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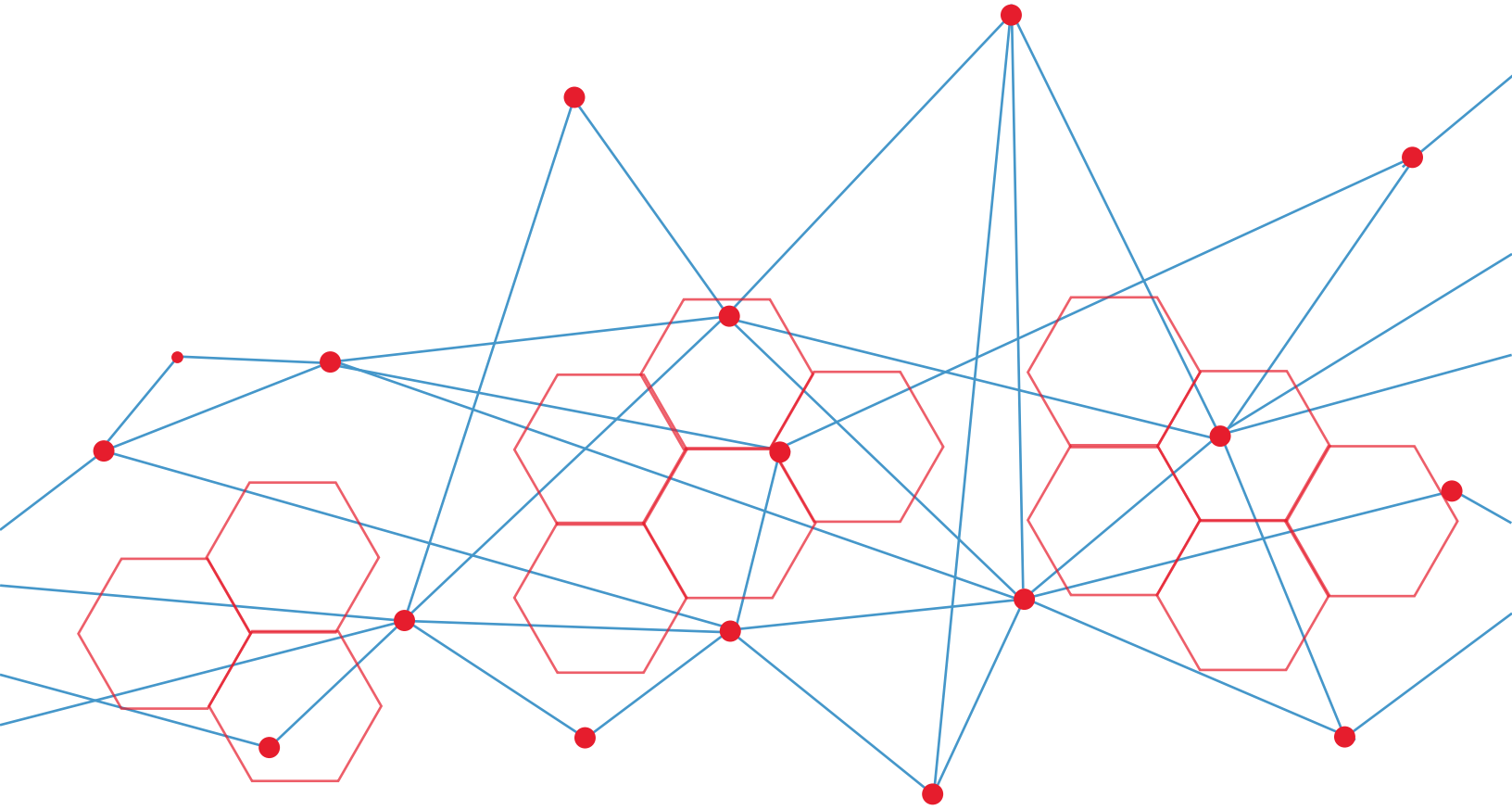
Bioreactor Monitoring: A New Application for the ChemDetect™ Liquid Analyzer

Prepared by

Dr. Miles Weida, Shaam Noble, Bill De Costa, Dave Arnone, Bruce Coy



16465 Via Esprillo • San Diego, CA 92127 • 858.432.7500 • daylightsolutions.com

Executive Summary

The ChemDetect™ Analyzer is coupled to a novel micro flow cell that allows repeatable quantitative spectroscopic determination of liquid components. The system is calibrated to determine concentrations of relevant chemicals used in bioreactor feedstocks. Exercise of a test protocol for characterizing bioreactor feedstocks is carried out. It is found that the ChemDetect™ is sensitive to the relevant chemicals at better than 50 mg/L concentration over two decades of concentration measurements for 90 second measurements. The analyzer can also differentiate between mixtures of analytes, and was characterized to be stable to less than 4% drift over three days without calibration.

