SEC Analysis of MAb Fragments with Increased Throughput and High Resolution

Size exclusion chromatography (SEC) is a common method for the separation of antibody monomer from dimer, aggregates, or degradation products on the basis of molecular size. YMC-Pack Diol-200 SEC columns with 2 µm exhibits reduced analysis time while achieving high resolution between monomer and Fab dimer. And with the new YMC-SEC MAB column an ideal analysis of fragments/degradation products of antibodies with high resolution can be achieved.

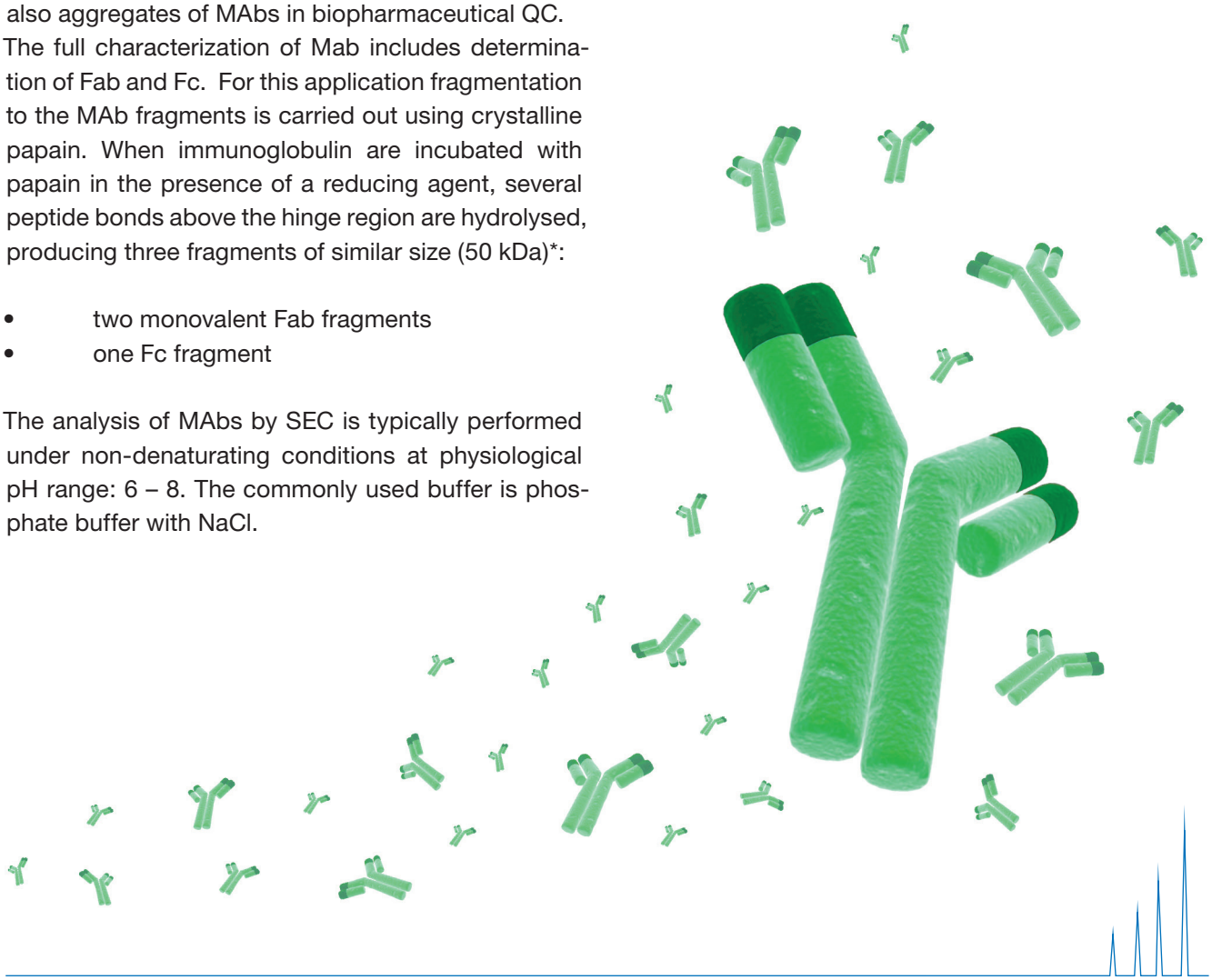
The characterisation of monoclonal antibodies (MAbs) is a major challenge in process monitoring and quality control. Size exclusion chromatography (SEC) is the standard method for analysis of fragments and also aggregates of MAbs in biopharmaceutical QC.

