

Efficient Preparative Peptide Purification Using A Novel Dynamic Axial Compression System



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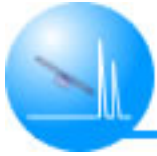
YMC CO., LTD. ¹

SEPARATION TECHNOLOGY

Kyoto, Japan

Seika Corp. of America ²

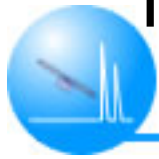
Bethlehem, PA, USA



Introduction

Reversed-phase HPLC is an invaluable tool also for the analytical and preparative separation of peptides and proteins. Owing to the availability of different pore sizes and particle sizes, the alkyl-bonded silica gel products are economically the first choice for both analytical and preparative separations.

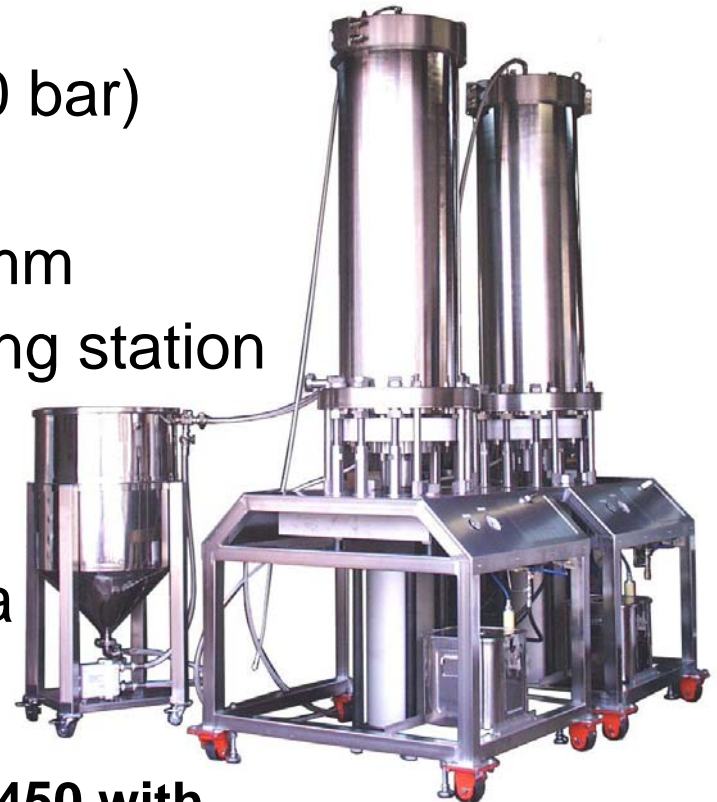
In purification of peptides and proteins, dynamic axial compression system is a very useful tool for purifying compound. Using the DAC column, mixtures are easily fractionated by changing the separation conditions. (e.g. packing material, column length, column volume, etc) We reported previously that choosing the optimum pore size of the gel gave good peak shape and separation in analytical scale. Based on these results, we applied the analytical conditions to preparative/process scale separations. Effective separation was obtained similar to the analytical separation of peptides and proteins.



New Dynamic Axial Compression Column

- **DAU series** -

- Easily packing and unpacking *via* inlet and outlet tube
- Unit is available for high (70 to 100 bar) pressure
- Column diameter extends to 600 mm
- Compact design with built in packing station
- Dynamic axial bed compression yields densely packed beds
- Recommended media: RPC media



