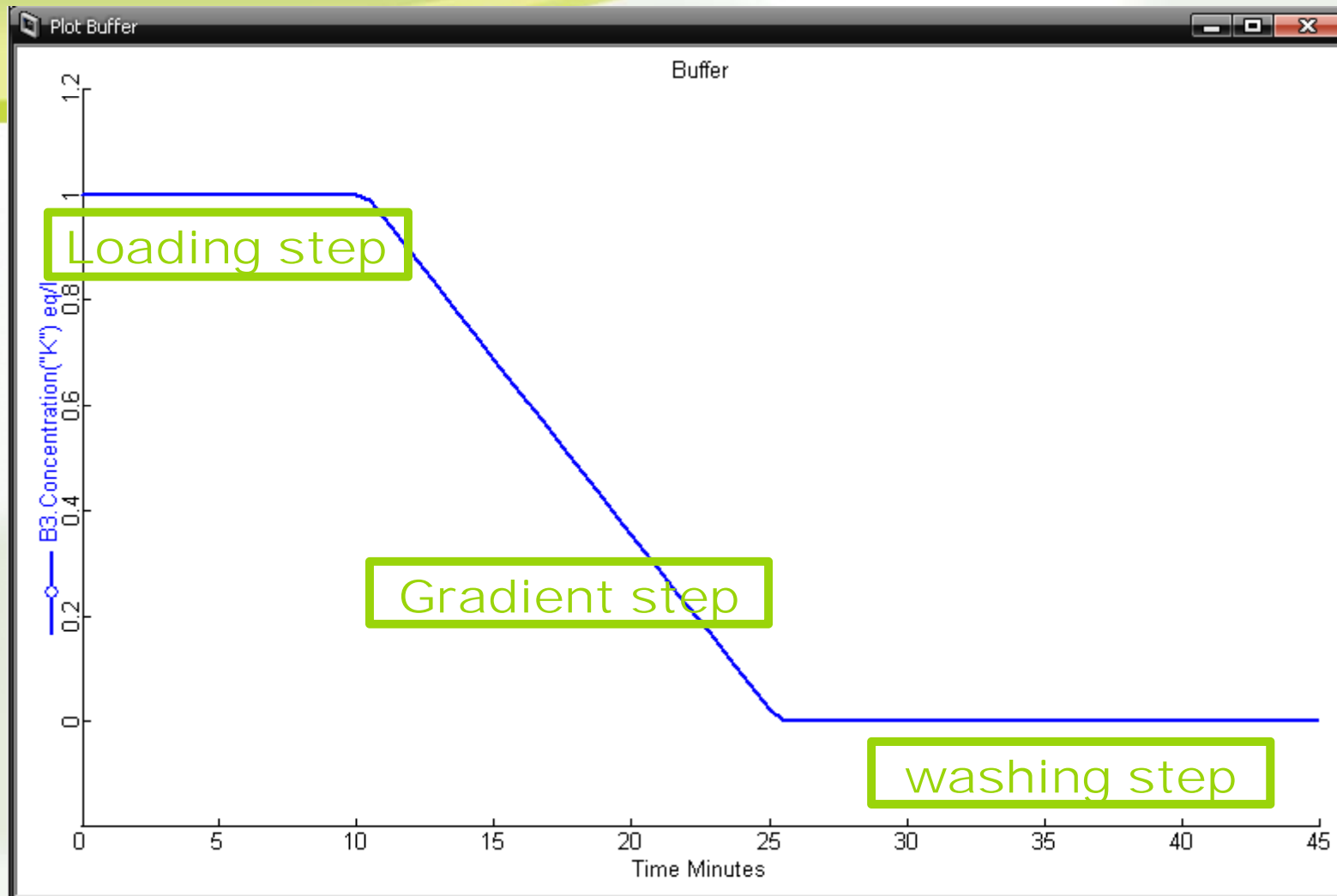
- **The step 1 (Loading step)**
 - **Time : 10 min**
- **The step 2 (Gradient step)**
 - **Time : 15 min**
- **The step 3 (Washing step)**
 - **Time : 30 min**

Step 1						
Manipulated Variables						
Variable	Value	Units	Spec	Ramped	Target	Time (min)
B2.Component_Concentration("A")	8.8e-5	eq/l	Fixed	No		
B2.Component_Concentration("B")	5.e-5	eq/l	Fixed	No		
B2.Component_Concentration("C")	6.5e-5	eq/l	Fixed	No		
B2.Component_Concentration("K")	1	M	Fixed	No		

