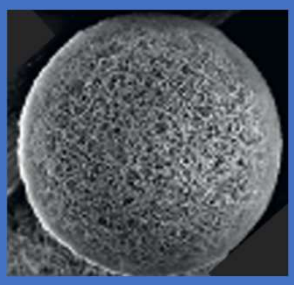
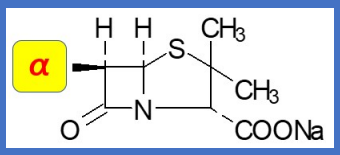
Rev.1

MITSUBISHI CHEMICAL

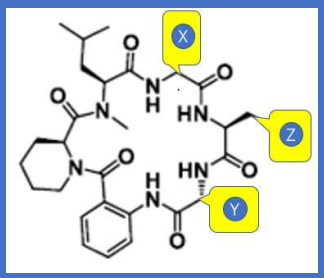
DIAION™ SEPABEADS™ MCIGEL™ Technical Guide



Polymeric Separation Media for Pharmaceutical Purification



Separation of 6-amino-penicilinic acid derivatives



Separation of cyclic peptide analogs

Mitsubishi Chemical's polymeric separation media have been used in pharmaceutical application from late 1960's and have become one of the standard resins in purification not only in pharmaceuticals but also juice, sugar and food segment.

Mitsubishi Chemical offers wide variety of polymeric separation media under the trade name "DIAION", "SEPABEADS" grades for industrial application and "MCIGEL CHP", "MCIGEL CMG" grades for analytical and preparative applications.

These media have been adapted in the separation/purification process of antibiotics, oligonucleotide, peptides, herb and Chinese medicines.

Separation and Purification of Pharmaceuticals

Peptides and nucleic acids used as drug substances for pharmaceuticals are produced by chemical synthesis (solid-phase or solution) or by recombinant procedures. In both cases, reversed-phase chromatography (RPC) is a common method for separation and purification of target molecules.
Mitsubishi Chemical's polymeric separation media such as DIAION HP20, SEPABEADS SP20SS and MCIGEL CHP20 series are commonly used as stationary phase of RPC process for industrial-scale pharmaceutical purification.

Principle of Reversed-Phase Chromatography (RPC)

In RPC, substances in mobile phase tend to be retained on stationary phase in chromatography column via hydrophobic interactions. The strength of the interaction generally depends on hydrophobicity of the stationary phase and substances and relationship between pore size of the stationary phase and molecular size of the substances. Substances with strong interaction have a longer retention time in the column and the difference in retention time leads to separation of each substance.
Polymeric separation media have covalently bound hydrophobic moieties such as aromatics in their chemical structure and are suitable as stationary phase for RPC.

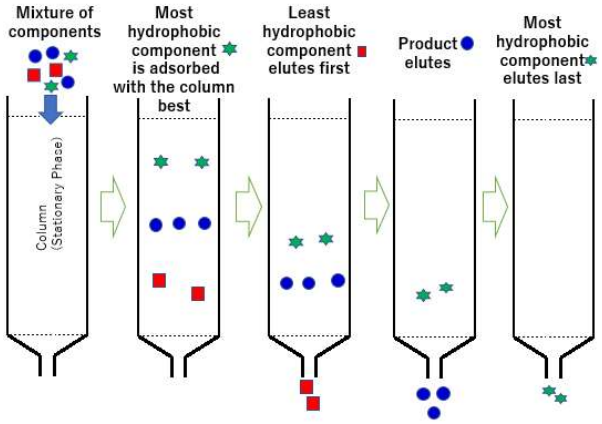


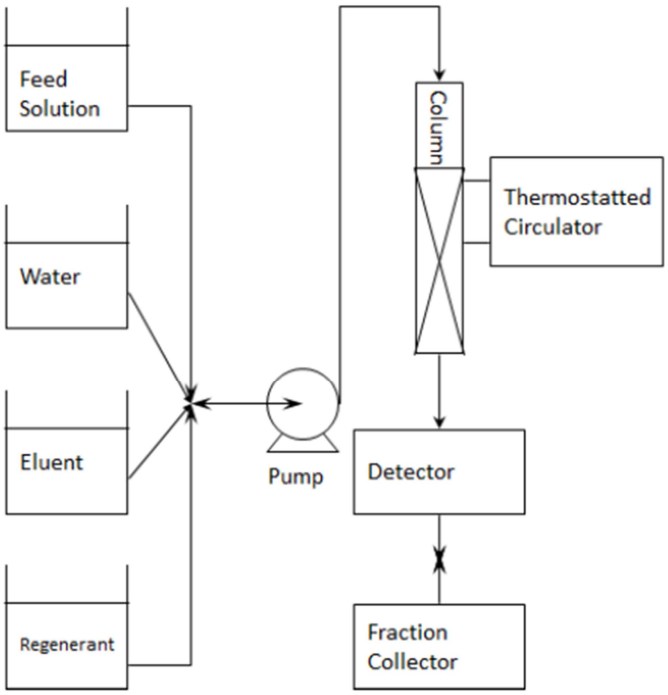
Illustration of principle of RPC

General procedure and equipment of RPC

The general procedure of RPC is as follows.

The solution that contains mixture of target molecules and impurities is loaded to the column packed with separation media (stationary phase), followed by elution with eluting solvent (mobile phase). The mobile phase should be able to dissolve the target molecules. For industrial-scale pharmaceutical purification process, aqueous solution of acetonitrile or alcohol is commonly used as mobile phase.

The schematic equipment used in RPC is shown in the figure. Component molecules in the feed solution travel through the stationary phase at different speeds depending on their individual natures and elute from the column separately. Elution of the molecules from the column is detected by UV detector etc. and purified target molecule is selectively fractionated.



Chromatographic separation equipment

Polymeric Separation Media for Pharmaceutical Purification

Mitsubishi's polymeric separation media are the spherical porous resins based on crosslinked polystyrene, crosslinked modified polystyrene or crosslinked methacrylate.
The advantages of the products can be summarized as follows;

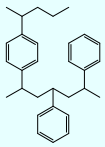
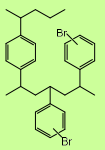
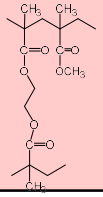
- High chemical stability over all pH range
- High physical strength
- Excellent cycle stability and long life
- Wide variety of degree in hydrophobicity based on polymer chemistry
- Wide variety of particle size from preparative to industrial application

How to select product grade

Mitsubishi's polymeric separation media can be roughly divided into three types based on the composition of base polymer resin. Depending on the nature of target molecule, the right product can be generally selected as follows.

