WEBINAR DIGEST

Precision in production: optimizing monitoring and quality control for high-value plasmids

Oleksandr Karpenko, Bioprocess Analytics Field Application Scientist, Repligen Corporation

Gene therapy analytics directly influence product quality, ultimately saving precious time and resources. However, traditional UV spectroscopy, commonly used for bioprocess analytics, can pose a range of challenges. This poster introduces a streamlined analytics process for gene therapy products utilizing variable pathlength technology (VPT) in place of traditional analytical tools. Case study data demonstrates that VPT-based technology can accurately determine pDNA purity ratios in human gene therapy products, automating the R-value calculations and eliminating the need for dilutions.

TRADITIONAL UV SPECTROSCOPY VERSUS VARIABLE PATHLENGTH SPECTROSCOPY

Traditional UV spectroscopy, commonly used for bioprocess analytics, has limitations such as lengthy assay times, labor-intensive procedures, and susceptibility to errors. VPT, also known as slope spectroscopy, addresses these issues by adjusting pathlengths to maintain a constant concentration (**Figure 1**). As a result, VPT eliminates the need for dilutions, significantly increasing process efficiency and reducing cycle times by providing instant analytical results, which ultimately enhances process understanding with real-time insights.

CASE STUDY: UTILIZING SLOPE SPECTROSCOPY TO DETERMINE PDNA PURITY RATIOS IN HUMAN GENE THERAPY PRODUCTS

In this case study, slope spectroscopy, namely SoloVPE©, was utilized to determine the pDNA purity ratios in human gene therapy products in place of traditional nucleic acid analytics. The aim of the study was to evaluate the impact of dilution on the measurement process and compare the reliability and accuracy of the SoloVPE method with traditional spectrophotometry techniques.

Figure 1. Traditional UV spectroscopy versus variable pathlength spectroscopy.

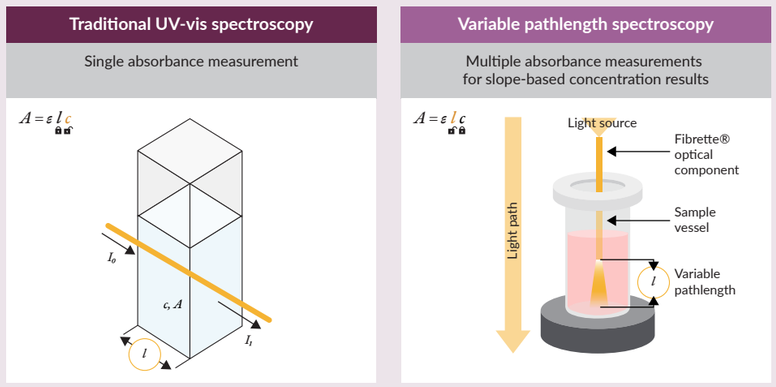


Table 1. pDNA purity study results.

Level	Theoretical purity ratio	Observed purity ratio	% Difference
1	0.62590	0.62723	0.21
2	0.87087	0.90315	−0.22
3	1.05311	1.06122	0.74
4	1.18483	1.17076	−1.14
5	1.28451	1.28847	0.30
6	1.42528	1.42358	−0.11
7	1.51996	1.52481	0.31
8	1.58798	1.58959	0.10
9	1.63927	1.65568	0.96
10	1.67930	1.67489	−0.25
11	1.71134	1.69203	−1.09
12	1.73770	1.71515	−1.25
13	1.75964	1.73533	−1.33
14	1.77821	1.77064	−0.41
15	1.79418	1.81956	1.36
16	1.80804	1.77874	−1.56
17	1.82026	1.80414	−0.85
18	1.83094	1.81184	−1.00
19	1.84046	1.82975	−0.56
20	1.84902	1.85457	0.29
21	1.85672	1.84667	−0.52
22	1.86028	1.88378	1.22
23	1.86365	1.85282	−0.56
24	1.86692	1.84941	−0.90
25	1.87000	1.87147	0.08

Firstly, 25 different purity levels for a mixture of DNA and protein were defined, and corresponding solutions for each level were prepared. These ratios ranged from 100% protein and 0% DNA to 100% DNA and 0% impurities. Triplicate measurements were taken at each purity level to obtain the 260/280 nm absorbance ratio and slope values. The SoloVPE software automatically calculated the R-values for each measurement. To compare the experimental R-values with theoretical values, theoretical purity ratios were calculated for each solution and compared with the R-values obtained from SoloVPE. Based on the measurements, the observed purity ratio results closely matched the theoretical values (**Table 1**).

Overall, the case study demonstrated that SoloVPE can be a critical analytical tool for determining plasmid DNA purity, expediting sample testing, providing immediate feedback, and expanding possibilities for meeting medical needs. The study also showed that the SoloVPE system provides significantly improved sensitivity compared to traditional spectrophotometers.

OTHER GENE THERAPY APPLICATIONS USING VPT

In addition to measuring the pDNA purity ratio in human gene therapy products, there are various other areas where VPT could be applied, including pDNA downstream processing monitoring, fermentation analysis, chromatography, mRNA purity ratio measurements, and AAV capsid and genome titer analysis.

SUMMARY

Adopting a unified analytical platform with VPT can offer a number of benefits, such as a broad concentration range without the need for baseline correction, robust and accurate measurements, and no requirement for dilution. Each result produced by VPT systems is based on multiple measurements, providing an R^2 value for every outcome, ensuring reliable and precise data in gene therapy analytics.

Watch the webinar here

Read the full transcript here

Copyright © 2025 Repligen. Published by *Nucleic Acid Insights* under Creative Commons License Deed CC BY C ND 4.0.

Nucleic Acid Insights 2025; 2(1), 11 • DOI: 10.18609/nuc.2025.003