**DAU- 450 with
slurry container**



DAU Products line

DAU-500



DAU-300



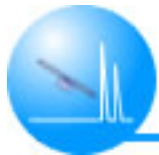
DAU-200



DAU-50

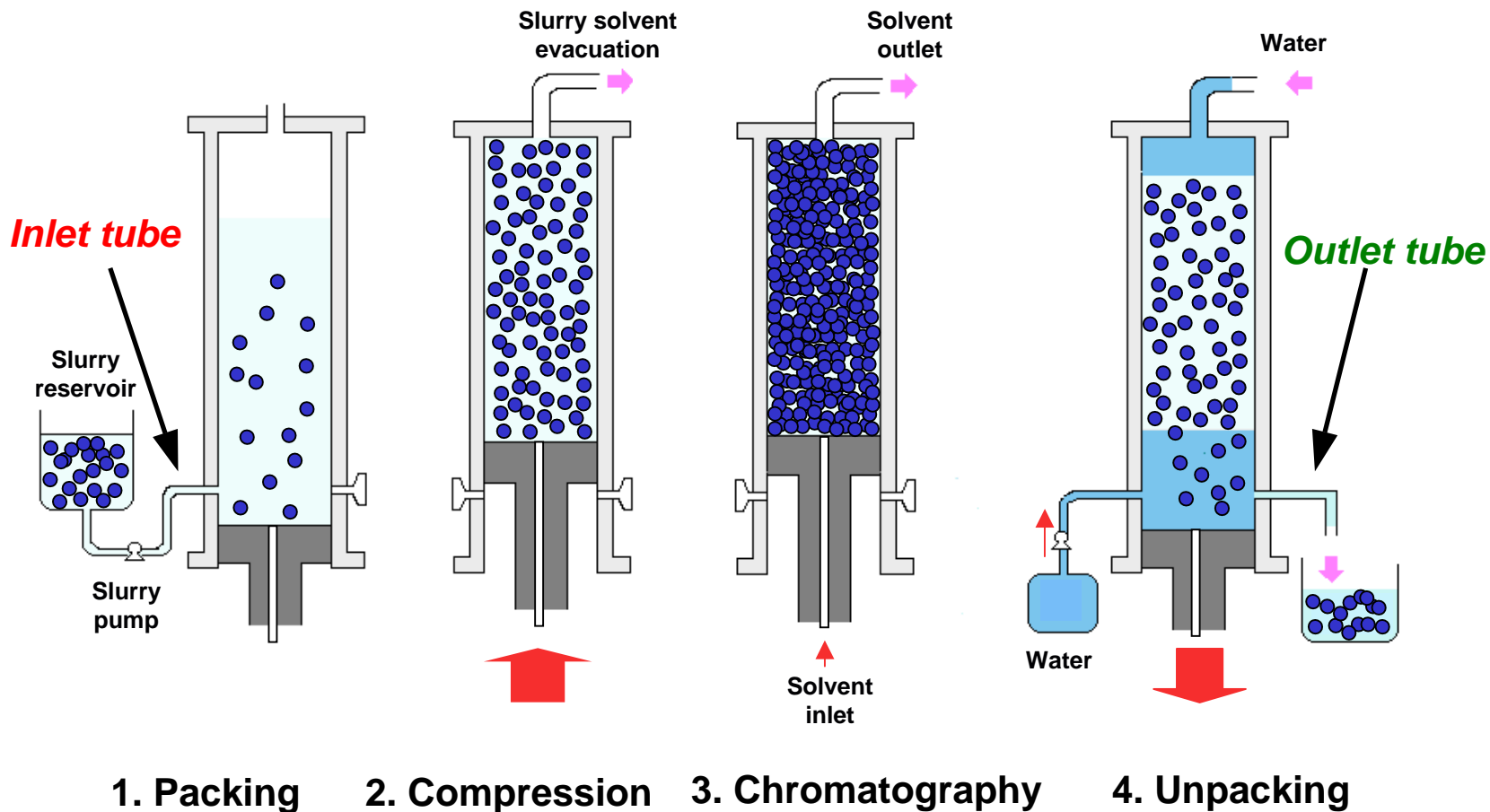
100 bar

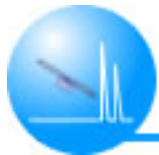
70 bar



Packing Steps for a DAU column

- Easily packing and unpacking *via inlet and outlet tube*





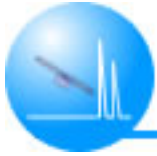
Optimum packing conditions for 50 mmI.D. DAU column

Packing conditions

Packing material	: YMC * GEL ODS-A 15 μ m, 120 Å
Weight of packing material	: 250 g
Column size	: 200 \times 50 mmI.D.
Packing pressure	: 6.4 MPa
Slurry solvent	: 100% methanol
Concentration of slurry	: 35%

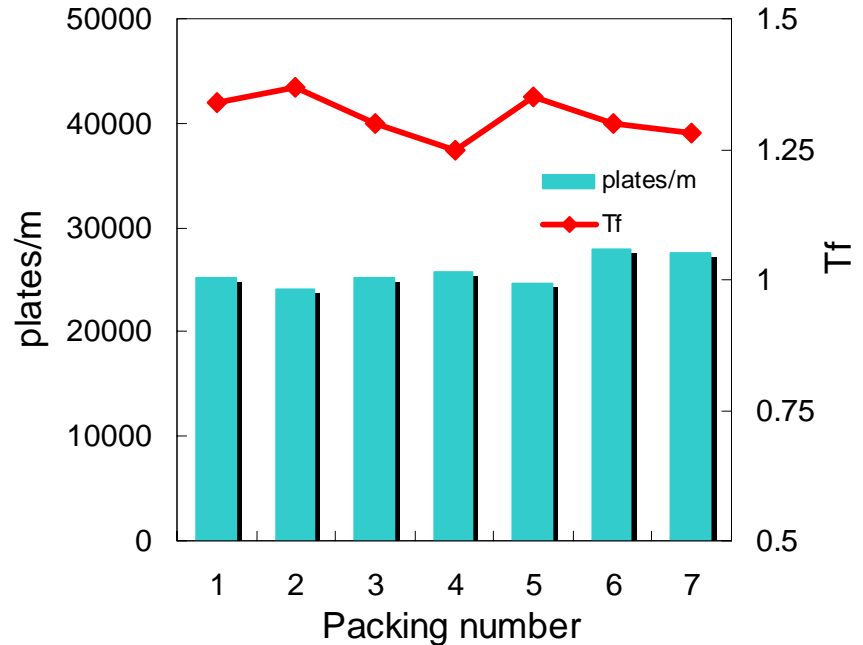
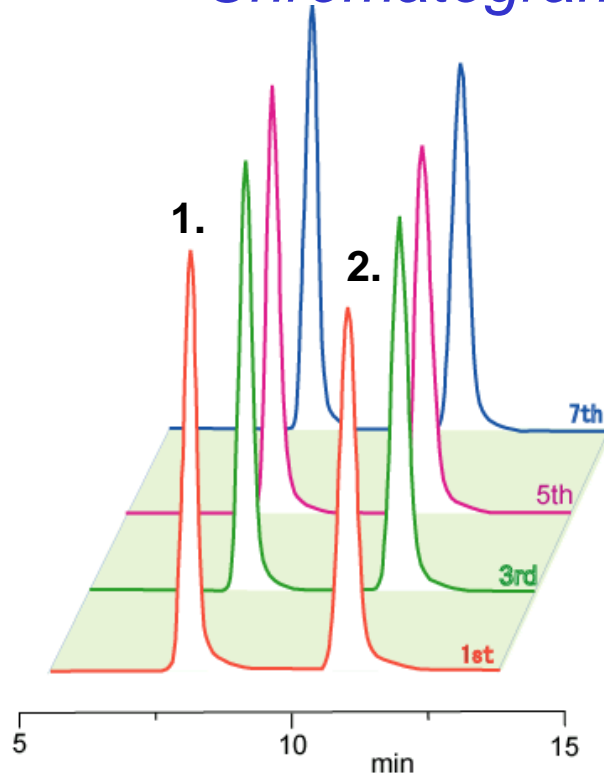
HPLC conditions for inspection of packing procedure

