Polymeric Ion Exchange Process Media for Biomolecule Separation with High Resolution

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Introduction

Ion exchange (IEX) chromatography enables the separation of native biological samples without disruption of high order structures. It has been widely used in the analysis and separation of ionizable pharmaceutical molecules. The method can be applied to all stages of the biological sample purification process. Currently commercially available IEX media are mostly based soft agarose and polymethacrylate, which only accommodates low pressure operation. Sepax has developed alternative polymethacrylate-based ion exchange media, which stands higher flow rate applications.

Sepax developed narrowly dispersed polymethacrylate-based ion exchange process media. The particle size of the resin is 30 µm with an average pore size of 1000 Å. The highly uniformly dispersed resin has a narrow particle distribution of D90/D10<1.3, as shown in **Figure 1**. The base resin is made of hydrophilic polymethacrylate. On the resin surface is covalently linked with different ion exchange functional groups, strong cation exchange group (sulfonic acid), strong anion exchange group (trimethyl quaternary amine) and weak anion exchange group (diethylamine), as shown in **Figure 2**.

Monomix MC30 Resin, Structure & Features

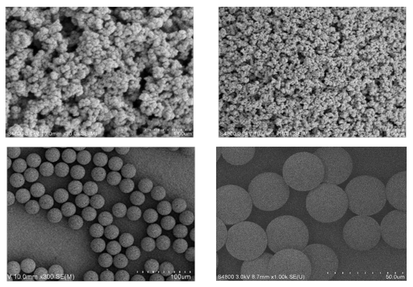


Figure 1. SEM images of Monomix MC30 Resin. The highly uniformly dispersed resin has a narrow particle distribution of D90/D10<1.3.

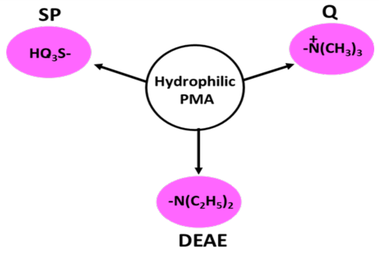


Figure 2. Monomix HC/MC30 IEX resin structures. Strong cation exchange group is sulfonic acid. Strong anion exchange group is trimethyl quaternary amine, while weak anion exchange group is diethylamine.

Features

- Monomix IEX resins are narrowly dispersed particles
- Well controlled pore structure
- Wide pH range
- Rigid beads can be operated at higher flow rates and higher pressure
- High dynamic binding capacity and high loading capacity
- High separation efficiency and resolution

Resin Technical Specifications

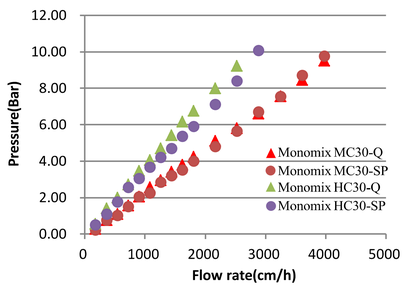
Depending on the resin surface structure and ion exchange group density, the dynamic binding capacity (DBC) of the resins varies as shown in the table below. High DBC is achieved with 105 mg Lys/mL for strong cation exchange SP resin and 100 mg BSA/mL for strong anion exchange resin Q.

Resin Type	Monomix HC-Q	Monomix MC-Q	Monomix HC -DEAE	Monomix MC -DEAE	Monomix HC-SP	Monomix MC-SP
Functional Group	$-N^+(CH_3)_3$		$-N(C_2H_5)_2$		$-SO_3H$	
DBC*(/mL resin)	>100 mg BSA	>51 mg BSA	>90 mg BSA	>47 mg BSA	>105 mg Lysozyme	>52 mg Lysozyme
Max Linear Flow (cm/h)	1000					
Matrix	Hydrophilic polymethacrylate					
Particle Size (µm)	30					
Operation Temp.	≤40°C					
pH Range	2-12					
Pore Size (Å)	1000					
Max Pressure	≤1 Mpa（10 bar）					
Compatible Mobile Phases	Compatible with aqueous solutions, mixtures of water and acetonitrile, acetone, or methanol. Typical buffers: phosphate, tris, & acetate.					
Storage	70% (v/v) , stored in 20% ethanol					
Regeneration	1-2 M NaCl					
CIP	0.5 M HCl or 1.0 M NaOH					

* Dynamic Binding Capacity (DBC) measurement method: for Monomix HC/MC-Q and DEAE: 2.0 mg/mL of BSA in 50 mM Tris buffer, pH 8.5, column size 4.6 x 50 mm, linear flow rate 180 cm/h, 10% breakthrough; for Monomix HC/MC –SP: 1.0 mg/mL of lysozyme in 50 mM phosphate buffer, pH 6.0, column size 4.6 x 50 mm, linear flow rate 360 cm/h, 10% breakthrough.