The full characterization of Mab includes determination of Fab and Fc. For this application fragmentation to the MAb fragments is carried out using crystalline papain. When immunoglobulin are incubated with papain in the presence of a reducing agent, several peptide bonds above the hinge region are hydrolysed, producing three fragments of similar size (50 kDa)*:

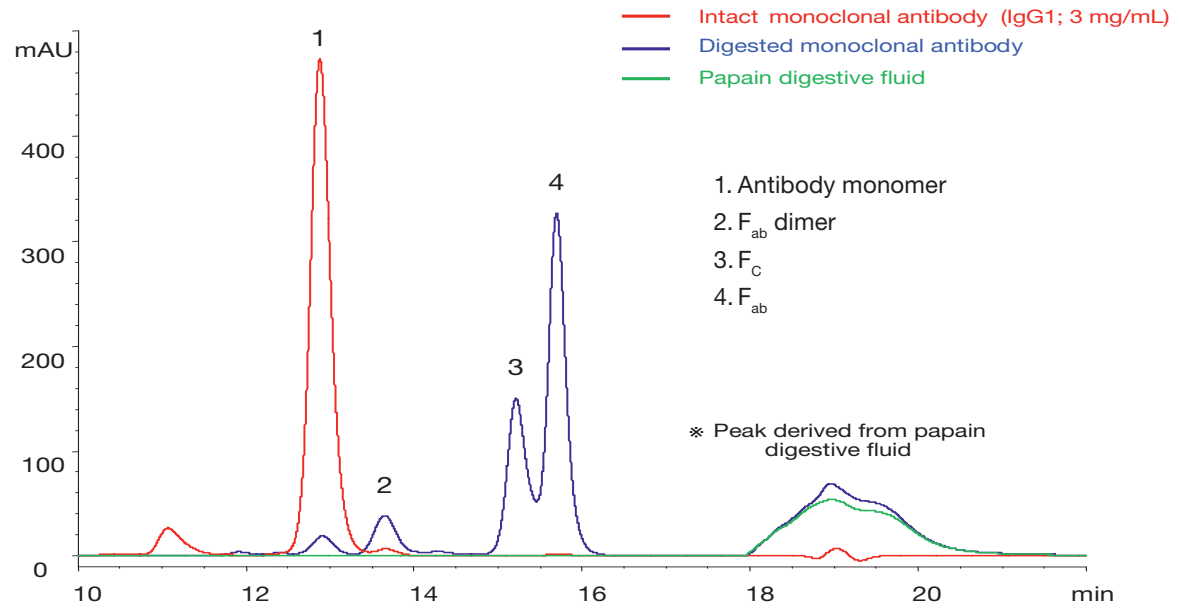
- two monovalent Fab fragments
- one Fc fragment

The analysis of MAbs by SEC is typically performed under non-denaturating conditions at physiological pH range: 6 – 8. The commonly used buffer is phosphate buffer with NaCl.

Figure 1 shows the analysis of intact MAb IgG1 and the separation of papain digested fragments Fab dimer, Fc and Fab using the UHPLC compatible YMC-Pack Diol-200 column. The elution conditions used were phosphate buffer (pH 7.0) with 0.2 M NaCl at ambient temperature (see details in table 1). YMC-Pack Diol-200, a silica-based phase with 2 µm particles greatly improves the resolution between aggregates and the monomer peak in contrast to 3 or 5 µm particles. The fragments Fc and Fab with similar molecular weight of 50 kDa are almost completely separated.



YMC Europe GmbH · Schöttmannshof 19 · 46539 Dinslaken · Phone +49 (0)2064 427-0 · Fax +49 (0) 2064 427-222 · Email: info@ymc.de · www.ymc.de



Column:	YMC-Pack Diol-200 (2 µm, 20 nm) 300 x 4.6 mm ID
Part no:	DL20S02-3046PTH
Eluent:	0.1 M KH_2PO_4-K_2HPO_4 (pH 7.0) containing 0.2 M NaCl
Flow rate:	0.2 mL/min
Temperature:	ambient
Detection:	UV at 280 nm
Sample:	IgG1 (3 mg/ml)