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Overview

Bioreactor feedstocks and broths are essential for the production of pharmaceuticals and chemicals via cell cultures or fermentation. In particular, the chemical composition, acidity, and dissolved gas content must be carefully controlled to ensure optimal production. Infrared liquid spectroscopy has shown promise [1, 2] as a tool for monitoring the sugars and other chemicals present in bio-reactors. This study is meant to demonstrate the potential of the ChemDetect™ sensor for monitoring the components of bioreactor feedstocks.

Bioreactors typically consist of a temperature controlled liquid feedstock held in aseptic conditions to prevent growth and contamination by unwanted micro-organisms. A sensor for monitoring the feedstock would ideally be able to monitor the liquid in situ without contamination. Attenuated total reflectance (ATR) infrared probes are promising in this regard, but first fundamental ChemDetect™ performance with liquid spectroscopy must be demonstrated. Hence this study focusses first on determining performance for liquid transmission spectroscopy. These results can then be extended in a straightforward fashion for the design of an ATR probe that has the necessary performance for the application.

The remainder of the study is organized as follows: First, the liquid sample cell and its use with the spectrometer is described. Next, data acquisition and analysis are covered. Tests designed for bioreactor feedstock characterization are then described, followed by measured results with the ChemDetect™ Analyzer. Finally, conclusions are presented.

Experiment Setup

Liquid spectroscopy presented unique challenges compared to both gas phase and solid spectroscopy. First, the main liquid component, water, is highly absorbing throughout the infrared. Liquid water has its own spectral features as well, so the amount of absorption also changes with wavelength. The strength of the water absorption is so large that previous infrared studies using non-laser based techniques have been restricted to path lengths of no more than about 25 µm. This not only reduces the sensitivity to analytes in the liquid, but also makes it near impossible to probe feedstock components such as cells that can be larger than this dimension.

Another challenge of liquid spectroscopy is designing sample cells that allow easy filling with a known concentration. Sample cells designed for spectrometers using incandescent light sources, such as the globar in Fourier transform infrared (FTIR) spectrometers, must have very large (~1 cm) optical throughput, meaning that the sample volume in the cell can become quite large and take significant time to completely clear out with introduction of a new analyte. This is a significant limitation when coupling to liquid chromatography columns or for the analysis of expensive analytes that are available in only small quantities. Moreover, transition from small tubing size to big sample chambers makes it very difficult to remove bubbles or completely fill the sample cell.

These factors were all taken into consideration when designing the sample cell for the ChemDetect™ Liquid Analyzer. The advantages of the quantum cascade laser are utilized in two

ways. First, the clear aperture is reduced to 1 mm. This is possible by focusing the laser beam, making small volume sample cells a possibility. Second, the optical path through the liquid is increased to 130 µm. The high power of the laser beam makes this possible, since significant signal is still present even after losing several orders of magnitude of power to water absorption. The longer absorption path means that a quantum cascade laser based system can be ten times more sensitive to analytes. Such an increase would require 100 times more averaging for an FTIR system, which can be prohibitively long in many measurement situations.

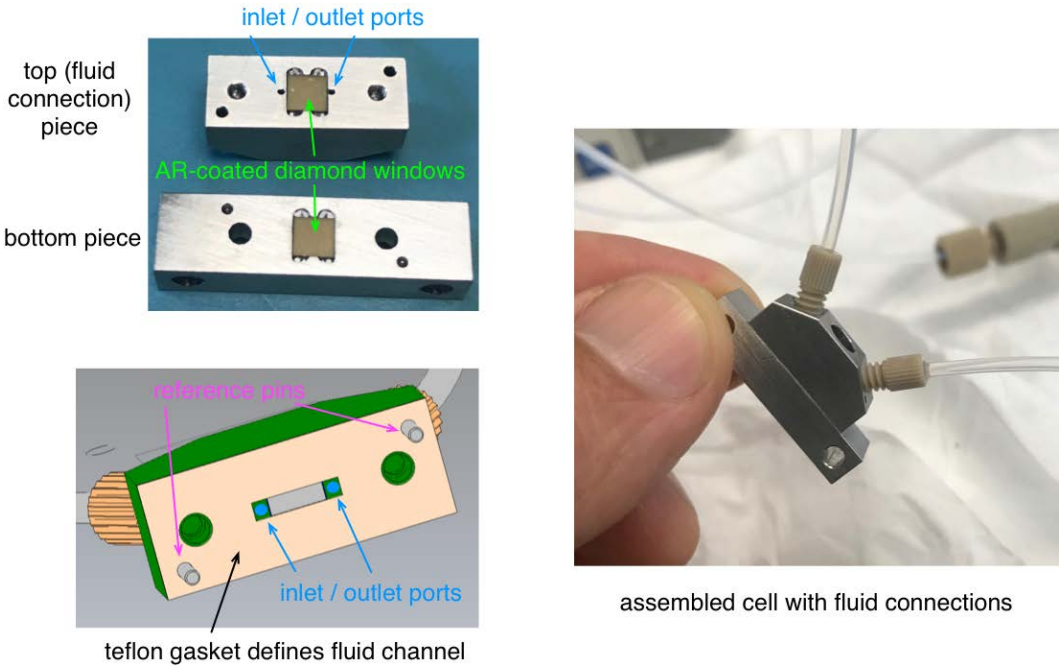
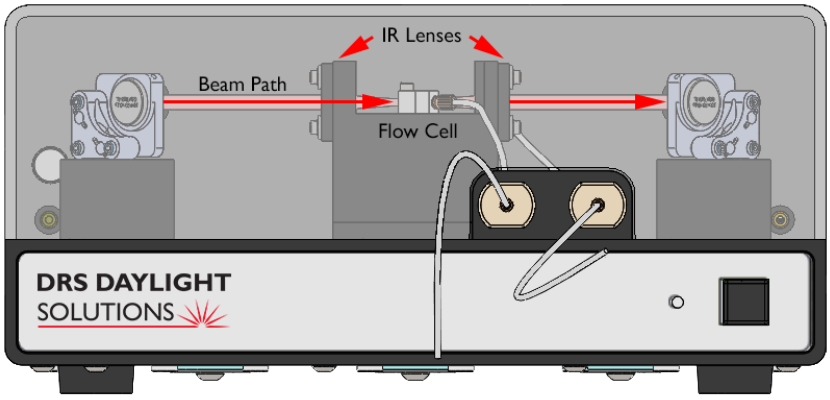