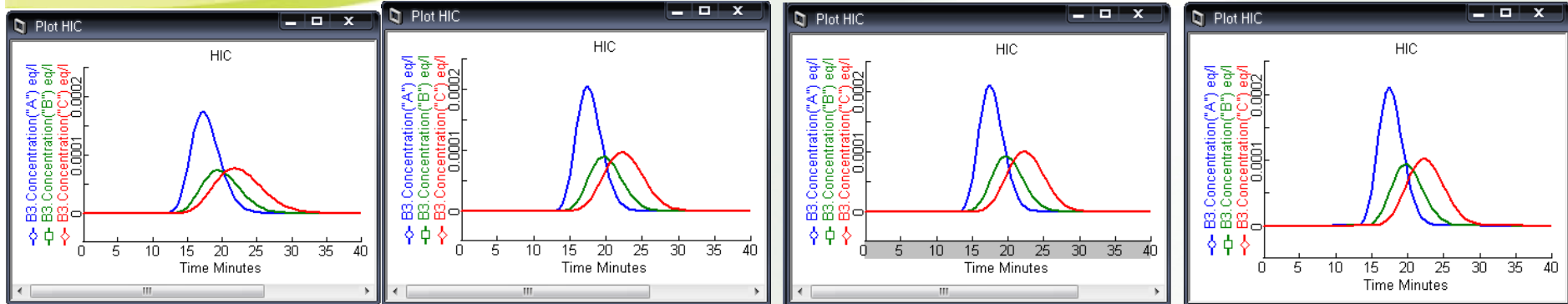
Step 2						
Manipulated Variables						
Variable	Value	Units	Spec	Ramped	Target	Time (min)
B2.Component_Concentration("A")	0.0	eq/l	Fixed	No		
B2.Component_Concentration("B")	0.0	eq/l	Fixed	No		
B2.Component_Concentration("C")	0.0	eq/l	Fixed	No		
B2.Component_Concentration("K")	1	M	Fixed	Linear	1.e-4	15

Step 3						
Manipulated Variables						
Variable	Value	Units	Spec	Ramped	Target	Time (min)
B2.Component_Concentration("A")	0.0	eq/l	Fixed	No		
B2.Component_Concentration("B")	0.0	eq/l	Fixed	No		
B2.Component_Concentration("C")	0.0	eq/l	Fixed	No		
B2.Component_Concentration("K")	1.e-4	M	Fixed	No		

Buffer Simulation



NP값변화



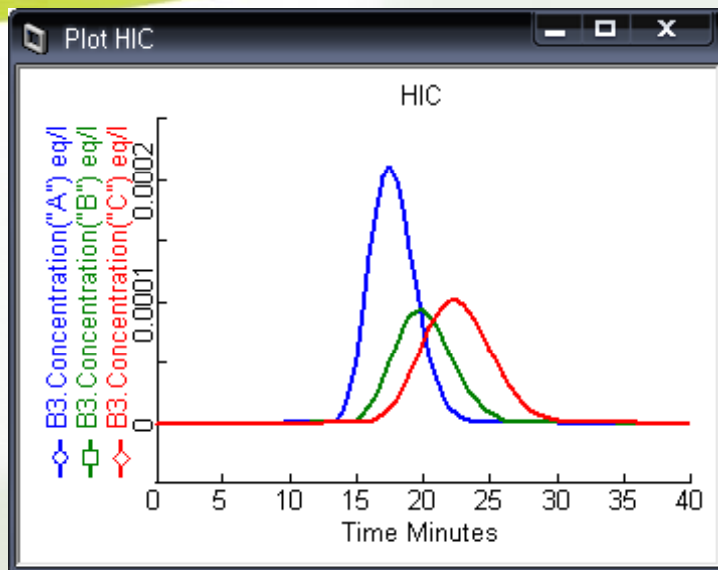
IP1(Lac-Trn-Tsh) : 10-15-20
IP2 : 30
IP3 : 8
NP : 30
MTC : 100000