Sample	: 1. toluene 2. methyl benzoate
Flow rate	: 50 mL/min
Temperature	: ambient
Detection	: UV at 254 nm
Eluent	: methanol / water (85/15)

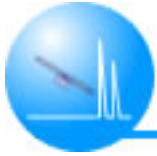


Reproducibility of repacking the gel

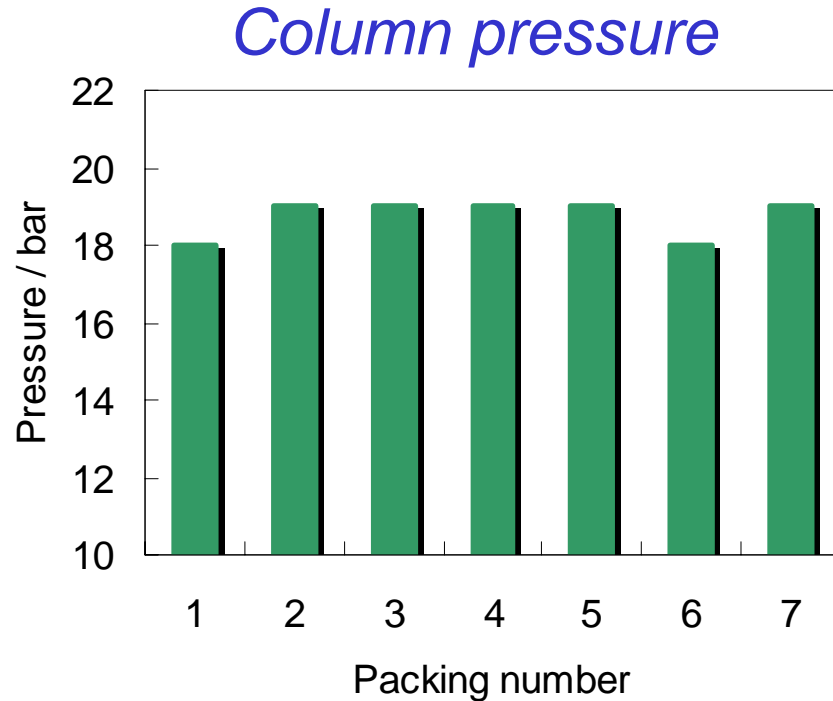
Chromatograms and data of inspection



- By using a 50 mmI.D. DAU column, repacking procedure was attempted. After 7 times repacking, plates/m and Tf are still good as the initial state.

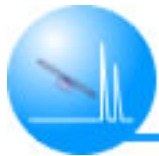


Mechanical stability of the gel



Eluent: methanol/water (85/15), Flow rate: **150 mL/min**

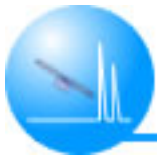
- After 7 times repacking, column pressure is almost same as initial state. It would be no formation of fines. This shows the gel is stable under the flow rate.
- High mechanical stability ensures longer lifetime of the gel.



