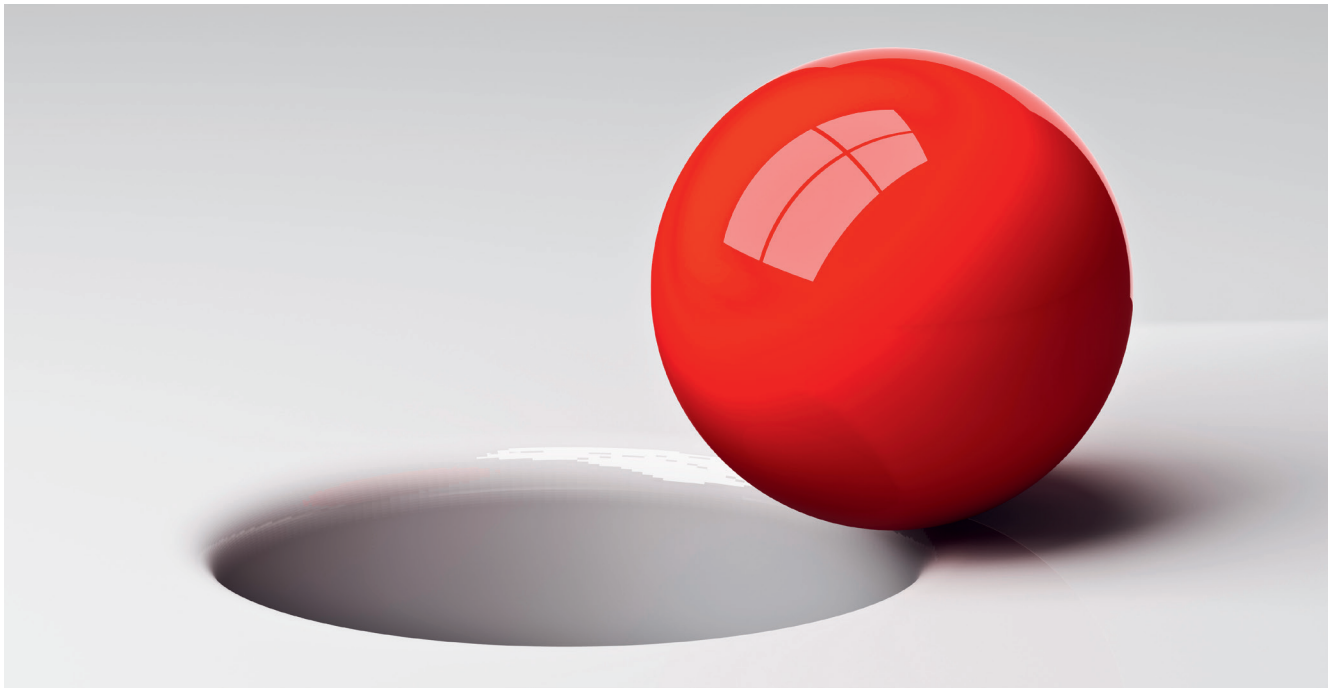
APPLICATION NOTE

Pore size selection for separation of oligonucleotides by YMC-Pack Diol columns

Oligonucleotides cover a broad field of applications ranging from molecular biology to therapy and diagnosis. Some oligonucleotides, such as antisense oligonucleotides, act as single-strands, but also double stranded oligonucleotides exist, such as the case of small interfering RNA (siRNA) duplexes. So far, the oligonucleotide length usually ranges from 12–25mer; however, the demand for longer oligonucleotides with lengths up to 200mer is increasing. These extremely long oligonucleotides are used in a variety of molecular biology applications including site directed mutagenesis.



