Glucose and Lactate Raman-Based Monitoring with MAVERICK: Performance in Spent Media

Introduction

MAVERICK™ offers the advantages of in-line Raman process analytical technology (PAT) without the cost and headaches inherent to the implementation of conventional process spectroscopy-based methods. Unlike conventional Raman analyzers, which rely on empirical calibration of spectral data to off-line reference measurements, MAVERICK utilizes a *de novo* model that does not require empirical trial-and-error 'training' on bioprocess data.[1] In other words, the levels of glucose and lactate are measured by their chemically specific Raman scattering signal. For a technology with a traditionally complex setup built on process-specific conditions and the combined signals (specific or unspecific) from a complex cell culture, MAVERICK offers an accessible, plug-and-play solution. This document shows the results from experiments to demonstrate the selectivity of glucose and lactate measurements from MAVERICK in complex spent media samples from CHO and HEK293 cultures.

Evaluation of MAVERICK Linearity and Accuracy in Spent Media Spiking Experiments

The measurements obtained from the MAVERICK typically provide a precision of <0.1 g/L for both glucose and lactate.

To evaluate the consistency, linearity, and specificity of MAVERICK measurements in the complex media environment of bioreactor runs, known quantities of glucose and lactate were serially spiked into spent CHO medium and spent HEK293 medium samples. These media already contained glucose and lactate in the sample. The glucose and lactate offline measurements of the fresh and spent media were performed using a BioProfile FLEX2 analyzer (Nova Biomedical). The spent media samples were collected from cell culture as described in table 1.

The pooled spent media samples contained a range of 0–4g/L of glucose and 1–5g/L lactate, and standard addition method was used to calculate measurement accuracy in the spiked spent media samples. Figures 1 and 2 plot the actual added concentration of glucose and lactate in each spiked sample (as determined by off-line measurements) against the change measured by MAVERICK.

The spent media and spiked sample measurements showed good linearity across the media and cell culture timepoints tested, demonstrating the ability of MAVERICK to report glucose and lactate concentrations accurately in diverse media conditions.

Media Type	Spent Media Cell Culture Description
Fed-batch CHO cell culture in commercially available chemically defined media	• Pool 1: Samples taken on cultivation days 2–7 • Pool 2: Samples taken on cultivation days 7–13 • Pool 3: Harvest sample at cultivation day 14
Fed-batch HEK293 cell culture in commercially available chemically defined media	• Pool 1: Samples taken on cultivation days 0–4 • Pool 2: Samples taken on cultivation days 4–6 • Pool 3: Harvest sample at cultivation day 6

Table 1: Spent media samples used in spiking experiment.

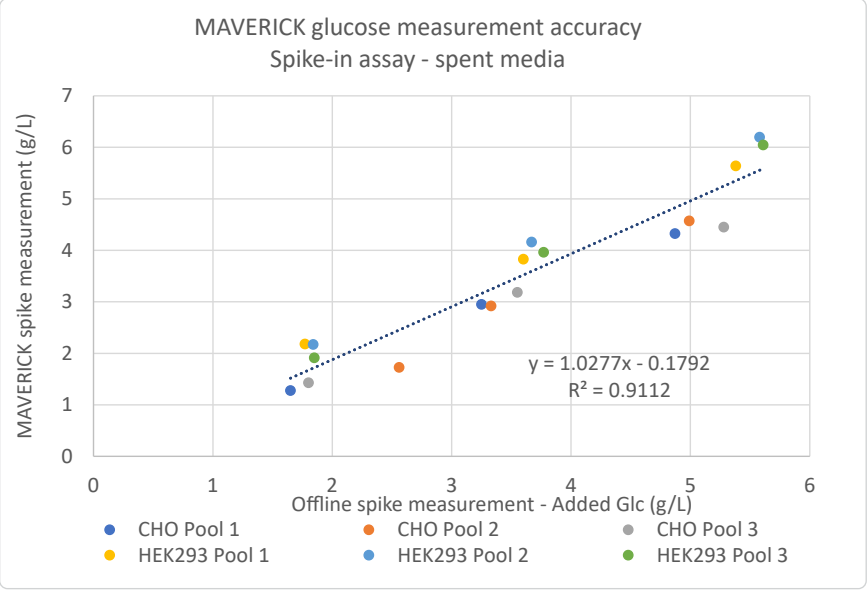

Figure 1. Spiking known concentrations of glucose into spent CHO medium and HEK293 media, at different time points of the cell culture (please see table 1). Actual spike concentrations (as determined by offline measurements; x axis) plotted against the MAVERICK measured values (y axis).

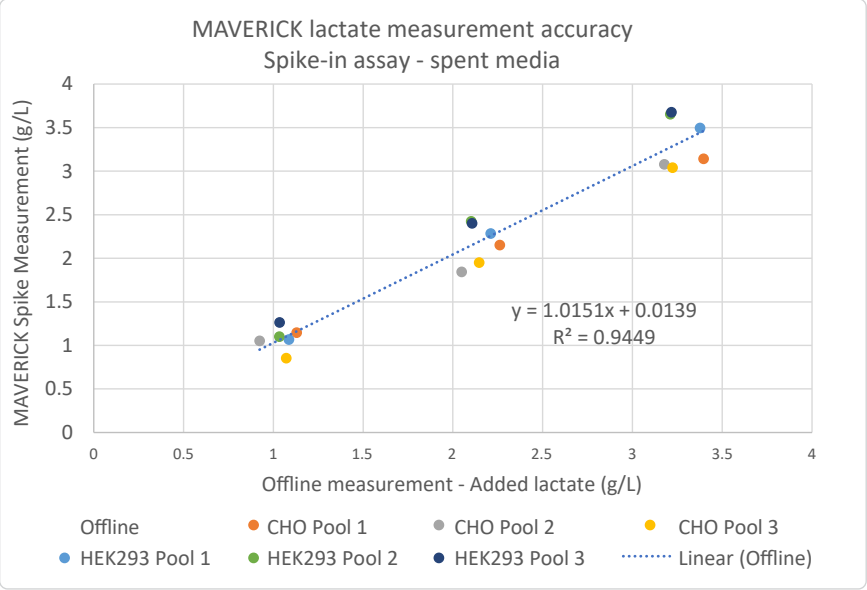

Figure 2. Spiking known concentrations of lactate into spent CHO medium and HEK293 media, at different time points of the cell culture (please see table 1.) Actual spike concentrations (as determined by offline measurements; x axis) plotted against the MAVERICK measured values (y axis).

The spiking experiments also provide quantitative information about the selectivity of the MAVERICK measurements. The International Union of Pure and Applied Chemistry (IUPAC) definition of selectivity is "the extent to which a method result is influenced by other interferences in the matrix." In ideal conditions, the addition of a particular interferent would have a near-zero effect on the reported analyte concentration. The selectivities determined from the spiking experiments were -0.040 g/L glucose per 1 g/L lactate, and -0.015 g/L lactate per 1 g/L glucose . These values are very close to theoretically ideal (zero), demonstrating very high selectivity.

Conclusions

MAVERICK provides a plug-and-play, in-line, Raman-based PAT solution for monitoring and control of glucose, lactate, and biomass in up to 6 bioreactors. MAVERICK is easy to set up and enables instant implementation of a PAT tool generating accurate, precise, linear, and specific in-line measurements of glucose, and lactate throughout cell culture conditions in fed-batch bioprocess, across different types of media used in mammalian (CHO, HEK293) cell culture.

Reference

1.Brown, C.D. Discordance between net analyte signal theory and practical multivariate calibration. *Analytical Chemistry.* 2004; 76:4364–4373