- DIAION HP20, SEPABEADS SP20SS and MCIGEL CHP20 series are the standard styrene-DVB resins with moderate hydrophobicity, so these can be selected as a first choice for new process.
- SEPABEADS SP207 and other brominated-styrenic resins have higher hydrophobicity which is suitable for purification of high polarity molecules. Also, these grades have high specific density and are easy to handle.
- DIAION HP2MGL and other methacrylic polymer resin have less hydrophobicity than styrenic resins and are suitable for purification of highly hydrophobic molecules. These can also be used for purification of oil soluble molecules using normal phase chromatography.

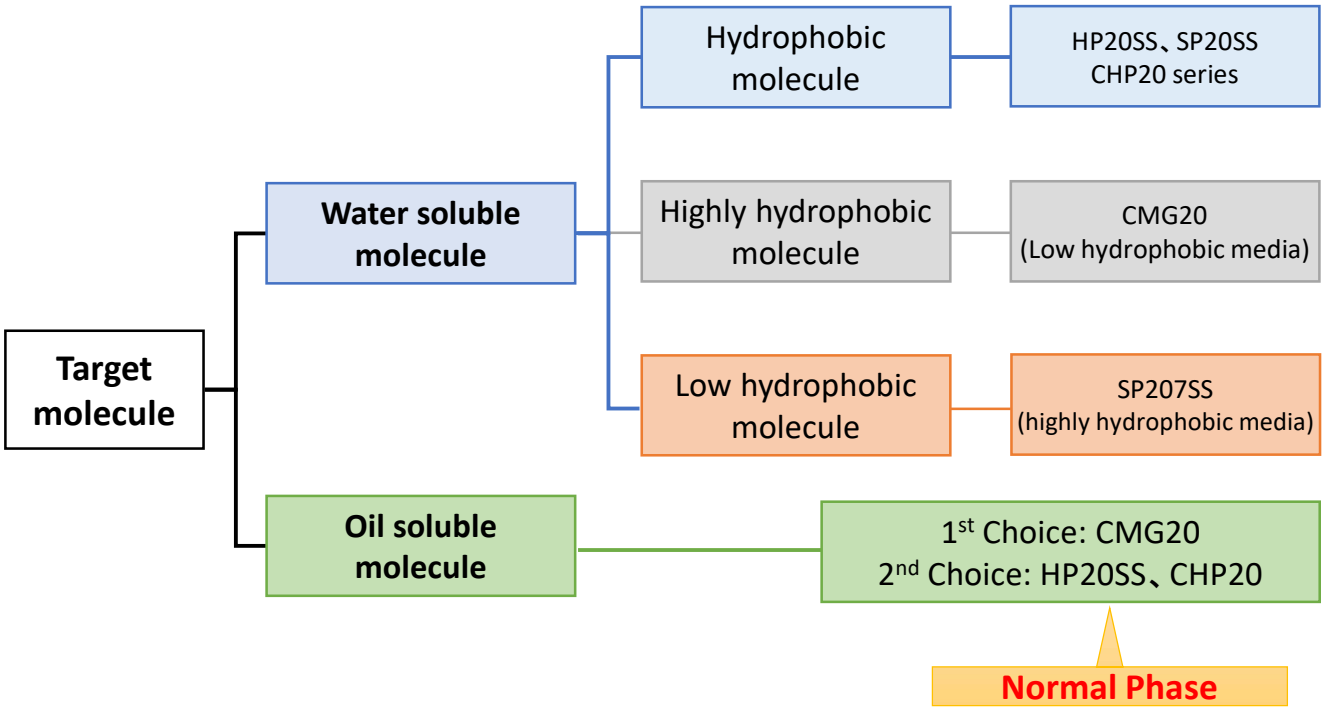
Also, each type of product has a variety of particle size grades and can be selected according to the balance between separation performance and pressure restriction.

Polymer structure	Resin grades	Particle size (μm)	Application (extraction, separation, purification)
Polystyrene (hydrophobic) 	HP20,HP21 SP825L,SP850 SP70、SP700	>250	Antibiotics (Cephalosporin, Vancomycin, Carbapenem and others.) Anti-cancer drug, immunosuppressive drug
	HP20SS SP20SS	120 70	Antibiotics (Vancomycin, Daptomycin) Anti-cancer drug, immunosuppressive drug
	CHP20 series	20,30,50	Peptide, Oligo-nucleotide
Modified Polystyrene (Highly hydrophobic) 	SP207	>250	Antibiotics (Cephalosporin and others) Low hydrophobic drug
	SP207SS	120	Low hydrophobic drug
	CHP07 (to be launched)	30	Peptide, Oligo-nucleotide
Methacrylate (Low hydrophobic) 	HP2MGL	>250	Cyclic Peptide (anti-cancer drug) Highly hydrophobic drug
	HP2MGSS (to be launched)	120	Highly hydrophobic drug Oil soluble drug (Normal phase chromatography)
	CMG20	30	Peptide , Oligo-nucleotide

Product Selection Guide

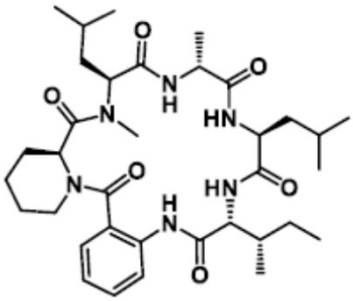
・Selection by nature of target molecule

The type of separation media can be selected based on the nature of target molecule to be separated. In general, highly hydrophobic media are suitable for separation of low hydrophobic (high polarity) molecules, and low hydrophobic media are suitable for highly hydrophobic molecules.

