Asahi KASEI BIOPROCESS

Mastering the Fundamentals of Oligo Development

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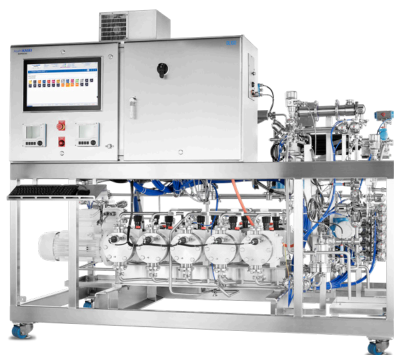
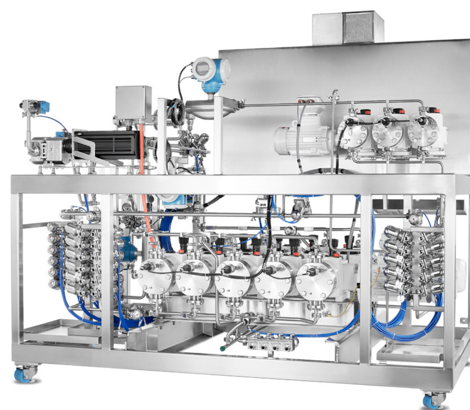
With over 300 candidates in clinical trials, oligonucleotides are one of the most promising therapeutic modalities and for the foreseeable future – and solid phase synthesis remains the gold standard approach for manufacturing.
Benefits include the relative ease of developing a synthesis route, the straightforward path to scale-up and improved in-process controls. For all the complexity that exists in oligonucleotide development – from end to end – a mastery of synthesis fundamentals can go a long way toward a successful run.



SYNTHESIS

As a chemical synthesis process, the reactor resides at the heart of oligonucleotide synthesis. In mid-scale to large-scale processes, that reactor takes the form of the flow-through synthesis column. Primarily, a synthesis column has two functions: (1) retain a well-packed bed of solid support during a turbulent and lengthy synthesis process; and (2) ensure an equal distribution of phosphoramidites and reagents to all beads in the packed bed.
Two of the main solvents required in oligonucleotide synthesis are acetonitrile (ACN) and toluene. As polymeric-based solid supports swell significantly in the presence of toluene and then shrink in the presence of ACN, the synthesis column must be able to accommodate these back-and-forth swings in column volume over the course of the synthesis cycle.
In order to obtain the highest yield of crude oligonucleotide during synthesis, it is critically important that all bead surfaces of the solid support are contacted by amidite and reagent. As solid phase processes scale up to larger column diameters, robust column flow distribution becomes an important driver for yield.

CLEAVAGE & DEPROTECTION



Once a full-length oligonucleotide has been successfully synthesized on the column, another set of chemical processes await to prepare the oligo for purification: cleavage of the molecule from the solid support, and removal of the protecting groups from the now-dissolved oligo. These processes require harsh chemicals and elevated temperatures. A typical cleavage may involve an amine wash of the column to remove phosphate groups followed by concentrated ammonia to free the oligo from the support.
Typically, the cleaved oligo is transferred into a deprotection vessel. For the deprotection step, the oligo must be heated in the vessel for several hours to temperatures between 30 and 60 C, depending on the molecule and the process. In the case of RNA, deprotection may also require the addition of TEA·3HF (triethylamine trihydrofluoride) at elevated temperatures.

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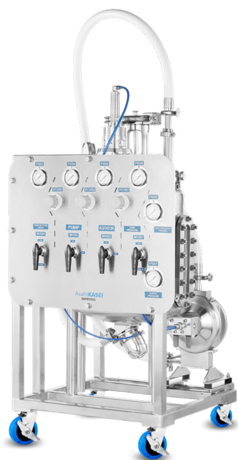

PURIFICATION: SYSTEMS

After cleavage and deprotection, the liberated crude oligonucleotide is ready for chromatographic purification. Reversed phase (RP) purification offers higher recoveries but requires organic solvent mobile phase. Ion exchange (IEX) chromatography benefits from largely aqueous eluents, but recoveries tend to be lower.

Whether purifying in RP mode, IEX mode, or both, the mechanical design of the MPLC or HPLC System is crucial. On one hand, the biologic complexity of an oligonucleotide molecule together with the potential aqueous environment of IEX purification demands a sanitary design with reduced bioburden risk. On the other hand, the chemical nature of nucleic acids and the flammable solvent-based RP purification technique necessitates a safety rating in compliance with regionally mandated fire protection and explosion proof regulations.

The purification system must be capable of delivering consistent and precise gradients to enable shortmer and longmer impurity removal while maintaining the highest level of recovery. The MPLC or HPLC System will also, ideally, offer user-friendly control software that can connect to the plant-wide automation platform.

PURIFICATION: COLUMNS

With its automated pumps and fractionation routines, the MPLC or HPLC System might be the brains of the purification process, but the separation of oligonucleotide from impurities occurs in the heart of the unit operation – the packed chromatography column.

Mid-sized oligonucleotides favor the use of spherical chromatography media with relatively small pore size to permit migration through the pores and small particle size to promote resolution. These small, spherical particles are best packed with dynamic axial compression, a technique in which the bulk media is slurried, transferred into the empty column and compressed into a packed bed by an axial piston. The axial compression ensures consolidation of the media into a uniform bed with no gaps or channels, and the dynamic nature of the compression ensures longer bed lifetimes by maintaining the integrity of the bed over time. These resins also generate backpressures of 10 to 50 bar, depending on bed height, temperature, eluent conditions, and flow rate. Therefore, the column must be able to reliably withstand these pressures.

PURIFICATION: SLURRY PREPARATION

In order to implement an RP or IEX resin for clinical and commercial scale oligonucleotide purification, you must first successfully pack the bulk media into the DAC column and qualify the packing via one or more column performance tests. Using a reproducible system platform, versus a manual process, allows for much more efficient removal of small fine particles that tend to clog column frits and prematurely increase column backpressure. Additionally, such a platform should be able to transfer the complete volume of prepared slurry into the empty column quickly to avoid any preferential settling of large particles in the column prior to compression.

OLIGO EXPERIENCE & EXPERTISE

Since 1998, Asahi Kasei Bioprocess has been delivering the fundamentals – and then some – to oligonucleotide developers around the globe, with novel equipment offerings from synthesis through purification. We apply time-tested ingenuity to amplify manufacturer capabilities – helping to meet growing demand with columns, systems and automation solutions that are Built For You.

Questions?

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