Application of In-Line Variable Pathlength Chromatography for High Concentration Oligonucleotide Solutions

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Introduction

Antisense oligonucleotides (ASOs) have become a group of interest in the field of oligonucleotide therapeutics due to successful implementation for diseases such as spinal muscular atrophy and hereditary transthyretin amyloidosis. Now, many pharmaceuticals aim to assess the viability and capabilities of ASOs to combat diseases such as Alzheimer's disease, Parkinson's disease, and other neurodegenerative diseases. With the increased focus on oligonucleotides within biopharmaceutical projects, real time measurements of product concentration can lead to increased efficiency within bioprocessing and manufacturing. A collaboration between Repligen and Ionis Pharmaceuticals assessed the viability of in-line measurement of oligonucleotide concentration during reversed-phase chromatography. The experiment incorporated a variable pathlength to measure highly concentrated ASO eluate. In order to test the reliability of the in-line variable pathlength spectrophotometer, the system data was then compared to a 1 mm fixed ultraviolet (UV) absorbance chromatogram traditionally used within a variety of bioprocesses.

Hypothesis

The common use of fixed pathlength UV absorbance sensor may not be the optimum application for in-line chromatography and concentration reading of oligonucleotides. Incorporating in-line variable pathlength technology will allow for high concentration oligonucleotide data to be integrated in real time and provide accurate pool mass in real time. The current purification system uses in-line 1 mm fixed pathlength sensor, which records all flow data for the system with the elution occurring at approximately 42 minutes. Typical UV sensors using fixed pathlengths lose linearity at around 2 Au with an absorption limit at around 3 Au. With the fixed pathlength sensor, the absorbance of the eluted ASO solution is consistently out of the linear range of detection when using either the standard lambda max 260 nm wavelength or the 295 nm wavelength at which the operation was previously optimized. As noted during our experiment, the absorbance numbers during elution maintained a number outside the traditional limits for more than 20 minutes (see Figure 1). Accurate quantification of integrated mass using this sensor would not be possible.

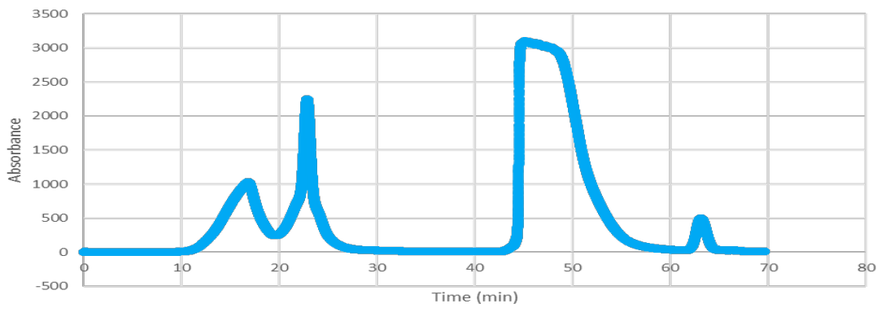

Figure 1. Standard 1 mm fixed pathlength at 295 nm production and elution.

Methods and Materials

The experiment was conducted using the in-line variable pathlength spectrophotometer measuring at the target wavelength of 260 nm. The System was protected by a splash guard to be Class I, Division 2 Zone 2 compliant (Figure 2). The System was then connected to the fraction outlet on the bioprocess system with a reversed phase HPLC column (see Figure 3). The in-line variable pathlength spectrophotometer System recorded the elution profile in real time. The eluted product was collected in a LDPE carboy. The traditional 1 mm built-in chromatography system within the bioprocess was compared against the in-line variable pathlength spectrophotometer. The in-line spectrophotometer was loaded with an adjustable 10 mm Flow Cell with a flow throughput of 2.7 l/min. The in-line variable pathlength spectrophotometer incorporated Beer's law linearization of five pathlengths per data point to calculate flow concentration and pool mass. Pathlengths ranged from 0.001 mm to 0.005 mm.

Figure 2. Oligonucleotide HPLC system setup.

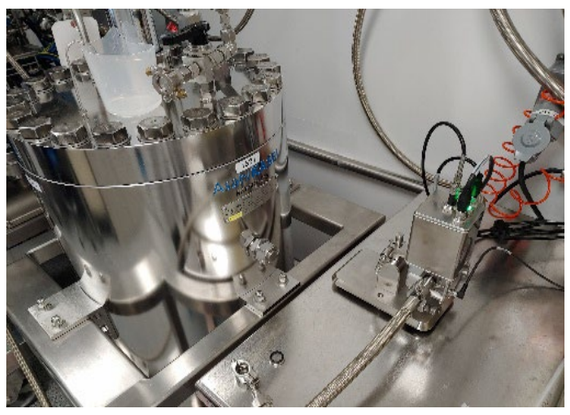

Figure 3. In-line variable pathlength sensor connected to HPLC elution line.