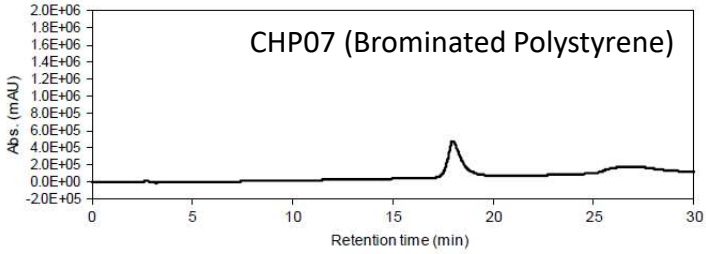


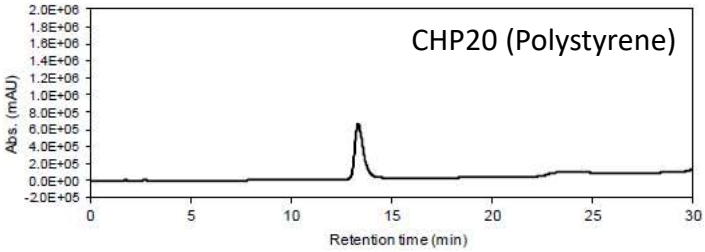
Comparison of retention time of cyclic peptide using polymeric media with different hydrophobicity

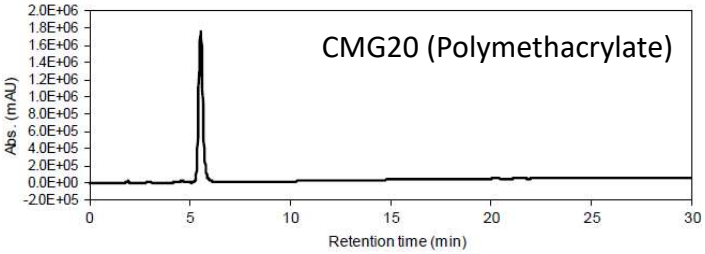
Under the same analytical conditions, the separation media with higher hydrophobicity shows longer retention time.



CF: $C_{35}H_{54}N_6O_6$
MW: 654.8530







Column: 4.6 mm I.D. x 250 mm
Temperature: 35℃
Flow rate: 1 mL/min
Solvent: 65-95% CH_3CN in H_2O, 20 min linear gradient

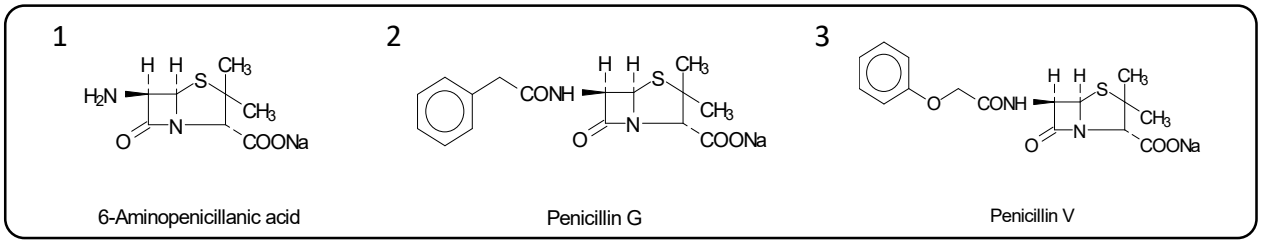
・Selection by particle size

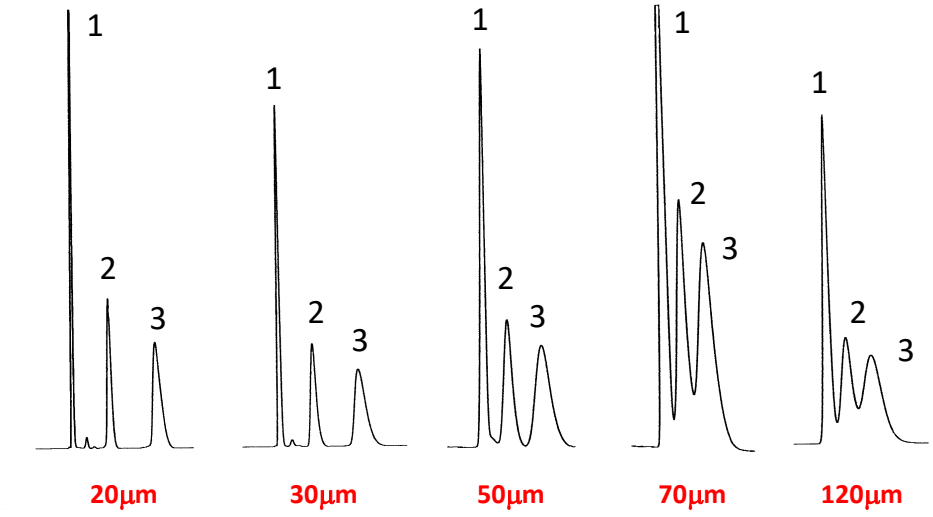
The particle size of separation media can be selected based on the required separation performance and pressure characteristics. In general, small particles are suitable for precision purification due to high separation performance, and large particles are suitable for large-scale refining due to low pressure.

	Small particle (20 - 50 µm)	Middle particle (70 - 120 µm)	Large particle (over 250 µm)
Polystyrene (hydrophobic)	CHP20 (20, 30, 50 µm)	SP20SS (70 µm) HP20SS (120 µm)	HP20, HP21. SP825L, SP850 SP70, SP700
Modified Polystyrene (Highly hydrophobic)	CHP07 (30 µm) (to be launched)	SP207SS (70 µm)	SP207
Methacrylate (Low hydrophobic)	CMG20 (30 µm)	HP2MGSS (120 µm) (to be launched)	HP2MGL

Separation of penicillanic derivatives using polystyrenic media with different particle size

The chromatographic separation data below shows the effect of particle size on the separation of three penicillanic derivatives using polymeric media with same chemistry. The smaller particle shows sharper peaks and better separation performance.



