A New Dynamic Axial Compression System for the Purification of Peptides and Proteins

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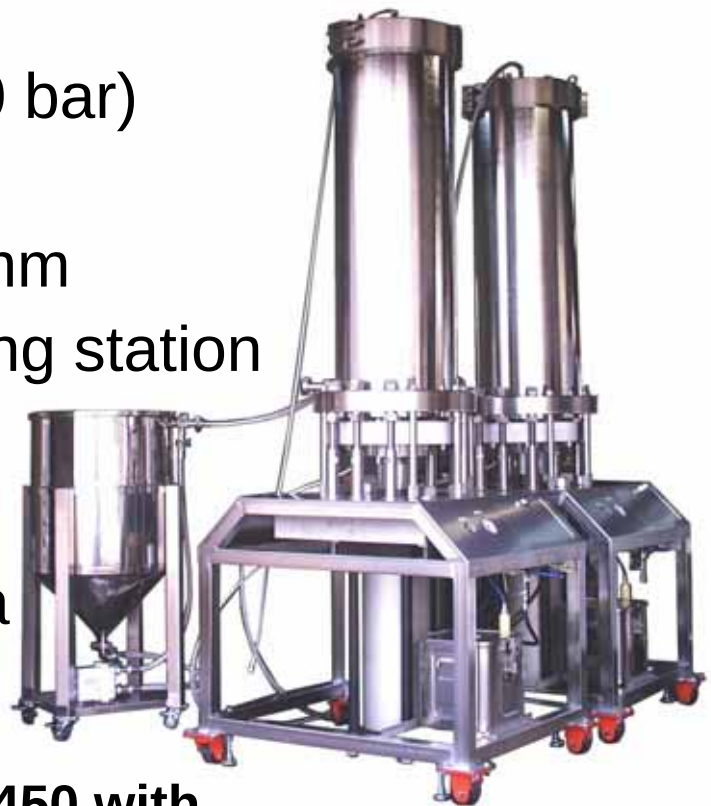
Introduction

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 the most useful method to purify the compound. Using the DAC column, it is easily fractionated by changing the separation conditions. (e.g. packing material, column length, column volume, etc) We reported that optimum pore size of gel gave good peak shape and separation in analytical scale. Based on the results, we referred the analytical conditions to preparative/process scale separation. Effective separation was obtained similarly 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-300