Flow – Pressure Characteristics

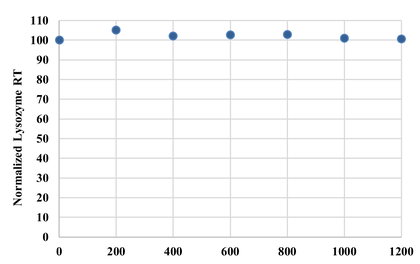
Figure 3. Back pressure vs. linear flow rate for Monomix IEX resins. The resins have shown excellent flow-pressure characters. HC resins showed higher back pressure than MC series, due to the resin surface structure difference.



Instrument: Sepax FPLC Generik HP36
Column: 10 x 150 mm
Mobile Phase: 20 mM Sodium Phosphate (pH 7.0) for SP columns, 50 mM Tris (pH 8.0) for Q columns
Column Temperature: Ambient

CIP Effects on Lysozyme Retention Time

Figure 4. The Monomix MC resin not only has higher physical strength, but shows caustic stability towards 1.0 M NaOH, which is routinely used in CIP. As illustrated in **Figure 4** there is negligible change in the retention time of lysozyme throughout CIP with 1.0 M NaOH for 1,200 column volumes.



Resin: Monomix HC30-SP (30 µm, 1000 Å)
Column: 4.6 x 50 mm (Stainless Steel)
Instrument: Agilent 1260
Mobile Phase: *A:* 20 mM Sodium Phosphate (pH 6.0) *B:* A + 1.0 M NaCl
Flow Rate: 0.4 mL/min
Detector: UV 214 nm
Column Temperature: RT (20°C)
Injection Volume: 20 µL
Sample: Lysozyme, 1 mg/mL in water
Lysozyme Test: Gradient 0-75% B in 10 min
CIP: 1.0 M NaOH

Non-Specific Binding Test from Monomix HC30-SP and Q Resin

The surface of Monomix IEX resin contains multi hydrophilic domains which minimizes the nonspecific binding of the biological analytes with the resins. As exemplified in **Figures 5 and 6**, both Monomix HC30-Q and SP showed minimal NSB toward Lysozyme and Bovine Serum Albumin (BSA).

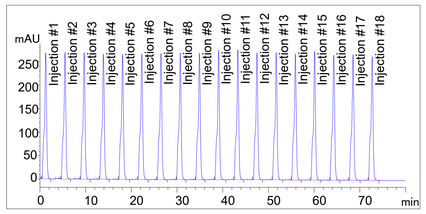


Figure 5. 18 injections of BSA through a Monomix HC30-SP column consecutively.

Resin: Monomix HC30-SP (30 µm, 1000 Å)
Column: 4.6 x 50 mm (Stainless Steel)
Instrument: Agilent 1260
Mobile Phase: 20 mM Sodium Phosphate + 0.3 M NaCl, pH 7.0
Flow Rate: 0.5 mL/min (180 cm/h)
Detector: 214 nm
Column Temperature: RT
Injection Volume: 5 µL
Sample: BSA (1.0 mg/mL)

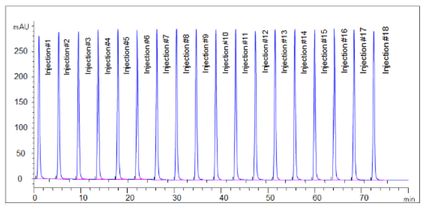


Figure 6. Consecutive 18 injections of Lysozyme through a Monomix HC30-Q column.

