Separation of Half-mAb and Half-mAb Equivalents with High Resolution Using Size Exclusion Chromatography Packed with a Unique Controlled Pore Technology

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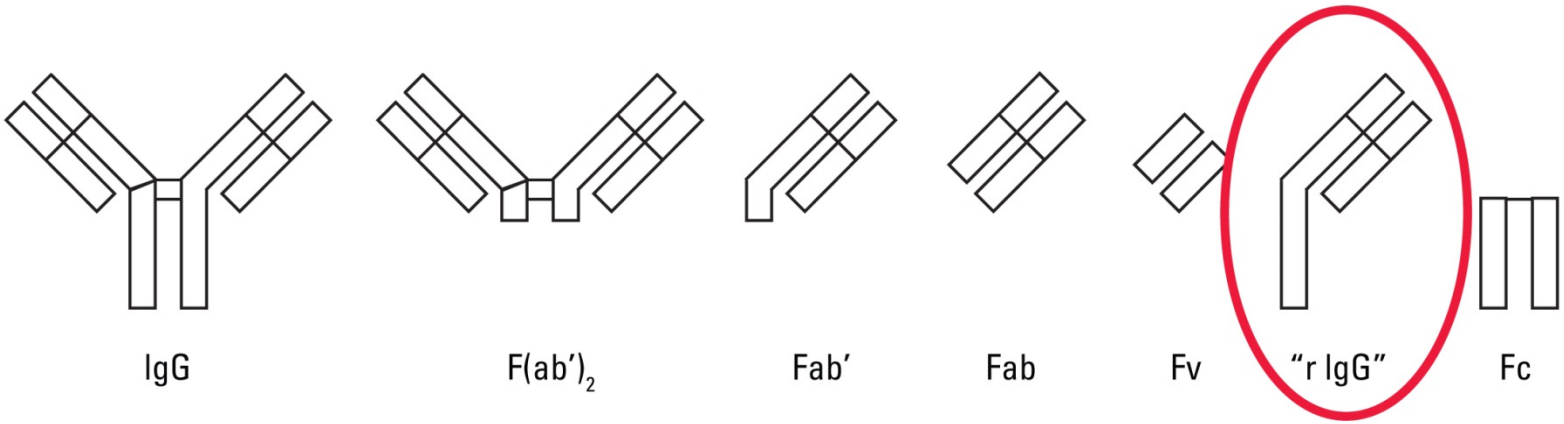
Introduction

- Monoclonal antibody research continues to grow in an effort to develop effective biotherapeutics for a wide range of diseases.
- Recent research has shown an interest into mAb half-bodies as therapeutic vectors as they can be further targeted for conjugation, enzyme labeling, or antibody immobilization.
- Monoclonal antibody half-bodies can be generated through genetic engineering of cells or by selective reduction of hinge-region disulfide bonds present in the monoclonal antibody by mild reducing agents such as 2-MEA, DTT or TCEP.
- TCEP (tris (2-carboxyethyl) phosphine) is a mild reducing agent commonly used in the reduction of hinge region disulfide bonds.
- Unlike 2-MEA or DTT, TCEP is odorless and more resistant to oxidation in the presence of air, making it a more stable reducing agent and a commonly used reagent in mAb half-body formation.
- As expected, mAb half-bodies have a molecular weight of approximately 70 kDa, or one half of that of the intact monoclonal antibody.
- Bovine transferrin, with a molecular weight of 78 kDa, is expected to simulate the half-mAb species when chromatographically separated using SEC and can be used as a molecular weight marker.

Introduction



Types of antibody fragmentation (image courtesy of piercenet.com):