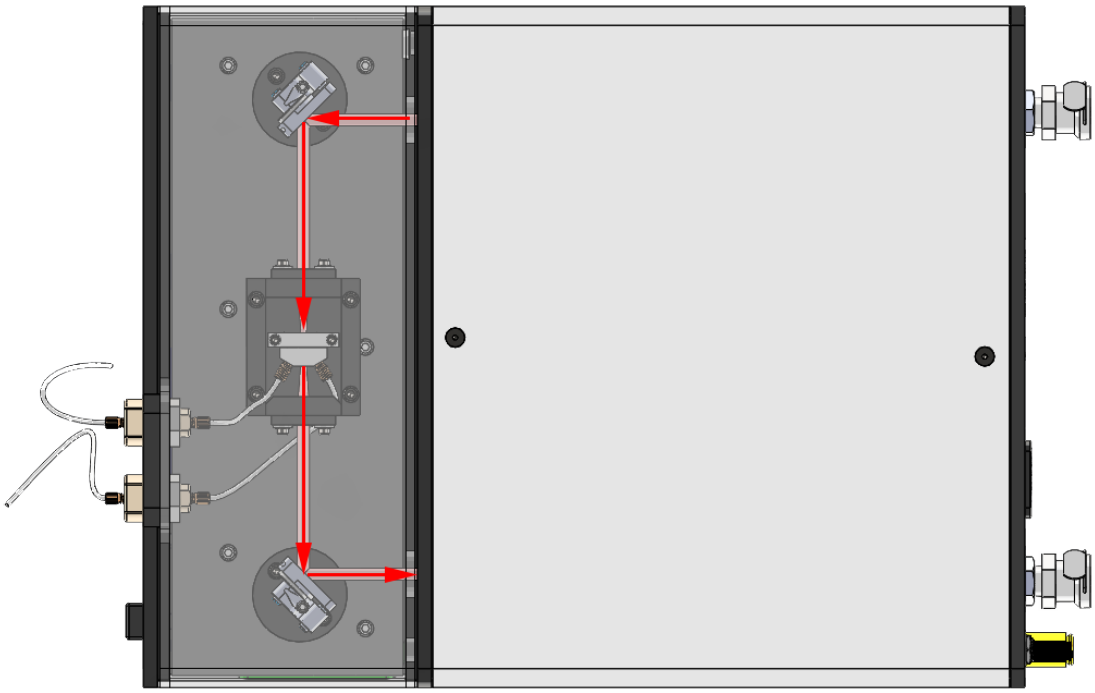
FIG. 1: Flow cell and components (Patents pending). Upper left shows two halves of cell with AR-coated diamond windows glued in place. Windows are 300 µm thick. Bottom left shows Teflon gasket that sets up fluid channel. The fluid channel is 1 mm wide and the gasket is 130 µm thick. Right side picture shows assembled flow cell with 1/16" tubing. Final flow cell volume is < 1 µL.

The flow cell for the ChemDetect™ Liquid Analyzer is shown in Fig. 1. Diamond windows are used for their optical properties and durability. The finished flow cell has a cell volume of less than 1 µL, and the cross-sectional flow area is relatively constant through the entire flow cell path. This ensures that sample changeover is quick, reduces potential for bubbles, and allows use with online chromatography systems. Finally, the cell is easily disassembled for cleaning or gasket changeover (e.g. to test different optical path lengths).

The flow cell is integrated into the ChemDetect™ Liquid Analyzer as shown in Fig. 2. Lenses are used to couple the laser into and out of the cell. Proprietary low-noise detectors were used to enable longer path lengths through the flow cell to overcome the associated increase in attenuation due to liquid water absorption.