Peptide and Protein purification by reversed phase silica gel

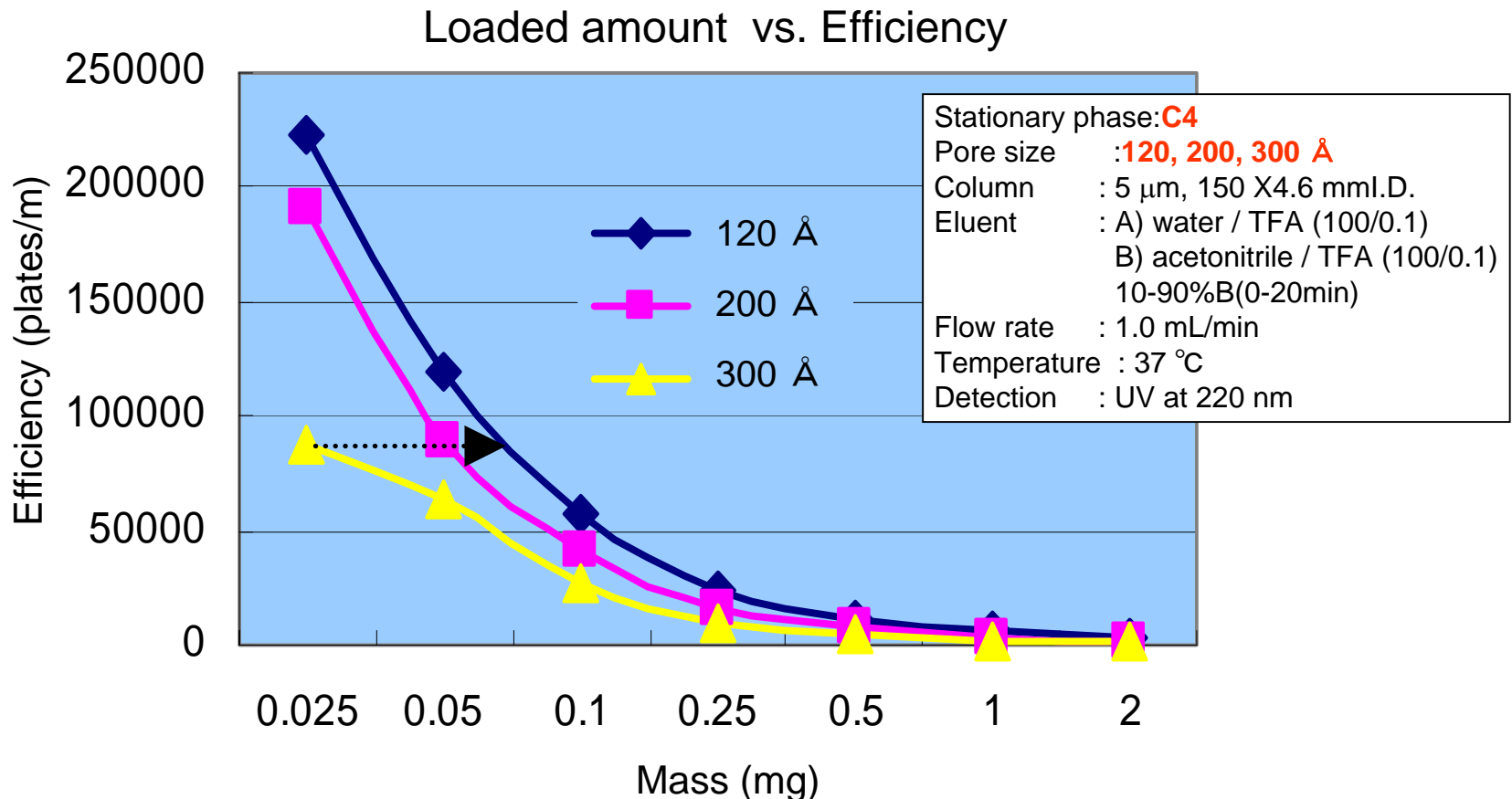
Small organic molecules are retained/eluted by a distribution mechanism. On the other hand, peptides and proteins are retained/eluted by an adsorption-desorption (on-off) mechanism. Due to this mechanism, the pore size plays a key role in determination of the resolution and loading amount in the separation of peptides and proteins.

Based on the results in analytical separation as we reported previously, we attempted to separate peptides and proteins in preparative conditions. This study shows scalability of the separation using DAU column.

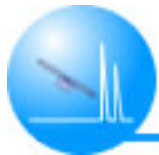


Impact of Pore Size on Efficiency (1)