rIgG – reduced IgG or Half IgG

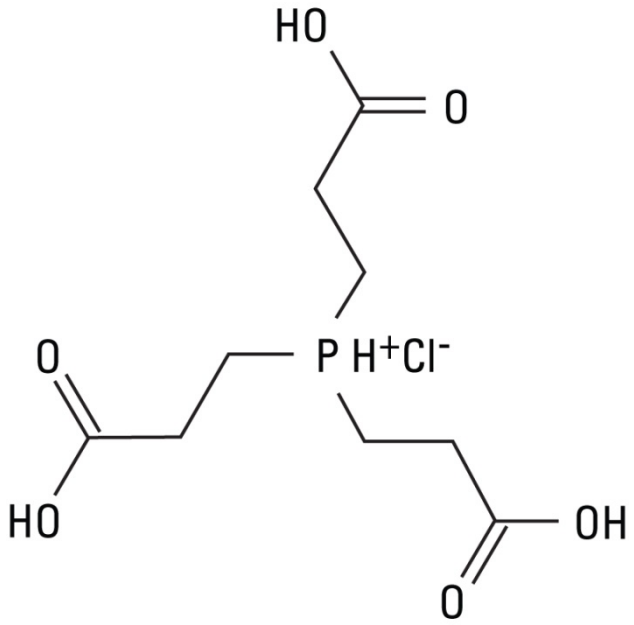
Material and Methods

Column:	TSKgel SuperSW mAb HR, 4 µm, 7.8 mm ID × 30 cm
Instrument:	All analyses were carried out using an Agilent 1100 HPLC system run by Chemstation (ver B.04.02).
Mobile phase:	100 mmol/L KH_2PO_4/K_2HPO_4, pH 6.7, 100 mmol/L Na_2SO_4 + 0.05% NaN_3
Flow rate:	1.0 mL/min
Detection:	UV @ 280 nm
Temperature:	ambient
Injection vol.:	10 µL (approximately 46 µg of total protein content per injection)
Samples:	mAb 01, 4.6 mg/mL - a gift from Tosoh Bioscience GmbH mAb 02, 4.7 mg/mL - a gift from Tosoh Bioscience GmbH human IgG, 4.6 mg/mL - Sigma bovine transferrin, 4.6 mg/mL - Sigma

All the representative chromatograms shown in this presentation are verified by 3 consecutive injections.

