Implementation of Variable Pathlength Technology at Boehringer-Ingelheim Fremont

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Introduction

Absorption spectroscopy is used to quantify biomolecules using Beer's Law: Absorbance **(A)** = Molar Absorptivity **(ε)** * Pathlength **(L)** * Concentration **(c)**. **A = ε L c**

At high concentrations, samples must be diluted due to limitations of traditional spectroscopy instrumentation utilizing a fixed pathlength of 1 cm. A gravimetric correction is applied to ensure accuracy of dilution. The process of diluting, applying the gravimetric correction and washing the cuvette is time consuming and can take several hours in a controlled setting such as Quality Control (QC) or Manufacturing. The use of Variable Pathlength Extension eliminates the need to dilute samples by taking absorbance measurements at multiple pathlengths using a disposable optical fibrette and sample vessel. The software plots absorbance vs. pathlength. Using Linear regression, the software calculates a slope for the points and determines the concentration by rearranging Beer's Law to: **A/L = slope = ε c.** Use of Variable Pathlength for concentration measurements results in time savings of greater than 80%.

Variable Pathlength Technology

Based On Beer's Law

A = ε L c

slope = A/L

c = m/ε

Where:
- **A :** Absorbance
- **e :** Extinction Coefficient
- **L :** Pathlength
- **C :** Concentration
- **m :** Regression Slope (ΔA/ ΔL)

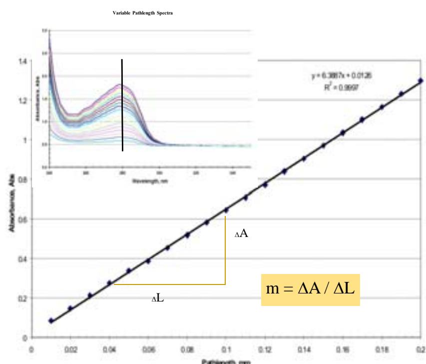

Figure 1: Measurement principle based upon Beer-Lambert Law
The instrument measures the absorbance over multiple pathlengths. The slope calculation uses multiple data points, improving accuracy over single point absorbance measurements. This slope measuring capability combined with the pathlength range of 0.005 mm to 15.000 mm allows the system to measure both highly concentrated and very dilute samples directly without sample preparation. This allows for rapid determination of concentration when the Extinction Coefficient is known.

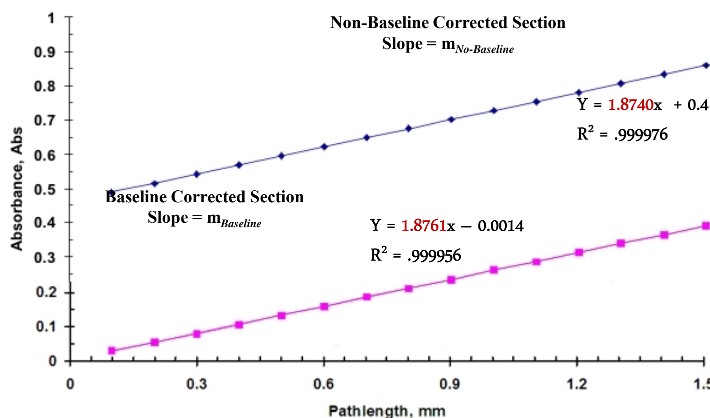

Figure 2: Blank subtraction not necessary
The change in absorbance relative to the change in pathlength is buffer independent. Concentration measurements are determined using slope rather than absorbance. The slope of the sample is the same with or without a buffer blank subtraction. Consequently, buffer blanks are not necessary unless the buffer has an absorbance at 280nm.