Resin: Monomix HC30-Q (30 µm, 1000 Å)
Column: 4.6 x 50 mm (Stainless Steel)
Instrument: Agilent 1260
Mobile Phase: 20 mM Sodium Phosphate + 0.3 M NaCl, pH 7.0
Flow Rate: 0.5 mL/min (180 cm/h)
Detector: 214 nm
Column Temperature: RT
Injection Volume: 5 µL
Sample: Lysozyme (0.5 mg/mL)

Lot-to-lot Consistency

Monomix IEX resins are manufactured with well controlled processes and are of high lot-to-lot consistency. Separations of standard protein mixtures on the Monomix IEX resins are illustrated in **Figure 7** and **Figure 8**.

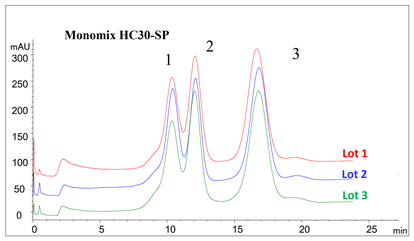


Figure 7. Lot-to-lot consistency for Monomix HC30-SP resins.

Resin: Monomix HC30-SP (30 µm, 1000 Å)
Column: 4.6 x 50 mm (Stainless Steel)
Instrument: Agilent 1260
Mobile Phase: *A:* 20 mM Sodium Phosphate (pH 6.0) *B:* A + 1.0 M NaCl
Flow Rate: 1.0 mL/min (360 cm/h)
Gradient: 0-75% B 25 min
Detector: UV 214 nm
Column Temperature: RT
Injection Volume: 20 µL
Sample: 1) Ribonuclease A 2) Cytochrome C 3) Lysozyme (1mg/mL)

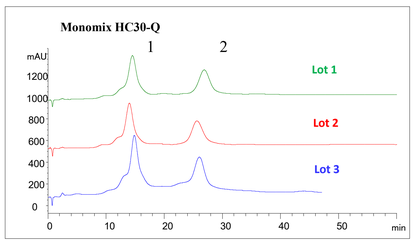
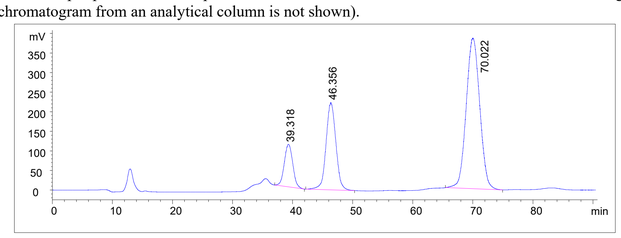


Figure 8. Lot-to-lot consistency for Monomix HC30-Q resins.

Resin: Monomix HC30-Q (30 µm, 1000 Å)
Column: 4.6 x 50 mm (Stainless Steel)
Instrument: Agilent 1260
Mobile Phase: *A:* 50 mm Tris pH 8.5 *B:* A + 0.5 M NaCl
Flow Rate: 1.0 mL/min (360 cm/h)
Gradient: 0-75% B 25 min
Detector: UV 214 nm
Column Temperature: RT
Injection Volume: 20 µL
Sample: 1) Ovalbumin 2) Trypsin inhibitor (5 mg/mL)

Easy to Scale Up for Monomix IEX Resins

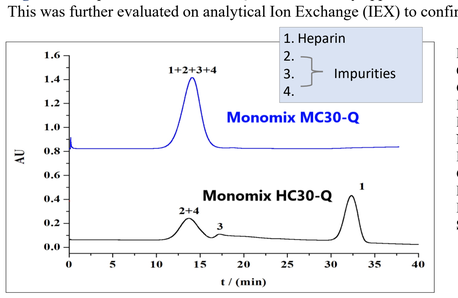
Figure 9. Scale up from a small analytical column to a larger semi-preparative column is easy and straightforward. As shown in the **Figure 9**, a scaled-up separation of three protein mixture was achieved on a 50 x 220 mm column with high resolution and efficiency (chromatogram from an analytical column is not shown).



