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CAPTURE THE ESSENCE

Development of GFC column for Antibody Drug Analysis

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Abstract

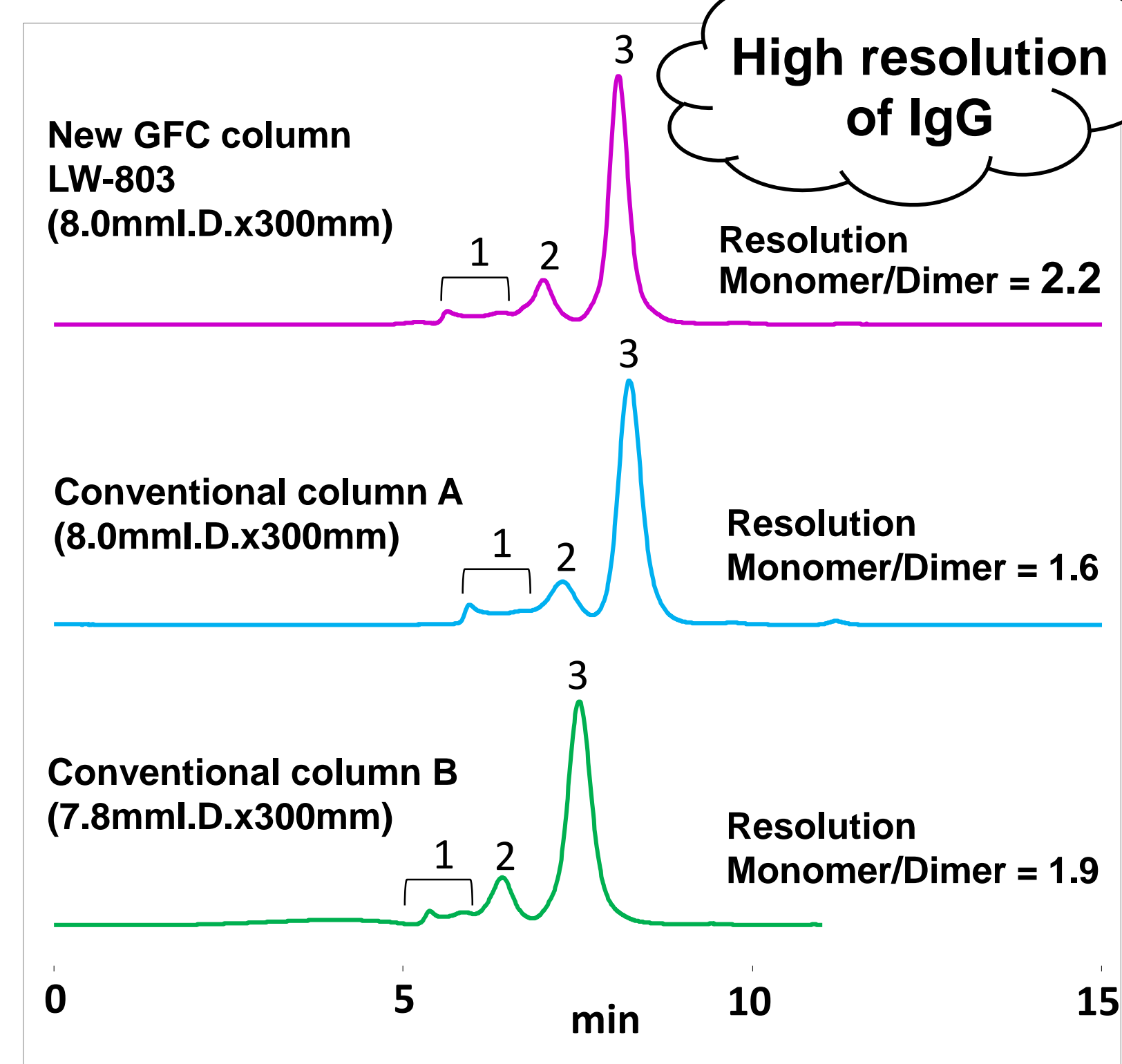
Antibody drugs are an important class of highly potent biopharmaceutical drugs designed for targeted therapy. Antibody drugs work with the immune system, allowing antibody drugs to cause few side effects, unlike conventional drugs.

