Advancing Raman spectroscopy for process analysis: methodology, data processing and instrumentation

Principal Investigators:

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Executive Summary: Raman spectroscopy is a well-established vibrational spectroscopy technique for determining both qualitative and quantitative molecular information from almost any type of sample (e.g. solid, liquid or gas). It is highly suitable for process analysis and control applications because it provides direct information about chemical bonds that change during reactions. No sample preparation is required and the measurements can be carried out conveniently with a simple back-scattering probe. This project has been focused on addressing fundamental research and development challenges faced when applying Raman to various process applications. Noteworthy achievements have been the development of a robust quantitative process Raman immersion probe (Ballprobe), a small-diameter Ballprobe, high sensitivity waveguide sampling for liquid systems, a variety of fluorescence reduction protocols and data processing algorithms.

Goals and Objectives: The main goal of the proposed project is to address fundamental and practical challenges when using Raman spectroscopy to monitor bio-processes and other absorbing or scattering samples. Recently we have demonstrated the applicability of Raman spectroscopy for monitoring sugar formation in complex highly fluorescent biomass mixtures using direct measurements with an immersion probe. However, several sampling challenges remain such as deconvolution of signals from the solid and liquid phases of the slurry and agitation difficulties of highly inhomogeneous viscous samples. We propose to address these challenges by developing a continuous flow filtration system and optimize it for samples with high solid content. Another part of the proposed research is optimizing the optical interface to minimize attenuation of the Raman signal due to scattering. And finally, we propose to continue the development of various data analysis approaches to ensure effective and reliable conversion of data into relevant information about the sample.

Budget for 2014-2015: $25,000 - Funds would support research staff, the analytical work and supplies/travel needs for the research.

Background and progress to date: Sustainable and economical production of liquid fuels from biomass involves chemical processes in batch or continuous flow reactors that need to be optimized, monitored and controlled. Raman spectroscopy can be a useful tool for these tasks. The key feedstock in biofuel industry is ligno-cellulosic biomass derived from various sources including hay, wood and corn. The goal is to hydrolyze polysaccharides into fermentable sugars that are subsequently fermented to ethanol. The hydrolysis is performed enzymatically as well as using steam, acid treatment or any combination of the above. Due to chemical complexity and diversity of lingo-cellulosic feed stock, monitoring kinetics of the hydrolysis is a challenging task.

In the past year we have been developing a PAT for monitoring formation of glucose and xylose during enzymatic hydrolysis of acid pre-treated corn stover. Mid-IR absorption and Raman spectroscopy were identified as the most promising techniques. To recognize the chemical complexity of the corn stover and multiple sampling challenges that we could face during the development, it was necessary to split the project into several phases and start from characterization of synthetic mixtures of the main corn stover ingredients (lignin, cellulose and xylan). Numerous mixtures were made with increasing complexity. Both IR absorption and Raman spectroscopy measurements were performed concurrently to investigate the strengths and limitation of each method as well as to perform cross-validation of the results. Thanks to the availability of the compact and sensitive 1/8" ballprobe developed in our lab in the past 2 years, the measurements were performed in a cost-effective way using small batches of only 20 mL (**Figure 1**).

Figure 1. Compact sample interface: IR and Raman probes in a 20 mL vial filled with water.

The results produced by the IR and Raman instruments were highly correlated across all tested mixtures: from simple binary suspensions (e.g. 10% of cellulose in water) to complex mixtures of lignin, xylan and cellulose. We have demonstrated the ability to perform selective and sensitive measurements independently tracking formation of individual sugars during hydrolysis with detection limits below 10g/L even in highly fluorescing samples with large amounts of lignin (**Figure 2**).