Angiotensin II (MW 1046) *in analytical*

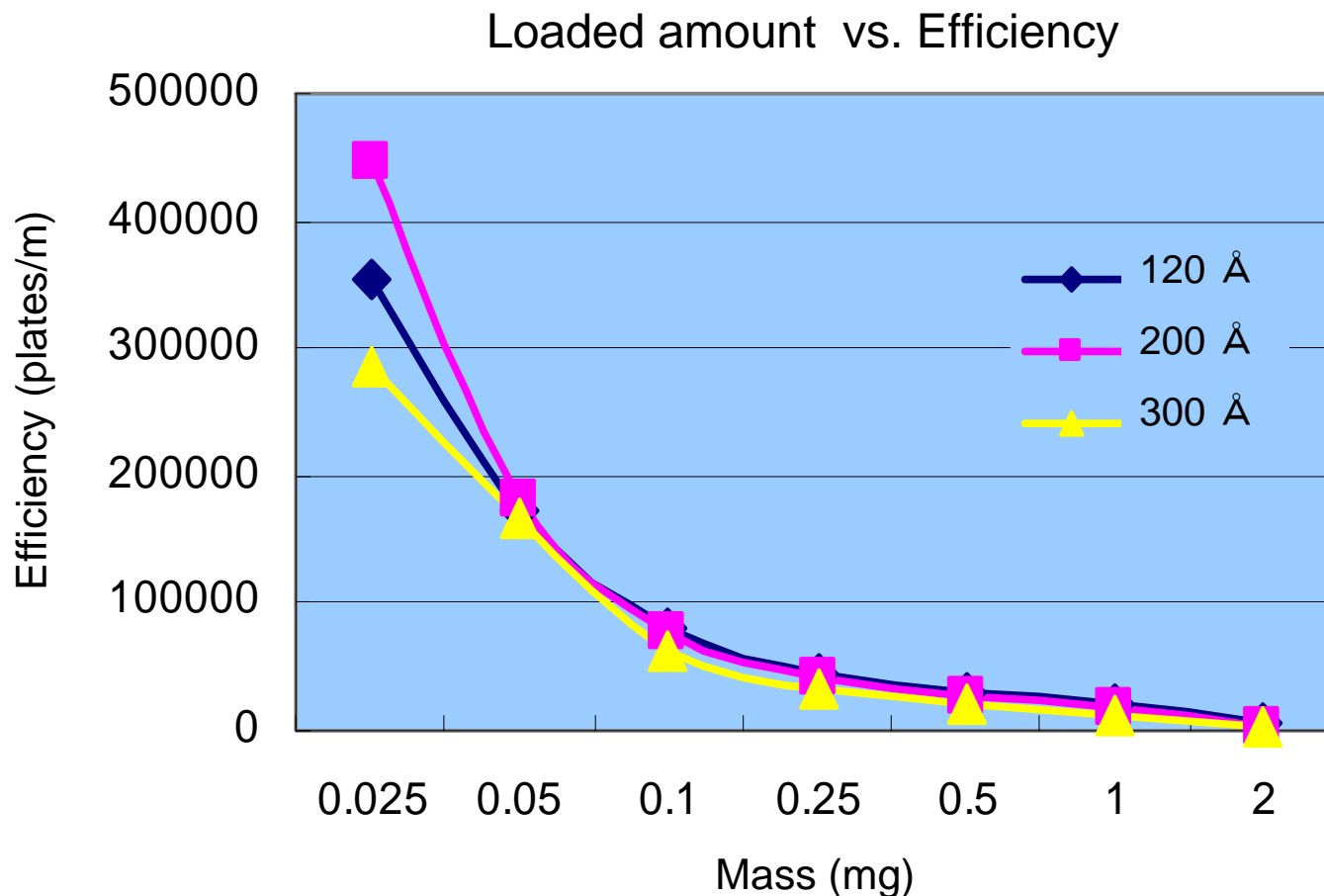


- **120 Å** pore size is most efficient at all the loading levels.
- **120 Å** pore size enables a threefold loading level compared with 300 Å pore size. (.....▶)

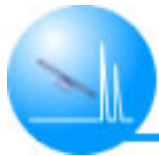


Impact of Pore Size on Efficiency (2)

Insulin (MW 5700) *in analytical*



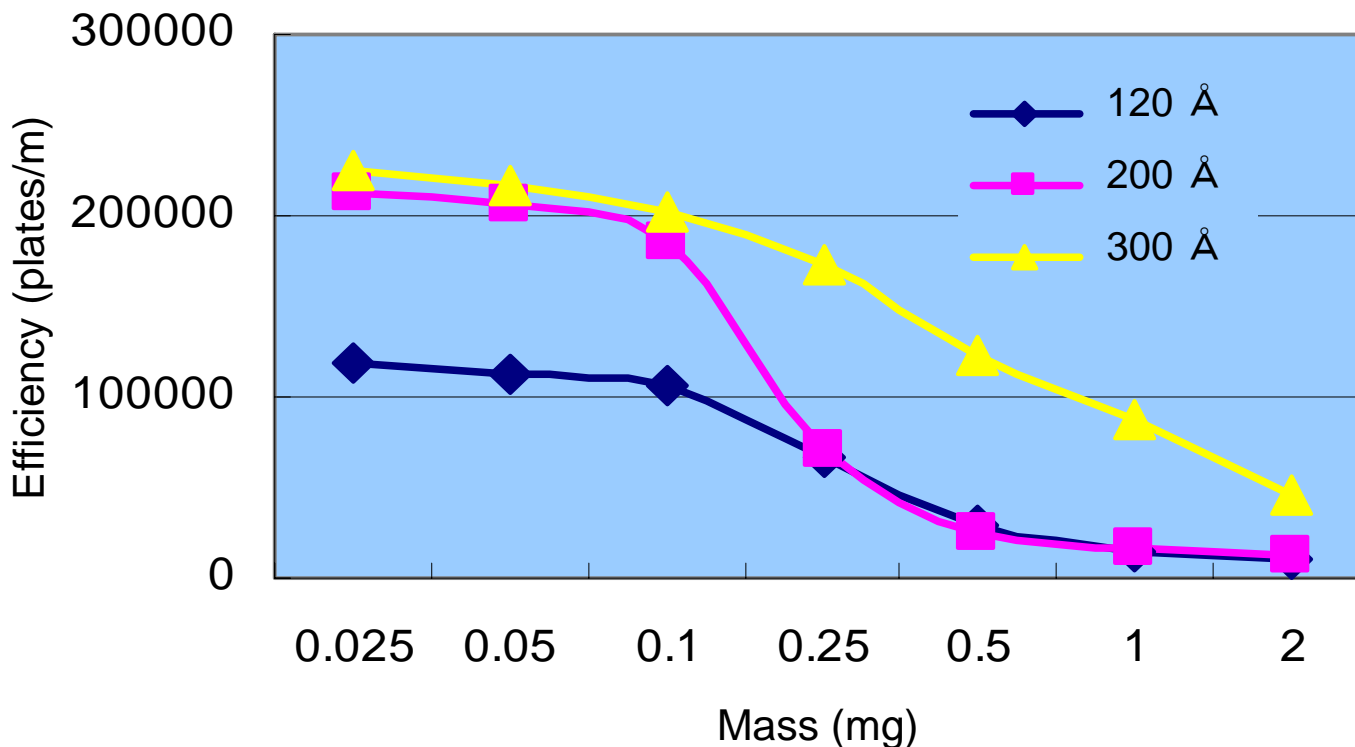
- **200 Å** pore size is the best choice for samples up to the 0.1 mg loading level.



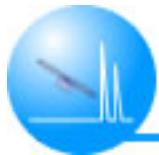
Impact of Pore Size on Efficiency (3)

Ovalbumin (MW 45000) *in analytical*

Loaded amount vs. Efficiency



- **300 Å** pore size is most efficient at all the loading levels.
- At low loading levels, **200 Å** pore size also shows good efficiency.

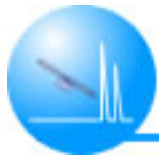


Optimized stationary phase for separation

MW		C18	C8	C4
 5000 20000 100000	120 Å	⊙	○	△
	200 Å	○	⊙	○
	300 Å	△	○	⊙

⊙ : excellent
○ : good
△ : moderate

- C18 column with 120 Å pore size is suitable for small peptides up to MW 5000, similar to the analyses of ordinary small molecules. In the case of large peptides or small proteins up to MW 20000, the C8 column with 200 Å pore size gives the best efficiency. Most of proteins are eluted effectively by C4 column with 300 Å.

