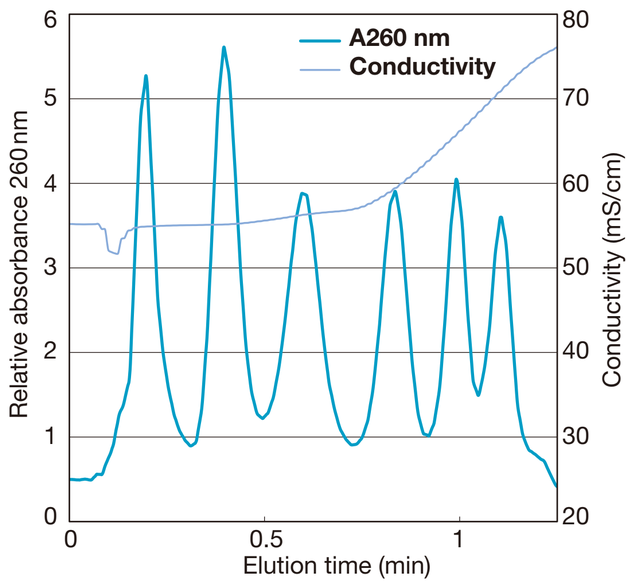
CIM™ Monolith : Purification of Nucleic Acid Molecules

- A purification of synthetic oligonucleotides by using CIM™ monolith was evaluated.
- In this case study, the CIM™ anion exchange column had the capability to resolve oligonucleotides with small difference in comparative chain length.
- A crude reaction mixture of synthetic oligonucleotide was loaded onto the CIM™ anion exchange column. Sample elution was achieved by salt concentration gradient. In comparison with conventional media, CIM™ monolith indicated higher resolution for major impurities.
- Advantages of the characteristic properties of the CIM™ monolith were evaluated based on the high throughput purification of oligonucleotides under the identified gradient separation conditions. Over 99 % HPLC purity for the target oligoDNA was achieved by one-step purification from the crude reaction mixture.

Separation of Synthetic Oligonucleotides



- This application evaluates the separation of an 8 to 16 mer synthetic oligonucleotide mixture utilizing the CIMmultus™ DEAE weak anion exchange column.
- Oligo DNA molecules having only small differences in base length from one another were separated by NaCl concentration gradient within 1.5 minutes.
- Target oligonucleotide elution conductivity was identified via linear gradient. Once appropriate target elution conditions were determined, step gradient conditions can be implemented to increase the efficiency of the target molecule purification. The significantly reduced run time of the CIM™ monolith allows for quick method optimization and an overall reduction in total method evaluation time. Optimizing parameters like ionic strength and mobile phase composition are easily achieved in a fraction of the time compared to currently available products. The intrinsic CIM™ monolith properties of excellent scalability and reproducibility ensure easy method transfer from the laboratory to larger scale preparative and manufacturing processes.

Column	: CIM™ monolith ;CIMmultus™ DEAE-1 bed volume 1 mL
Sample	: sample: 8 to 16 mer synthetic oligoDNA (indicated in right)
Buffer	: A : 20mM Tris-HCl (pH7.4) B : A + 1M NaCl
Gradient	: As indicated in figure (conductivity: mS/cm)
Flowrate	: 16 mL/min
Detection	: UV 260 nm
Instrument	: Liquid chromatography system (manufacturer A) with max. 100 mL/min pumping capacity
Temperature	: Ambient

Chain length	5'-3' sequence
8	CCATGTCT
10	GTCCATGTCT
12	AGGTCCATGTCT
14	CGAGGTCCATGTCT
15	CCGAGGTCCATGTCT
16	GCCGAGGTCCATGTCT

Purification of Synthetic Oligonucleotide

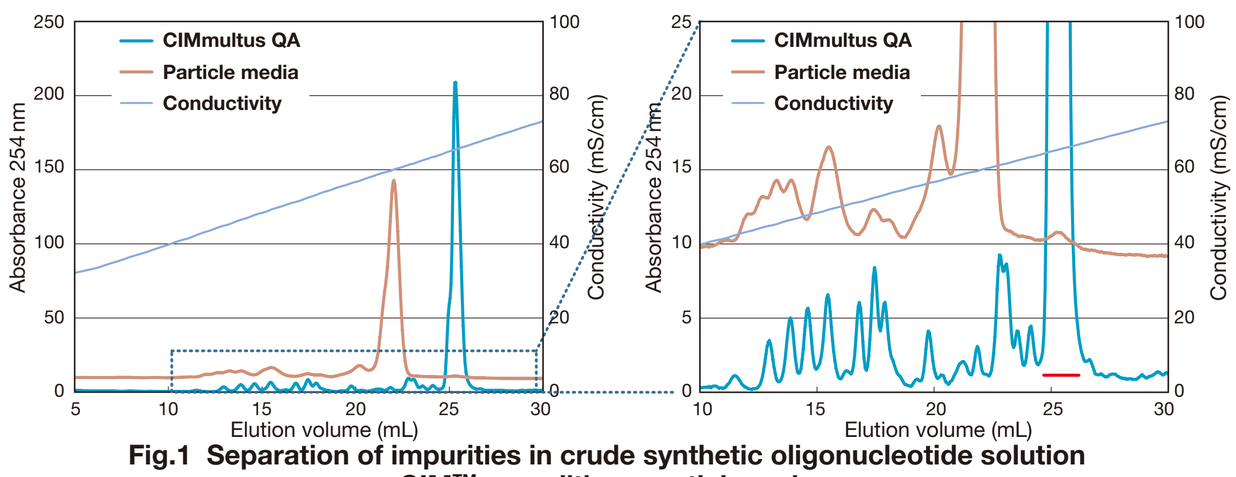


Fig.1 Separation of impurities in crude synthetic oligonucleotide solution CIM™ monolith vs particle resin.