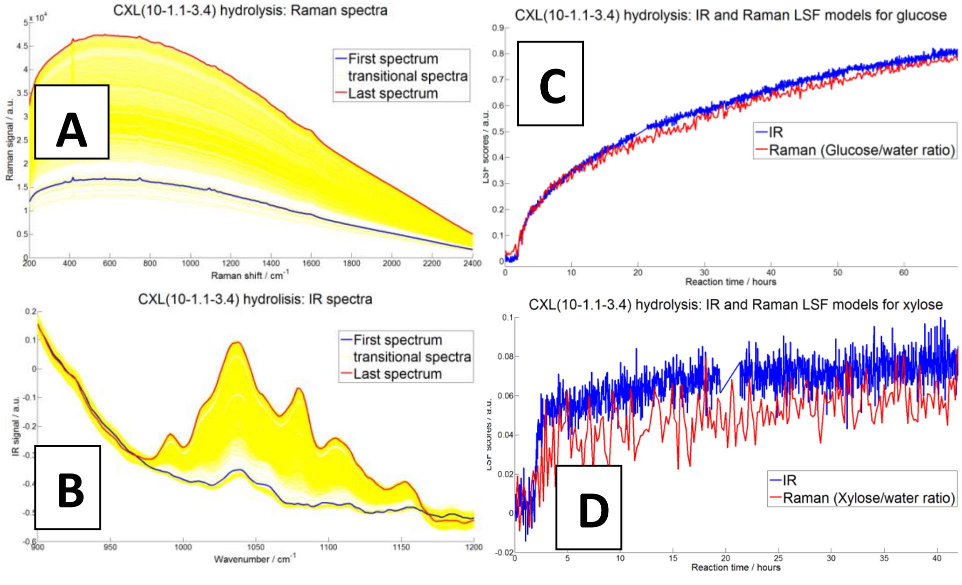

Figure 2. Hydrolysis of of a mixture of cellulose (10%), xylan (1.1%) and lignin (3.4%). (A) - Raman spectra; (B) - IR spectra; (C, D) - formation of glucose (C) and xylose (D) over time determined by IR and Raman measurements. The high noise level on plot D is due to the initially low levels of xylan. Note the correlation between the results.

Our work offers evidence that Raman spectroscopy is a viable tool for monitoring and control of complex biomass processes. With better spectral resolution, maintenance free operation and high stability of modern CCD and array detector sensors, Raman spectroscopy can complement or replace IR absorption spectroscopy. Using real-time IR and Raman measurements together is a great way to validate the results and determine whether the process occurs in the liquid or solid phase. The success of this project is largely attributed to a number of fundamental and practical insights gained in our lab during the last year:

1. A method for accurate intensity calibration of an InGaAs array detector was developed and was essential for detection and analysis of Raman peaks on top of broadband fluorescence background.

2. Innovative data analysis approaches were implemented. Specifically, we developed and optimized a curve fitting method based on the EMSC algorithm. Using this theory-driven approach allowed us to correct for scattering and fluorescence interference and model chemical reactions in the sample that are expected and understood. The subsequent data-driven multivariate modeling using PCA revealed side reactions and allowed us to study poorly understood phenomena. This combination of inductive and deductive approaches was found very powerful in analysis of complex processes such as biomass processing.

Proposed Research: Our goal in the coming year is to continue to optimize the application of Raman spectroscopy for detection and quantification of fermentable sugars during biomass hydrolysis.

Recently we have found that depending on the pre-treatment method, biomass samples differ widely in particle size and distribution of lignin between solid and liquid phases. The variable amount of lignin in the solid phase adds another component to the measurement complexity – the interplay between light scattering and absorption during generation of the Raman signal. We propose to address this challenge and evaluate theoretical and practical approaches to account for this phenomenon.

We will work on development of a continuous flow filtration system and collect a large set of spectra from filtered and unfiltered samples. Using this dataset we will attempt to account for the variability in biomass particles and thus perform Raman measurements of the liquid phase without any filtration. Alternatively, developments in the continuous filtration hardware will allow us to filter the sample in real time and perform direct measurements using simpler models.

In addition, we propose to continue tests and optimization of the theory-based data analysis methodology. Optimization of existing data analysis algorithms as well as possible development of new approaches will allow fast and efficient processing of Raman data.

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Brian J. Marquardt	Senior Principal Engineer

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Ripon College	A.B.	1989-1993	Chemistry
University of South Carolina	Ph.D.	1993-1997	Analytical Chemistry

A. Positions and Honors.

Positions and Employment

01/98 - 10/99 Post-Doctoral Fellowship, University of Washington (Seattle, WA)

11/99 - 10/01 Center for Process Analytical Chemistry, University of Washington (Seattle, WA) Associate Research Scientist

10/01- 01/07 Center for Process Analytical Chemistry, University of Washington (Seattle, WA) Senior Research Scientist/Principal Investigator

02/07 – 09/2012 Applied Physics Laboratory, University of Washington (Seattle, WA) Senior Engineer and Director of Applied Optical Sensing Lab

09/12 – Present Applied Physics Laboratory, University of Washington (Seattle, WA) Senior Principal Engineer and Director of Applied Optical Sensing Lab