Resin: Monomix HC30 SP (30 µm, 1000 A) **Column:** 50 mm × 220 mm FPLC **Instrument:** Sepax FPLC Generik HP36 **Mobile Phase:** *A:* 20 mM PB (pH 6.0) *B:* 20 mM PB + 1 M NaCl (pH 6.0) **Flow Rate:** 20 ml/min (61 cm/h) **Detector:** 214 nm **Column Temperature:** RT **Injection Volume:** 4 mL **Sample:** 1) Ribonuclease A (5 mg) 2) Cytochrome C (5 mg) 3) Lysozyme (10 mg)

Separations of Heparin from Impurities in Production Mixture

Figure 10. Sepax Monomix HC-30Q was successfully applied for the purification of heparin and related production impurities. This was further evaluated on analytical Ion Exchange (IEX) to confirm.

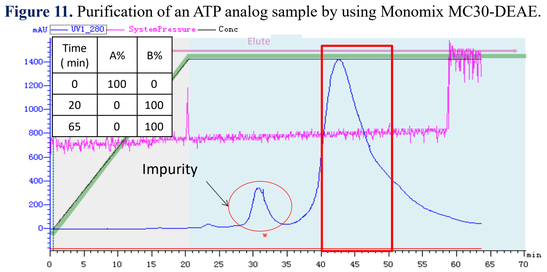


Resin: Monomix HC30-Q (30 µm, 1000 Å) and Monomix MC30-Q (30 µm, 1000 Å)
Column: 4.6 x 250 mm (Stainless Steel)
Instrument: Agilent 1260
Mobile Phase: *A:* 4% NaH_2PO_4, pH 3.0 *B:* 4% NaH_2PO_4, 14% $NaClO_4$, pH 3.0
Flow rate: 0.22 mL/min (80 cm/h)
Gradient: 25%-100% B from 10 min to 35 min
Detection: 202 nm
Injection Volume: 10 µL
Sample: Heparin (25 mg/mL) production sample

Purification of the ATP Analog on Monomix MC30-DEAE Column

The diversified pore structures, binding capacities as well as sturdiness of the resin provide excellent choices for separation of different bio molecules The following are some case studies. **Figure 12** shows the crude analysis of an ATP analog sample by using GP-C18 (3µm, 120 Å). Purification of the crude sample was then performed, as illustrated in **Figure 11.** The purity of the crude ATP sample is 88.9% analyzed by HPLC, as shown in **Figure 13**. After one step of ion-exchange, it achieved purity of 98.6% with 98% recovery.

Figure 11. Purification of an ATP analog sample by using Monomix MC30-DEAE.



Time (min)	A%	B%
0	100	0
20	0	100
65	0	100

Resin: Monomix MC30-DEAE (30 µm, 1000 Å) **Column:** FPLC 6.6 x 100 mm, AF
Mobile Phase: *A:* 20 mM Sodium Phosphate (pH 7.5) *B:* A + 1.0 M NaCl **Flow Rate:** 1.0 mL/min (175 cm/h)
Detector: UV 280 nm **Column Temperature:** Ambient **Injection Volume:** 100 µL **Instrument:** Sepax FPLC Generik HP36

Figure 12. Purity analysis of crude ATP analog by HPLC.

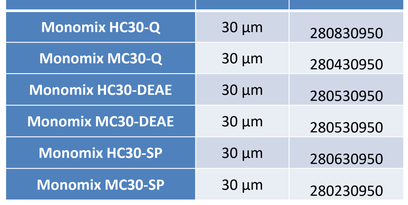
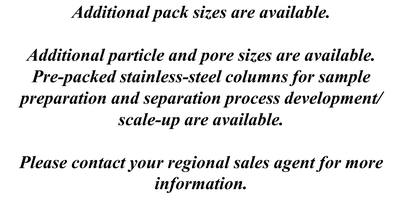


Figure 13. Analysis of ATP purified sample after one step of ion-exchange with Monomix MC30-DEAE.



