DUPONT™

Anion Exchange Resin for Oligonucleotide and Peptide Purification

Studies on agarose-based DuPont™ AmberChrom™ TQ1 resin

Introduction

Oligonucleotide and peptide therapeutics are gaining momentum with increasing drug approvals and expanding applications in various types of treatments.[1] While there are many differences in their manufacturing processes, the purification of these medium-sized biomolecules share certain features such as their use of reverse-phase high-performance liquid chromatography (RP-HPLC) and/or anion exchange chromatography (AIEX). This study provides an overview of DuPont™ AmberChrom™ TQ1 chromatography resin – an agarose-based AIEX resin designed for oligonucleotide and peptide purification. Table 1 showcases the typical properties of this resin. DuPont™ AmberChrom™ TQ1 resin complements DuPont's bioprocessing portfolio, which includes the DuPont™ AmberChrom™ CG and XT resins for the purification of similar drug modalities.

Table 1: Typical properties of DuPont™ AmberChrom™ TQ1 resin.

Property	DuPont™ AmberChrom™ TQ1 resin
Average particle size (D50)	50 – 60 µm
pH stability	2 – 12 (working pH), up to 14 (cleaning)
Temperature	4 – 30 °C
Functional group	Quaternary amine
Ion exchange capacity	0.19 – 0.25 mmol Cl^-/mL resin
Antisense oligonucleotide (20-mer) 10% Dynamic Binding Capacity (DBC); Residence time ~ 4 min; measured in 0.02 M NaOH	DMT-On: 51 mg/mL of resin DMT-Off: 62 mg/mL of resin
Insulin 10% DBC, measured at pH ~8	90 mg/mL of resin

Experimental Conditions

Purifications were performed on DuPont™ AmberChrom™ TQ1 resin, packed in Omnifit® columns, and DuPont™ Profile™ AmberChrom™ XT20 column on ÄKTA pure™ 25 M or 150 M at 10 – 20 mg oligo or peptide loads per mL of resin (room temperature). Oligonucleotide purification was performed by loading the oligonucleotide feed in 0.02 M NaOH and eluting in 0.02 M NaOH with 2 M NaCl (Fisher Scientific™). Analysis was performed on Agilent™ 1260 Infinity II HPLC system using anion exchange (IEX) (DNAPac™ PA200 column, 8 µm) for oligonucleotides. Peptide purification was performed in basic conditions in the anion exchange step and in 0.1% trifluoroacetic acid (TFA) in the reverse phase step. Liraglutide elution was performed with a gradient of 0.1% TFA in acetonitrile (Fisher Scientific™). Liraglutide analysis was performed on Waters™ XSelect™ CSH C18 column (2.5 µm) in acidic conditions on the same Agilent™ system.

Results

Oligonucleotide purification

Oligonucleotides are typically produced by the synthetic phosphoramidite method, which utilizes a solid support typically loaded with a first base where the 3'-hydroxil is blocked by a protecting group. The synthesis consists of four main steps that include deblocking the protective dimethoxytrityl (DMT) group and the coupling of the next base. Any hydroxyl groups that fail to couple are then capped to prevent further chain propagation. An oxidation step follows to ensure the production of the phosphate triester linking group.[2] This cycle repeats in a stepwise fashion until the target number of bases have been added in the desired sequence. When the oligonucleotide is detached from the resin, impurities in the mixture include shorter failure sequences and other small molecule byproducts.

In other studies, we showcase the performance of the reverse phase polymeric DuPont™ AmberChrom™ XT resins in removing these impurities and isolating the full-length product (FLP). These resins can be used to purify various oligonucleotide modalities ranging from 20-mer antisense oligonucleotides (ASOs) to greater than 100-mer single guide RNAs (sgRNAs) both in DMT-On mode and DMT-Off mode with ion-pairing agent. Here, we showcase the benefits of the DuPont™ AmberChrom™ TQ1 resin performing similar purifications in anion exchange mode.

Results

High-load oligonucleotide purification on DuPont™ AmberChrom™ TQ1 resin

Anion exchange resins are typically used with detritylated (DMT-Off) oligonucleotide feeds. This purification step can be the core of the downstream process of certain modalities after detritylation on a solid support or in solution. It can also be used as a polishing step following a crude purification using reverse phase resins.