The quality control, specifically the aggregate concentration, of antibody drugs is very important. The aggregates have a potential impact on pharmacological activity and safety of antibody drugs.

This poster introduces the separation performance of the new GFC column, PROTEIN LW-803, especially on analysis of immune globulin G (IgG), most important ingredient of antibody drugs.

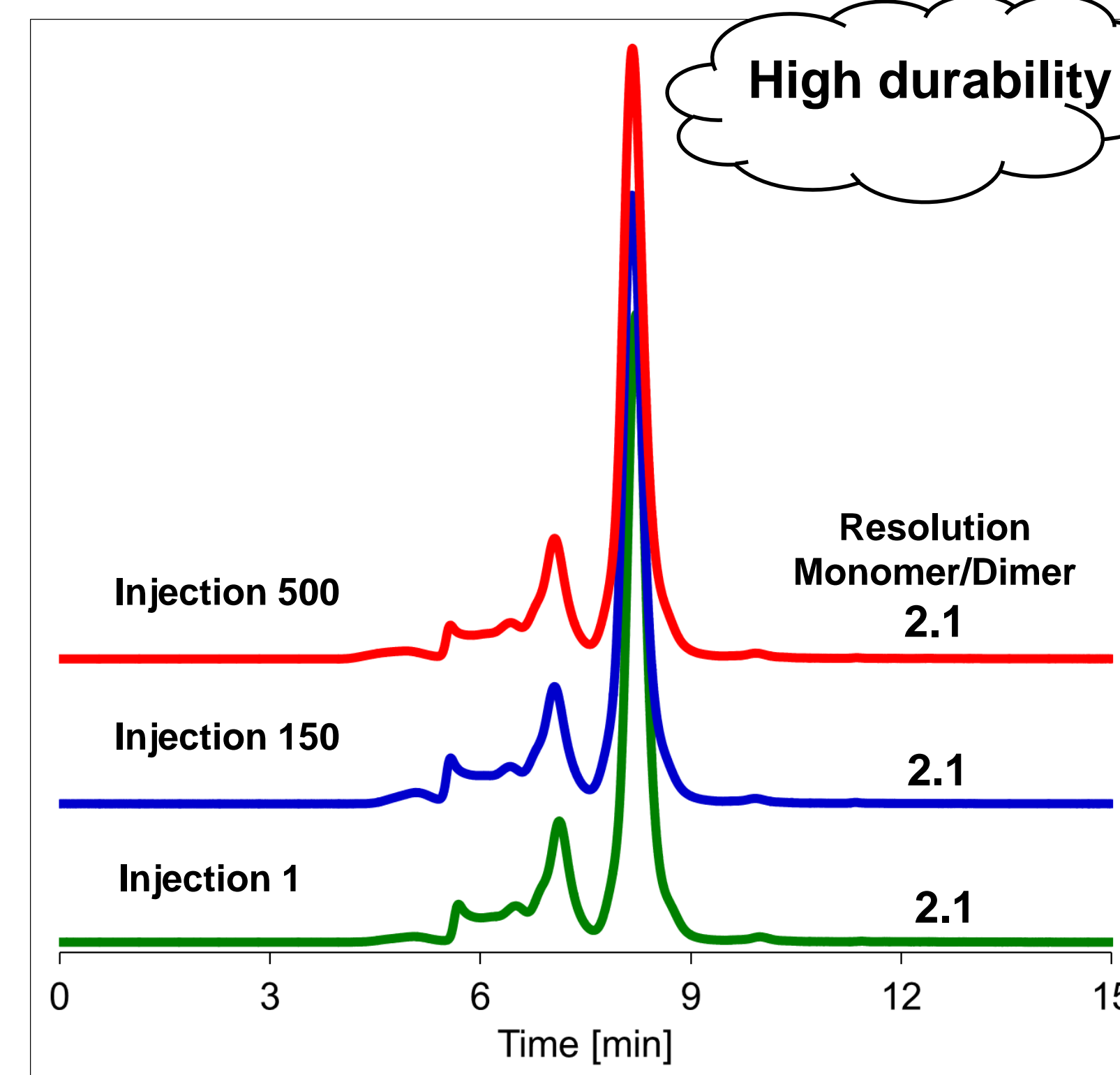