FRONT VIEW

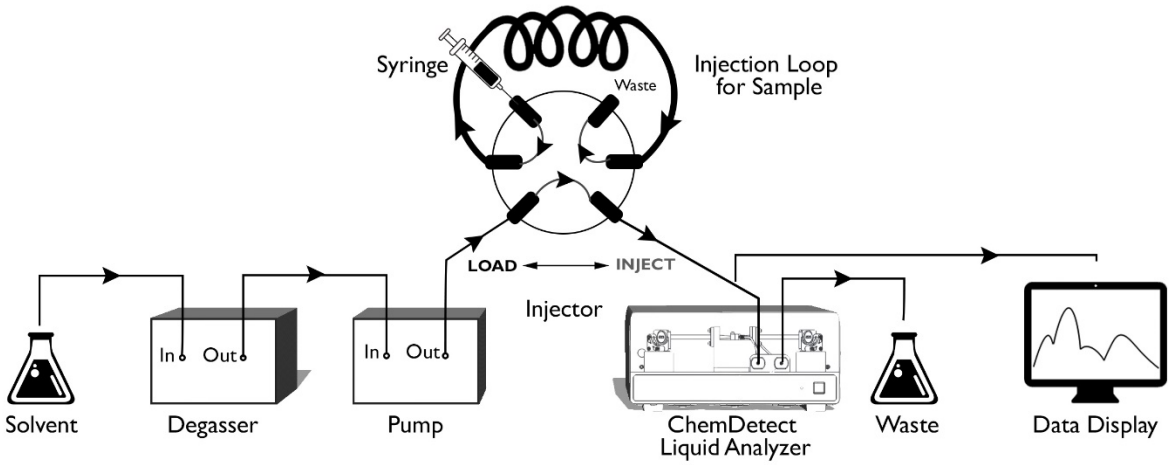


TOP VIEW

FIG. 2: Front and top views of the ChemDetect™ Liquid Analyzer system. The flow cell is centered within the modular and customizable sample compartment surrounded by the precision beam coupling optics. The inlet and outlet fluidic ports are easily accessible for connecting and disconnecting.

Sweeps are made with 0.2 cm^{-1} steps, then averaged down to 2 cm^{-1} resolution. A single sweep was collected at a 5 second update rate. The measured noise level on the final spectrum, even after passing through 130 µm of water, is approximately 0.1%transmittance.

(a) Load Configuration



(b) Inject Configuration

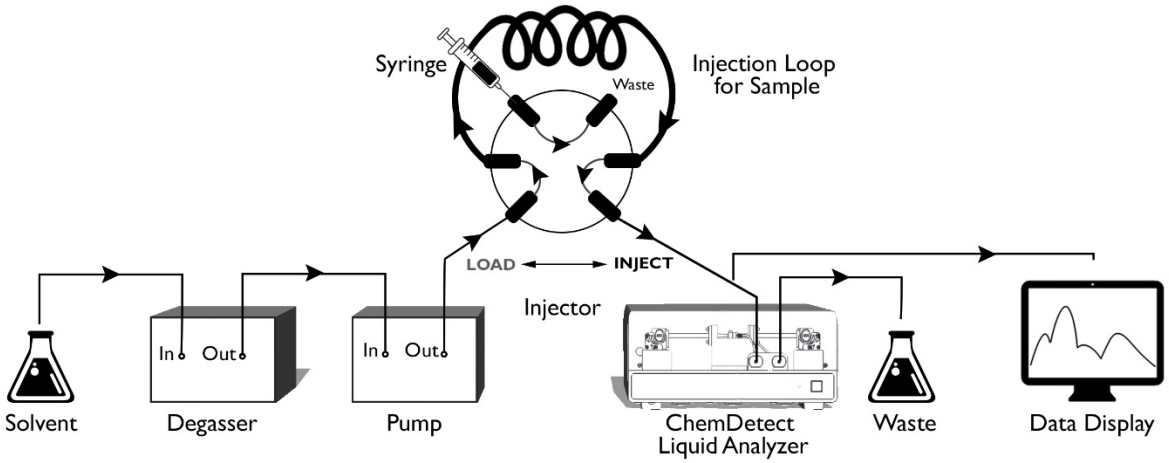


FIG. 3: Liquid sample delivery system. Clean solvent is drawn from a reservoir and sent first to the degasser. It then travels to the pump, which sets the flow rate. From the pump, the solvent is sent to the auto-injector. The auto-injector is a six port valve with two configurations. In the load configuration (a), the solvent goes directly to the flow cell, while sample can be introduced into the 600 uL sample loop with an injection syringe. In the inject configuration (b), the auto-injector valve is switched so that the solvent is driven through the sample loop before being sent to the flow cell. In this way a controlled 600 uL slug of sample can be sent to the flow cell. After the flow cell, waste tubing routes sample / solvent to a waste container.

