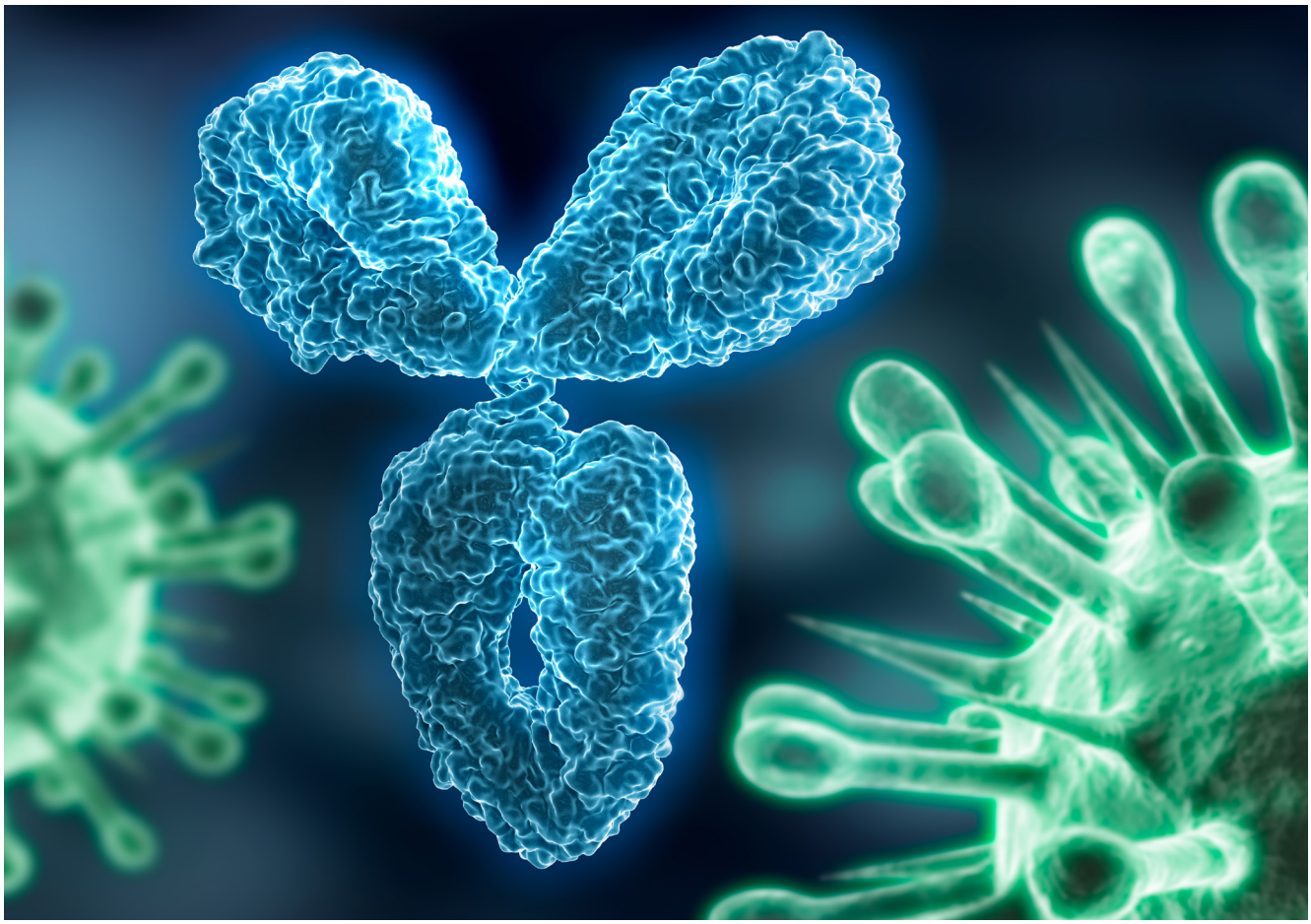
Analysis of Bevacizumab (Avastin®) by SEC-MALLS

Size exclusion chromatography (SEC) is a standard technique for analysing monoclonal antibodies (MAbs) such as Bevacizumab (Avastin®).
It is also a standard separation mode used in quality control to obtain information about aggregation and/or fragmentation of the MAb. Detection by light scattering, e.g. multi-angle laser light scattering (MALLS), can provide additional information, as the signal intensity is also influenced by the molar mass in contrast to usual concentration detectors such as UV or RI.