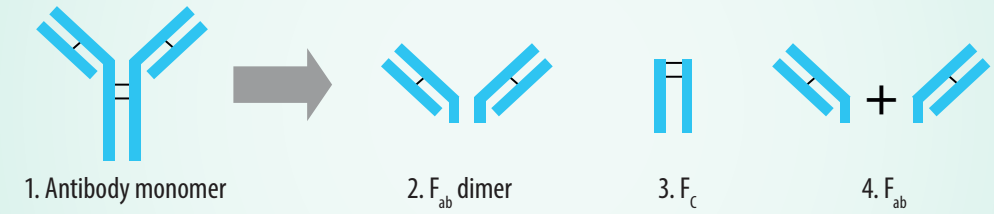
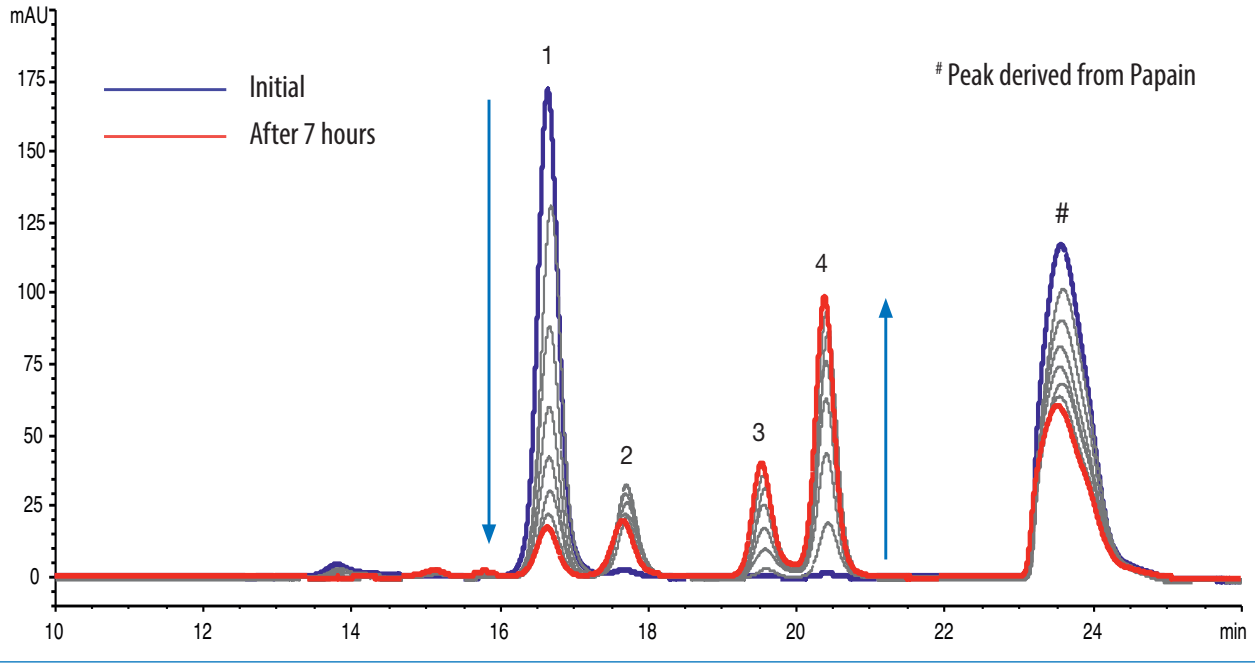


Figure 1: SEC analysis of antibody monomer and digest fragments using UHPLC compatible YMC-Pack Diol-200.

With the new YMC-SEC MAB column specifically designed for antibody analysis, the separation of the fragments Fc and Fab can also be achieved. It is possible to easily monitor the digestion of a monoclonal antibody with papain using similar conditions as before (see figure 2). The peak of the monomer decreased as the digestion proceeded, while peaks for the degradation products increased. For the fragments Fc and Fab a baseline separation can be achieved due to the excellent resolution*.



Column:	YMC-SEC MAB (3 µm, 25 nm) 300 x 4.6 mm ID
Part No.:	DLM25S03-3046WT
Eluent:	0.1 M phosphate buffer (pH = 7) cont. 0.2 M NaCl
Flow rate:	0.165 mL/min
Temperature:	25 °C
Detection:	UV at 280 nm
Injection:	2 µL (3 mg/mL)
Sample:	Humanised monoclonal IgG1 + Papain

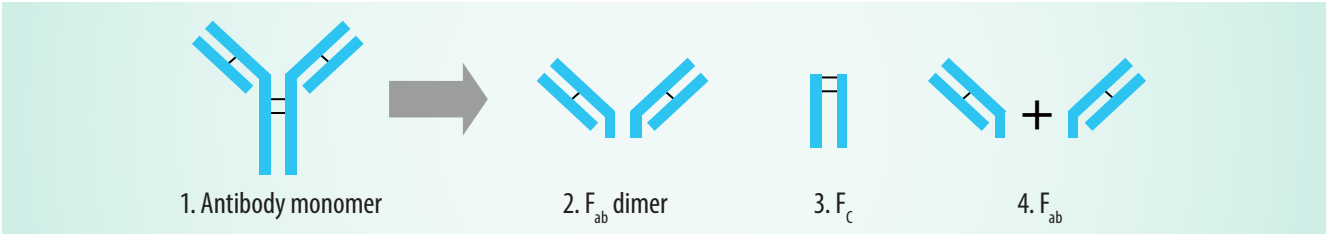


Figure 2: SEC analysis of digested antibodies using YMC-SEC MAB.

* The molecular weight (MW) of Fab and Fc give theoretical values of about 50 kDa. Often antibody glycosylation is a common post-translational modification which results in higher MW being observed. In other cases, the hydrodynamic radius can be different, giving an apparent change in MW.