Another significant challenge for liquid spectroscopy is sample introduction and repeatability. This is accomplished through the use of HPLC (high-performance liquid chromatography) equipment such as carefully controlled pumps, fluid degassers, and injection valves. The fluid delivery

and sample injection system is shown and described in Fig. 3. This system allowed reliable, repeatable introduction of sample to the flow cell, and eliminated problems with bubbles and sample changeover that were seen with the larger sample cells from FTIR-based equipment.

Data Acquisition and Analysis

To ensure the best results, it is essential to introduce the same sample volume into the flow cell under constant pressure and flow rate, and to do so repeatedly. Therefore, a constant flow method based on injection and sample arrival times was preferred over a stop-and-measure based approach. .

The fluid sample injection and data collection method was as follows: First, the sample arrival and time to completely fill the flow cell were measured using results such as those shown in Fig. 4. It was found that a flow rate of 200 µL/min and a sample injection loop of 600 µL resulted in a stable plateau of constant sample concentration in the flow cell for 90 seconds. A data collection algorithm was then defined that automatically controlled the sample injector and acquired a final reference background of the carrier solvent just before the sample arrival. Data was then collected as shown in Fig. 4 for the complete evolution of the sample through the flow cell. The 90 second plateau region was then used to average together spectra (18 spectra averaged) to obtain the final spectra used for the results below.

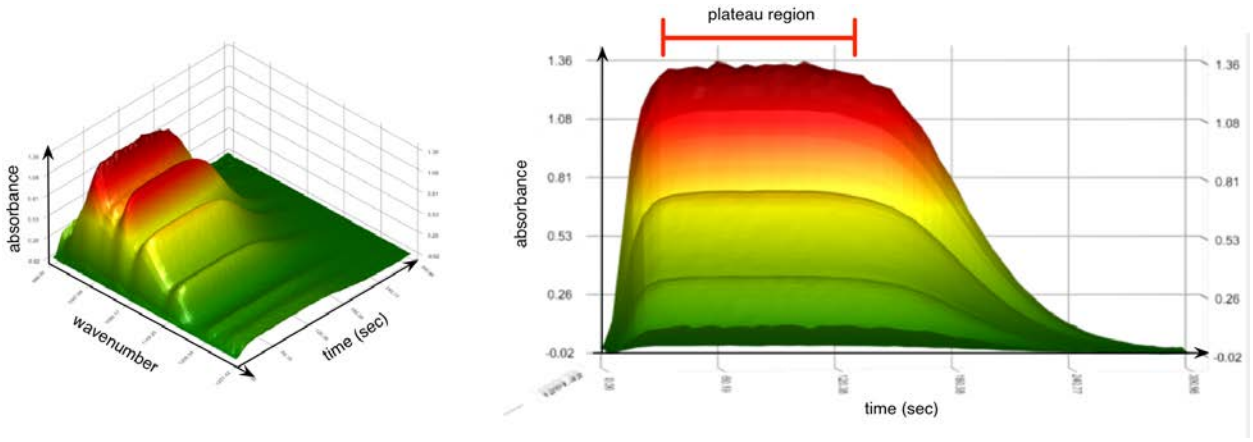


FIG. 4: Time evolution of spectrum with injection. Left: Glucose spectrum evolution as a function of time after injection. Right: Side view shows absorbance dependence on time. Plateau region defined as shown. The plateau region indicates that the flow cell is completely filled with undiluted injection sample. Spectra are averaged over the plateau time range to determine a final spectrum for the injected sample.

Data analysis consisted of first acquiring reference spectra for known pure analytes and concentrations, and then using these reference spectra in a linear-least squares fit of components. For weighting purposes, system noise was set to the measured value of 0.001 rms transmittance noise, then scaled according to the level of measured absorbance following the Bier-Lambert law.

Tests and Results

First, the sample preparation and introduction steps are described, followed by the measured reference spectra. Then, individual tests and results for sensitivity and applicability to bioreactor monitoring are covered.

Sample Preparation

Samples are prepared either in neutral (pH 7) de-ionized water, or buffered as described in the tests below. Samples are obtained from standard scientific catalog stores, and are as described in Table I. Samples are weighed out on a Mettler PC 440 digital scale, 10 mg resolution, then mixed with known volumes of water, measured with both a graduated cylinder and pipette (20 to 1000 µL) controlled volumes.

Table I: Dry analytes used for sample preparation.