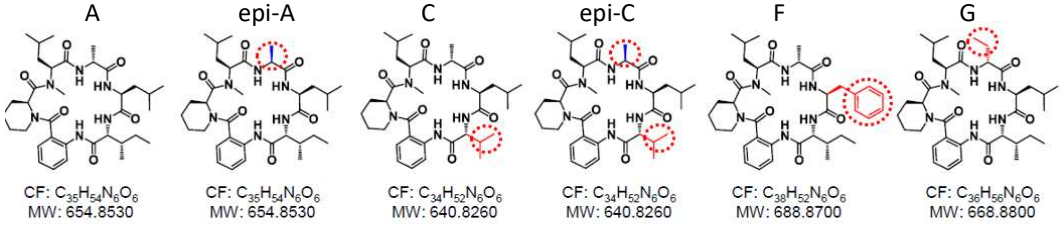


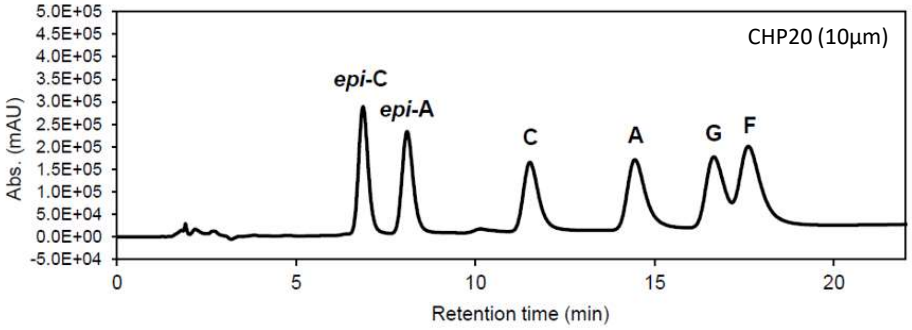
Column size: 250 x 10mm I.D., Eluent: MeOH/50mM phosphate (pH8.0) = 60/40
Flow rate: 2.18ml/min, Detection: UV 254nm
Samples: 1: 6-aminopenicillanic acid (1g/L)
2: penicillin G (1g/L)
3: penicillin V (1g/L).
Injection: 100µL

Examples of Separation using Polymeric Separation Media

Separation of cyclic peptide analogs

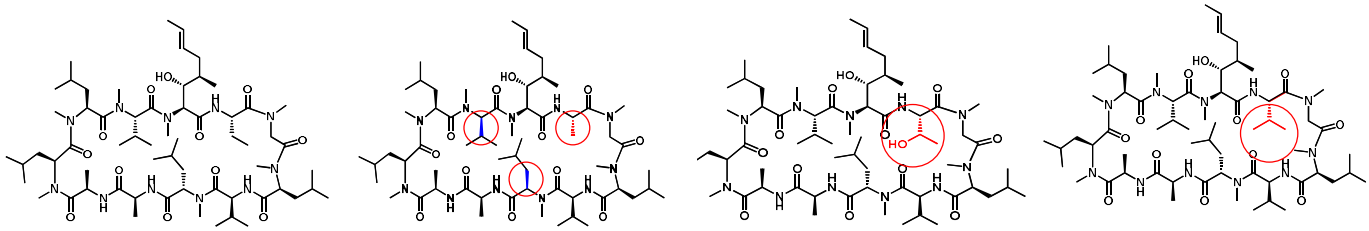
CHP20 can separate cyclic peptides with six similar chemical structures as shown below.
The separation is carried out using acetonitrile/water gradient as eluent.





Sample loadability of polymeric separation media

Compared to ODS-silica media, CHP20 maintains good separation behavior even at high loadings in cyclosporine analogs separation.



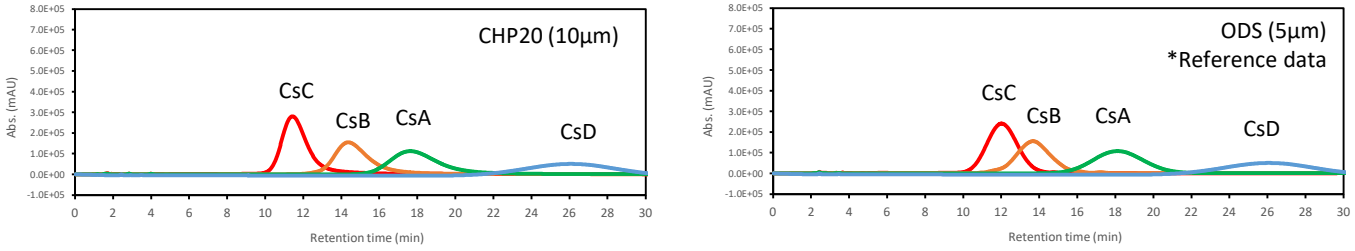
Cyclosporin A (CsA)
MW: 1202.64

Cyclosporin B (CsB)
MW: 1188.61

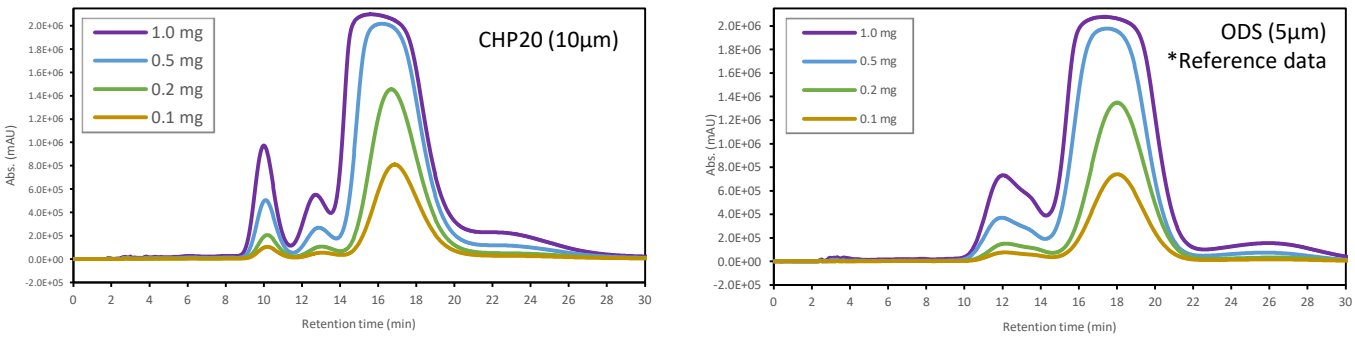
Cyclosporin C (CsC)
MW: 1218.63

Cyclosporin D (CsD)
MW: 1216.66

Low loading condition (Sample injection: 0.01 mg each of CsA, CsB, CsC, and CsD)



High loading condition (Sample injection: Total 0.1, 0.2, 0.5 and 1.0 mg (content ratio: CsA 90%, CsB 3.3%, CsC 3.3%, CsD 3.3%))