Data

The in-line variable pathlength UV System utilized an average of five pathlength readings per concentration measurement and recorded a total of 42 measurements for Elution 1 and 248 measurements for Elution 2. The data recorded for each regression was found to have an R^2 ≥0.999. The in-line System recorded product masses of 562 g for Elution 1 and 526 g for Elution 2 with a 11% and 2.9% difference, respectively (see Table 1), compared to the determination of the product mass via an analytical HPLC method. These masses were obtained over a 45-minute runtime and automatically integrated within the in-line variable pathlength System software. The data was automatically updated and saved per cGMP standards using secured software. Secured standards for the in-line variable pathlength spectrophotometer considers readings with R^2 ≥0.999 as acceptable data sets and indicates any data points that fall out of that criteria. Two different acquisition methods were utilized, auto-ranging Quick Slope mode and Fixed Pathlength mode.

Table 1. In-line real-time chromatography data for variable and set pathlengths

Elution #	Fraction Volume (l)	Fraction Weight (kg)	Total Oligo Concentration (mg/g)	Total Oligo (g)	FlowVPX mass (g)	%Diff
1	20.06	17.58	28.8	506	562	11%
2	19.47	17.14	29.8	511	526	2.9%

Elution #	Time (min)	Volume (l)	Time (min)	Volume (l)
1	44.55	118.52	51.98333	138.59
2	44.42167	118.17	51.64167	137.67

Results

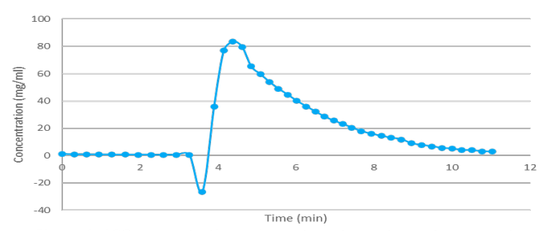

Figure 4. ASO concentration using automated pathlength chromatography.

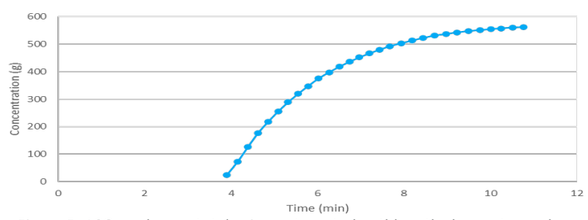

Figure 5. ASO pool mass total using automated pathlength chromatography.

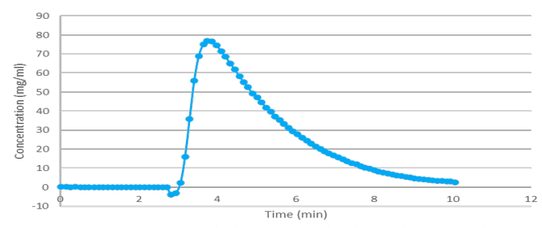

Figure 6. ASO concentration using set pathlengths chromatography.

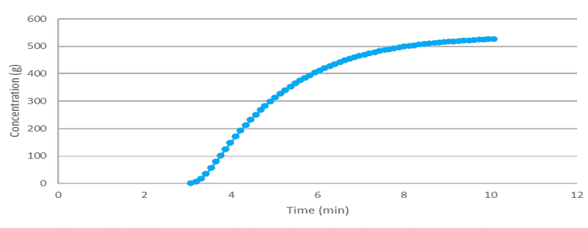

Figure 7. ASO pool mass total using set pathlength chromatography.

The data gathered is automatically integrated into a graph based on current flow concentration and total pool concentration. The data points within the graphs are dependent upon the parameters set prior to the run. The first elution graph is a Kinetics Quick software operation incorporating a fixed set of data points and instead uses the in-line variable pathlength spectrophotometer's automated pathlength finder to optimize data collection (see Figures 4 and 5). The second elution incorporating a set pathlength displays greater number of data points, though the efficacy for each run remains at R^2 ≥0.999 (see Figures 6 and 7). When compared to the 1 mm traditional chromatography, the in-line variable pathlength spectrophotometer was able to record the total elution at the target wavelength for the automated and set pathlength settings. Maximum peak concentration was determined at around 80 g/l of ASO.

Conclusion

The experiment supports the implementation of in-line variable pathlength chromatography as a viable alternative for in-line variable pathlength spectrophotometer real-time integration of high concentration oligonucleotide solutions. When compared to the traditional 1 mm fixed pathlength chromatography, the variable pathlength chromatography was able to read real-time high concentration ASO solutions at the target wavelength without going over the readable range. Within the growing field of ASO and oligonucleotide therapeutics, the need for reliable in-line data will become a stressor on projects regarding high concentration solutions. The capabilities of the in-line variable pathlength spectrophotometer demonstrated in this experiment provide a reliable alternative for biopharmaceutical laboratories to streamline drug development and manufacturing without needing to perform additional analyses to quantify in-process intermediates.

Acknowledgements

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