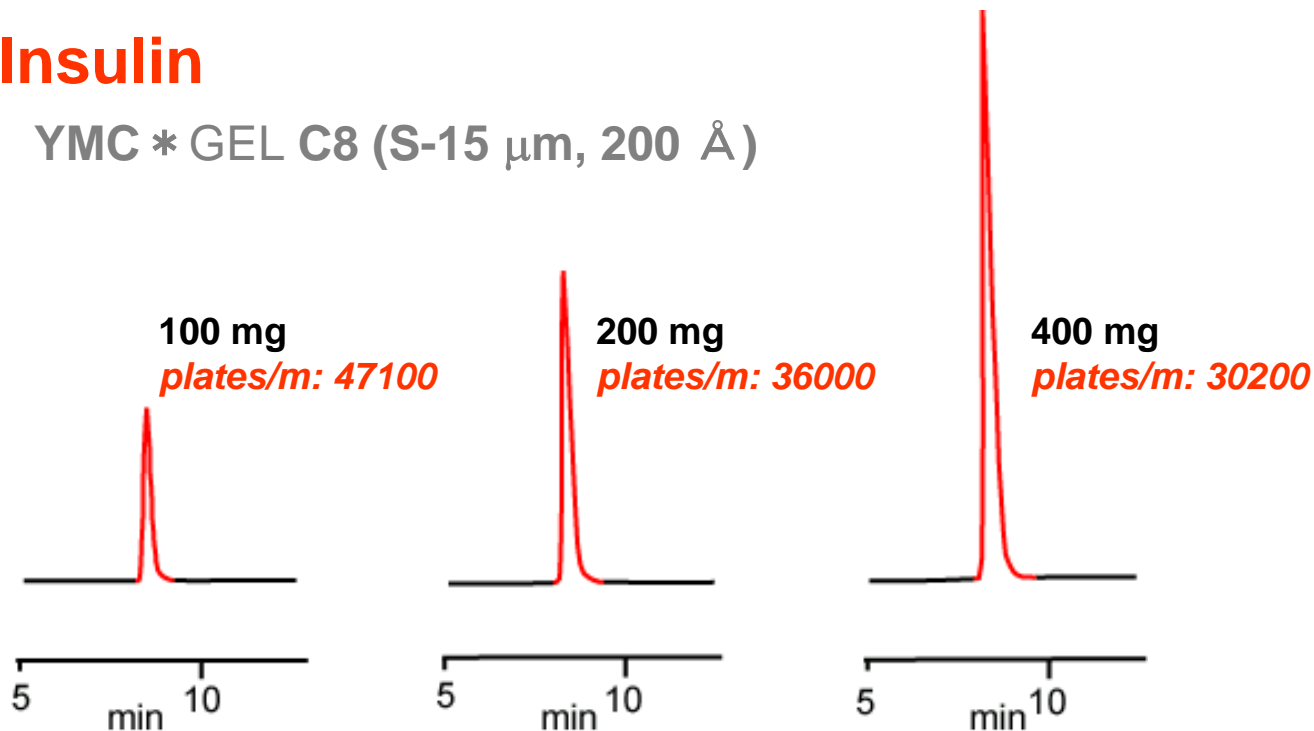


Loadability (1)

Insulin (MW 5700) *in preparative*

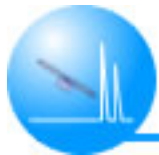
Insulin

YMC * GEL C8 (S-15 μm , 200 \AA)



Column	: 150 X50 mmI.D.
Eluent	: A) water / TFA (100/0.1) B) acetonitrile / TFA (100/0.1), 10-90%B(0-20min)
Flow rate	: 100 mL/min, Temperature: ambient, Detection: UV at 220 nm

- Up to 400 mg loaded amount, peak shapes are sharp as the analytical separation.