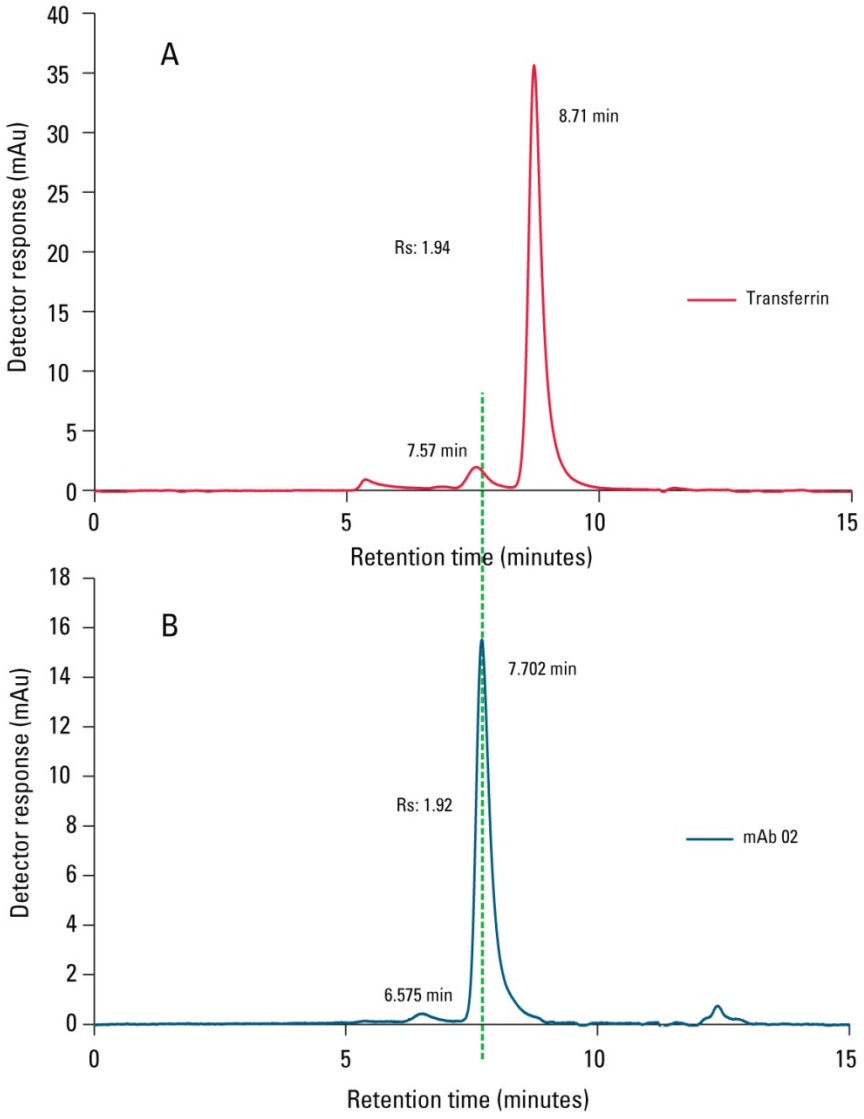
Material and Methods

- mAbs were reduced using TCEP (tris (2-carboxyethyl) phosphine) - Thermo Scientific
- Reduction procedure was optimized to 150 mmol/L TCEP with sample incubation at 37 ºC for 20 hrs.
- Separated fragments were collected and concentrated using protein precipitation by acetone.
- Dried protein precipates were reconstituted in SDS PAGE running buffer prior to gel electrophoresis sizing.



TCEP•HCl
MW 286.65

Figure 1: Evaluation of Bovine Transferrin as a Half-mAb Equivalent to mAb 02

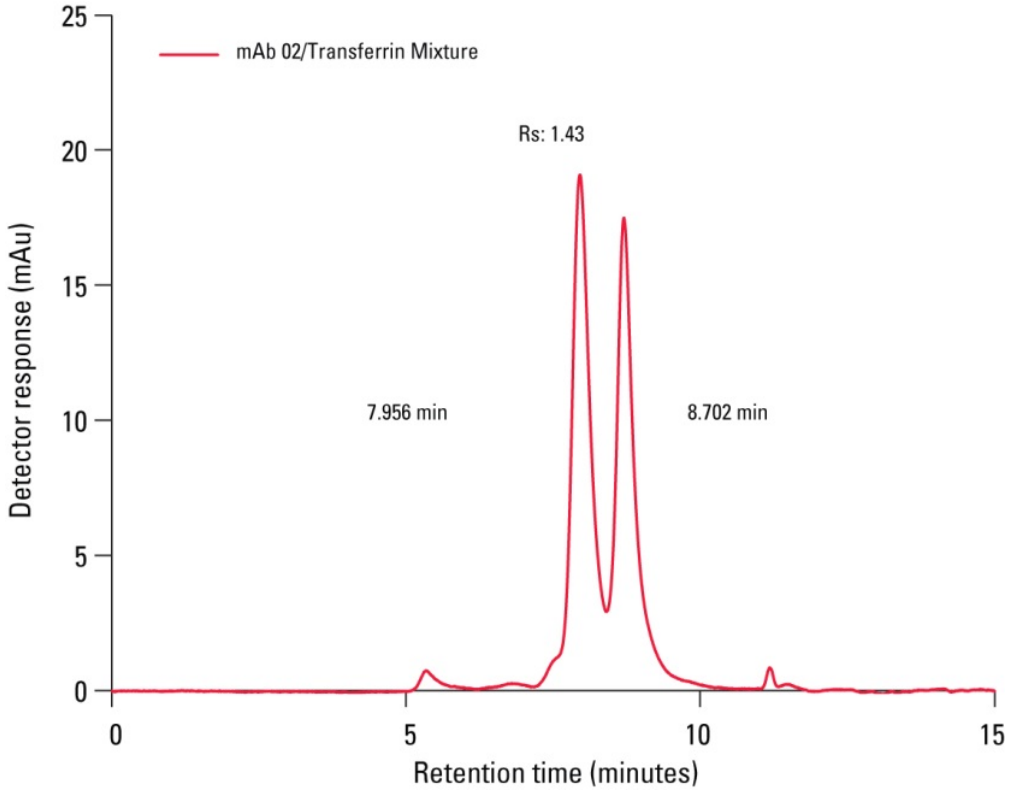


Panel A: Separation of the bovine transferrin monomer and dimer using the TSKgel SuperSW mAb HR column