Analyte	Dry Sample Info
glucose	D-Glucose, Anhydrous, CAS 492-62-6 Catalog number: C4130, Lot: 161121-3C Supplier: Lab Connections, St. Augustine FL 32085
fructose	Fructose, Purified, CAS 57-48-7 Catalog number: C4000, Lot: 161121-2A Supplier: Lab Connections, St. Augustine FL 32085
maltose	Maltose, Practical, CAS 6363-53-7 Catalog number: C4130, Lot: 161121-1C Supplier: Lab Connections, St. Augustine FL 32085
lactose	Anhydrous Lactose, CAS 63-42-3 Catalog number: LA103, Lot: 1FF0606 Supplier: Spectrum Chemical Mfg, Gardena, CA 90248
sucrose	Sucrose, Reagent, CAS 57-50-1 Catalog number: C7970, Lot: 161121-4A Supplier: Lab Connections, St. Augustine FL 32085
BSA	Albumin, Bovine (BSA), CAS 9048-46-8 Catalog number: 0332-25G, Lot: 0466C262 Supplier: VWR Life Sciences
yeast extract	Yeast Extract Powder, CAS 8013-01-2 Catalog number: 103303, Lot: Q2896 Supplier: MP Biomedicals, LLC, Solon, OH 44139
phosphate buffer	Phosphate Buffered Saline (PBS) Tablets, CAS: n/a 137 mM sodium chloride + 2.7 mM potassium chloride + 10 mM phosphate buffer Catalog number: E404-200TABS, Lot: 1855C484 Supplier: VWR Life Sciences

Reference Spectra

One of the biggest challenges of liquid spectroscopy is obtaining quantitative reference spectra. As compared to gas phase spectroscopy where sample preparation is easily reproduced, liquid spectroscopy is influenced by a host of factors from concentration to pH to measurement technique (ATR vs transmission) that can affect the final spectrum. Therefore, reference spectra are acquired on the system to be tested using the chemicals for testing. Pure samples are prepared using the chemicals in Table I with neutral de-ionized water, and three different test runs are averaged for the final spectra (shown in Fig. 5).

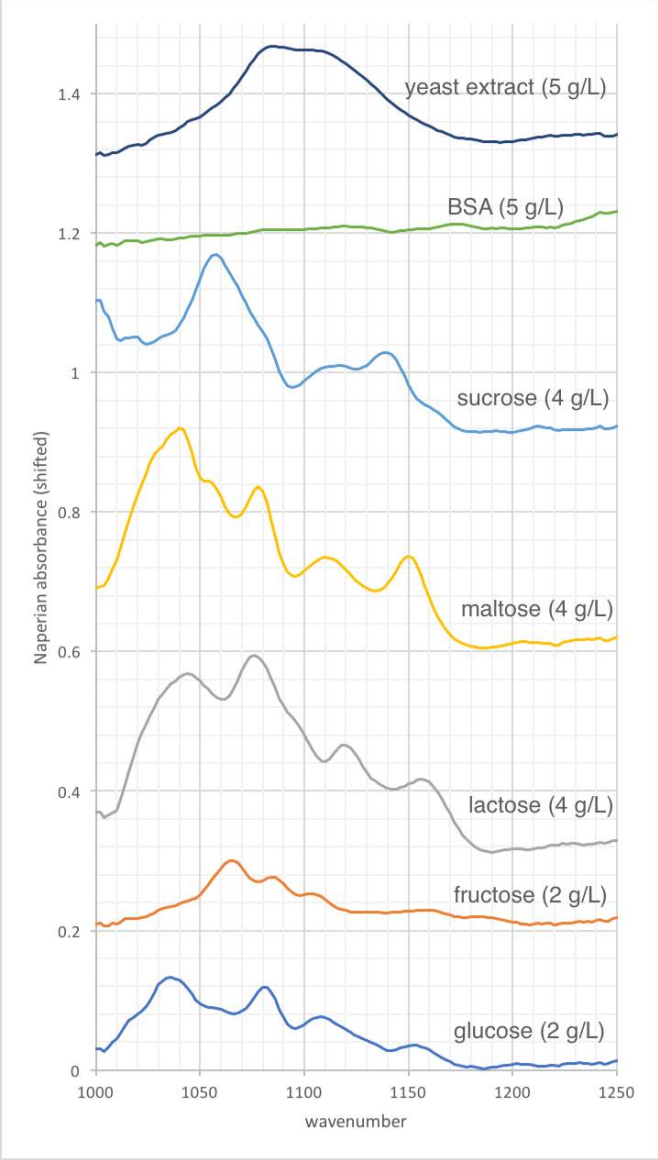


FIG. 5: Reference spectra recorded with ChemDetect sensor for individual components. Absorbance offset for comparison.

These reference spectra are used as the fit components for quantitative analysis used in the remaining tests. Note that there is a high degree of spectral similarity between glucose and maltose in the 8 to 10 µm spectral range, so these two species might be more correlated in the least squares fit analysis then the other species. This can be corrected by expanding the spectral range. Also note that the BSA spectrum lacks strong identifying features in the 8 to 10 µm spectral range. BSA selectivity and sensitivity can be enhanced also by extending the spectral range to include stronger absorption features.

Accuracy and Sensitivity for Pure Analytes

For the first test, repeated measurements of the same pure analytes are made and then analyzed either against a fit of all the reference spectra shown Fig. 5, or just against the known spectrum for the pure analyte. The exception is for BSA, which is omitted from the analysis except when analyzing BSA itself, due to the inferior 8 to 10 µm spectral region for BSA analysis.