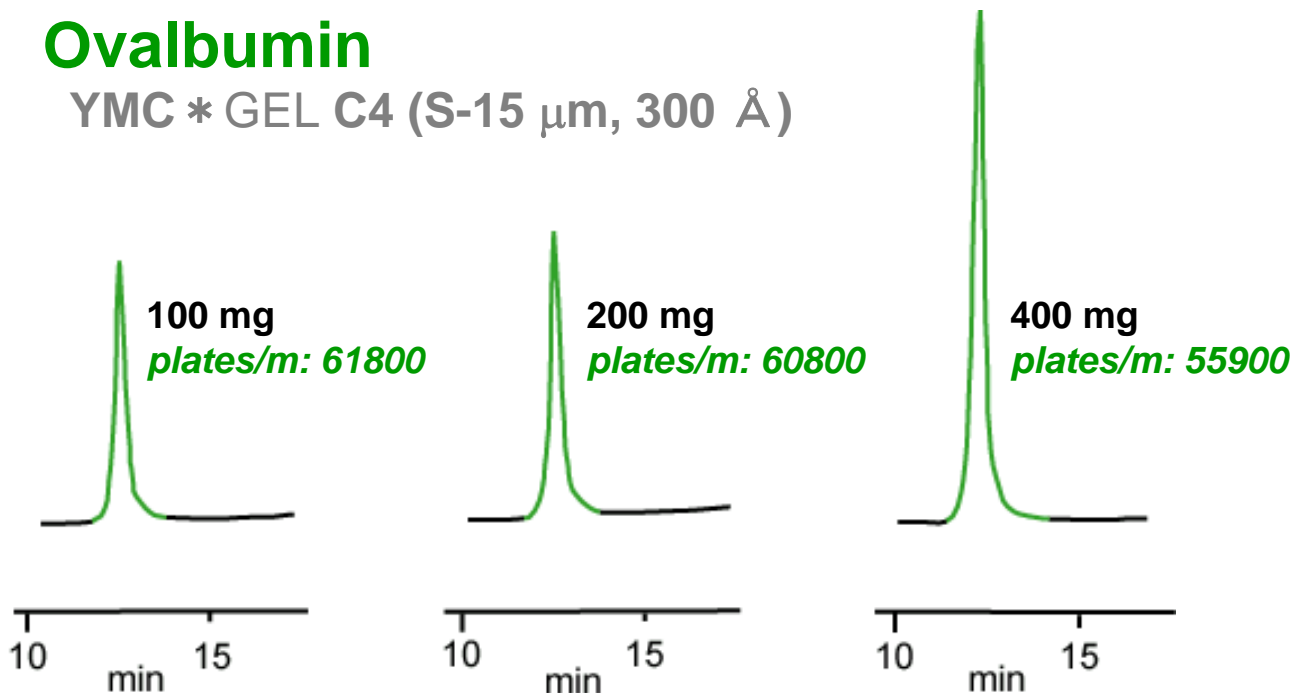


Loadability (2)

Ovalbumin (MW 45000) *in preparative*

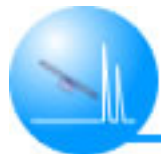
Ovalbumin

YMC * GEL C4 (S-15 μm , 300 \AA)

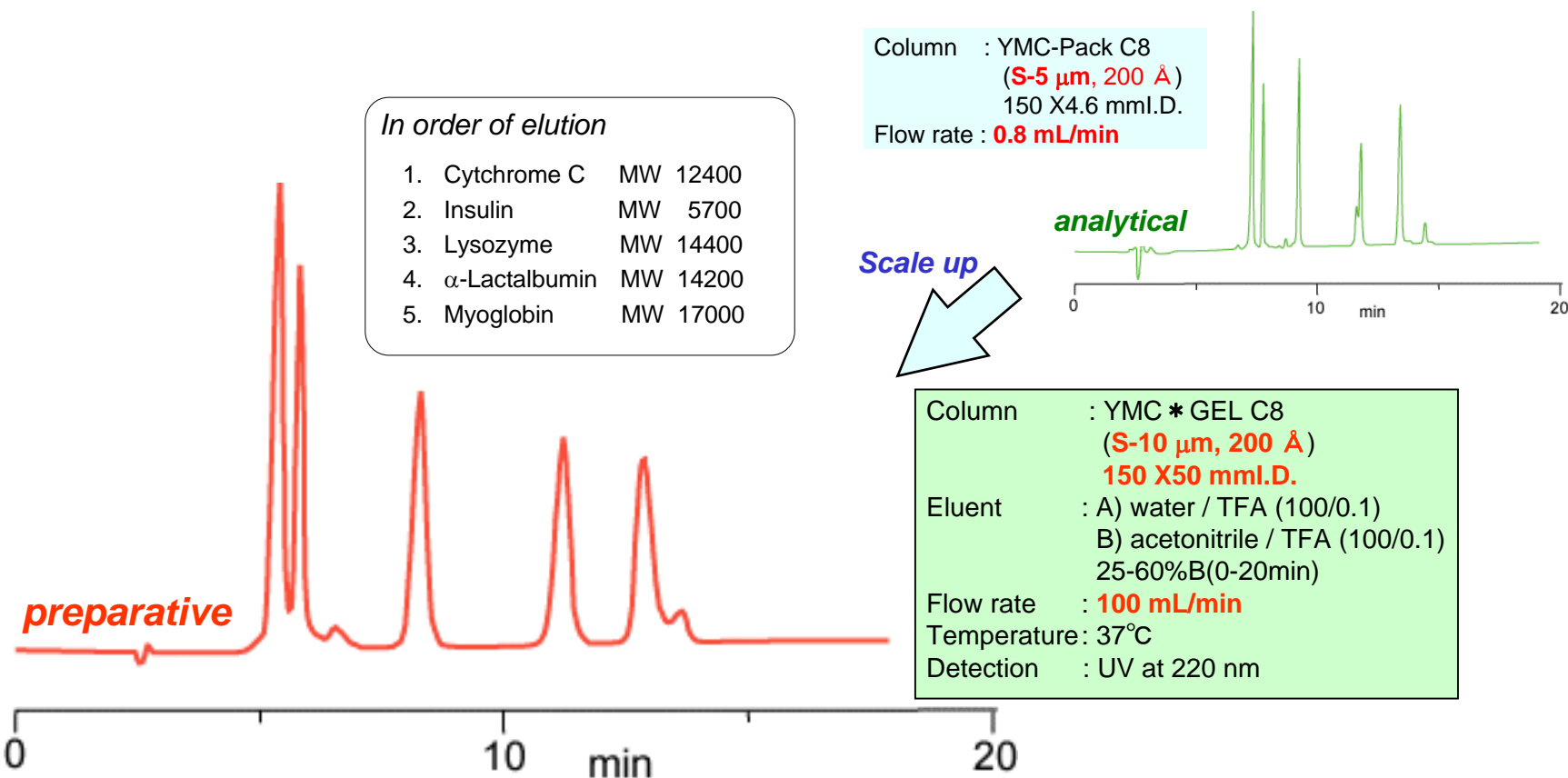


Column	: 150 X50 mml.D.
Eluent	: A) water / TFA (100/0.1) B) acetonitrile / TFA (100/0.1), 10-90%B(0-20min)
Flow rate	: 100 mL/min, Temperature: ambient, Detection: UV at 220 nm

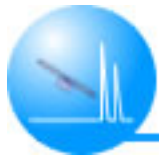
- At all loading levels, high efficiency was obtained.