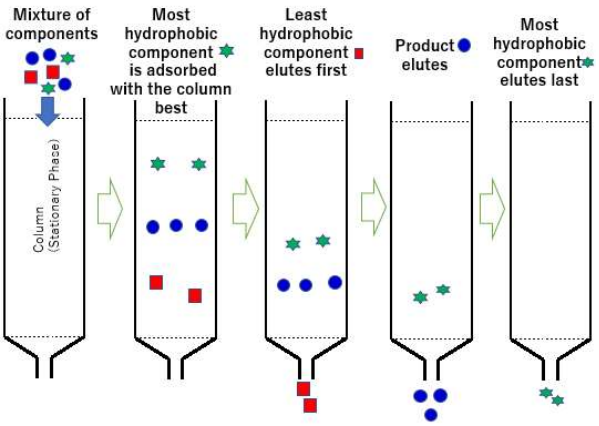
Column: 4.6 mm x 250 mm, Temp: 35℃, Flow rate: 1 mL/min, Detection: 214 nm
Solvent: (CHP20) 69% CH_3CN in H_2O, 30 min isocratic elution, (ODS) 75% CH_3CN in H_2O, 30 min isocratic elution

医薬品の分離精製

医薬品の原薬として使用されるペプチドや核酸は、化学合成(固相・溶液)または組換え法によって製造されます。 いずれの場合も、対象分子の分離精製の一般的な方法として逆相クロマトグラフィー(RPC)が使用されます。
DIAION HP20、SEPABEADS SP20SS、MCIGEL CHP20シリーズをはじめとするの三菱ケミカルのポリマー系分離剤は、工業スケールでの医薬品精製におけるRPCプロセスの固定相として一般的に使用されています。

逆相クロマトグラフィー(RPC)の原理

RPCでは、移動相の物質が疎水性相互作用によってクロマトグラフィーカラム内の固定相に保持されます。 強い相互作用を示す物質は、カラム内での保持時間が長くなり、その保持時間の違いにより各物質が分離されます。
ポリマー系分離剤は、共有結合された芳香族などの疎水性官能基を有しており、RPCの固定相に適しています。



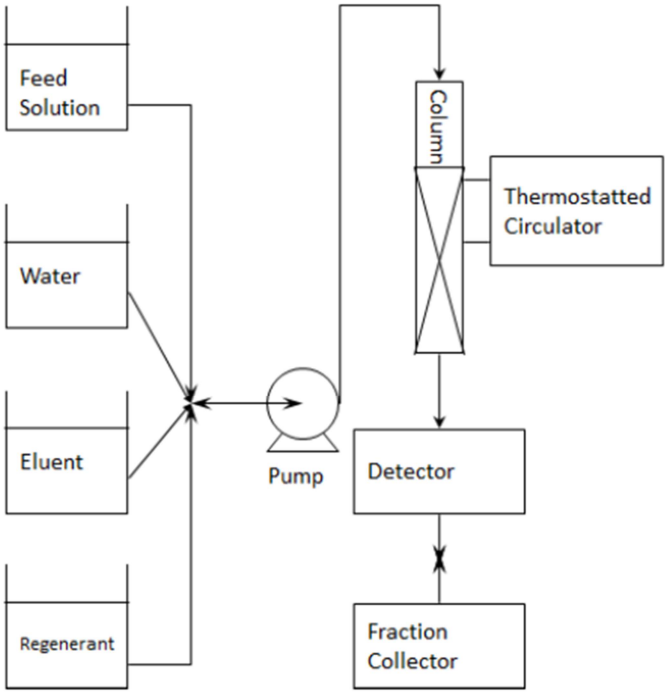
RPCの原理

RPCの一般的な手順と装置

RPCの一般的な手順は以下の通りです。

標的分子と不純物の混合物を含む溶液を分離剤(固定相)が充填されたカラムにロードし、続いて溶出溶媒(移動相)で溶出します。移動相は標的分子を溶解できる必要があります。 工業規模の医薬品精製プロセスの場合、移動相としてアセトニトリルまたはアルコールの水溶液が一般的に使用されます。

RPCで使用される装置の概略図を右に示します。フィード溶液中の各成分分子は、個々の物性に応じて異なる速度で固定相中を移動し、カラムから別々に溶出します。 カラムから溶出された分子はUV検出器などによって検出され、精製されたターゲット分子が選択的に分画されます。



クロマトグラフィー分離装置

医薬精製用ポリマー系分離剤

三菱ケミカルのポリマー系分離剤は、架橋ポリスチレン、架橋修飾ポリスチレンまたは架橋メタクリレートをベースとした球状多孔質樹脂です。製品の主な特徴は以下の通りです。

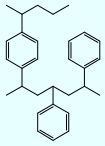
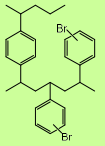
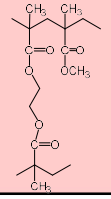
- 幅広いPH範囲での高い化学的安定性
- 高い物理的強度
- 優れたサイクル耐久性
- 多様に調節された表面疎水度
- 分取から工業スケールまでに対応可能な粒径ラインナップ

製品グレードの選択方法

三菱ケミカルのポリマー系分離剤は、ベースポリマー樹脂の組成により大きく3つのタイプに分類できます。用途に適した製品グレードは分離・精製の対象となる分子の性質に応じて以下のように選択されます。

- DIAION HP20、SEPABEADS SP20SS、MCIGEL CHP20シリーズは、中程度の疎水性をもつスチレン-DVB系樹脂であるため、新規精製プロセス検討における第１選択肢となります。
- SEPABEADS SP207及びその他の臭素化スチレン系樹脂は疎水性が高いため、高極性分子の精製に適しています。また、これらのグレードは比重が高く、取り扱いが容易です。
- DIAION HP2MGL及びその他のメタクリル系樹脂はスチレン系樹脂よりも疎水性が低いため、疎水性の高い分子の精製に適しています。また、これらは順相クロマトグラフィーによる油溶性分子の精製にも使用できます。