IP1(Lac-Trn-Tsh) : 10-15-20
IP2 : 30
IP3 : 8
NP : 300
MTC : 100000

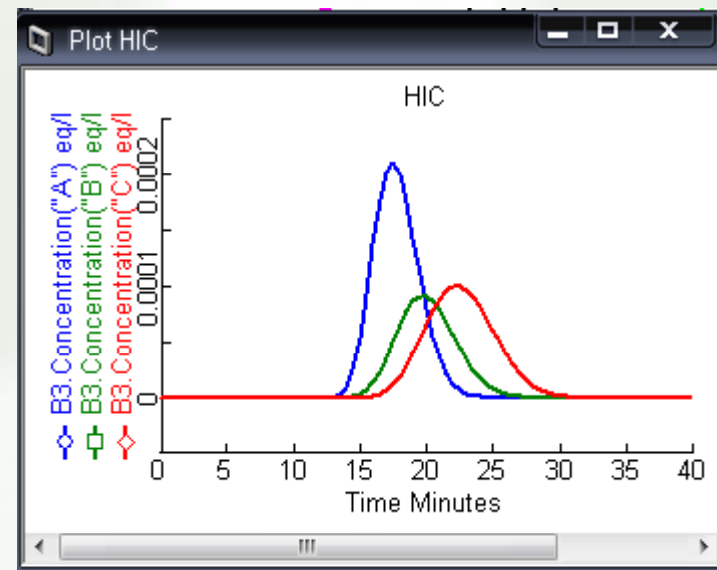
IP1(Lac-Trn-Tsh) : 10-15-20
IP2 : 30
IP3 : 8
NP : 30000
MTC : 100000