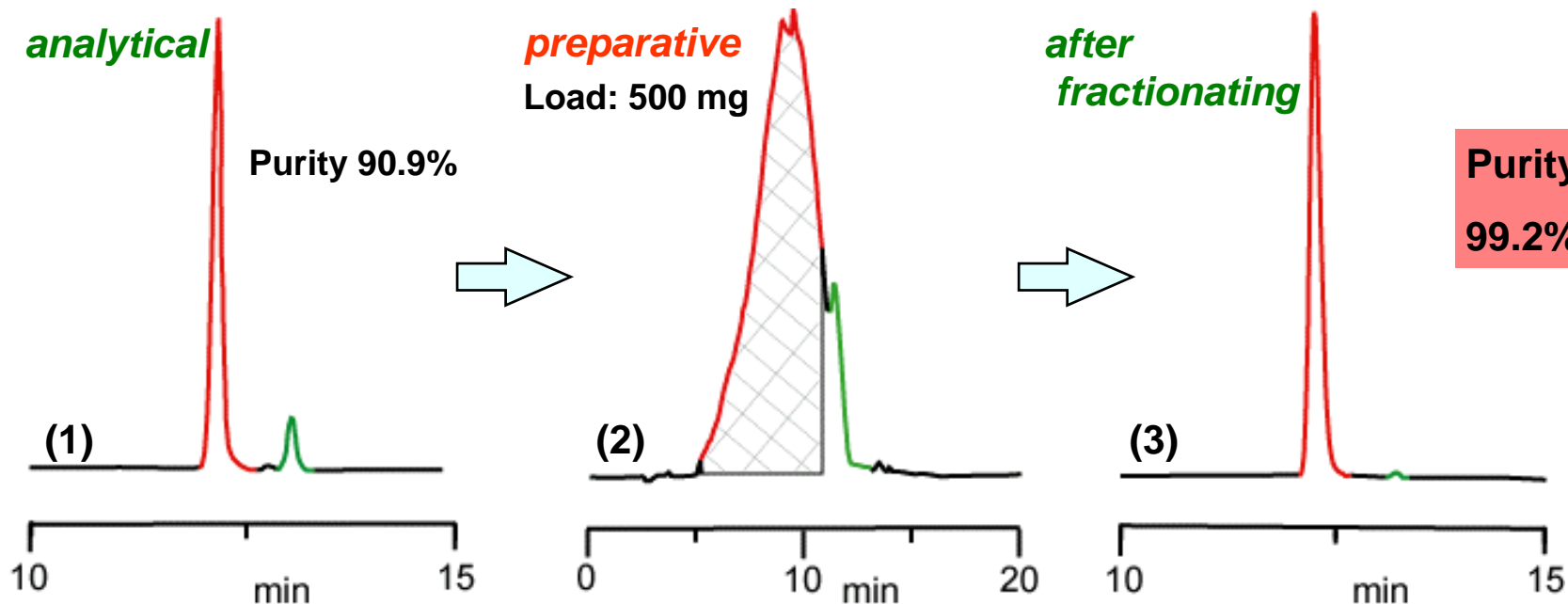
Scale up from analytical to preparative



- It is possible to scale up from analytical to preparative.
- Each samples are separated in preparative conditions, similarly to analytical scale.



Preparative separation of insulin (1)



Analytical conditions (1), (3)

Column : YMC-Pack C8
(S-5 μm , 200 \AA)
150 X4.6 mm I.D.
Eluent : A) water / TFA (100/0.1)
B) acetonitrile / TFA (100/0.1)
27%B (0-5min)
27-40%B (5-20min)
Flow rate : 0.8 mL/min
Temperature: ambient
Detection : UV at 220 nm

Preparative conditions (2)

Column : YMC * GEL C8
(S-10 μm , 200 \AA)
150 X50 mm I.D.
Eluent : A) water / TFA (100/0.1)
B) acetonitrile / TFA (100/0.1)
27%B (0-5min)
27-40%B (5-20min)
Flow rate : **100 mL/min**
Temperature: ambient
Detection : UV at 220 nm

Sample: **insulin bovine** /
insulin human = 10/1(w/w)

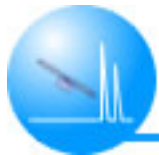


Preparative separation of insulin (2)

Loadability of insulin

<i>Loaded amount / mg</i>	<i>Purity / %</i>	<i>Recovered amount / mg</i>	<i>Recovery / %</i>
500	99.2	441	88.9
700	99.3	561	80.7

- Choosing the optimum gel, high purity and high yield of recovered insulin was obtained.
- Effective separation was obtained similarly to the analytical separation. It could be scaled up to gram scale purification.



Summary

- After 7 times repacking procedure, peak shapes and column performance are as good as the initial state.
- In preparative scale, it is also important to choose the optimum pore size and the right ligand to achieve optimal separation of peptides or protein, similarly to analytical separation.
- You can use a wide variation of gels from YMC Co. Ltd. [e.g. three pore sizes (120, 200, 300 Å), several particle sizes, several ligand groups] We also offer several dimensions of dynamic axial compression systems.