Ion pair reversed phase liquid chromatography (IP-RP) and anion exchange chromatography (AEX) are the most commonly used methods for oligonucleotides analysis. However, separation of oligonucleotides by size exclusion chromatography (SEC) is a valuable alternative for analysis of oligonucleotides. A major advantage of SEC is the absence of secondary interactions of the analytes with the stationary phase. This is especially beneficial for oligonucleotide analysis due to their negatively charged phosphate backbone. In SEC the choice of an optimal pore size is crucial to successfully analysing oligonucleotides of different lengths. In this application note, columns of the YMC-Pack Diol series, which have distinct pore sizes (table 1) were tested for their ability to separate DNA of various lengths. Single-stranded DNAs (ssDNA) with lengths ranging from 10–120mer were compared to double stranded DNA (dsDNA) with lengths of 10–300mer as well as to proteins of different sizes (table 2).

Table 1: Chromatographic conditions.

Columns:	YMC-Pack Diol-120 (5 µm, 12 nm) 300 x 4.6 mm ID YMC-Pack Diol-200 (5 µm, 20 nm) 300 x 4.6 mm ID YMC-Pack Diol-300 (5 µm, 30 nm) 300 x 4.6 mm ID
Part Nos.:	DL12S05-3046WT DL20S05-3046WT DL30S05-3046WT
Eluent:	0.1 M KH_2PO_4-K_2HPO_4 (pH 7.0) containing 0.2 M NaCl
Flow rate:	0.17 mL/min
Detection:	UV at 260 nm
Temperature:	25 °C
Injection:	1.0 µL (each 5 nmol/mL)
Sample:	ssDNA dsDNA Protein mix

Table 2: DNA samples and proteins used.

Sample		Size
DNA	Single-stranded DNA	10mer, 15mer, 20mer, 25mer, 30mer, 40mer, 60mer, 80mer, 100mer, 120mer + dTMP
	Double-stranded DNA	10mer, 20mer, 35mer, 50mer, 75mer,100mer,150mer, 200mer, 300mer
Proteins	IgM (Mouse myeloma)	900,000 Da
	Thyroglobulin (Bovine thyroid)	670,000 Da
	IgA (Human colostrum)	390,000 Da
	Fibrinogen (Human plasma)	340,000 Da
	IgG (Human serum)	150,000 Da
	Transferrin (Human)	75,000 Da
	BSA	66,000 Da
	α1-Antitrypsin (Human plasma)	50,000 Da
	Ovalbumin	45,000 Da
	Carbonic anhydrase (Bovine)	30,000 Da
	Trypsin inhibitor (Soybean)	20,000 Da
	Myoglobin (Horse skeletal muscle)	17,000 Da
	α-Lactalbumin (Human milk)	14,000 Da
	Ribonuclease A (Bovine pancreas)	13,700 Da
	Cytochrome c (Horse heart)	12,400 Da
	L-Valine	117 Da

YMC-Pack Diol-120 (12 nm pore size) was best suited for shorter oligonucleotides (10–40mer), whereas YMC-Pack Diol-200 (20 nm pore size) showed the best resolution for oligonucleotides of medium size (30–80mer, figure 1). Longer oligonucleotides of 60-120mer in length were separated most effectively by YMC-Pack Diol-300 (30 nm pore size). Similar results were obtained when analysing dsDNA (figure 2). Small oligonucleotides were separated with higher resolution when smaller pore sizes of 12 and 20 nm were used. Above a length of 50mer, oligonucleotides were unable to penetrate the small pores and eluted at the same time. YMC-Pack Diol-200 (20 nm) was able to resolve oligonucleotides up to a size of 100mer. dsDNA of 150–300mer were only separated by YMC-Pack Diol-300 with the largest pore size of 30 nm. This column also shows the best resolution over a wide range of oligonucleotide lengths.