DAU-500

100 bar

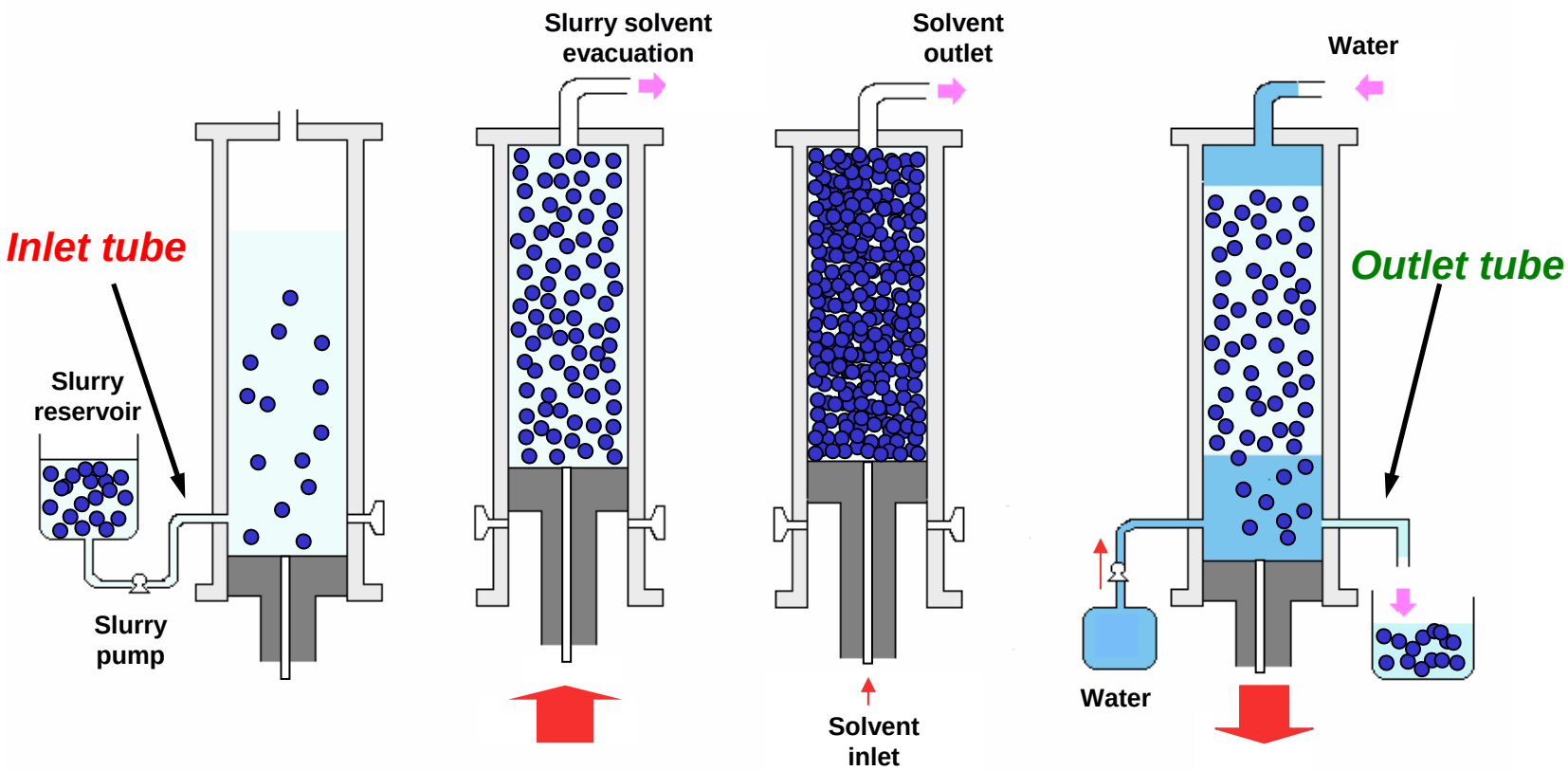
DAU-50

DAU-200

70 bar

Packing Steps for a DAU column

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



1. Packing **2. Compression** **3. Chromatography** **4. Unpacking**

Optimum packing conditions for 50 mmI.D. DAU column

Packing conditions

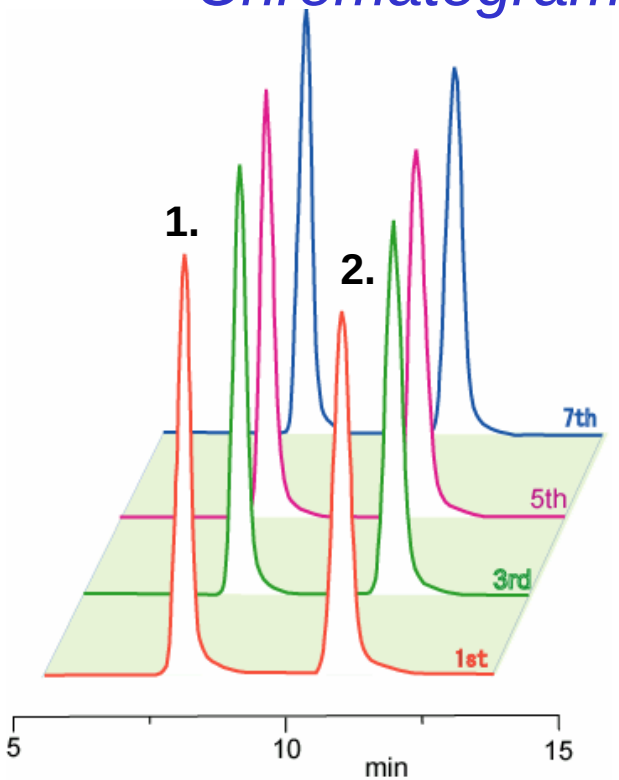
Packing material	: YMC∗GEL ODS-A 15 μm, 120 Å
Weight of packing material	: 250 g
Column size	: 200✕50 mmI.D.
Packing pressure	: 6.4 MPa
Slurry solvent	: 100% methanol
Concentration of slurry	: 35%

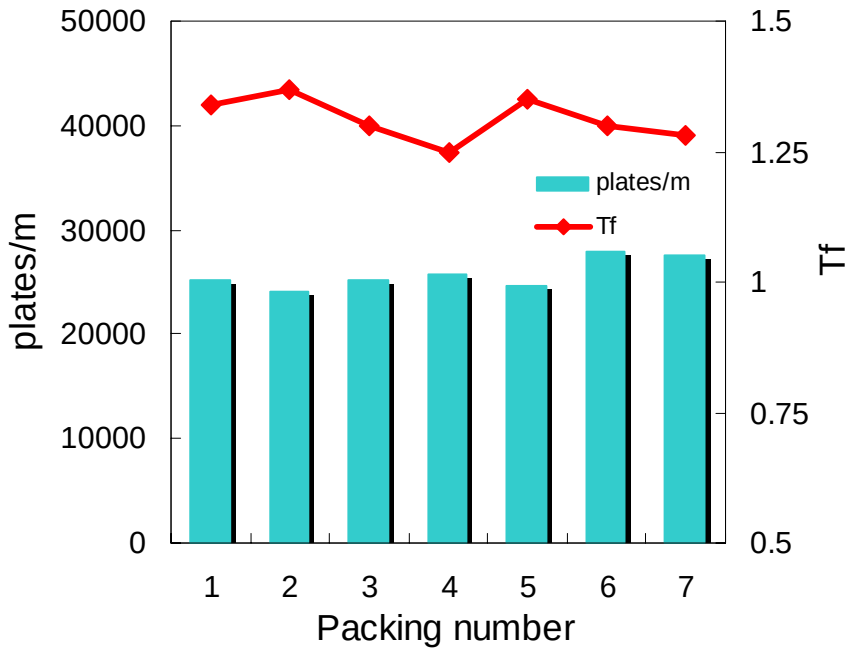
HPLC conditions for inspection of packing procedure