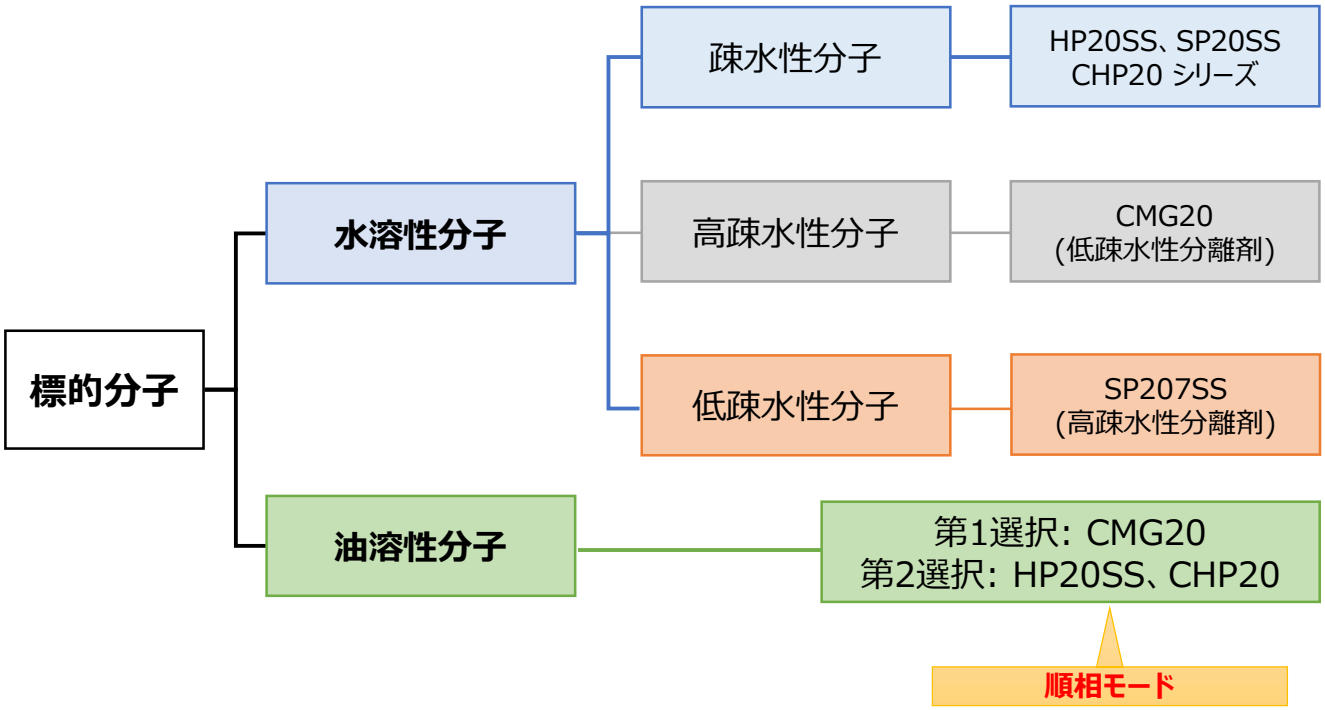
各タイプの製品は複数の粒径グレードを持つため、分離性能と圧力のバランスに合わせて選択できます。

化学構造	製品名	粒径 (μm)	適用用途
ポリスチレン系 （疎水性） 	HP20,HP21 SP825L,SP850 SP70、SP700	>250	セファロスポリン、バンコマイシン、 カルバペネムなどの抗生物質の抽出・分離 抗がん剤、免疫抑制剤の抽出・分離
	HP20SS SP20SS	120 70	バンコマイシン、ダプトマイシンの分画 抗がん剤、免疫抑制剤の分画
	CHP20 シリーズ	20,30,50	中分子ペプチド、オリゴ核酸の精密分離
修飾ポリスチレン系 （高疎水性） 	SP207	>250	セファロスポリンなどの抗生物質の抽出・分離 低疎水性低分子医薬の抽出・分離
	SP207SS	120	低疎水性低分子医薬の分画
	CHP07 (上市予定)	30	中分子ペプチド、オリゴ核酸の精密分離
メタクリル系 （低疎水性） 	HP2MGL	>250	抗がん剤(環状ペプチド)の抽出・分離 高極性医薬の抽出・分離
	HP2MGSS (上市予定)	120	高極性医薬の分画 油溶性医薬品の順相クロマト分画
	CMG20	30	中分子ペプチド(高分子)の精密分離 オリゴ核酸の精密分離

製品選択ガイド

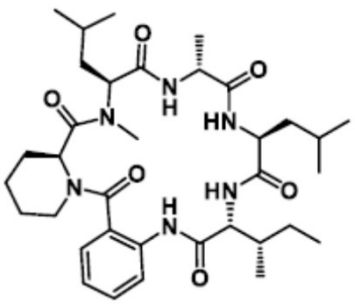
・標的分子の性質による選択

分離剤のタイプは、分離する標的分子の性質に基づいて選択できます。一般に、疎水性の高い分離剤は疎水性の低い(極性の高い)分子の分離に、疎水性の低い分離剤は疎水性の高い分子の分離に適しています。

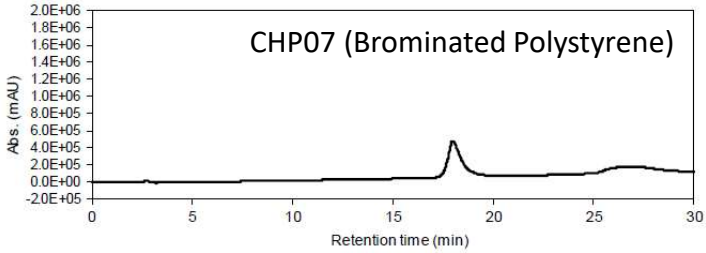


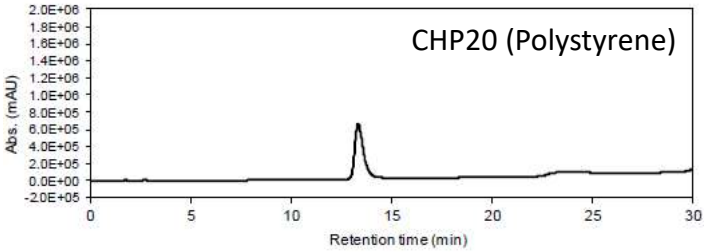
疎水性の異なる分離剤による環状ペプチドの保持時間比較

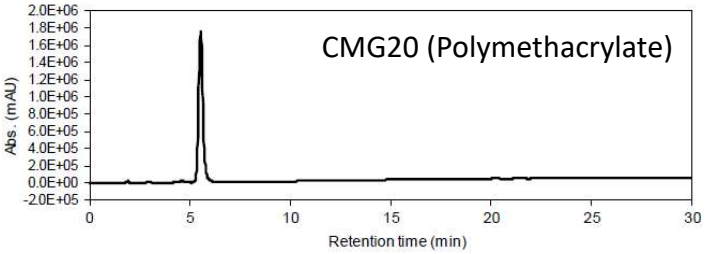
同じ分析条件下では、疎水性の高い分離剤はより長い保持時間を示します。



CF: $C_{35}H_{54}N_6O_6$
MW: 654.8530







Column: 4.6 mm I.D. x 250 mm
Temperature: 35℃
Flow rate: 1 mL/min
Solvent: 65-95% CH_3CN in H_2O, 20 min linear gradient