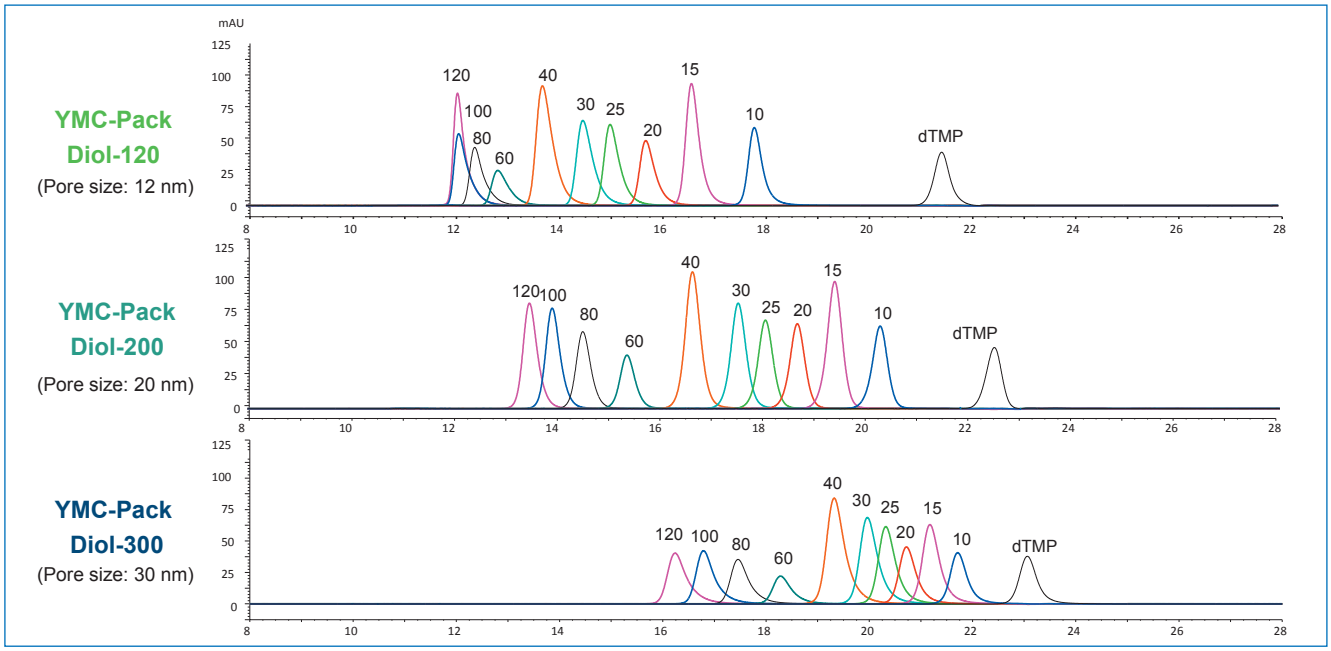


Figure 1: SEC analysis of ssDNA of 10–120mer length using YMC-Pack Diol columns with different pore sizes.