IP1(Lac-Trn-Tsh) : 10-15-20
IP2 : 30
IP3 : 8
NP : 300000
MTC : 100000

MTC값변화

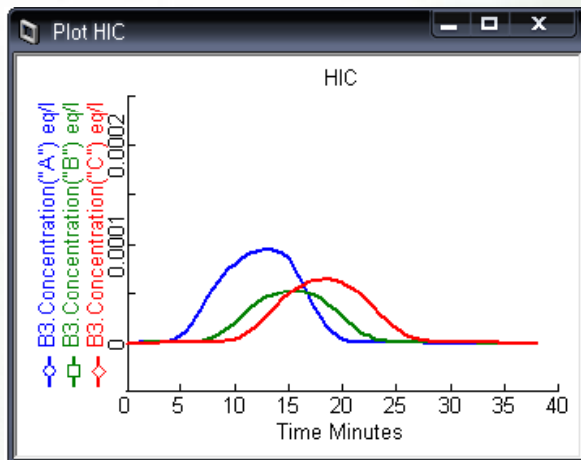


IP1(Lac-Trn-Tsh) : 10-15-20
IP2 : 30
IP3 : 8
NP : 30
MTC : 100000

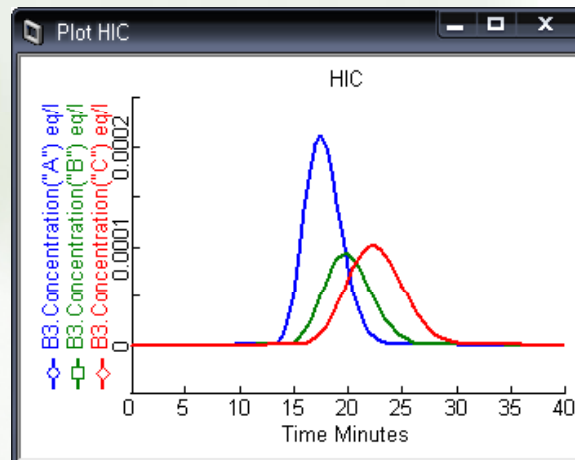


IP1(Lac-Trn-Tsh) : 10-15-20
IP2 : 30
IP3 : 8
NP : 30
MTC : 1000

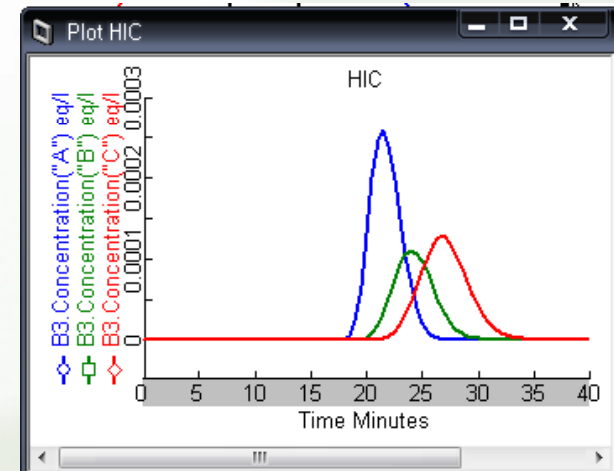
IP2값변화



IP1(Lac-Trn-Tsh) : 10-15-20
IP2 : 1
IP3 : 8
NP : 30
MTC : 100000

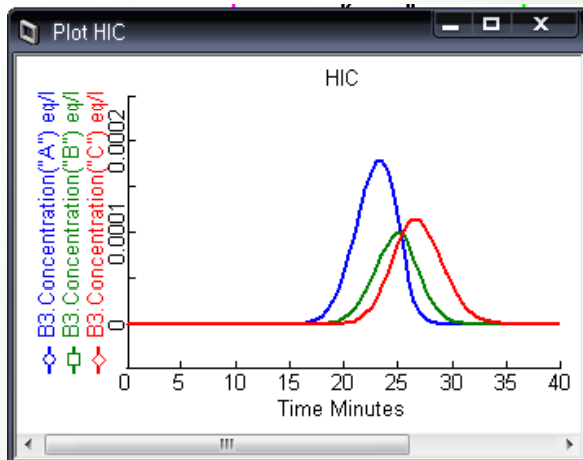


IP1(Lac-Trn-Tsh) : 10-15-20
IP2 : 30
IP3 : 8
NP : 30
MTC : 100000

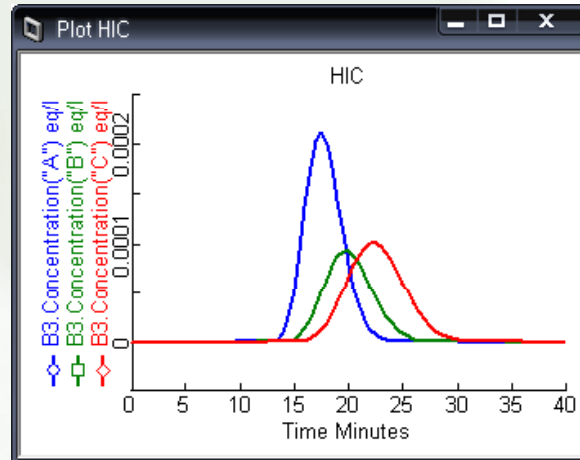


IP1(Lac-Trn-Tsh) : 10-15-20
IP2 : 300
IP3 : 8
NP : 30
MTC : 100000

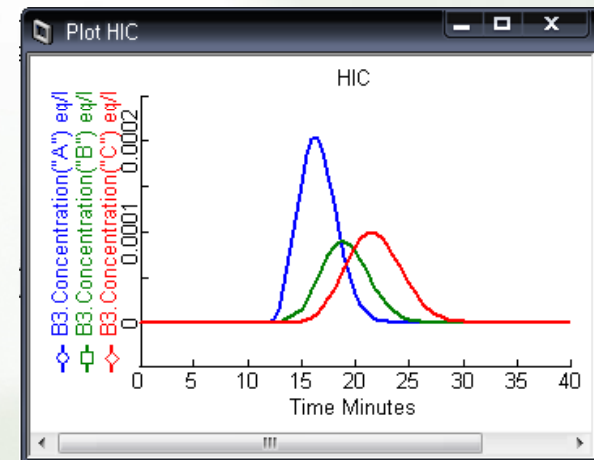
IP3값변화



IP1(Lac-Trn-Tsh) : 10-15-20
IP2 : 30
IP3 : 1
NP : 30
MTC : 100000