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

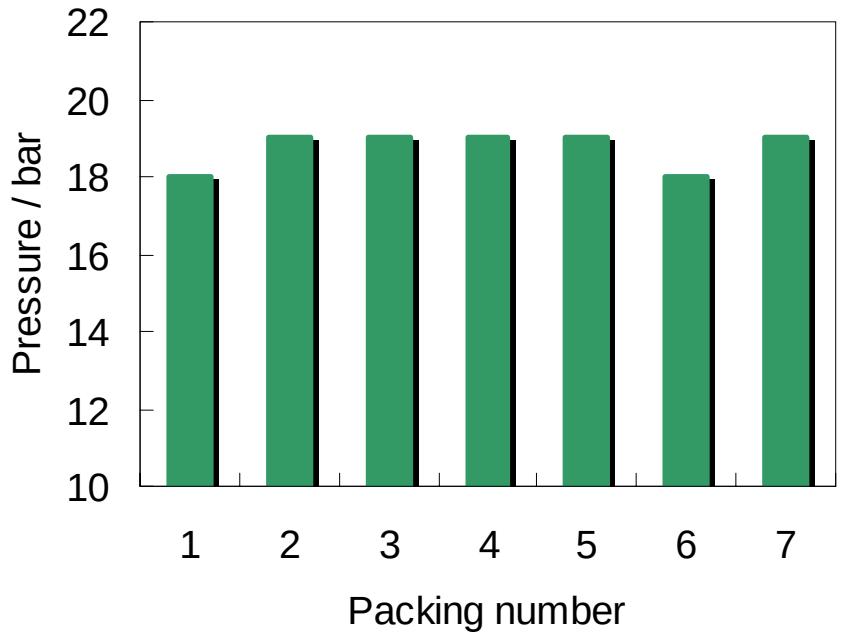




- **By using a 50 mmI.D. DAU column, repacking procedure was attempted. After 7 times repacking, plates/m and Tf are still good as the initial state.**

Mechanical stability of the gel

Column pressure



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.**

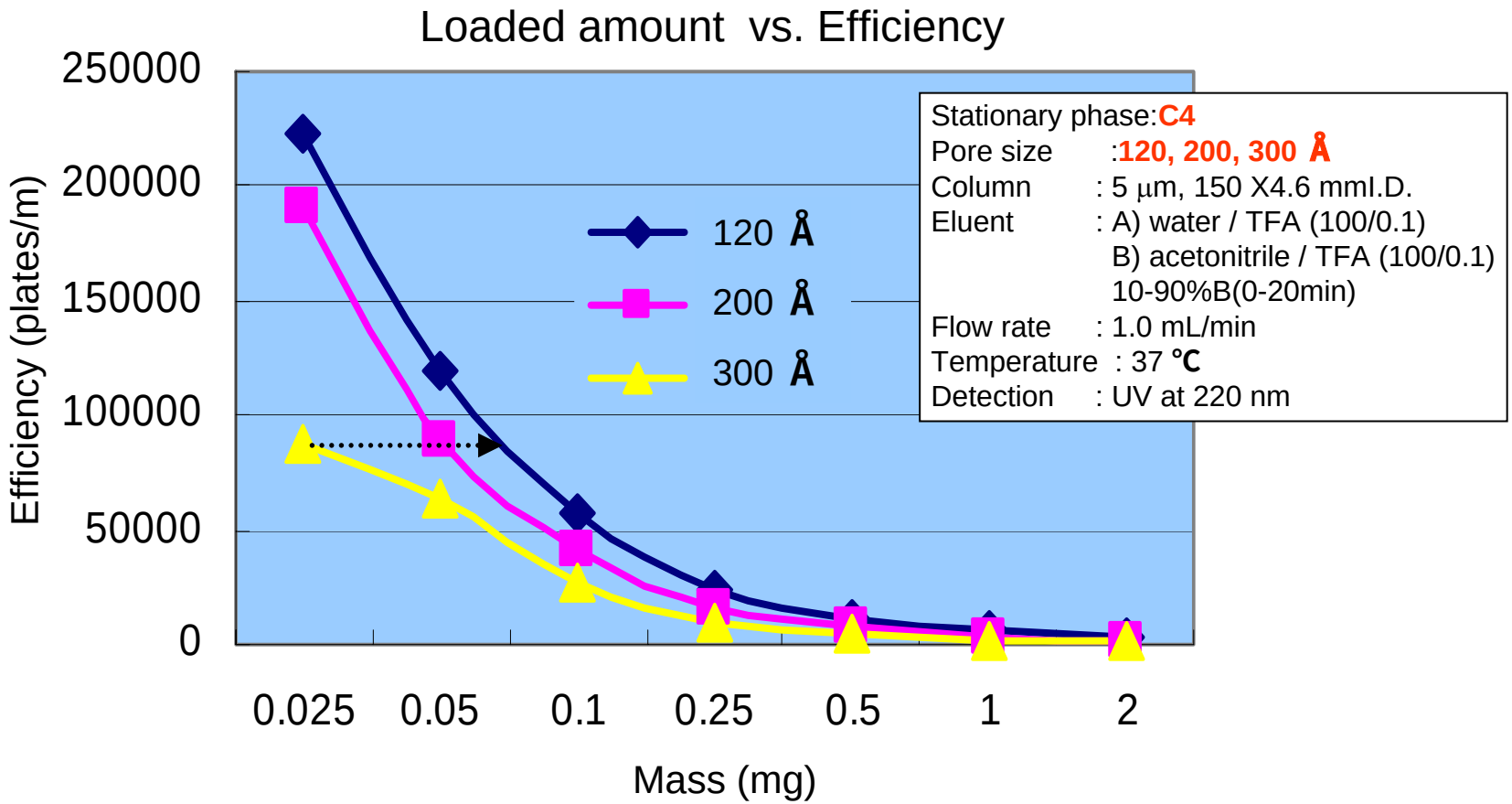
Peptide and Protein purification by reversed phase silica gel

