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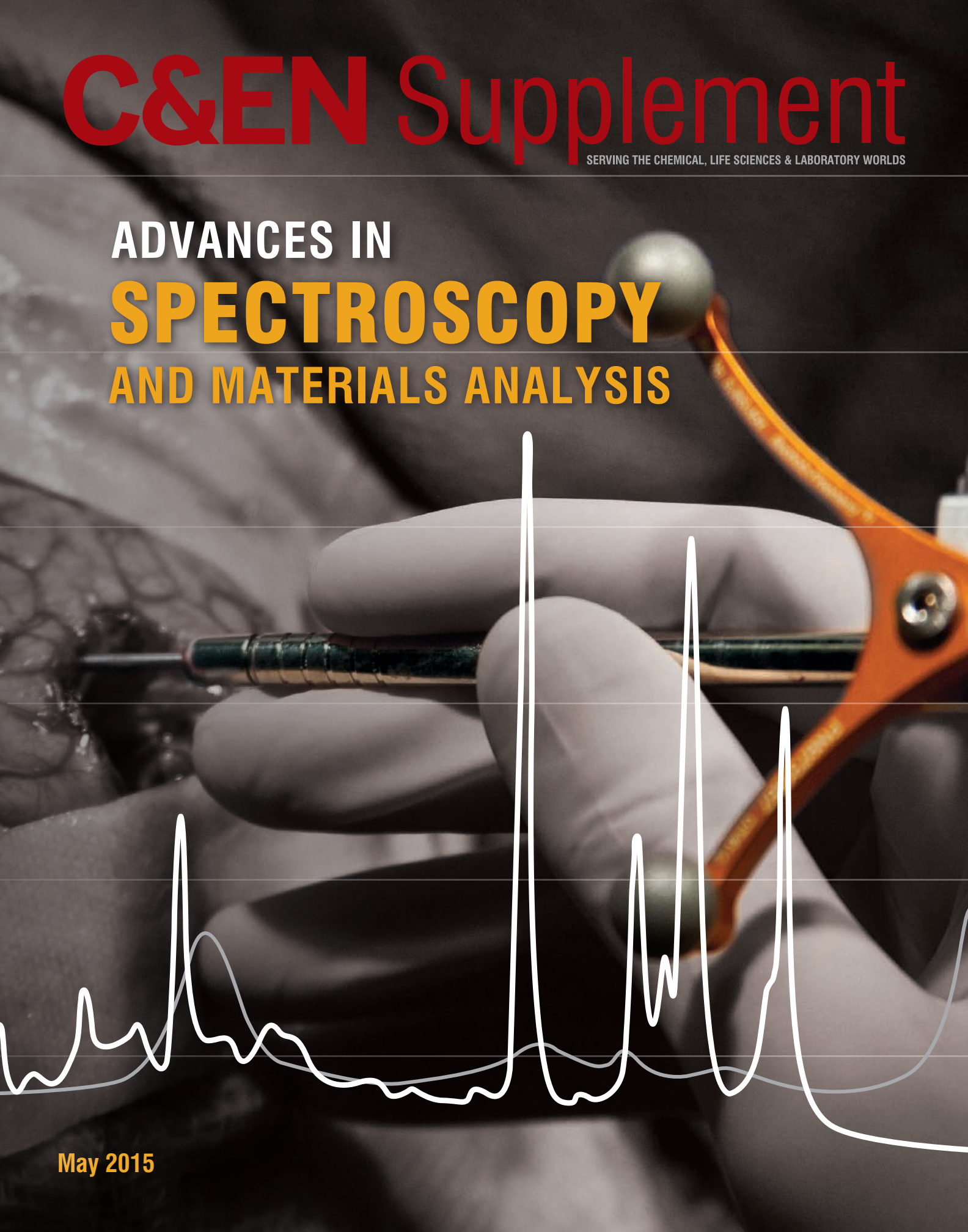
# ADVANCES IN SPECTROSCOPY AND MATERIALS ANALYSIS