Panel B: Separation of mAb 02 monomer and dimer using the TSKgel SuperSW mAb HR column

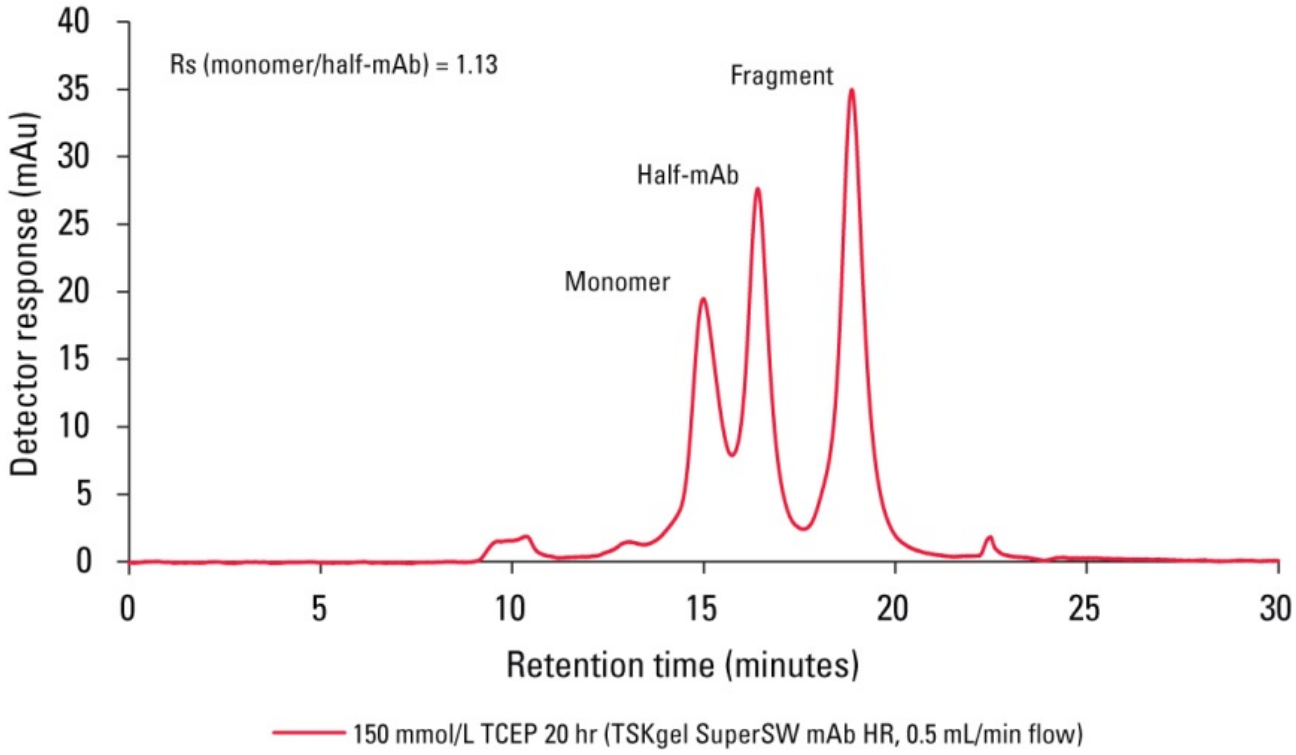
As shown, retention time of the bovine transferrin dimer (156 kDa) corresponds to nearly the same retention time as the mAb 02 monomer (150 kDa), illustrating equivalency of transferrin monomer (78 kDa) to the mAb 02 half-mAb species.