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 resolution and loading amount in 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)

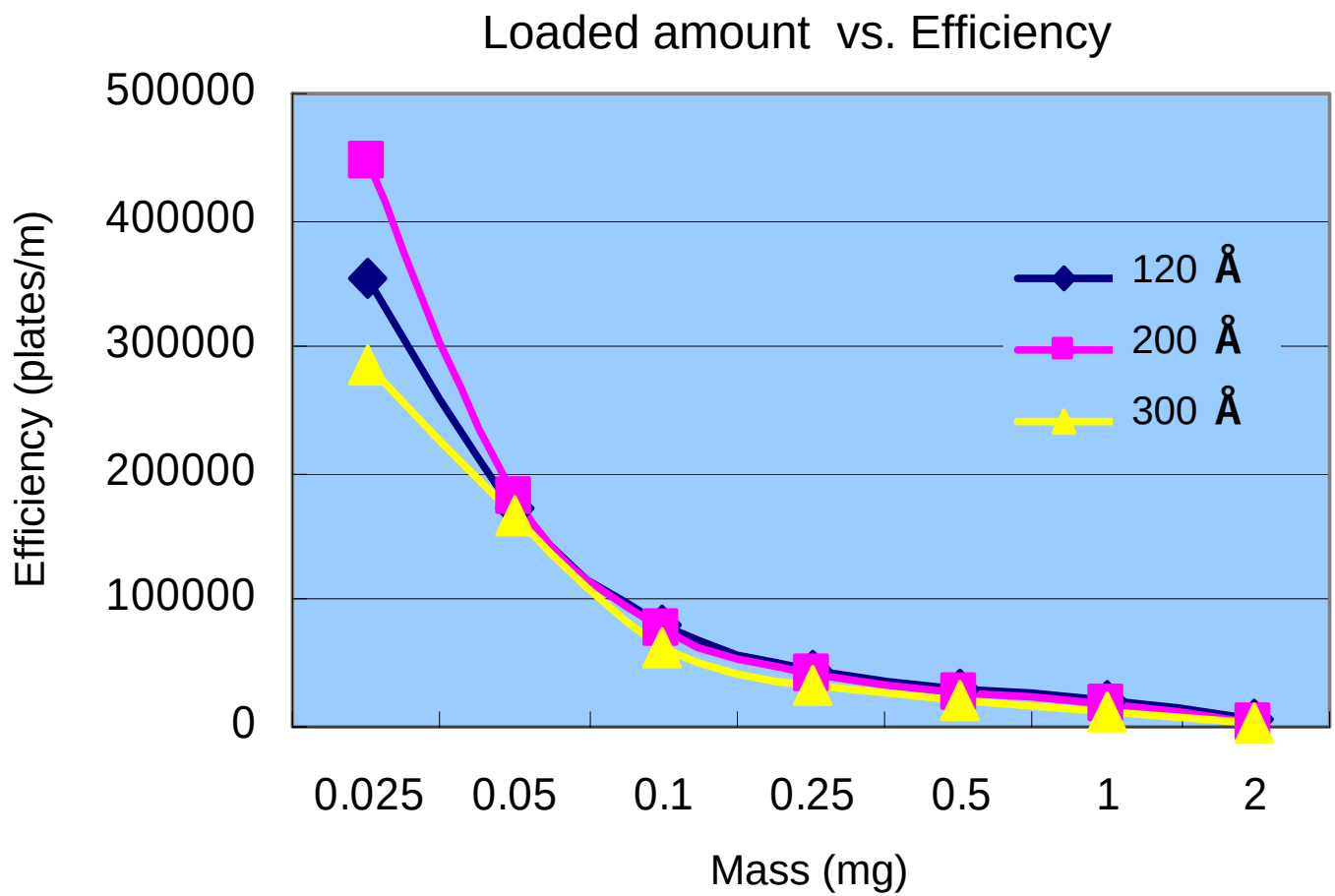
Angiotensin II (MW 1046) *in analytical*