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CHEMICAL & ENGINEERING NEWS

# Advances in Spectroscopy And Materials Analysis



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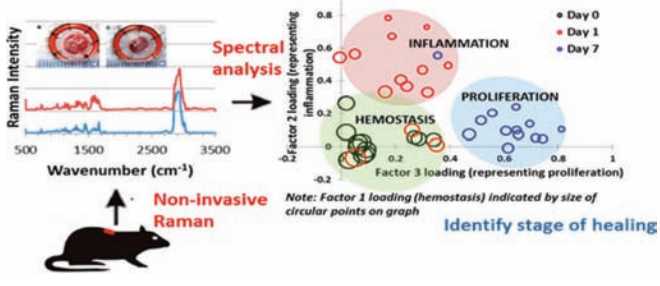


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# PUBLISHER'S NOTE

## Materials World

**WELCOME TO THE** second C&EN Supplement for 2015. This supplement is devoted to "Advances in Spectroscopy and Materials Analysis," and features a selection of application notes from leading instrumentation companies that are pushing the boundaries of spectroscopy analysis. Many of these tools were on display at recent conferences including Pittcon in New Orleans and the ACS National Meeting in Denver.

These vendor contributions are complemented by an editorial selection of abstracts from ten of the most impactful research articles relevant to this field, as seen over the past 12 months in the peer-reviewed journal *Analytical Chemistry,* published by the American Chemical Society.

Thanks to our contributing editor, Victoria Mountain, who shepherded this supplement to fruition with the help of C&EN's digital production manager Renee Zerby and her staff. Our thanks also to *Analytical Chemistry* managing editor Antonella Mazur for gathering the Top Ten list and to the companies that contributed to this C&EN Supplement.

We have an exciting series of supplements in store for the rest of this year, including a special edition on technology in BRICS countries and an in-depth issue looking at the Top 20 Drugs in the Pipeline later this summer.

If you are interested in participating in future C&EN supplements or any of our print, digital or lead-generation media offerings, please visit the C&EN media site—http://acsmediakit.org/—and don't hesitate to contact me.

Best wishes,

Kevin Davies, PhD
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*For the record: The editorial content in this supplement was created without direct involvement of C&EN reporters or editors.*

# LIGHT ON MY MIND

**Vicki Mountain, Ph.D.**

**GREETINGS, AND** welcome to this latest special supplement to *Chemical & Engineering News* magazine. The prominent theme of this issue is spectroscopy, a technique founded on the study of visible light, although it has since expanded to encompass other forms of radiative energy. The connection to light is fortuitous, especially for those of us in the Northeast who endured a long, arduous winter and are now delighting in the arrival of spring, and the increasing hours of daylight this season brings.

This year more than others, throughout the world, light has been at the forefront of popular science news, grabbing the attention of the general public. In March 2015, a solar eclipse, where the moon passes between the Earth and the Sun, was observed throughout Europe, as well as in parts of Northern and Eastern Asia, and Northern and Western Africa. In some places a total solar eclipse could be seen, turning day into night by blocking all direct sunlight for a time.

The greatest celebration of light this year, however, is taking place across the globe: 2015 has been designated the International Year of Light by the United Nations Educational, Scientific, and Cultural Organization, UNESCO. A series of events is planned throughout the year with the intention of raising public awareness and understanding of the importance of light and optical technologies to people throughout the world. More information on the International Year of Light can be found at http://www.light2015.org.

Within the American Chemical Society, Harry A. Atwater, Editor in Chief of the journal ACS Photonics, noted in his editorial from January 20151, "As proud sponsors of the International Year of Light, in this issue, and during this year, we will join together with other professional societies around the world to celebrate the role of light in science and technology."

In February this year, C&EN Senior Editor Celia Arnaud highlighted a brilliant example of researchers using light for the direct benefit of human health[2], specifically a tool that enables neurosurgeons to differentiate between healthy and cancerous cells during surgery to remove brain cell tumors.[3] As Arnaud explains in her story, this is a breakthrough for neurosurgeons who currently face the dual challenges of first finding the cancer cells—an especially difficult task for invasive cancers—and second, making sure they have removed all the cancerous tissue knowing that if they leave these cells behind, tumors may regrow and impact the patients life. The tool, developed by a team of Canadian researchers from Montréal Polytechnique and McGill University, is a handheld contact Raman spectroscopy probe that detects cancer cells locally in live human brains, during surgery. In the C&EN report, Petrecca, one of the team leaders, notes that the next step is to run clinical trials to demonstrate that the Raman technique can improve surgery outcomes, and that such a trial will begin soon. We will be certain to revisit this story as the results of the clinical trial are revealed.