The results of this analysis are shown in Table II. As can be seen, there is greater spread in the fit values (taken for three runs of each sample) when including all fit components. Based on this analysis, the limit of quantification (ten times the signal to noise) is probably very close to the spread in the complete fit uncertainty, while the limit of detection is close to the self fit uncertainty. These tests neglect uncertainty in actual sample on a run-to-run basis, so this approximation should be close. That being said, the limit of quantification is approximately 110 mg / L for all of the analytes except BSA (weaker spectrum in this region), and the limit of detection is approximately 50 mg / L. This is for a single 90 second measurement.

Table II: Spread in fit values for fit of individual analytes.

Analyte	Complete Fit Uncertainty (g/L)	Self Fit Uncertainty (g/L)
glucose (2 g/L)	0.132	0.097
fructose (2 g/L)	0.042	0.025
lactose (4 g/L)	0.104	0.044
maltose (4 g/L)	0.112	0.011
sucrose (4 g/L)	0.093	0.057
yeast extract (5 g/L)	0.080	0.071
BSA (5 g/L)	0.255	0.259

Dilutions

The goal of this test is to demonstrate linearity in response to changes in concentration. Glucose concentrations are created in neutral (pH 7) de-ionized water at the following levels: 0.2, 0.6, 1.0, 2.0, 4.0, 8.0, 14.0, and 20.0 g/L. The sensor is calibrated at 2.0 g/L and then the concentration measured. The results and a linear fit are shown in Fig. 6.

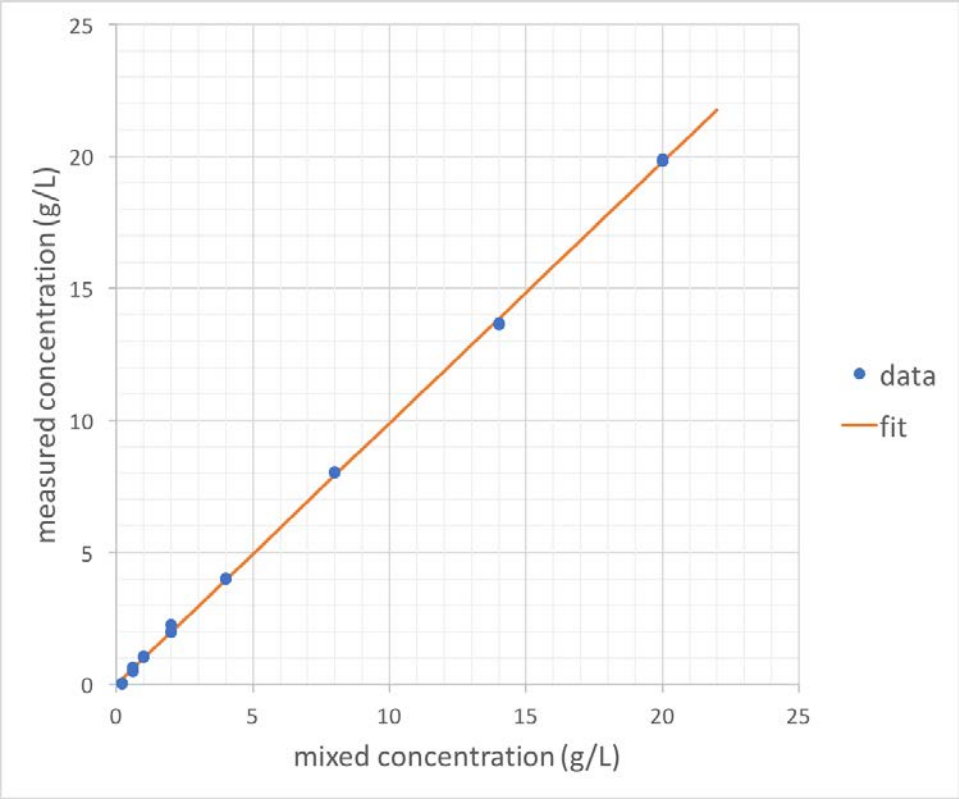


FIG. 6: Measured glucose concentrations vs. prepared values.

The raw results from the measurement sequence are presented in Table III. The fitted linearity is 0.9630(36). This 3% deviation from linearity could be due to sensor response over two decades of concentration, or from some systematic error in the preparation of the concentrations. The offset of the fit is 53(34) mg/L, consistent with the limit of detection value of approximately 50 mg /L determined above.

Table III: Measured vs. prepared glucose concentrations.

Prepared Concentration (g/L)	Measured Concentration (g/L)
0.2	0.220
0.6	0.625
1	1.027
2	1.911
4	3.980
8	7.849
14	13.435
20	19.340

Mixtures

The goal of these tests is to demonstrate the selectivity and specificity of the sensor for an analyte being measured without overdue influence by the other possible interferences. Mixtures of fructose, maltose, lactose, sucrose, BSA serum, and yeast extract are prepared in a solution of neutral (pH 7) de-ionized water and varying degrees of glucose. Data analysis and calibration is performed using the reference spectra collected above.