Other Experience and Professional Memberships

- Federation of Analytical Chemistry and Spectroscopy Society (FACSS) active member – Process Section Chairman 2007 -2009, Session Leader for Process Spectroscopy and Process Raman, Instructor for Process Raman Short Courses.
- Instructor and advisor for FDA Process Analytical Technology (PAT) initiative and certification program for reviewers and inspectors
- Visiting Scholar – Matforsk (Food Research Institute of Norway)
- Reviewer for*: Talanta*, *Applied Spectroscopy, Analytical Chemistry, Spec. Chimica Acta*
- Reviewed proposals for: *NSF, NSF SBIR, CPAC, DOE, NIH, NASA*
- Society for Applied Spectroscopy (1997 – Present)
- Coblentz Society (1997 - Present)

Honors

1992 ACS President, Ripon College Chapter
1997 Department of Energy Graduate Student Practicum, Los Alamos National Laboratory, Advisor: Dr. David A. Cremers

1997 Guy Lipscombe Award, Outstanding Graduate Student Research, Department of Chemistry, University of South Carolina

1998 Tomas Hirschfeld Scholarship Award, Department of Chemistry, University of Washington

2002 Nominated as a candidate for "Outstanding Young Spectroscopist" by the Journal for Vibrational Spectroscopy

2003 Elected to scientific advisory board to IFPAC – International Forum of Process Analytical Chemistry

2006 Nominated for Coblentz award as an "Outstanding Young Molecular Spectroscopist" by the Coblentz Society

2007 Elected as Board Member of Coblentz Professional Society of Vibrational Spectroscopists

2007 Nominated for Coblentz award as an "Outstanding Process Spectroscopist" by the Coblentz Society

2008 Awarded Applied Physics Lab Director's 2009 Science and Engineering Excellence Award

B. Selected peer-reviewed publications (in chronological order).

(Publications selected from 38 peer-reviewed publications)

1. K. R. Mann, J. R. Burney, K. A. McGee, B. J. Marquardt, "Crystalline Oxygen Sensors that Contain Ruthenium Complexes," *JACS*, **129(49)**, 15092, **2007**.
2. N. K. Afseth, B.J. Marquardt, and J.P. Wold, "A Chromatographic Approach for Fluorescence Rejection in Raman Analysis," *Appl. Spectrosc*, **61(12)**, 1283, **2007**.
3. K.A. McGee, B.J. Marquardt and K.R. Mann, "Concurrent Sensing of Benzene and Oxygen by a Crystalline Salt of Tris(5,6-dimethyl-1,10 phenanthroline) ruthenium(II)", *Inorg. Chem.*, **47**, 9143, **2008**.
4. C. Smith, C. Branham, B. Marquardt, K. Mann, "Oxygen Gas Sensing by Luminescence Queching with Crystals of Cu(xantphos)(phen)+ Complexes", *JACS*, 132(40), 14079-14085, **2010**.
5. E. Gunn, L. Wong, C.W. Branham, B. Marquardt and B. Kahr, "Extinction mapping of polycrystalline patterns," *CrystEngComm*, 13(4), 1123-1126, **2011.**
6. T. Dearing, W. Thompson, C. Rechsteiner and B.J. Marquardt, "Characterization of Crude Oil Products Using the Data Fusion of Process Raman, IR and NMR Spectra," *Appl. Spectrosc.*, 65(2), 181-186, **2011.**
7. M. Roberto, S. Martin, T. Dearing, B.J. Marquardt, "Integration of Continuous Flow Reactors and in-line Raman Spectroscopy for Process Optimization", *Journal Pharm. Innov*., 7(2), 69 – 75, **2012**.
8. W. Blaser, C. Branham, B. Marquardt, R. Chrisman, "Sampling Strategy for Process Control", (Eds) J, Pawliszyn, *Comprehensive Sampling and Sample Preparation*, Elsevier, Chapter 1.34, **2012**
9. S. Mozharov, A. Nordon, D. Littlejohn and B. Marquardt "Automated Cosmic Spike Filter optimized for Process Raman Spectroscopy". *Applied Spectosc.*, 66(11), 1326, **2012**
10. S. Ewanick, W. Thompson, B. Marquardt and R. Bura, "Real-time Understanding of Lignocellulosic Bioethanol Fermentation by Raman Spectroscopy", *Biotechnology for Biofuels*, 6:28, **2013**