- **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)

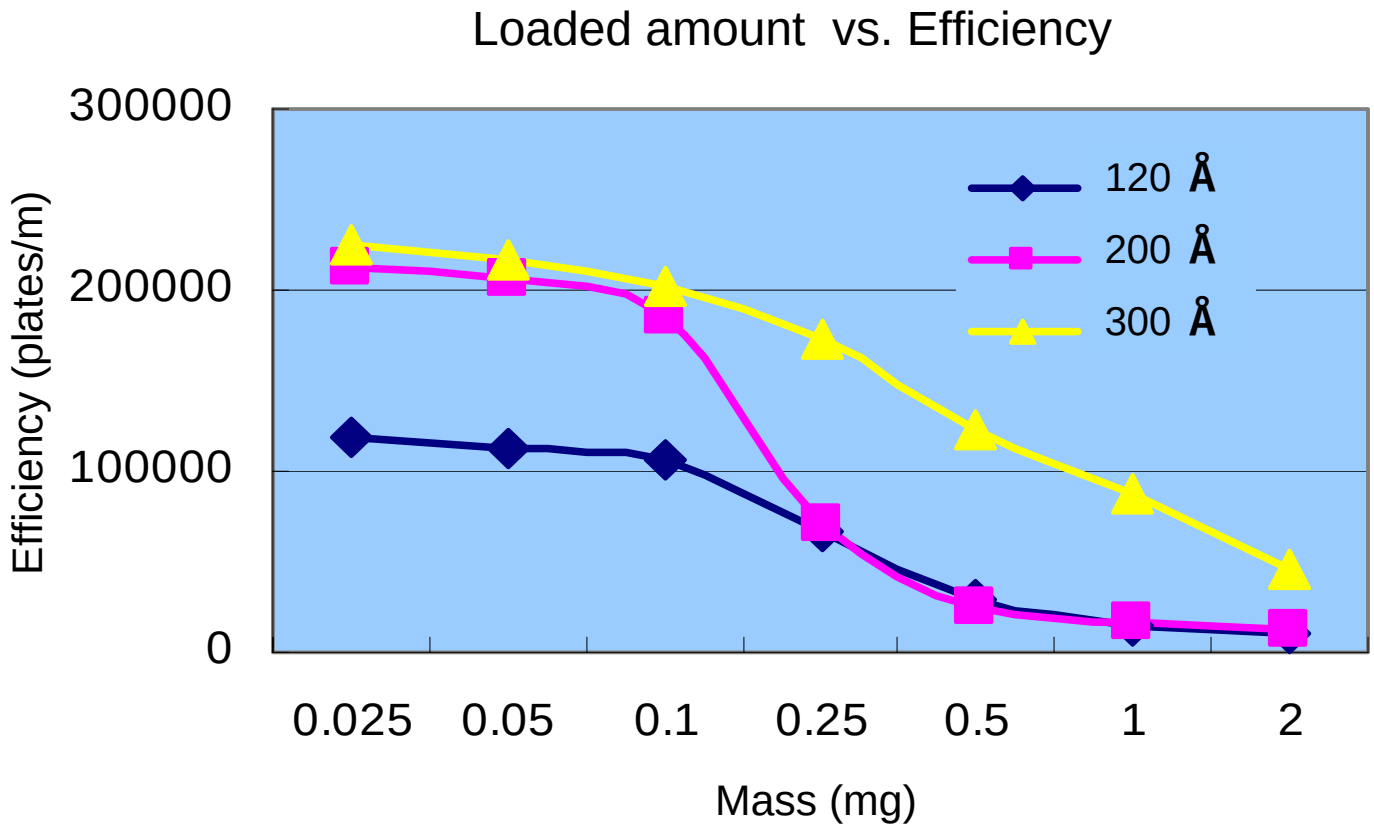
Insulin (MW 5700) *in analytical*



- **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*



- **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	120 Å	◎	○	△
20000	200 Å	○	◎	○
100000	300 Å	△	○	◎