Fundamental IgG separation performance

IgG analysis



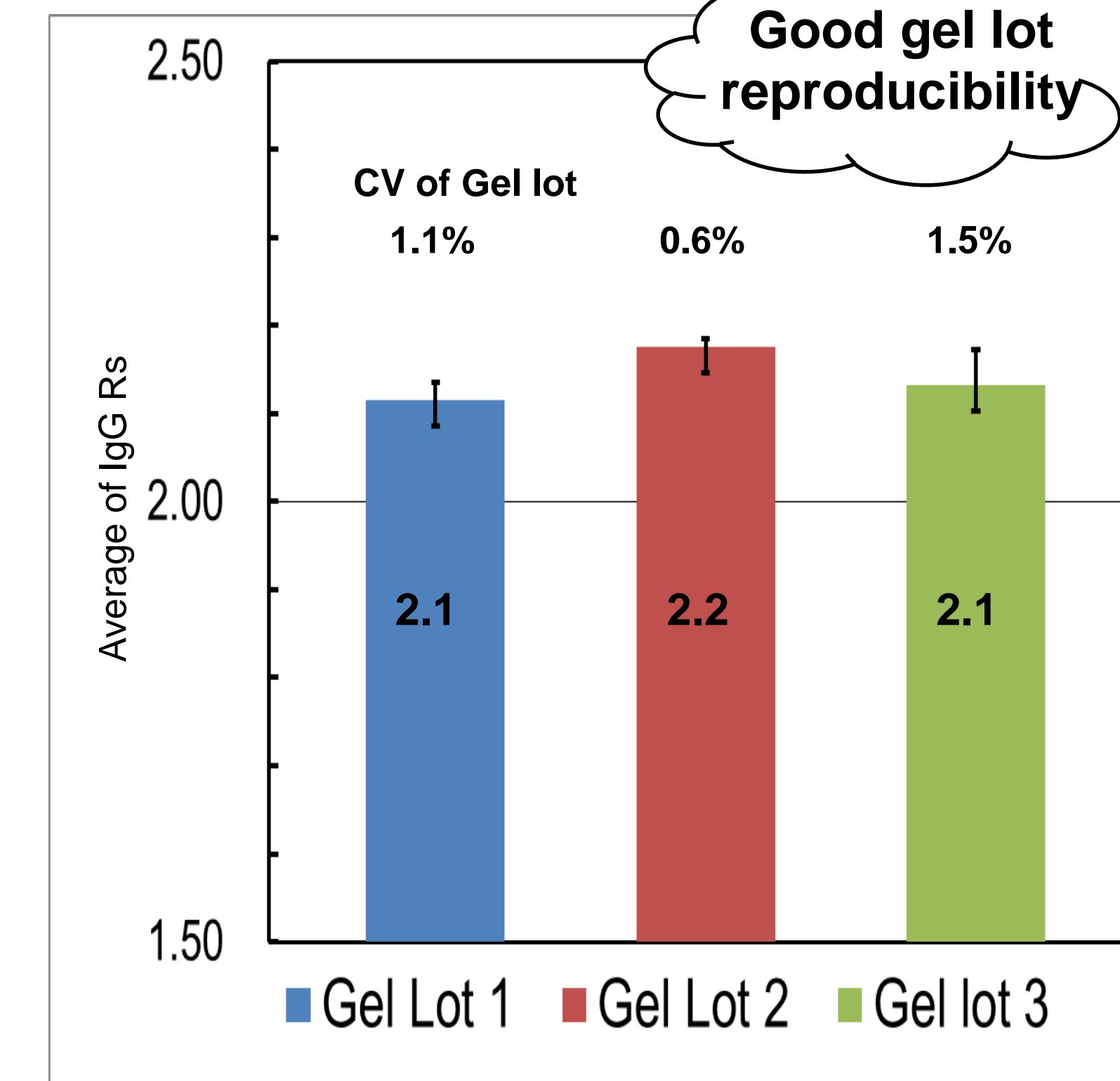
Sample : IgG from human serum
 1. Aggregate
 2. Dimer
 3. Monomer
 System : Prominence (Shimadzu)
 Eluent : 50mM Sodium phosphate buffer + 0.3M NaCl (pH7.0)
 Injection volume : 5µL (manual)
 Injector : Rheodyne manual injector P/N 8125 (Semi micro)
 Flow rate : 1.0mL / min
 Detector : UV (280nm) (Conventional type)
 Column temp : Room temp