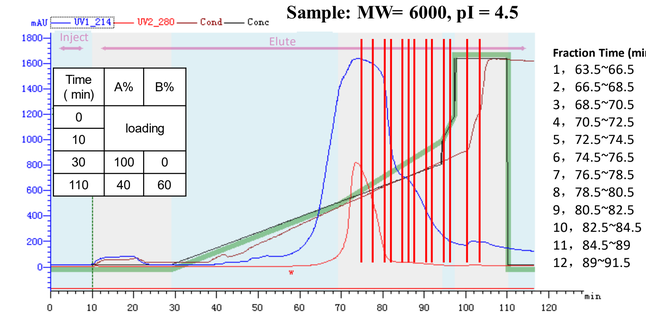
Resin: GP-C18 (3µm, 120 Å) **Column:** 4.6×250 mm (Stainless Steel) **Instrument:** Agilent 1260 **Mobile Phase:** *A:* Sodium Phosphate, pH 8.5 *B:* Acetonitrile **Flow Rate:** 1.0 mL/min (360 cm/h) **Detector:** UV 254 nm **Column Temperature:** 30°C **Injection Volume:** 2 µL **Sample:** Purified ATP Analog, 22 mg/mL in water

Purification of Insulin Analog on Monomix MC30-DEAE

Purification of Insulin analogs was achieved with Monomix MC30-DEAE column. Purity was increased from 92% to 97.9%. Results were repeatable. Both purity and recovery yield meet the expectation. **Figure 15** shows the crude analysis of an Insulin Analog sample by using GP-C18 (5µm, 120 Å). Purification of the crude sample was then performed as illustrated in **Figure 14** and the analysis of the purified sample is illustrated in **Figure 16**.

Figure 14. Purification of an Insulin Analog sample by using Monomix MC30-DEAE, total sample loading was ~10 g/L.

Sample: MW= 6000, pI = 4.5



Time (min)	A%	B%
0	loading	
10		
30	100	0
110	40	60

Fraction Time (min)
1，63.5~66.5
2，66.5~68.5
3，68.5~70.5
4，70.5~72.5
5，72.5~74.5
6，74.5~76.5
7，76.5~78.5
8，78.5~80.5
9，80.5~82.5
10，82.5~84.5
11，84.5~89
12，89~91.5

Resin: Monomix MC30-DEAE (30 µm, 1000 Å) **Column:** FPLC 6.6x150 mm, AF
Mobile phase: *A:* 20 mm sodium phosphate（pH 7.0) *B:* A + 1.0 M NaCl **Flow rate:** 1.0 mL/min (175 cm/h)
Detector: UV 214/280 **Column temperature:** Ambient **Injection volume:** 10 mL **Sample:** Insulin Analog, 5 mg/mL (pH 7.0)
Instrument: Sepax FPLC Generik HP36

Figure 15. Purity analysis of crude Insulin Analog by HPLC.

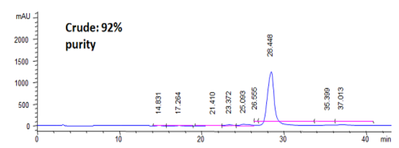
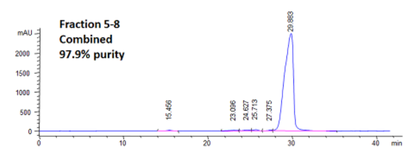


Figure 16. Analysis of Insulin Analog purified sample



Resin: GP-C18 (5µm, 120 Å) **Column:** 4.6×250 mm (Stainless Steel) **Instrument:** Agilent 1260 **Mobile Phase:** *A:* 0.2 M Na_2SO_4 (pH 3.6) *B:* water : acetonitrile =5:5. Isocratic, 42% B **Flow Rate:** 1.0 mL/min (360 cm/h) **Detector:** UV 214 nm **Column Temperature:** 50°C **Injection Volume:** 20 µL **Sample:** Insulin Analog 5 mg/mL (pH 7.0)

Product Order Information

Resin	Particle Size	Part Number
Monomix HC30-Q	30 µm	280830950
Monomix MC30-Q	30 µm	280430950
Monomix HC30-DEAE	30 µm	280530950
Monomix MC30-DEAE	30 µm	280530950
Monomix HC30-SP	30 µm	280630950
Monomix MC30-SP	30 µm	280230950

Standard packing size:
1L, 5L, 10L, 25L, 50L, 100L
Additional pack sizes are available.

Additional particle and pore sizes are available. Pre-packed stainless-steel columns for sample preparation and separation process development/ scale-up are available.

Please contact your regional sales agent for more information.

Authored by: Ke Yang, Xinmei Hu, Huiming Mao and Xueying Huang

Better Surface Chemistry for Better Separation