◎ : excellent

○ : good

△ : moderate

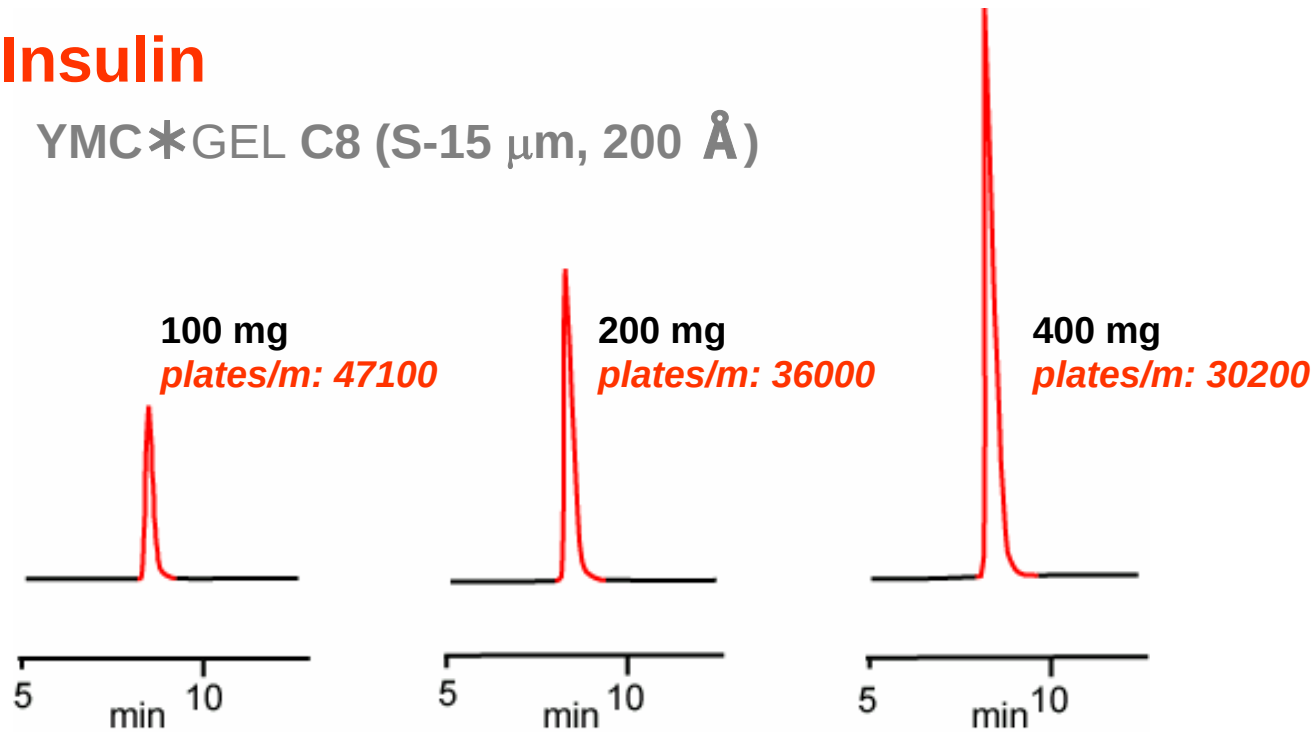
- C18 column with 120 Å pore size is suitable for small peptides up to MW 5000, similar to the analyses of ordinary small molecules. In the case of large peptides or small proteins up to MW 20000, the C8 column with 200 Å pore size gives the best efficiency. Most of proteins are eluted effectively by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

Loadability (1)

Insulin (MW 5700) *in preparative*

Insulin

YMC∗GEL C8 (S-15 μm, 200 Å)