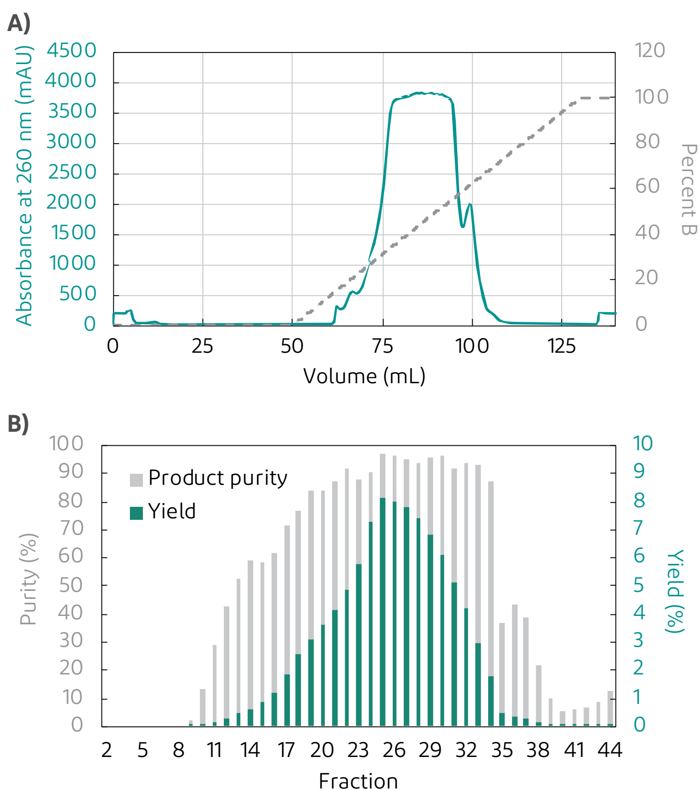

Figure 1:
A) Purification of a 20-mer DMT-Off antisense oligonucleotide loaded at 20 mg/mL of resin on DuPont™ AmberChrom™ TQ1 resin and eluted with a gradient of 2 M NaCl in 0.02 M NaOH.
B) Analysis of fractions under the product peak in (A) with the yield and purity of each fraction.

Figure 1A shows the purification of a 20-mer DMT-Off antisense oligonucleotide on DuPont™ AmberChrom™ TQ1 resin. The feed had a crude purity around 86%. The fractions obtained under the main peak are analyzed and plotted in Figure 1B. The analysis shows one of the key features of this resin: the fractions obtained are high in purity, with the highest purity reaching approximately 98%, but also high in yield or product concentration. Highly concentrated fractions translate into easier downstream processing and analytical work, especially as processes are scaled-up during drug manufacturing.

Results

High-loading capacity with advantageous pressure-flow behavior

To further explore the performance of DuPont™ AmberChrom™ TQ1 resin in purification experiments, the same oligonucleotide was progressively loaded on the resin and the breakthrough capacity was measured at 10% of the absorbance saturation value. Table 2 shows that the 10% DBC value in these conditions for this crude oligonucleotide feed is around 51 mg/mL of resin for the DMT-On oligonucleotide and even higher – 62 mg/mL of resin – for the DMT-Off oligonucleotide. This is another advantageous feature of this resin as AIEX resins are generally known to have a relatively lower loading capacity compared to RP resins. In comparative studies, a series of other AIEX resins exhibited a range of loading capacities of around 30 ± 10 mg/mL of resin in the same conditions with the same feed.

This high loading capacity likely underpins the high yields observed in the purification performed in Figure 1. With the results observed in Table 2, the purification of the same DMT-Off oligonucleotide was performed at an even higher load, 50 mg/mL, which surpasses the 10% DBC of many AIEX resins used for oligonucleotide purification. Figure 2 shows that even at this high load, the recovered product purity remains high in pooled fractions. This observation points to the resin's high-resolution purification, leading to an effective separation between the full-length product and the impurities.

Table 2: Dynamic binding capacity (10% DBC) of 20-mer antisense oligonucleotide loaded in 0.02 M NaOH in water on DuPont™ AmberChrom™ TQ1 chromatography resin.

Product	DMT-On oligonucleotide	DMT-Off oligonucleotide
DuPont™ AmberChrom™ TQ1 resin	51 mg/mL	62 mg/mL

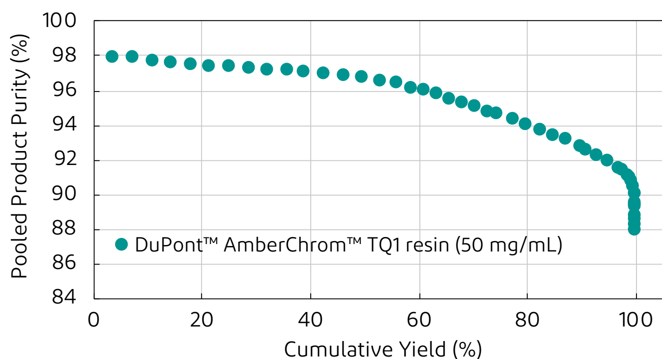

Figure 2: Yield-purity curve obtained by analyzing fractions eluted after loading the same oligonucleotide at 50 mg/mL of resin.

The DuPont™ AmberChrom™ TQ1 resin is also compatible with large scale processes. Figure 3 shows the resin bed pressure build-up in a larger column (5 cm internal diameter x 15 cm bed height) is at or below 1 bar in the typical running range of 200 – 300 cm/hour.