Injection stability of IgG separation



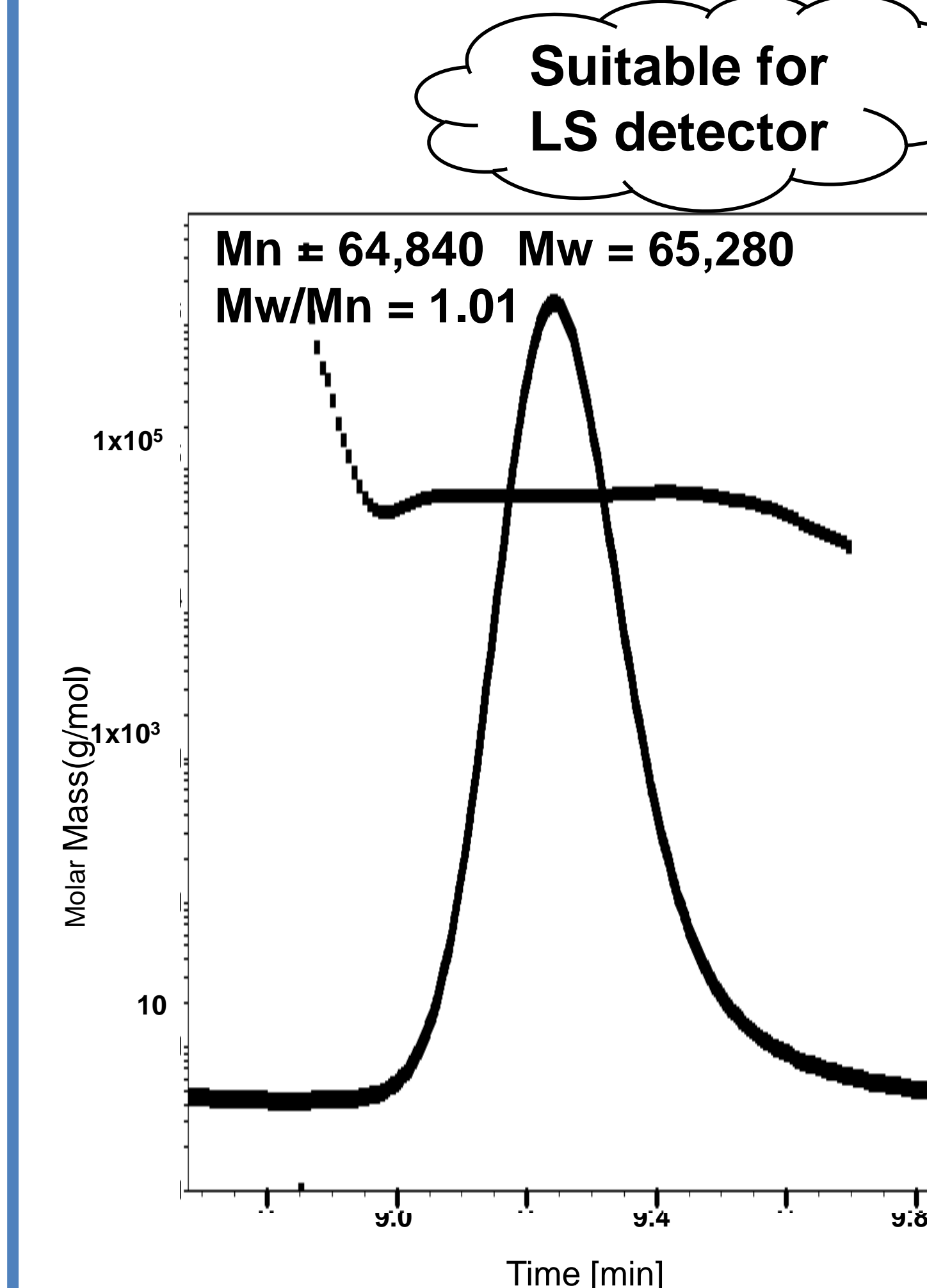
Column : LW-803 (8.0mmI.D.x300mm)
 Sample : IgG from human serum
 System : Prominence-i (Shimadzu)
 Eluent : 50mM Sodium phosphate buffer + 0.3M NaCl (pH7.0)
 Injection volume : 5µL (auto)
 Injector : Conventional
 Flow rate : 1.0mL / min
 Detector : UV (280nm) (Conventional type)
 Column temp : Room temp