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

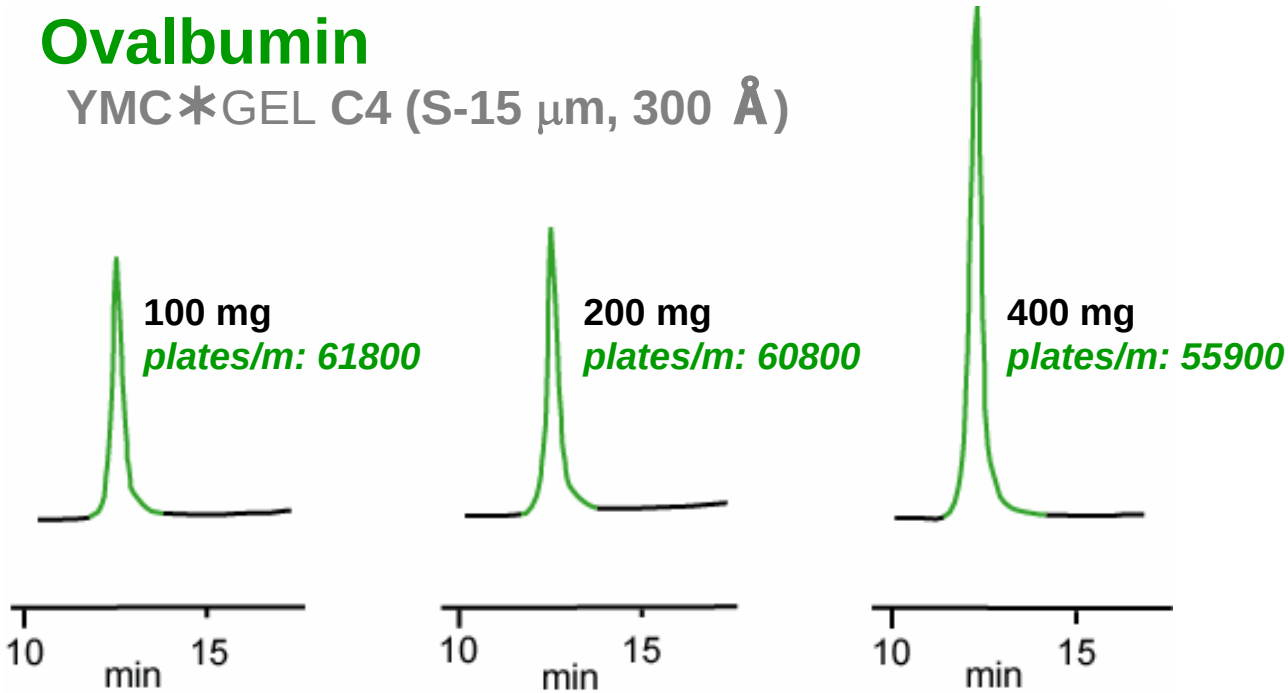
- **Up to 400 mg loaded amount, peak shapes are sharp as the analytical separation.**

Loadability (2)

Ovalbumin (MW 45000) *in preparative*

Ovalbumin

YMC∗GEL **C4 (S-15 μm, 300 Å)**

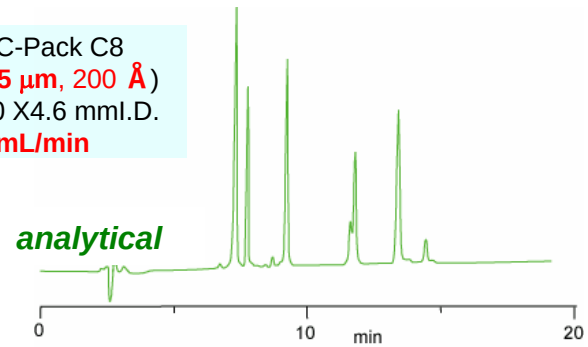


Column	: **150 X50 mmI.D.**
Eluent	: A) water / TFA (100/0.1)
	B) acetonitrile / TFA (100/0.1), 10-90%B(0-20min)
Flow rate	: **100 mL/min,** Temperature: ambient, Detection: UV at 220 nm

- **At all loading levels, high efficiency was obtained.**

Scale up from analytical to preparative

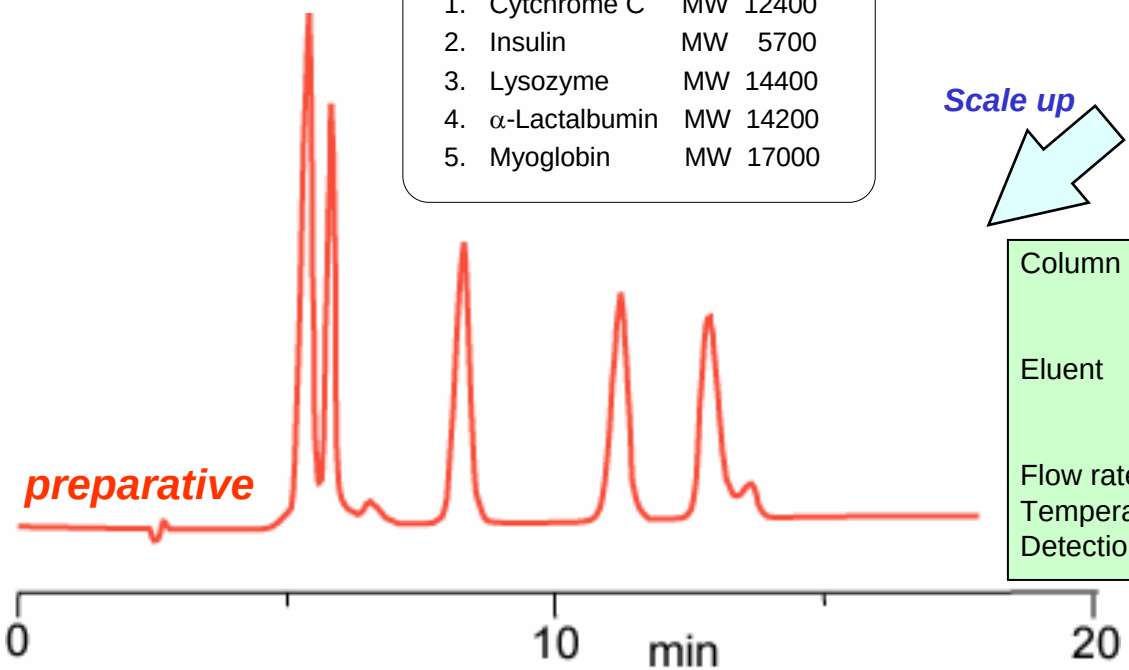
Column : YMC-Pack C8
(**S-5 μm**, 200 **Å**)
150 X4.6 mmI.D.
Flow rate : **0.8 mL/min**