Figure 2: Separation of mAb 02 Monomer and Bovine Transferrin Monomer using a TSKgel SuperSW mAb HR Column



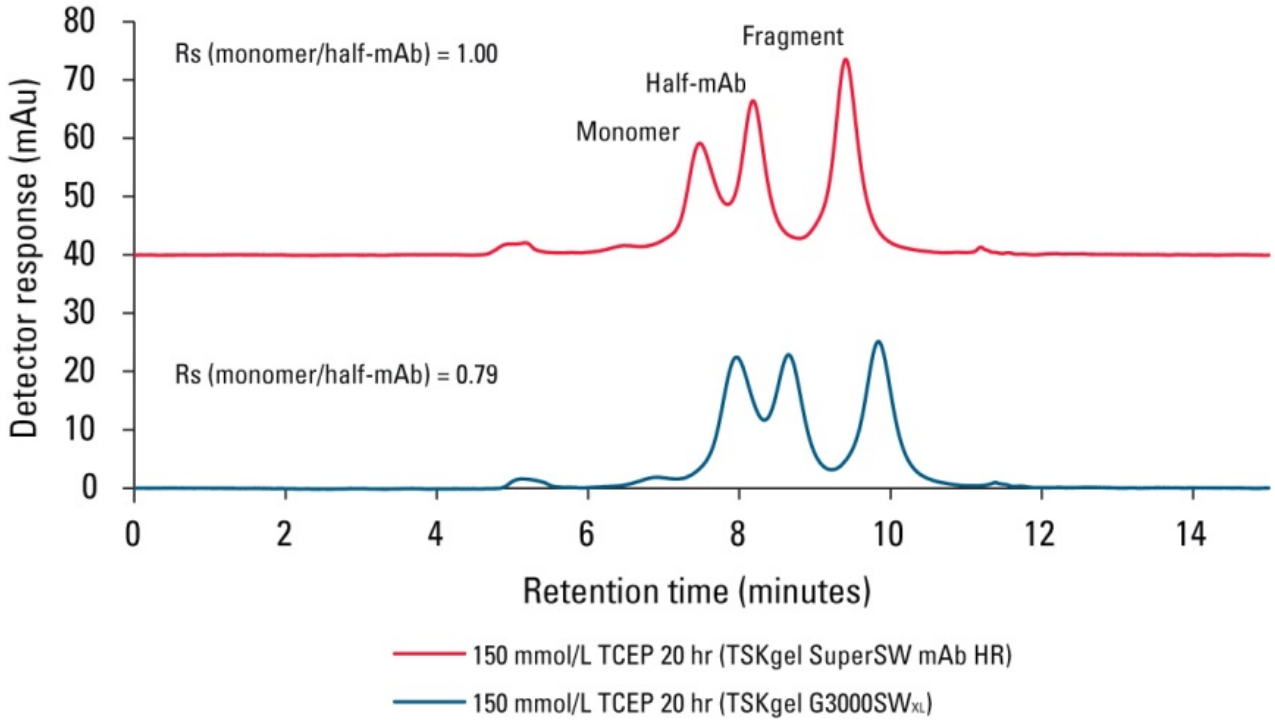
- The mAb 02 monomer and transferrin monomer are clearly separated using the TSKgel SuperSW mAb HR column.
- A reduction in the expected resolution between the molecules is most likely due to a distribution of monomer, dimer, and intermediate species present in the transferrin sample.

Figure 3: Separation of mAb 01 Monomer From its Half-mAb and Low Molecular Weight Fragment