Time Savings Per Year

Variable Pathlength vs. Gravimetric	Hrs Saved / Sample	Total Hrs Saved
Quality Control	0.7	> 48
Manufacturing	1.5	> 450
Process Science	0.5	> 75
Process Science **	0.2	>1800

** Time Saved Compared to Volumetric Method

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Qualification Parameters

Table 1: Precision (Repeatability)

Conc. mg/mL	Measured Concentration mg/mL	Avg Conc, mg/mL	STDev	%CV
65.0	65.9	66.3	0.4	0.6
	66.4			
	66.7			
50.0	51.8	51.9	0.3	0.6
	51.6			
	51.2			
10.0	10.1	10.1	0.2	0
	10.1			
	10.1			
1.0	1.0	1.0	0	0.1
	1.0			
	1.0			
0.1	0.1	0.1	0	0.4
	0.1			
	0.1			

Graph 1: Dilutional Linearity

Single preparations of one sample at 0.1, 1.0, 10.0, 50.0, and 65.0 mg/mL concentrations were measured. The average slope for each sample concentration was plotted against the target sample concentration

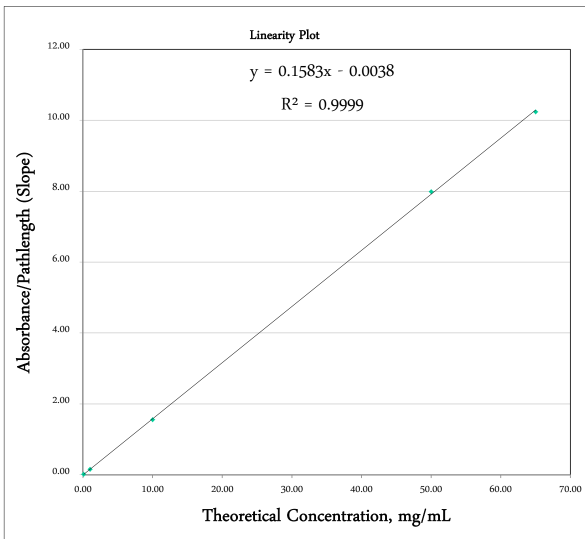

Table 2: Intermediate Precision

Samples	Group	Variable Pathlength Measured Conc. mg/mL	Avg Conc. mg/mL	% CV	Avg Conc. mg/mL	Avg %CV
In Process Purification Step 2	Analytical Science	6.7	6.7	0.1	6.7	0.1
		6.7				
		6.7				
	Manufacturing	6.7	6.7	0		
		6.7				
		6.7				
	Quality Control	6.7	6.7	0.1		
		6.7				
		6.8				
In process Purification Step 1	Analytical Science	19.3	19.3	0.2	19.3	0.3
		19.4				
		19.3				
	Manufacturing	19.2	19.2	0.2		
		19.3				
		19.2				
	Quality Control	19.3	19.3	0.1		
		19.3				
		19.3				
BDS	Analytical Science	10.0	10.0	0.1	10.2	3.4
		10.0				
		10.0				
	Manufacturing	10.0	10.0	0.2		
		10.0				
		10.0				
	Quality Control	10.7	10.7	0.1		
		10.7				
		10.7				

Comparability

Table 3: Gravimetric VS. Variable Pathlength

Sample (N = 3)	Gravimetric Conc. mg/mL	Gravi-metric %CV	Variable Pathlength Conc. mg/mL	Variable Pathlength %CV	% Difference Gravimetric vs. Variable Pathlength
In-Process Purification Step 1	2.8	2.2	2.7	0.1	1.8
In-Process Purification Step 2	2.7	0.9	2.6	0.1	2.6
In-Process Purification Step 3	49.8	0.6	50.5	0.3	1.4
In-Process Purification Step 4	62.4	2.5	62.5	0.3	0.1
BDS	50.4	1.9	50.5	0.3	0.3

Conclusions

Variable Pathlength Technology:

- ☞ Saves time
- ☞ Eliminates errors due to dilution
- ☞ Improves accuracy over single point absorbance measurements
- ☞ Functions over a wide range of protein concentrations
- ☞ Comparable to Gravimetric data