SEG-FLOW® 4800

Automated Sampling & Feed Control System

Seg-Flow® 4800 Sample Module: On-line Sample Dilution Performance

Introduction

The Seg-Flow 4800 system's Sample Module is an ancillary sample management device which increases Seg-Flow functionality such as automated on-line sample dilution, sample delivery precision, reagent addition and increased device output. In this study, the Sample Module was evaluated to determine dilution performance for on-line glucose analysis of chemically defined media. A range of dilution factors were tested to ensure on-line analytical analysis was not compromised through dilution imprecision and/or mixing inefficiency.

Performance Evaluation

For this study, four dilution factors were tested: 1:2, 1:5, 1:10 and 1:25. This dilution scheme was chosen for two reasons. First, the dilution factors evaluated are commonly used throughout the bioprocess industry for sample preparation. Second, the Sample Module's precision and performance should be assured over a broad range of dilutions.

All experimental runs were conducted using a Seg-Flow 4800 automated sampling system integrated with the Sample Module and a YSI® 2700 Select™ biochemistry analyzer. The YSI analyzer was configured for *d*-glucose analysis. A vessel containing a minimal salts media, having a *d*-glucose concentration of 10.0 g/L, was sampled on-line every 10 minutes by the SegFlow 4800 system. On-line samples were drawn from the vessel using a FISP® *in-situ* sampling probe (0.2 um microfiltered) and delivered to the Sample Module for sample dilution. Once the diluent (deionized H_2O) addition and mixing was completed, the prepared sample was sent to the YSI analyzer for *d*-glucose analysis. The YSI analytical data was subsequently communicated to the Seg-Flow 4800 for data acquisition. Each dilution test consisted of 10 analyses, having an undiluted control analyzed following each dilution sample analysis. Undiluted control samples were manually withdrawn from the vessel and analyzed using the same YSI analyzer.

The mean, standard deviation and coefficient of variation (CV) was computed for each sample set. The *d*-glucose concentration mean and CV values of the undiluted and diluted samples were compared to determine dilution precision and mixing efficiency of the Sample Module. The *d*-glucose measurement range for the YSI analyzer was 0 – 25 g/L (1).

Performance Data

YSI defines the analytical precision of the YSI 2700 Select analyzer using CV, which is the ratio of the standard deviation to the mean. The CV value is expressed as a percentage (CV%). The *d*-glucose analytical precision of the YSI 2700 Select analyzer is 2% (1). Therefore, the Sample Module's dilution precision could be determined by comparing the CV of the diluted sample sets to their respective control (undiluted) CV value.

Table 1. Mean (μ), standard deviation (δ) and coefficient of variation (CV) of glucose measurements for diluted and undiluted samples

Dilution Factor	Sample Type	Number of Samples Analyzed	Theoretical glucose concentration(g/L)	Measured glucose concentration (g/L) (μ ± δ)	Actual glucose concentration (g/L)* (μ ± δ)	CV%
2	Diluted	10	5.00	4.91	9.82 ± 0.19	1.93
	Undiluted	10	10.00	9.76	9.76 ± 0.18	1.81
5	Diluted	10	2.00	2.03	10.13 ± .20	1.98
	Undiluted	10	10.00	10.09	10.09 ± .20	1.98
10	Diluted	10	1.00	1.05	10.45± .21	2.03
	Undiluted	10	10.00	10.39	10.39 ± .21	2.00
25	Diluted	10	0.40	0.40	9.99 ± 0.20	2.00
	Undiluted	10	10.00	10.01	10.01 ± 0.20	1.96

*measured glucose value multiplied by dilution factor to obtain actual glucose concentration for diluted samples

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As shown in Table 1, the CV values for the diluted sample analyses were very comparable to their respective controls (undiluted), while ensuring the *d*-glucose assay's precision requirement of 2% CV was met. Thus, the Sample Module demonstrated a high degree of dilution precision over a wide range of dilution factors.

Figure 1 compares the variation of mean glucose concentration measurements between the diluted and undiluted sample sets. Comparing these values provides an indication of mixing efficiency and dilution precision (2). As shown in Figure 1 the mean glucose values were nearly identical for both the diluted and undiluted samples, regardless of the dilution factor. The data, in turn, signifies a high degree of mixing efficiency and dilution performance by the Sample Module.