Not surprisingly, Raman spectroscopy is used in several of the articles featured in our latest selection of Top 10 papers from Analytical Chemistry. Innovations in a range of other spectroscopic techniques are reported in our contributed Applications notes. We hope that you enjoy this selection, and as always welcome your comments.

## References

1. http://pubs.acs.org/doi/pdf/10.1021/acsphotonics.5b00001
2. http://cen.acs.org/articles/93/i7/Raman-Technique-Helps-Surgeons-Excise.html
3. *Sci. Transl. Med.* 2015, DOI: 10.1126/scitranslmed.aaa2384

*Vicki Mountain, Ph.D. is a contributing editor on this C&EN Supplement and is a freelance science editor and writer based in Medford, MA.*

# TOP TEN SPECTROSCOPY, AND MATERIALS ANALYSIS PAPERS

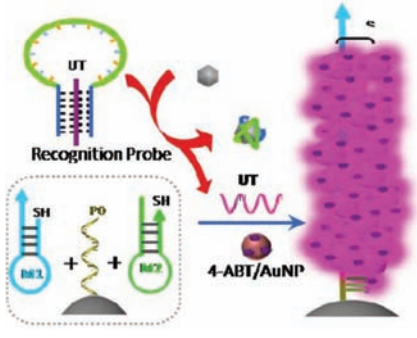
***Analytical Chemistry's* Most Popular Papers of 2014**

**HOW HAVE** the fields of spectroscopy and materials analysis changed it was last the focus of our special supplement to C&EN in June 2014? With their unique perspective on chemical research, the Editors of *Analytical Chemistry* have answered this question by selecting from their archives, reports that they consider representative of the 10 most significant advances in these areas from this last year. To whet your appetites, we have reproduced the abstracts of these noteworthy papers below, along with links to the articles to fill up on later. Keep current with the latest developments in this field throughout 2015 by reading the most recent publications at http://pubs.acs.org/journal/ancham

## Universal Surface-Enhanced Raman Scattering Amplification Detector for Ultrasensitive Detection of Multiple Target Analytes

**Jing Zheng, Yaping Hu, Junhui Bai, Cheng Ma, Jishan Li, Yinhui Li, Muling Shi, Weihong Tan, and Ronghua Yang**
State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, College of Biology, and Collaborative Innovation Center for Chemistry and Molecular Medicine, Hunan University, Changsha, Hunan 410082, China
*Anal. Chem.,* **2014,** *86* (4), 2205–2212
**DOI:** 10.1021/ac404004m

Here, we describe a novel "switch-on" biosensor based on quinonyl glycosides functionalized quantum dots (QDs) for the specific targeting and imaging of transmembrane glycoprotein receptors on the surface of cancer cells. The design of the quinonyl glycosides lies in that the quinone moiety serves as a quencher of QDs and the glycoside moiety as a biospecific




ligand for targeting a receptor. We observed that the quenched photoluminescence of the quinone glycosides functionalized QDs could be significantly recovered by a specific lectin that selectively binds to the glycosides clustering the QDs but was not affected by a panel of nonspecific lectins. Moreover, we determined that quinonyl galactoside functionalized QDs could optically image the asialoglycoprotein receptors of a hepatoma cell line in a target-specific manner. This system might provide new insights into the fabrication of photoluminogenic biosensors for the analysis of the universal ligand–receptor recognitions in nature. ■

## Target-Specific Imaging of Transmembrane Receptors Using Quinonyl Glycosides Functionalized Quantum Dots

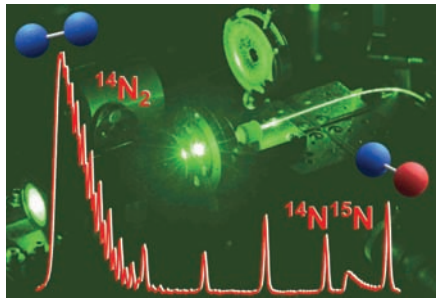
**Wei Ma †, Hui-Ting Liu †, Xiao-Peng He †, Yi Zang ‡, Jia Li *‡, Guo-Rong Chen †, He Tian †, and Yi-Tao Long †**
† Key Laboratory for Advanced Materials & Institute of Fine Chemicals, East China University of Science and Technology, Shanghai, P. R. China
‡ National Center for Drug Screening, State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Shanghai Institutes of Biological Sciences, Chinese Academy of Sciences, Shanghai, P. R. China
*Anal. Chem.*, **2014,** *86* (11), 5502–5507
**DOI:** 10.1021/ac501463u