In this application note YMC's dedicated SEC column for antibodies, YMC-SEC MAB, has been used to develop a method for Bevacizumab using MALLS detection.
Four different buffers, a phosphate buffered saline (PBS; containing 0.138 M NaCl, 0.027 M KCl) pH 7.4 and phosphate buffers pH 6.6 (Sigma-Aldrich, 0.034 M) with varying concentrations of NaCl (0.1, 0.3 and 0.5 M), were used.
A defined minimum ionic strength is necessary to achieve a robust method with good resolution, which is not fulfilled by the use of phosphate buffer with 0.1 M NaCl.

On the other hand, no significant difference can be determined between the chromatograms obtained using PBS buffer and phosphate buffer with 0.3 or 0.5 M NaCl (figure 1). Compared to UV detection, the MALLS signal shows 2 higher molar mass species, aggregates of Bevacizumab, at about 2.0 mL and 2.3 mL elution volume. In PBS another signal can be detected at about 4.0 mL.
Therefore, phosphate buffer with 0.3 M NaCl appeared to be the most suitable eluent and was used for further optimisation.

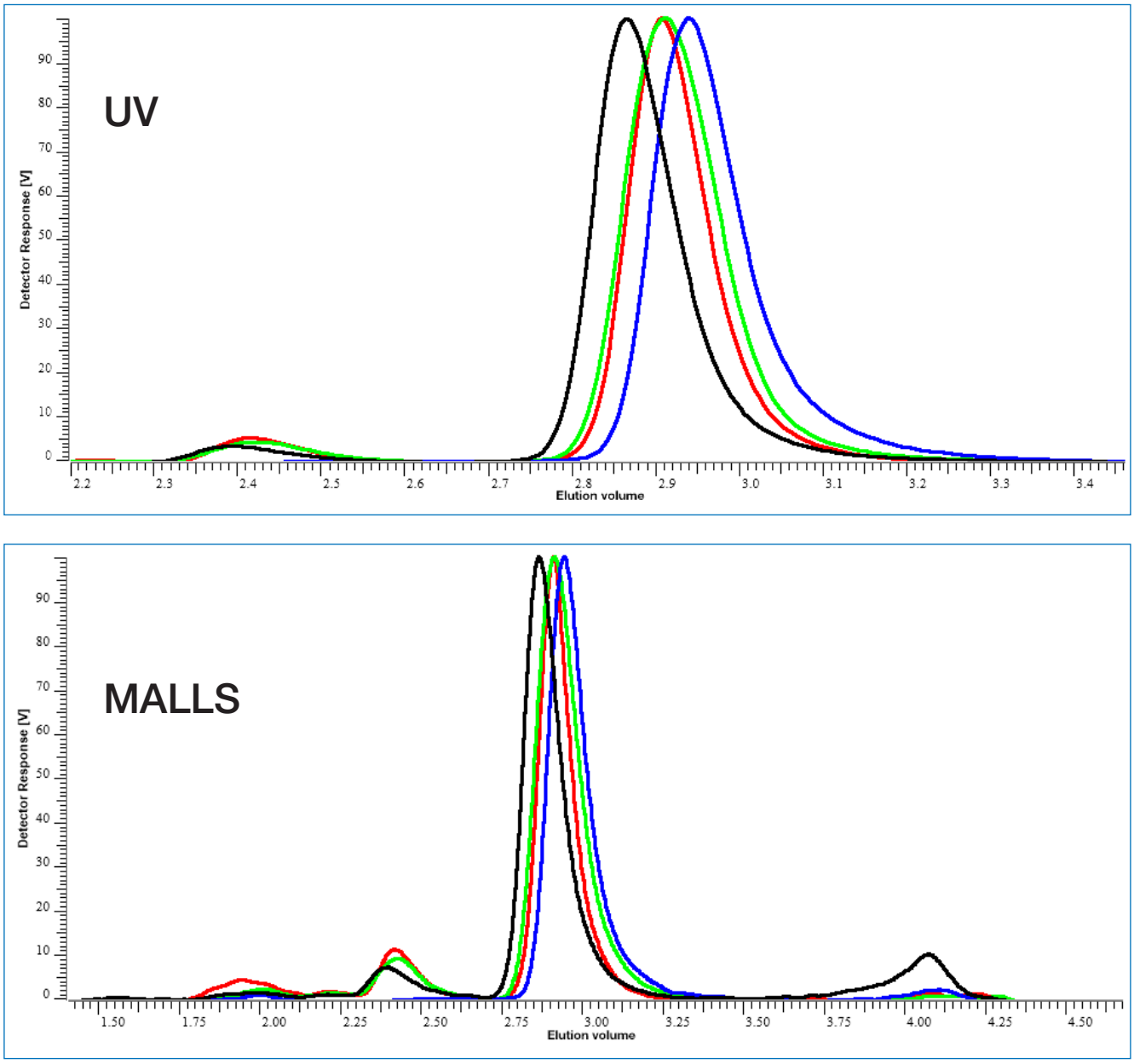


Figure 1: Chromatograms of Avastin® using different eluents. Top: UV at 280 nm. Bottom: MALLS detection trace (90° angle).
Black: PBS buffer; blue: phosphate buffer + 0.1 M NaCl; green: phosphate buffer + 0.3 M NaCl; red: phosphate buffer + 0.5 M NaCl.