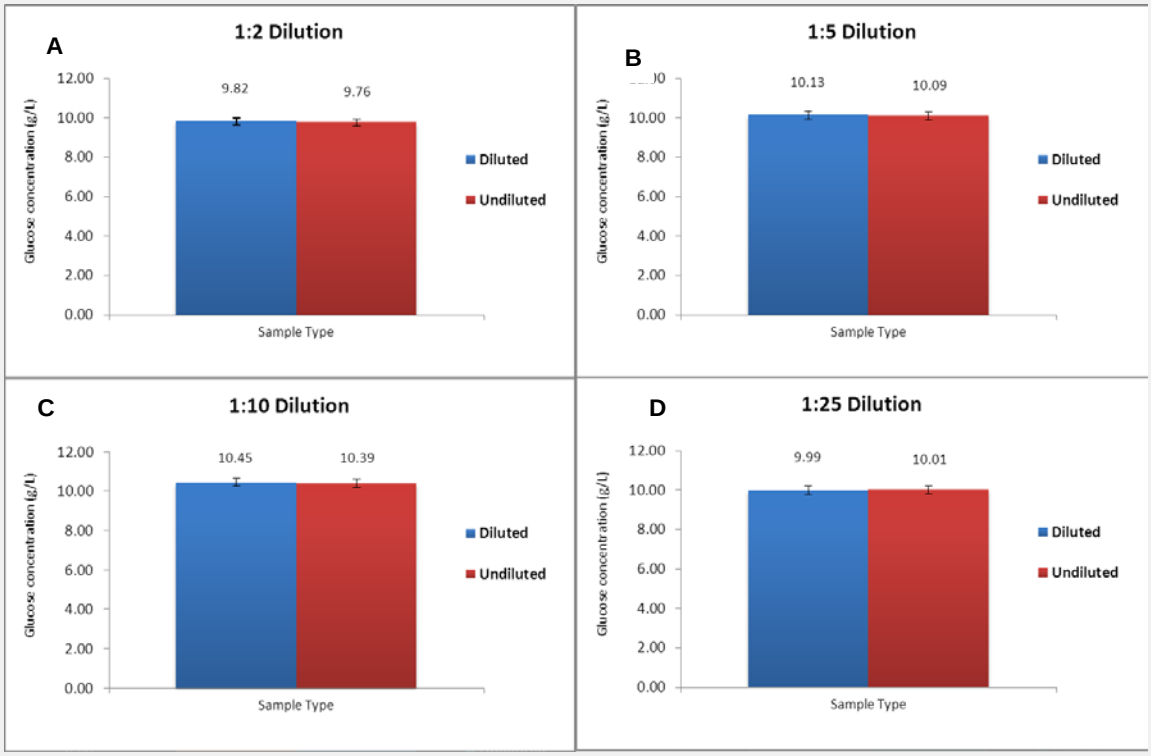

Figure 1. Impact of Sample Module mixing efficiency and dilution precision on automated on-line glucose analysis at different dilution factors (A) 1:2 dilution, (B) l1:5 dilution, (C) 1:10 dilution, (D) 1:25 dilution. Each bar represents the mean of 10 analyses with error bars of one standard deviation. Mean glucose concentration data for the diluted and undiluted samples are shown in blue and red, respectively.

Conclusion

The Seg-Flow 4800 Sample Module was evaluated for its dilution performance when used for on-line automated *d-g*lucose analysis. The testing of various dilution schemes indicated no mixing problems as evidenced by the comparability of the diluted and undiluted sample mean *d*-glucose values. Coefficients of variation for the diluted sample analyses were 2% or less, indicating a high degree of dilution precision while meeting the requirements of the analytical instrument's assay (1). More importantly, analytical fidelity was uncompromised due to the Sample Module's mixing efficiency and robust sample delivery system, even at a dilution of 1:25. The Seg-Flow Sample Module provides the accuracy and precision required for dilution, reagent addition and quantitative analytical purposes.

References:

1. YSI 2700 Select Biochemistry Analyzer User's Manual. (2009). Yellow Springs, OH.
2. May, T. W. & Wiedmeyer, R.H. (1998). The CETAC ADX-500 Autodiluter System: A Study of Dilution Performance with the ELAN 6000 ICP-MS and ELAN Software. *Atomic Spectroscopy, 19*, 143-149.