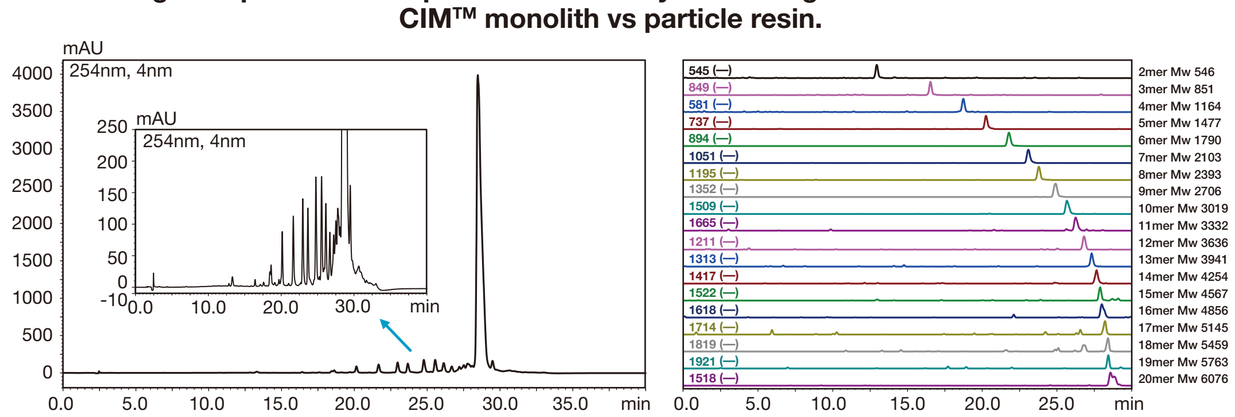
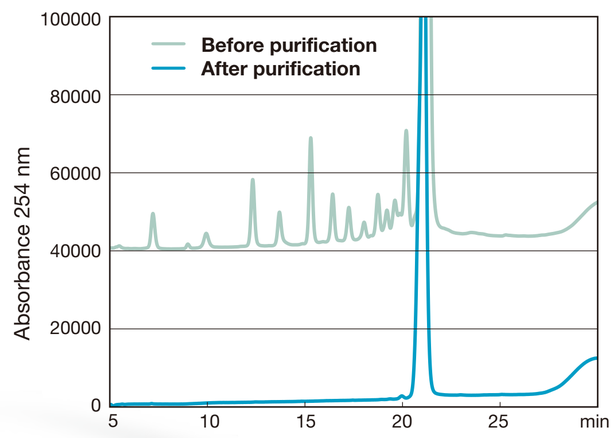


Fig.2 The identification of major impurities in crude synthetic oligonucleotide solution by LC-MS(RP-HPLC/MS).



[HPLC Purity (Abs. 254 nm)]

Before purification : 75.6 %

After purification : 99.5 %

Fig.3 Evaluation of the purity before/after purified by CIMmultus™ QA. (RP-HPLC).

- This application evaluates the purification of oligo DNA (20 mer) from a synthetic reactant extract utilizing the CIMmultus™ QA (Quaternery Ammonium) ion exchange column.
- Under the same gradient conditions, the resolution of impurities / target by the CIM™ monolith was compared to the result in existing particle based media (high-speed/resolution type polymer resin). The CIM™ monolith achieved higher resolution than traditional particle based media in a series of crude extract purifications.
- Each impurity peak observed in the crude extract was identified by using Reverse Phase (RP)-LC/MS (Fig.2). The molecular weight information retrieved from each peak clearly indicated the existence of intermediate length oligo DNAs (2 to 19 mer) in the crude extract.
- The main peak fraction of CIMmultus™ QA was recovered (red lined in Fig.1) and the HPLC purities before/after purification were estimated. The large amount of impurities existing in the crude extract was effectively removed, achieving a final product purity of over 99%. The CIMmultus™ QA is an effective tool for the fast one-step chromatographic purification of oligonucleotide.

[Fig.1] Prep. Chromatography

Column	: CIM™ monolith; CIMmultus™ QA-1, bed volume 1 mL Conventional media; polymer based high-speed/resolution resin with quaternary ammonium group, bed volume 1 mL (manufacturer A)
Sample	: Crude extract of synthetic oligoDNA (Automatically synthesized → desalted; target length 20 mer)
Buffer	: A : 10mM NaOH (pH 12) B : A + 1.5M NaCl
Gradient	: As indicated in figure (conductivity : mS/cm)
Flowrate	: 1.6 mL/min (conventional), 5 mL/min (CIM™ monolith)
Detection	: UV 254 nm
Instrument	: Liquid chromatography system (manufacturer A) with max. 100 mL/min pumping capacity

[Fig.2] LC-MS

Column	: Shodex Silica C18P 2D
Sample	: indicated above
Buffer	: A : 5mM DBAA B : A+ 50%Acetonitrile
Gradient	: 30 min B10-50
Flowrate	: 0.2 mL/min
Detection	: UV 254 nm

[Fig.3] Purity evaluation

Column	: Shodex Silica C18P 4E
Sample	: indicated above
Buffer	: A : 5mM DBAA B : A+ 50%Acetonitrile
Gradient	: 30 min B10-50
Flowrate	: 1 mL/min
Detection	: UV 254 nm

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