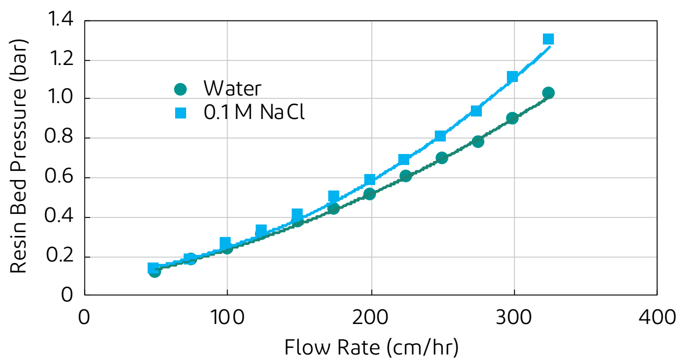

Figure 3: Pressure-flow performance of DuPont™ AmberChrom™ TQ1 resin in a 5 cm-diameter column with a resin bed height of 15 cm.

Results

Peptide purification

DuPont™ AmberChrom™ TQ1 resin is also compatible with peptide purification. In Figure 4, the purification of liraglutide, a glucagon-like peptide-1 (GLP-1) receptor agonist, was performed on this resin at a loading of 10 mg/mL of resin. The purification, which was performed in basic conditions (Tris-base pH 8.3), shows an increase in the purity from 60% to a maximum purity of more than 80%. This purification was followed by a polishing step with DuPont™ AmberChrom™ XT20 resin, a 20 μm reverse phase polymeric resin. The polishing step, performed in acidic conditions, led to a maximum purity greater than 97%.

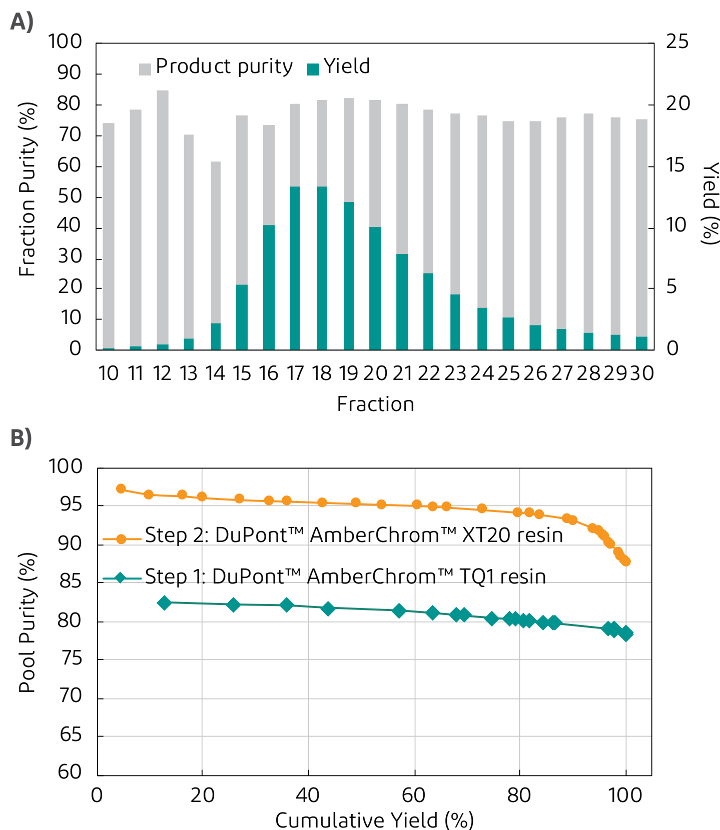

Figure 4:
A) Fraction yield and purity obtained from the purification of a liraglutide feed (crude purity approximately 60%) on DuPont™ AmberChrom™ TQ1 resin.
B) Yield-purity curve for the crude purification of liraglutide shown in (A) and the polishing step performed on the polymeric reverse phase DuPont™ AmberChrom™ XT20 resin.

Conclusions

The studies above introduce the DuPont™ AmberChrom™ TQ1 chromatography resin, a hydrophilic anion exchange resin compatible with purifications of oligonucleotides and peptides. They showcase the performance of this resin in purifying various types of biomolecules in different conditions where it delivers a balance between high yields and purities, highlighting well-separated products and impurities. The resin also provides a high loading capacity for these molecules and a well-balanced pressure flow performance in larger columns.

References

1) Moumné et al., Pharmaceutics 2022, DOI: 10.3390/pharmaceutics14020260
2) Andrews et al., The Journal of Organic Chemistry 2021, DOI: 10.1021/acs.joc.0c02291

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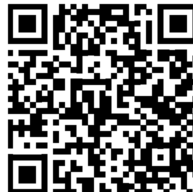

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