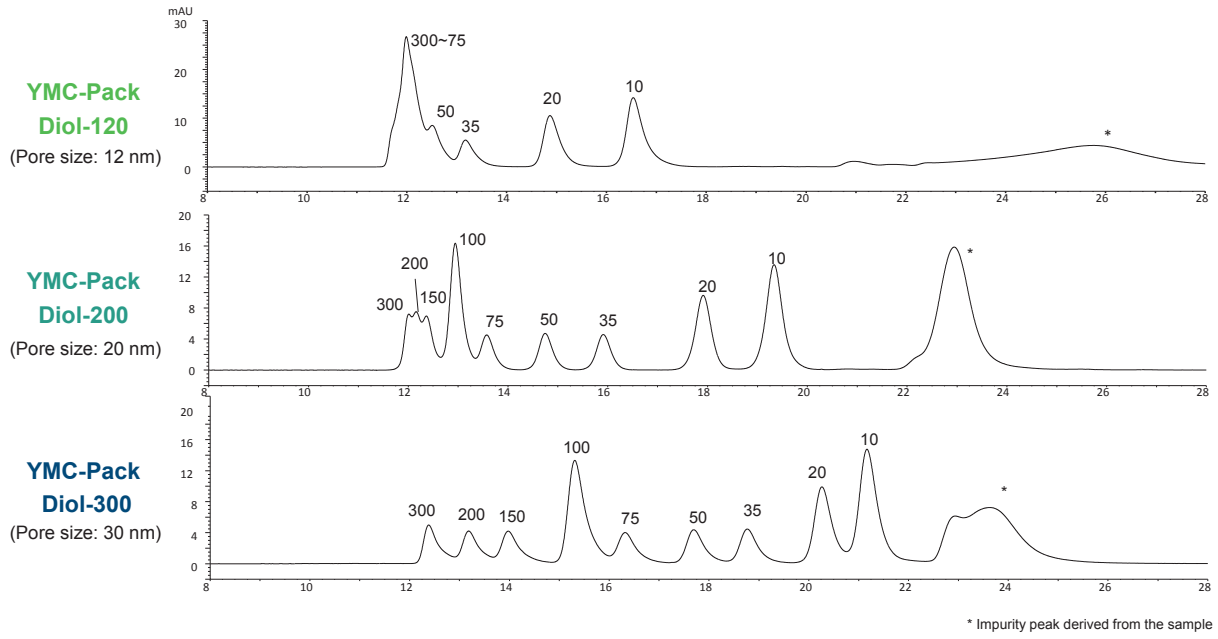


Figure 2: SEC analysis of dsDNA of 10–300mer length using YMC-Pack Diol columns with different pore sizes.

Although dsDNA has the same length as its single-stranded counterpart, the dsDNA elutes at lower elution volumes when separated by SEC (figure 3). This behaviour is most probably due to the larger hydrodynamic radius of dsDNA compared to ssDNA, which results in faster diffusion through the stationary phase. When comparing the elution volume of DNA with proteins of the same molecular weight separated using YMC-Pack Diol-200, it can also be seen that DNA elutes at a much lower volume than the proteins (figure 4). This indicates that the hydrodynamic radius of DNA is larger than of proteins leading to a distinct elution behaviour.

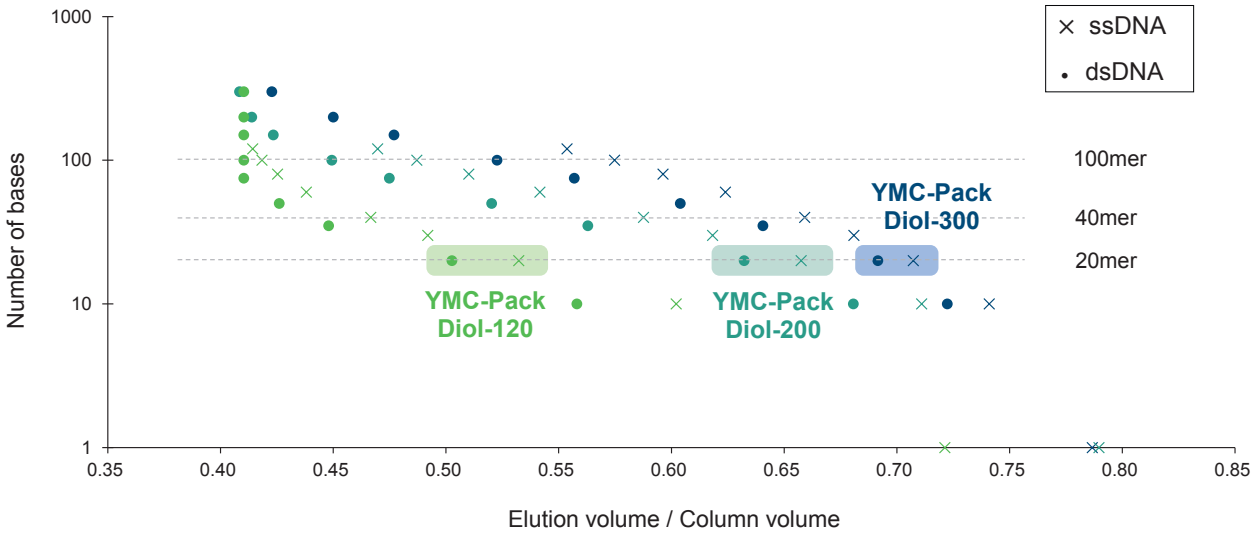


Figure 3: Comparison of the elution volume of ssDNA and dsDNA by using YMC-Pack Diol columns with different pore sizes. Selected corresponding ssDNA and dsDNA pairs of the same number of bases are marked.