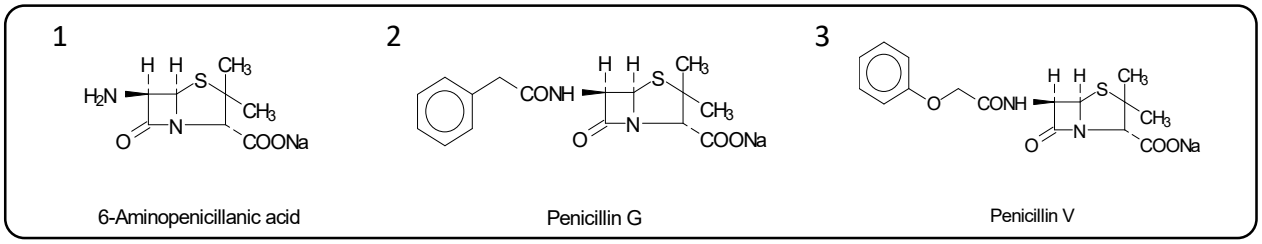
・粒子径による選択

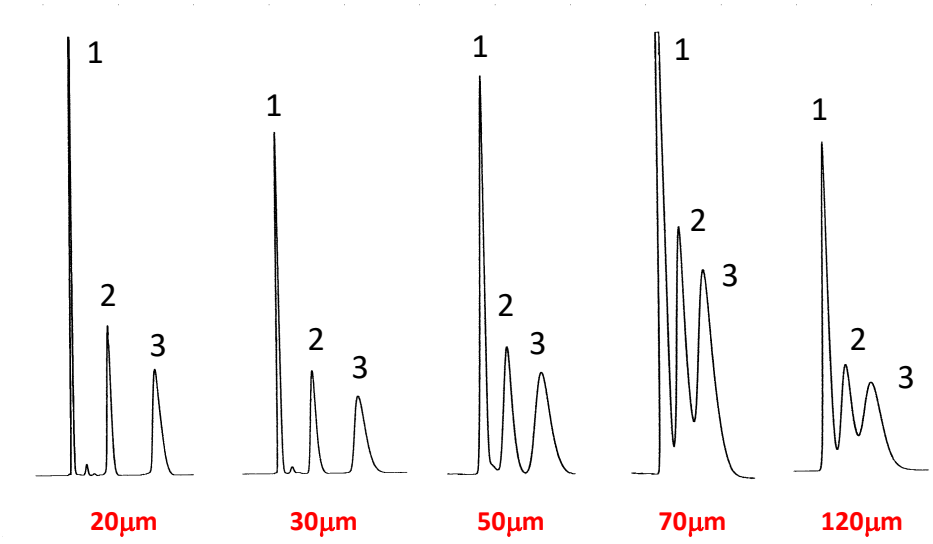
分離剤の粒径は、精製に必要な分離性能と圧力特性に基づいて選択できます。 一般に、小粒子は分離性能が高いため精密精製に、大粒子は低圧による大規模精製に適しています。

	小粒径 (20 - 50μm)	中粒径 (70 - 120μm)	大粒径 (250μm以上)
ポリスチレン系 (疎水性)	CHP20 (20, 30, 50μm)	SP20SS (70μm) HP20SS (120μm)	HP20, HP21. SP825L, SP850 SP70, SP700
修飾ポリスチレン系 (高疎水性)	CHP07 (30μm) (上市予定)	SP207SS (70μm)	SP207
メタクリル系 (低疎水性)	CMG20 (30μm)	HP2MGSS (120μm) (上市予定)	HP2MGL

異なる粒径のポリスチレン分離剤によるペニシリン誘導体の分離

以下の分離データは、同じ化学組成をもつポリマー系分離剤による3つのペニシリン誘導体の分離における粒径の影響を示しています。粒子が小さいほどピークがシャープになり、分離性能が向上します。



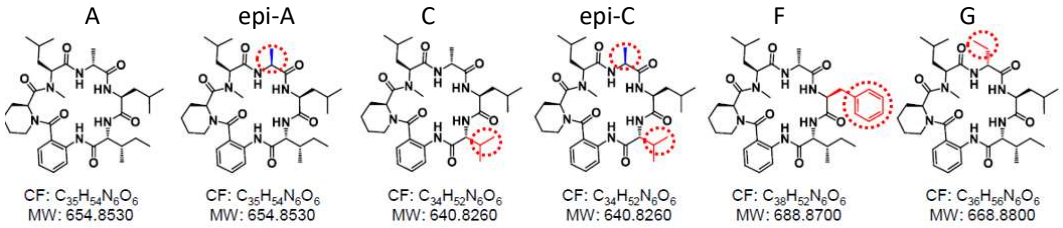


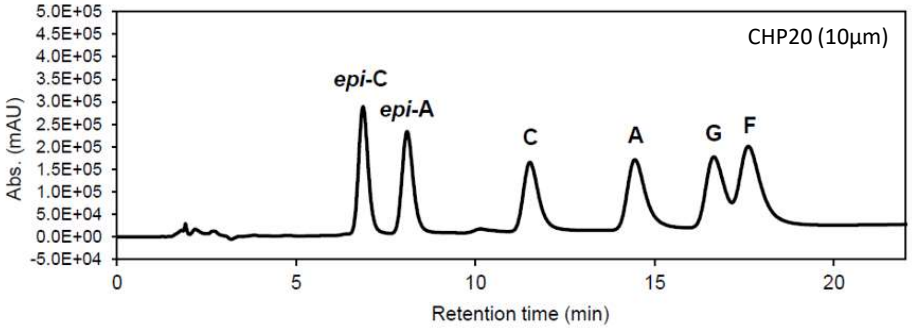
Column size: 250 x 10mm I.D., Eluent: MeOH/50mM phosphate (pH8.0) = 60/40
Flow rate: 2.18ml/min, Detection: UV 254nm
Samples: 1: 6-aminopenicillanic acid (1g/L)
2: penicillin G (1g/L)
3: penicillin V (1g/L).
Injection: 100μL