Tables V, VI, and VII show the test results for each component of the test matrix. Three runs are performed for each entry in the matrix, and the uncertainty is reported as the maximum spread in measured concentrations. In general, the results show the ability to resolve mixtures of concentrations, with a level of uncertainty consistent with the 50 mg/L sensitivity estimate from above. The absolute value of the measured concentrations is generally within 10% of stated values. Additional work needs to be done to validate that the prepared mixtures are accurate before a final assessment on accuracy can be made. It is expected that the accuracy should be similar to the level of quantification (100 mg/L) estimated above.

Table V: Fit results for mixture of 0 g/L glucose plus specified analyte. Value in parenthesis is spread in fit values.

Analyte	Analyte Fit (g/L)	Glucose Fit (g/L)
fructose (2 g/L)	2.000(0.006)	0.000(0.015)
maltose (4 g/L)	4.000(0.056)	0.000(0.054)
lactose (4 g/L)	4.000(0.029)	0.001(0.022)
sucrose (4 g/L)	4.000(0.091)	0.000(0.039)
BSA (5 g/L)	5.000(0.253)	0.000(0.010)
yeast extract (5 g/L)	5.000(0.029)	-0.007(0.032)

Table VI: Fit results for mixture of 0.4 g/L glucose plus specified analyte. Value in parenthesis is spread in fit values.

Analyte	Analyte Fit (g/L)	Glucose Fit (g/L)
fructose (2 g/L)	2.566(0.012)	0.406(0.007)
maltose (4 g/L)	3.987(0.032)	0.360(0.008)
lactose (4 g/L)	3.534(0.080)	0.368(0.016)
sucrose (4 g/L)	3.965(0.040)	0.406(0.013)
BSA (5 g/L)	4.988(0.368)	0.315(0.011)
yeast extract (5 g/L)	4.709(0.147)	0.387(0.007)

Table VII: Fit results for mixture of 2.0 g/L glucose plus specified analyte. Value in parenthesis is spread in fit values.

Analyte	Analyte Fit (g/L)	Glucose Fit (g/L)
fructose (2 g/L)	2.944(0.069)	2.014(0.050)
maltose (4 g/L)	3.337(0.048)	1.988(0.024)
lactose (4 g/L)	3.266(0.064)	1.999(0.031)
sucrose (4 g/L)	3.943(0.066)	2.003(0.033)
BSA (5 g/L)	5.547(0.383)	1.896(0.020)

Analyte	Analyte Fit (g/L)	Glucose Fit (g/L)
yeast extract (5 g/L)	5.286(0.032)	1.983(0.005)

Buffered Solution Measurements

A phosphate buffer (see Table I) was added to assess effects of changing pH. Initial tests indicate that the phosphate buffer adds an additional spectral peak similar in magnitude to the 2.0 g/L spectrum of glucose. However, once the spectrum for the phosphate buffer was accounted for, the results are quite similar to those obtained above.

The goal of these tests is to demonstrate the ability to attain the correct concentration reading for various conditions:

a) Detect minor changes in concentration by using separate solutions of standard addition in a buffered pH simple solution. One tablet of the phosphate buffer was used per 100 mL water. The test was run for the same concentration sequence described above for glucose, and the results presented in Table VIII. The results are almost identical regardless of the presence of the phosphate buffer.

b) Test for three different buffered solutions at pH values of (4, 6, and 7.5) with a 2.0 g/L glucose concentration. These tests were not performed.

Table VIII: Measured vs. prepared glucose concentrations in phosphate buffered solution.

Prepared Concentration (g/L)	Measured Concentration (g/L)
0.2	0.209
0.6	0.624
1	1.039
2	1.957
4	3.998
8	7.974
14	13.735
20	19.812

Sensor Stability and Repeatability

For this test, the stability of the sensor over several days was tested. An initial measurement of a known phosphate buffered glucose solution (2.0 g/L) was made on Friday afternoon, with a new baseline for the measurement being acquired. The measurement was repeated on Monday morning (three days later) without acquiring a new baseline. The measured glucose concentration changed by 3.3%. A measurement made immediately after the three day wait with a new zero of the system only differed by 1.0%, suggesting that some of this drift might have been due to changes in the measured solution.

Conclusions

The ChemDetect™ Liquid Analyzer and new flow cell have demonstrated the ability to analyze relevant components of bioreactor feedstocks. For example, glucose sensitivity and accuracy is shown to be better than 50 mg/L. Compare this to the level of accuracy required for commercial blood glucose monitors (+/- 150 mg/L). Linearity, reproducibility, and selectivity among relevant components is excellent. Any systematic differences between measured and prepared sample concentrations might be do either to sensor linearity or issues in sample preparation. Further tests would be required to determine the cause. Nonetheless, these trends are consistent, and could be calibrated out from the sensor if necessary.

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