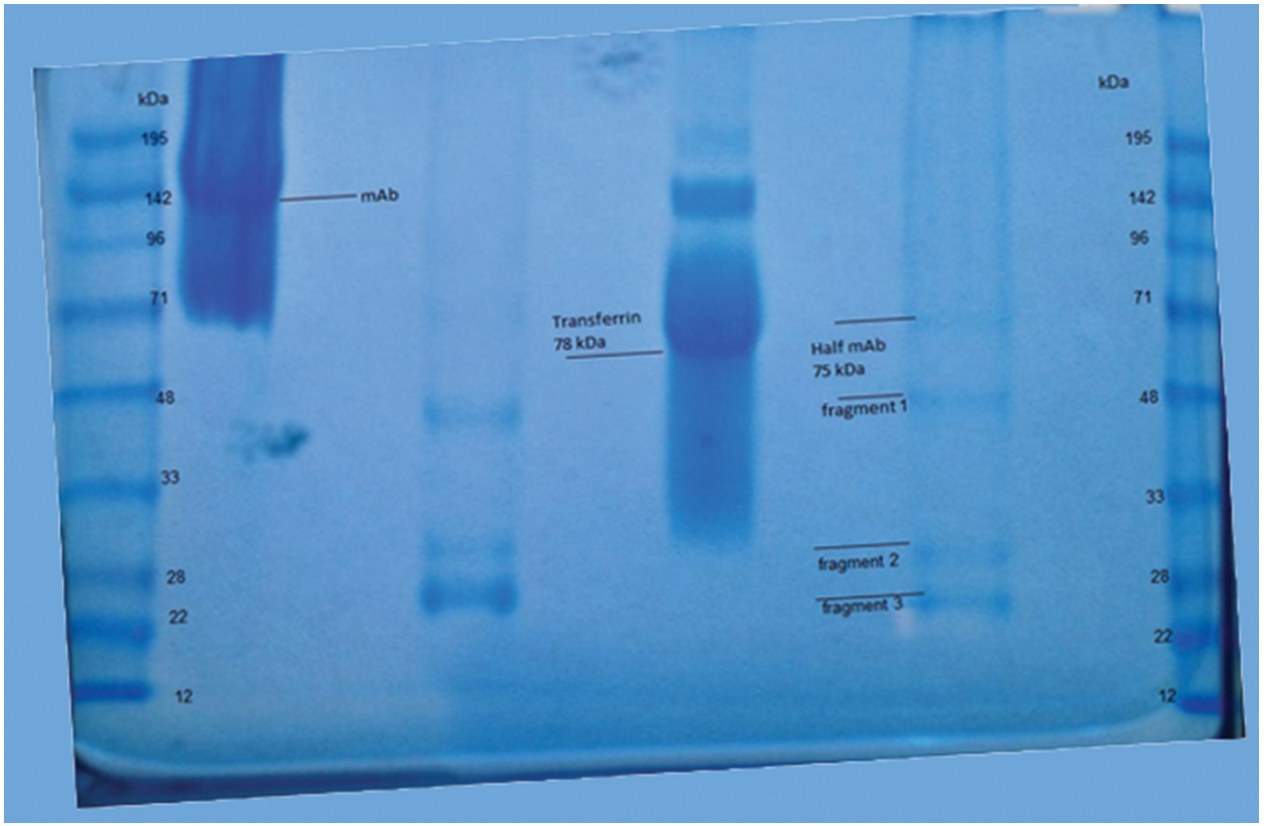
- All three species are clearly separated using the TSKgel SuperSW mAb HR column.
- Operation at 0.5 mL/min yields an increase in resolution while maintaining column efficiency ($N_{fragment,\ 0.5\ mL/min}$ = 3633 vs $N_{fragment,\ 1.0\ mL/min}$ = 3983)

Figure 4: Comparison of Human IgG Monomer and Half-mAb Resolution Using a TSKgel SuperSW mAb HR and TSKgel G3000SWXL Column



The 4 µm particle size and controlled pore technology of the TSKgel SuperSW mAb HR column leads to higher resolution of the half-mAb and monomeric species compared to the same separation performed on the TSKgel G3000SWXL column.

Figure 5: SDS-PAGE of Protein Reduction Products Collected From Separation on a TSKgel SuperSW mAb HR Column



- The collected half-mAb fraction corresponds with the 71 kDa marker and is equal to ½ the molecular weight of the intact mAb (142 kDa).
- Bovine transferrin (78 kDa) was used to further confirm the formation of the half-mAb species.

Conclusions

- Monoclonal antibodies mAb 01and mAb 02, and human IgG could be reduced by TCEP to form half body fragments.
- TCEP is an effective reducing agent for the formation of half-mAb species.
- Half body fragments could be separated from intact monomer by using size exclusion chromatography.
- Bovine transferrin has been shown as a half-mAb equivalent and could be separated from the mAb monomer.
- mAb monomer and transferrin monomer yielded better resolution relative to mAb monomer and TCEP-formed half-mAb species.
- TSKgel SuperSW mAb HR column with pore controlled technology and 4 µm particles yielded better resolution of the half-mAb from the intact mAb compared to a TSKgel G3000SWXL column.
- The intact monomer and TCEP-generated fragments, such as half-mAb and smaller fragments, could be verified by a molecular weight marker during SDS PAGE analysis.
- Further work is in progress to increase the resolution between the intact mAb and the half-mAb.