Lot reproducibility of IgG separation



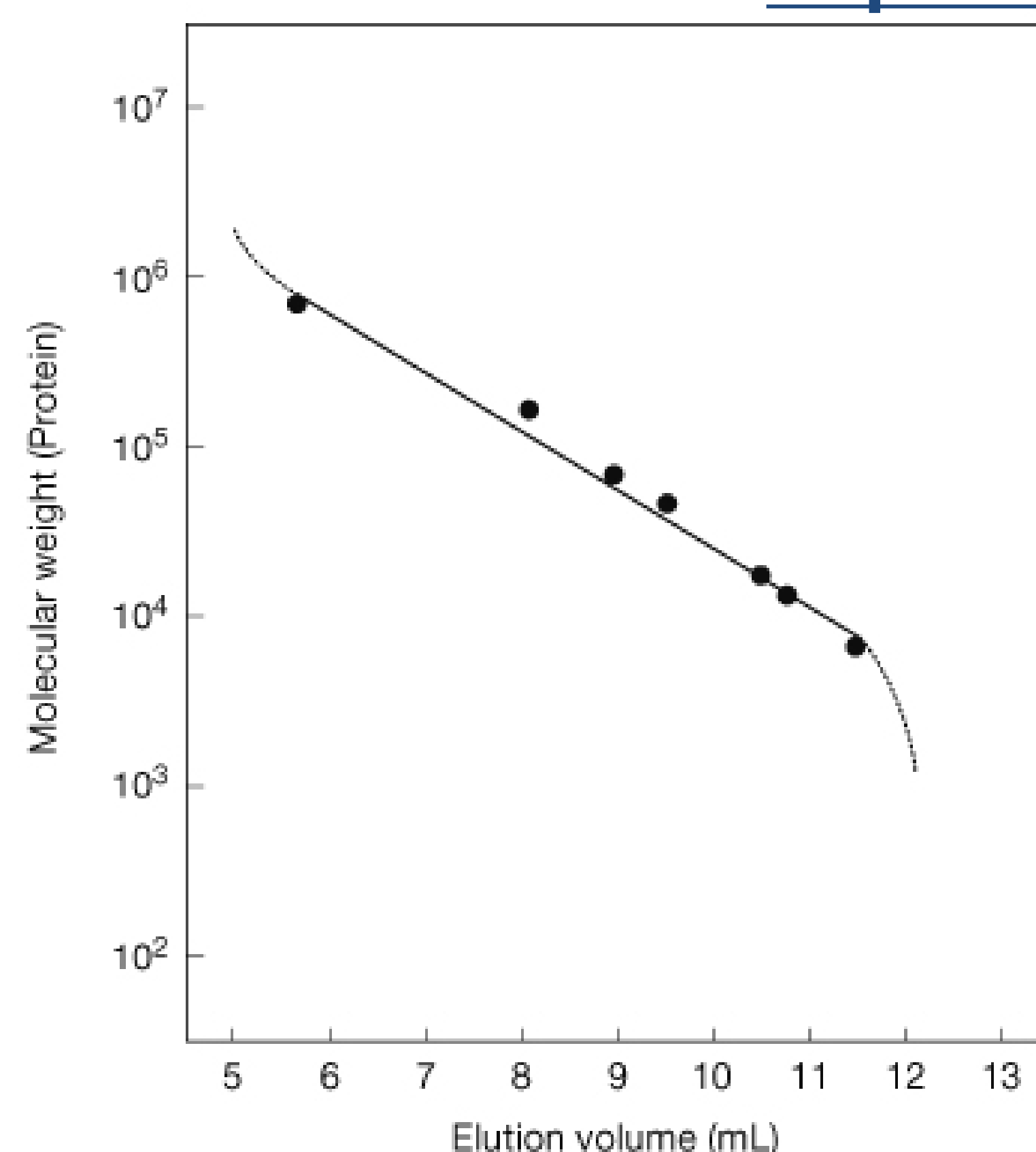
Column : LW-803 (8.0mmI.D.x300mm)
 Sample : IgG from human serum
 System : Prominence-i (Shimadzu)
 Eluent : 50mM Sodium phosphate buffer + 0.3M NaCl (pH7.0)
 Injection volume : 5µL (manual)
 Injector : Rheodyne manual injector P/N 8125 (Semi micro)
 Flow rate : 1.0mL / min
 Detector : UV (280nm) (Conventional type)
 Column temp : Room temp