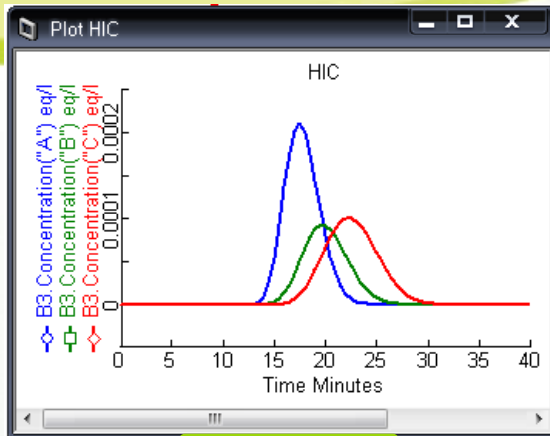


IP1(Lac-Trn-Tsh) : 10-15-20
IP2 : 30
IP3 : 8
NP : 30
MTC : 100000

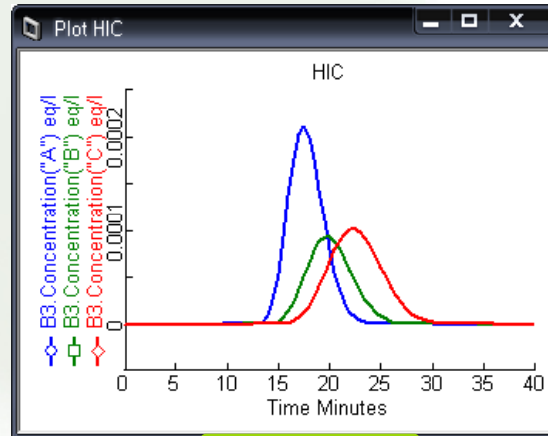


IP1(Lac-Trn-Tsh) : 10-15-20
IP2 : 30
IP3 : 20
NP : 30
MTC : 100000

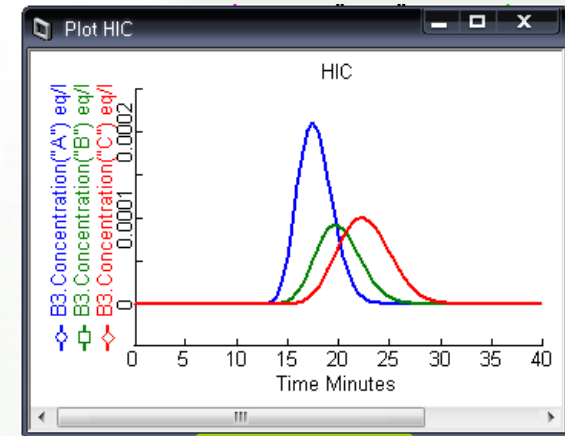
Q값변화



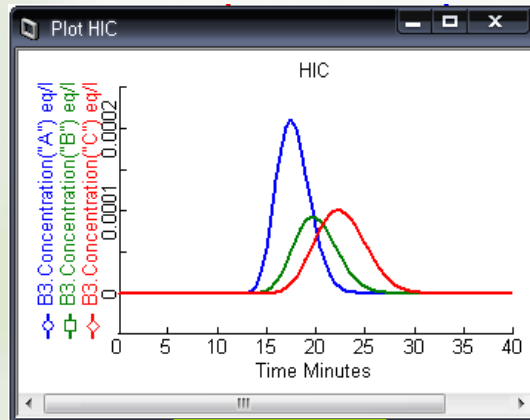
Q = 0.015



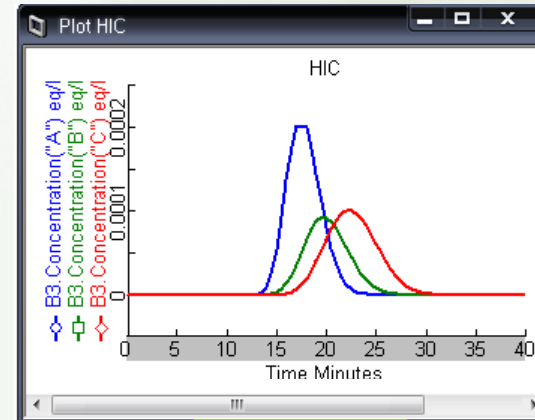
Q = 0.15



Q = 1.5

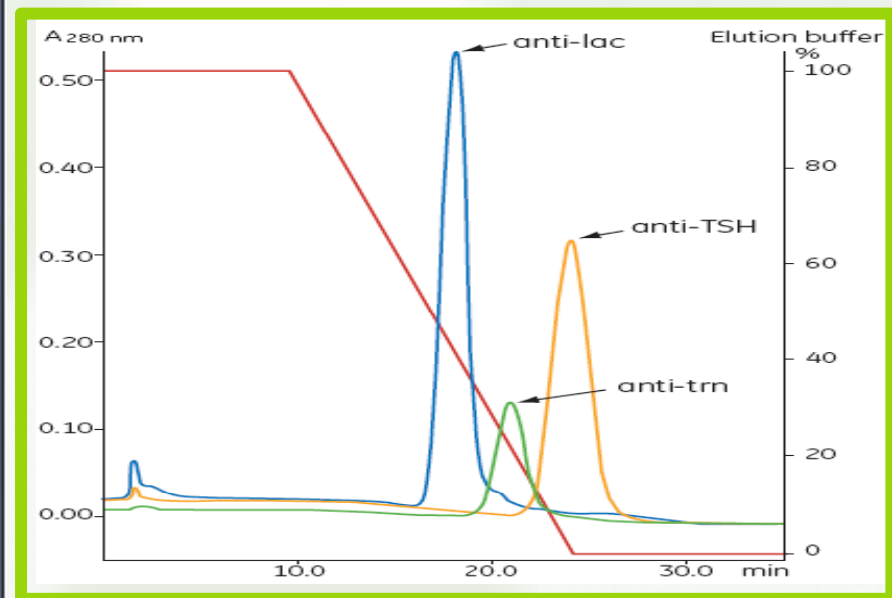
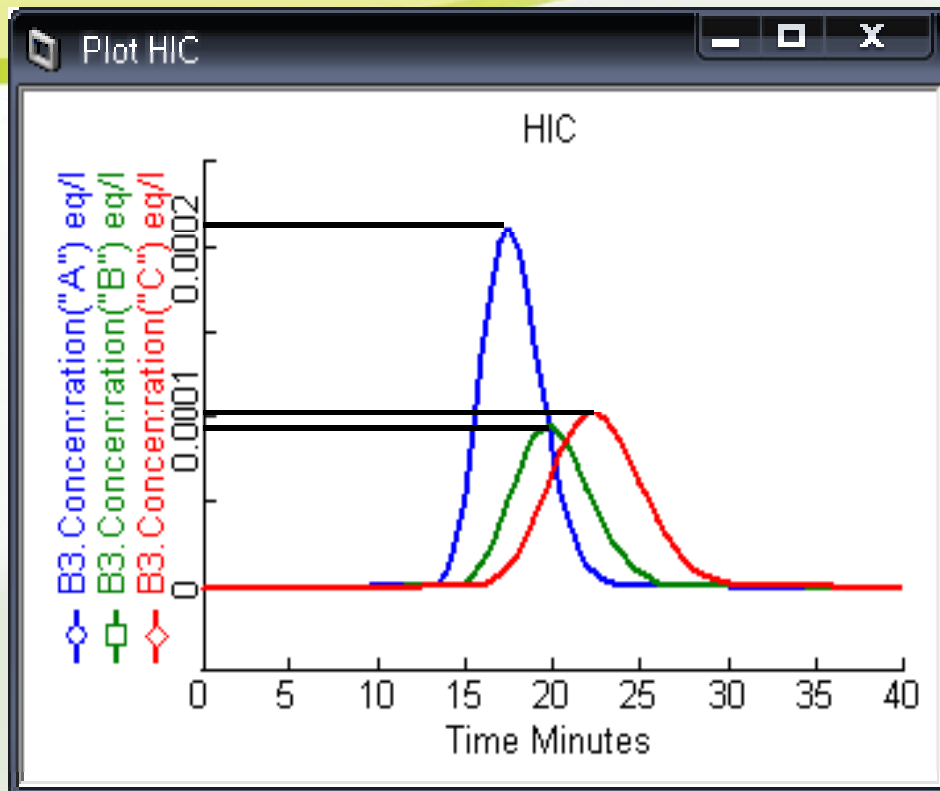


Q = 15



Q = 1500

HIC Simulation Interim Result



분자량의 변화

MW(molecular weight)
A, B, C = 10,000

	Value	Spec	Units	Description
Flowrate	1.0	Fixed	ml/min	Feed flowrate
Component_Concentration(*)				
Component_Concentration("A")	8.8e-005	Fixed	eq/l	Component concentration
Component_Concentration("B")	5.e-005	Fixed	eq/l	Component concentration
Component_Concentration("C")	6.5e-005	Fixed	eq/l	Component concentration
Component_Concentration("K")	1.0	Fixed	M	Component concentration
Pressure	3.0	Fixed	bar	Feed pressure



MW(molecular weight)
A = 40,000
B = 71,400
C = 36,100

	Value	Spec	Units	Description
Flowrate	1.0	Fixed	ml/min	Feed flowrate
Component_Concentration(*)				
Component_Concentration("A")	2.2e-005	Fixed	eq/l	Component concentration
Component_Concentration("B")	7.e-006	Fixed	eq/l	Component concentration
Component_Concentration("C")	1.8e-005	Fixed	eq/l	Component concentration
Component_Concentration("K")	1.0	Fixed	M	Component concentration
Pressure	3.0	Fixed	bar	Feed pressure

HIC Simulation and Experiment