In order of elution

1. Cytchrome C MW 12400
2. Insulin MW 5700
3. Lysozyme MW 14400
4. α-Lactalbumin MW 14200
5. Myoglobin MW 17000

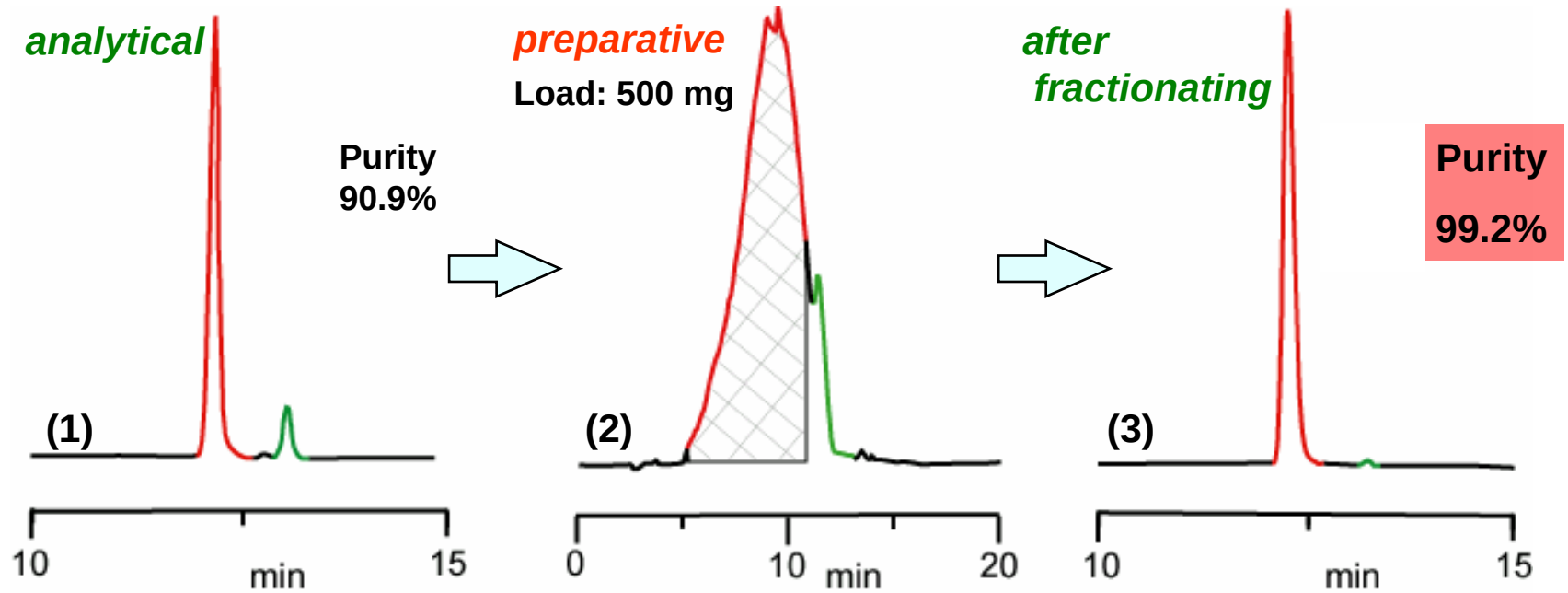
Scale up



Column : YMC✱GEL C8
(**S-10 μm, 200 Å**)
150 X50 mmI.D.
Eluent : A) water / TFA (100/0.1)
B) acetonitrile / TFA (100/0.1)
25-60%B(0-20min)
Flow rate : **100 mL/min**
Temperature : 37℃
Detection : UV at 220 nm

- **It is possible to scale up from analytical to preparative.**
- **Each sample 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 Å**)
150 X4.6 mmI.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 Å**)
150 X50 mmI.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

Loaded amount / mg	*Purity / %*	*Recovered amount / mg*	*Recovery / %*
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 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 some dimension of dynamic axial compression column.