MALS analysis



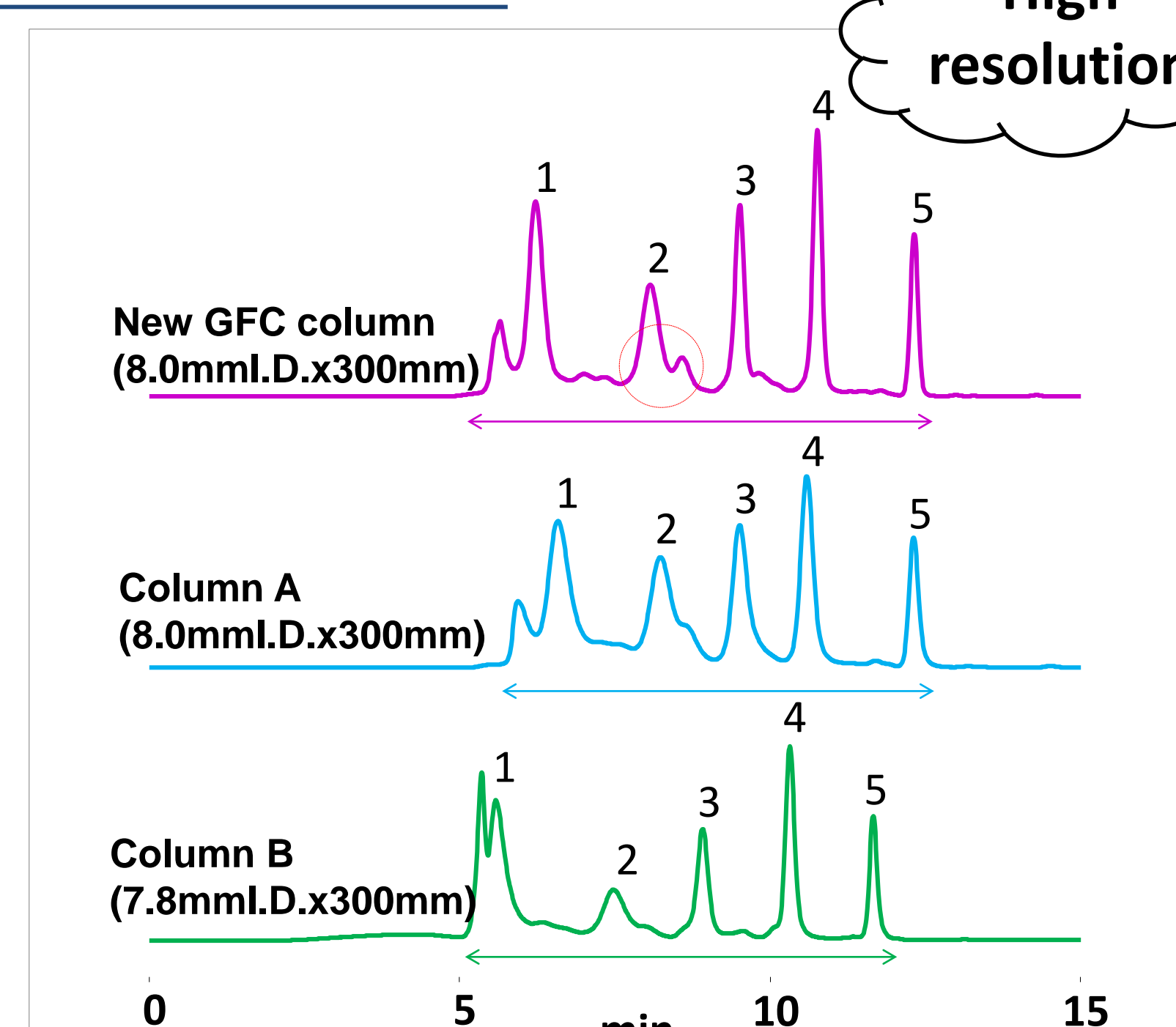
Column : LW-803 (8.0mmI.D.x300mm)
 Sample : 2.4mg/mL BSA (Mw: 66,430)
 Eluent : 50mM Sodium phosphate buffer + 0.3M NaCl (pH7.0)
 Injection volume : 10µL (auto)
 Flow rate : 1.0mL / min
 Detector : MALS + RI
 Column temp : 30°C