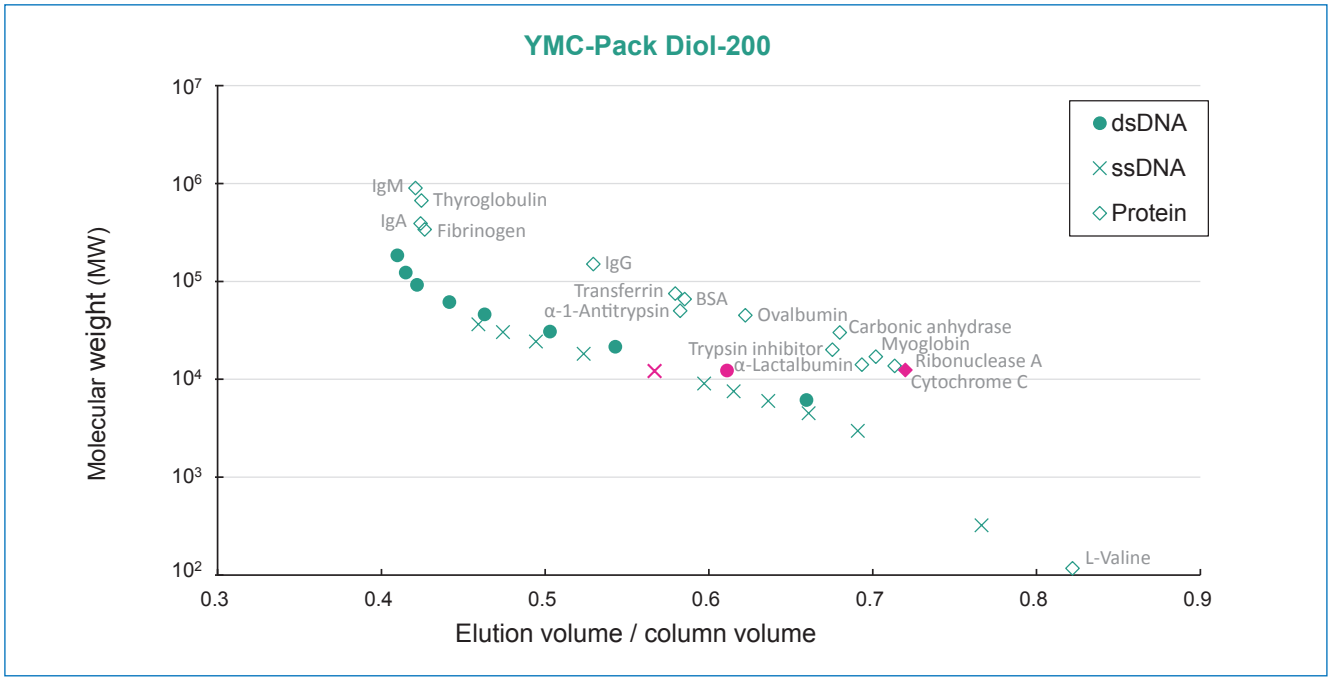


Figure 4: Comparison of elution volume of ssDNA, dsDNA and proteins of similar molecular weight separated by SEC using the YMC-Pack Diol-200 column. A triplet of the same MW of about 14,000 Da (a ssDNA, a dsDNA and Cytochrome C) is marked.

SEC can be a suitable method for separation of double stranded and single-stranded DNA of distinct lengths. However, the choice of an appropriate pore size suitable for the length of DNA analysed is critical. The recommended YMC-Pack Diol columns according to DNA length are shown below.