ポリマー系分離剤による分離例

環状ペプチド類縁体の分離

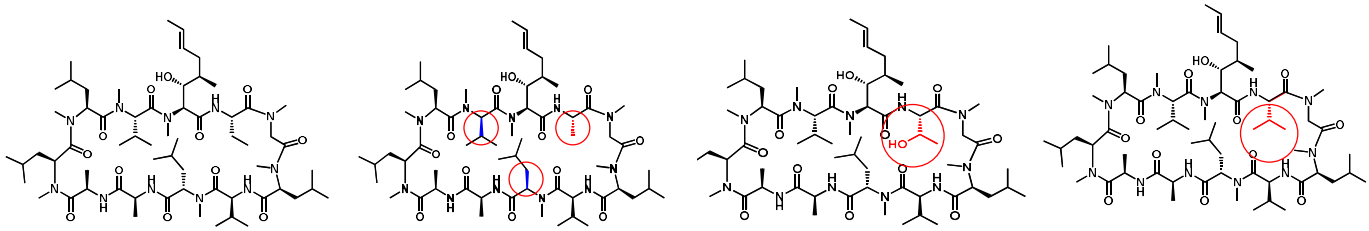
以下は化学構造が類似した6つの環状ペプチドをMCIGEL CHP20を用いて分離したデータです。分離移動相はアセトニトリル／水を使用しています。



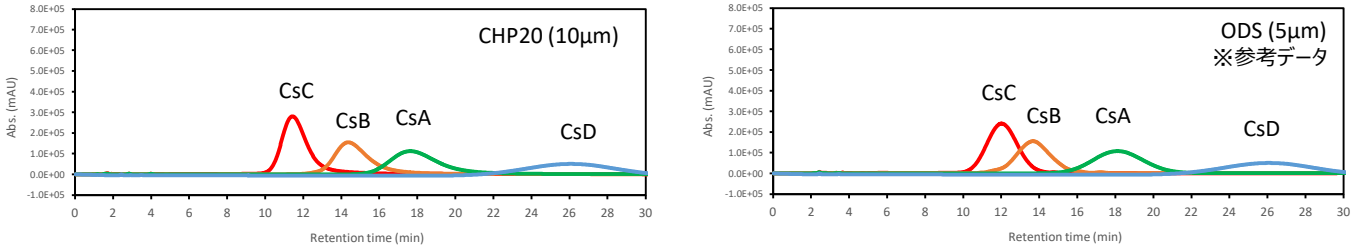


ポリマー系分離剤のサンプル負荷耐性

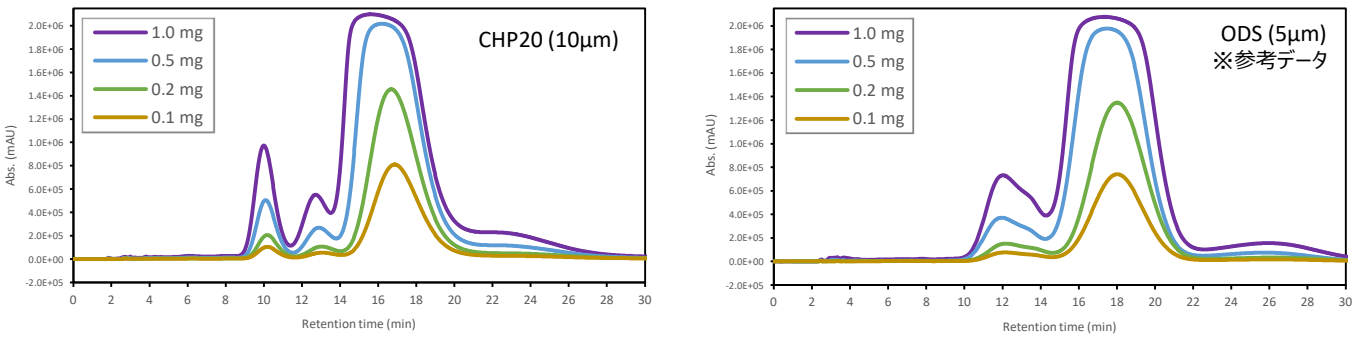
ODSシリカ分離剤と比較して、CHP20はシクロスポリン類縁体の分離において高いサンプル負荷条件でも良好な分離性を維持します。



低負荷条件 (サンプル注入量：CsA, CsB, CsC, CsD　各0.01mg)



高負荷条件 (サンプル注入量: 合計 0.1, 0.2, 0.5, 1.0 mg (サンプル組成比: CsA 90%, CsB 3.3%, CsC 3.3%, CsD 3.3%))



Column: 4.6 mm x 250 mm, Temp: 35℃, Flow rate: 1 mL/min, Detection: 214 nm
Solvent: (CHP20) 69% CH_3CN in H_2O, 30 min isocratic elution, (ODS) 75% CH_3CN in H_2O, 30 min isocratic elution

MITSUBISHI CHEMICAL

・Product List of Polymeric Separation Media

DIAION™	HP20, HP21, HP2MGL, HP20SS
SEPABEADS™	SP825L, SP850, SP70, SP700, SP207, SP20SS, SP207SS
MCIGEL™	CHP20/P20, CHP20/P30, CHP20/P50, CMG20/P30

[Affiliates and Distributor]

<Japan, Asia>
Mitsubishi Chemical Aqua Solutions Co., Ltd. (Tokyo)
www.mcas.co.jp E-mail: info@mcas.co.jp

<Europe, Africa, Middle East>
Resindion S.R.L. (Milan)
www.resindion.com E-mail: info@resindion.com

<Taiwan, China, SEA>
Tai-Young Chemical Co, Ltd. (Kaohsiung)
E-mail: service@diaion.com.tw

<China>
Mitsubishi Chemical (China) Co., Ltd (Shanghai)
E-mail: MCCN-DG-MCN_SH_AQUA@mchcgr.com

<India, Middle East>
Mitsubishi Chemical India Pvt. Ltd. (Haryana)
www.mc-india.co.in
E-mail: mcap-mbx-mcc_india_info@mchcgr.com

<N & S America>
Itochu Chemicals America Inc. (New York)
www.itochu-ca.com
E-mail: s&ptsales@itochu-ca.com

<Others>

Mitsubishi Chemical Corporation
www.diaion.com E-mail: #MCC-HO-DIAION-HP@m-chemical.co.jp