Separation of Protein



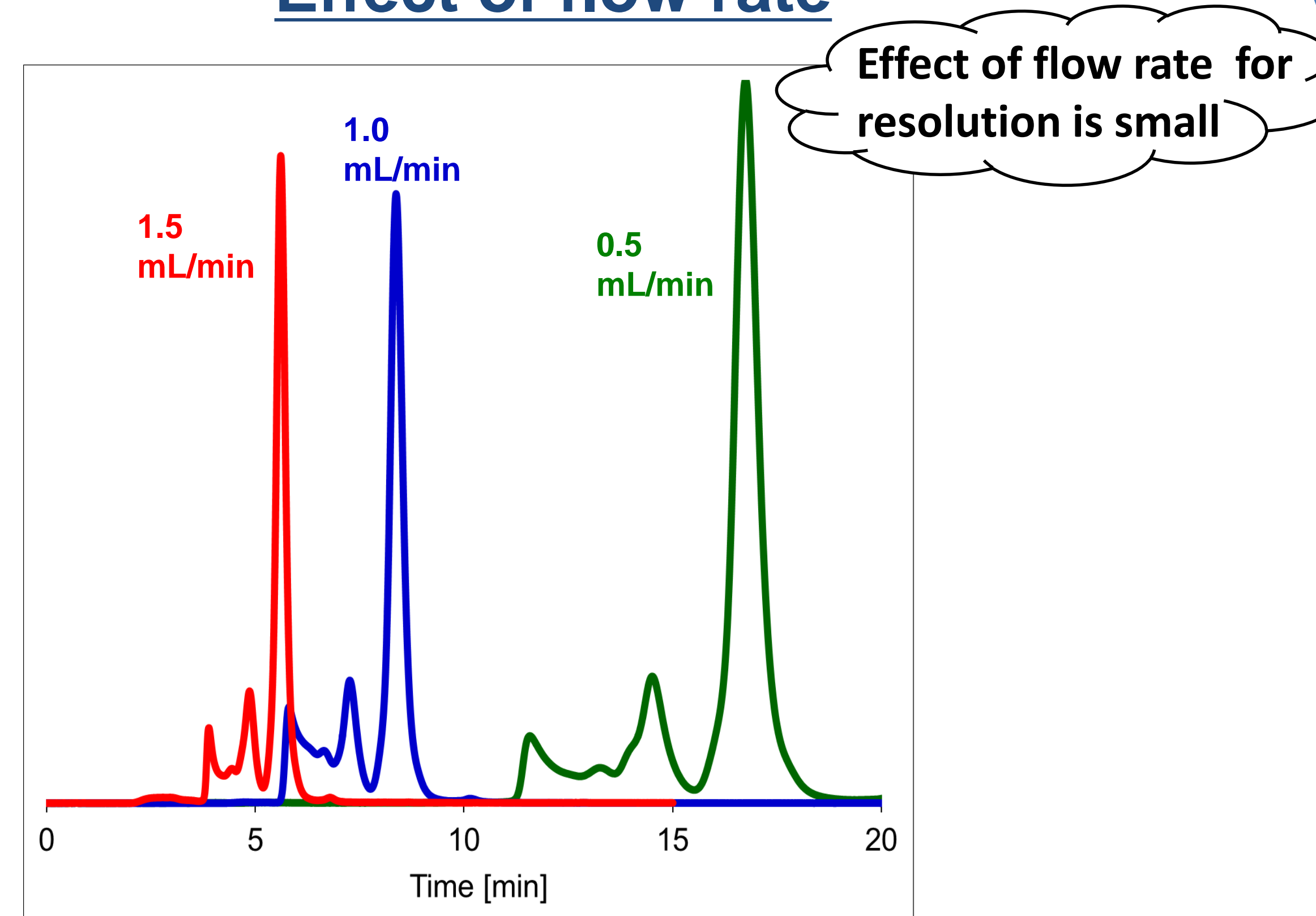
Column : LW-803 (8.0mmI.D.x300mm)
 System : Prominence (Shimadzu)
 Eluent : 50mM Sodium phosphate buffer + 0.3M NaCl (pH7.0)
 Flow rate : 1.0mL / min
 Detector : UV (280nm) (Conventional type)
 Column temp : Room temp.

High resolution



Column : LW-803 (8.0mmI.D.x300mm)
 Sample :
 1. Thyroglobulin (Mw:670,000),
 2. γ-Globulin (Mw:160,000)
 3. Ovalbumin (Mw:44,300)
 4. Ribonuclease A (Mw:13,700)
 5. Uridine (Mw:244)
 Eluent : 50mM Sodium phosphate buffer+ 0.3M NaCl
 Injection volume : 5µL (manual)
 Flow rate : 1.0mL / min
 Detector : UV (280nm) (Conventional type)
 Column temp : Room temp

Effect of flow rate



Flow rate [mL/min]	Rs (M/D)	analysis time [min]	analysis pressure [MPa]
0.5	2.2	30	3.9
1.0	2.0	15	7.4
1.5	1.9	10	11.1

Column : LW-803 (8.0mmI.D.x300mm)
 Sample : IgG from human serum
 Eluent : 50mM Sodium phosphate buffer + 0.3M NaCl (pH7.0)
 Injection volume : 5µL (auto)
 Flow rate : 0.5, 1.0, 1.5 mL / min
 Detector : UV (280nm) (Conventional type)

Conclusions

1. A new GFC column for analyzing antibody drug by optimizing control of the silica pore diameter and modification method was successfully developed.
2. The new GFC column, LW-803, 3µm packing material was packed into a column of 300 mm x 8 mm I.D. The sample can be analyzed within 15 minutes in with a flow rate of 1.0mL/min. At the flow rate, back pressure reaches 8.0MPa, which can be used with conventional HPLC system.
3. The new GFC column was accurate for the separation of protein and quality control of antibody drug, due to the large pore volume.