The injection volume was increased from 10 µL to 25 µL and 50 µL without any significant influence on the resolution being observed. This demonstrates the tolerance of the YMC-SEC MAB phase to larger injection volumes.

While a general flow rate of 0.33 mL/min was used, a lower flow rate of 0.165 mL/min was also tested. The reduced flow rate showed no benefit, in terms of an improved resolution (figure 2). Therefore, the higher flow rate was preferred for a higher sample throughput.

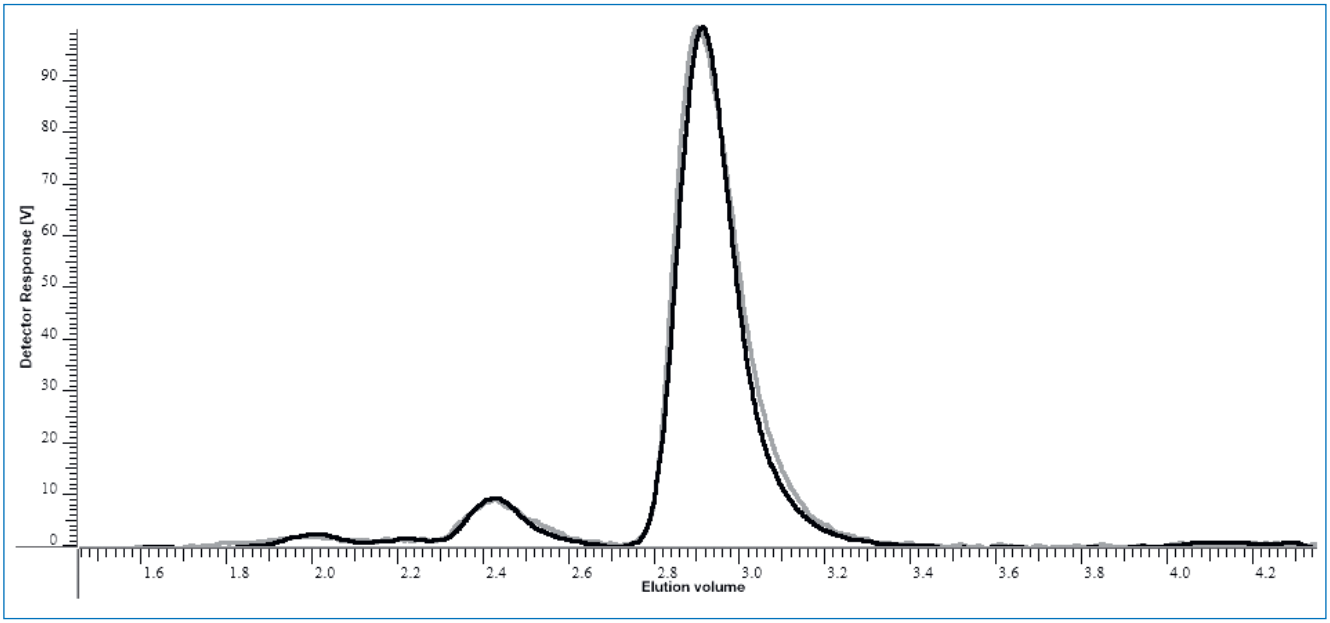


Figure 2: MALLS detection chromatograms (90° angle) at 0.33 mL/min /black and 0.165 mL/min (grey).

Table 1: Chromatographic conditions

Column:	YMC-SEC MAB (3 µm, 25 nm) 300 x 4.6 mm ID
Part No:	DLM25S03-3046WT
Eluent:	Phosphate buffer pH 6.6 containing 0.3 M NaCl
Flow rate:	0.33 mL/min
Temperature:	25 °C
Detection:	MALLS at 90° angle (PSS SLD7100)
Injection volume:	10µL
Sample:	Bevacizumab (Avastin®) dosage form (10 mg/mL, diluted to 1 mg/mL)
System:	PSS-SECcurity GPC systems, 1260 Infinity II
Software:	WinGPC Unichrom

Chromatograms courtesy of Thorsten Hofe, PSS Polymer Standards Service GmbH, Mainz, Germany.