Here, we describe a novel "switch-on" biosensor based on quinonyl glycosides functionalized quantum dots (QDs) for the specific targeting and imaging of transmembrane glycoprotein receptors on the surface of cancer cells. The design of the quinonyl glycosides lies in that the quinone moiety serves as a quencher of QDs and the glycoside moiety as a biospecific ligand for targeting a receptor. We observed that the quenched photoluminescence of the quinone glycosides functionalized QDs could be significantly recovered by a specific lectin that selectively binds to the glycosides clustering the QDs but was not affected by a panel of nonspecific lectins. Moreover, we determined that quinonyl galactoside functionalized QDs could optically image the asialoglycoprotein receptors of a hepatoma cell line in a target-specific manner. This system might provide new insights into the fabrication of photoluminogenic biosensors for the analysis of the universal ligand–receptor recognitions in nature. ■

## Fiber-Enhanced Raman Multigas Spectroscopy: A Versatile Tool for Environmental Gas Sensing and Breath Analysis

**Stefan Hanf †, Robert Keiner †, Di Yan †, Jürgen Popp †‡§, and Torsten Frosch ‡‡**
† Leibniz Institute of Photonic Technology, Jena, Germany
‡ Institute for Physical Chemistry, Friedrich-Schiller University, Jena, Germany
§ Abbe School of Photonics, Friedrich-Schiller University, Jena, Germany
*Anal. Chem.,* **2014,** *86* (11), 5278–5285
**DOI:** 10.1021/ac501463u

Versatile multigas analysis bears high potential for environmental sensing of climate relevant gases and noninvasive early stage diagnosis of disease states in human breath. In this contribution, a fiber-enhanced Raman spectroscopic (FERS) analysis of a suite of climate relevant atmospheric gases is presented, which allowed for reliable quantification of $CH_4$, $CO_2$, and $N_2O$ alongside $N_2$ and $O_2$ with just one single measurement. A highly improved analytical sensitivity was achieved, down to a sub-parts per million limit of detection with a high dynamic range of 6 orders




of magnitude and within a second measurement time. The high potential of FERS for the detection of disease markers was demonstrated with the analysis of 27 nL of exhaled human breath. The natural isotopes $^{13}CO_2$ and $^{14}N^{15}N$ were quantified at low levels, simultaneously with the major breath components $N_2$, $O_2$, and $^{12}CO_2$. The natural abundances of $^{13}CO_2$ and $^{14}N^{15}N$ were experimentally quantified in very good agreement to theoretical values. A fiber adapter assembly and gas filling setup was designed for rapid and automated analysis of multigas compositions and their fluctuations within seconds and without the need for optical readjustment of the sensor arrangement. On the basis of the abilities of such miniaturized FERS system, we expect high potential for the diagnosis of clinically administered $^{13}C$-labeled $CO_2$ in human breath and also foresee high impact for disease detection via biologically vital nitrogen compounds. ■

## Fabrication of Gold Nanoparticle-Embedded Metal–Organic Framework for Highly Sensitive Surface-Enhanced Raman Scattering Detection

**Yuling Hu *, Jia Liao, Dongmei Wang, and Gongke Li**
School of Chemistry and Chemical Engineering, Sun Yat-sen University, Guangzhou, Guangdong 510275, China
*Anal. Chem.,* **2014,** *86* (8), 3955–3963
**DOI:** 10.1021/ac5002355

Surface-enhanced Raman scattering (SERS) signals strongly rely on the interactions and distance between analyte molecules and metallic nanostructures. In this work, the use of a gold nanoparticle (AuNP)-embedded metal–organic framework was introduced for the highly sensitive SERS detection. The AuNPs were in situ grown and encapsulated within the host matrix of MIL-101 by a solution impregnation strategy. The as-synthesized AuNPs/MIL-101 nanocomposites combined the localized surface plasmon resonance properties of the gold nanoparticles and the high adsorption capability of metal–organic framework, making them highly sensitive SERS substrates by effectively preconcentrating analytes in close proximity to the electromagnetic fields at the SERS-active metal surface. We discussed the fabrication, physical characterization, and SERS activity of our novel substrates by measuring the Raman signals of a variety of model analytes. The SERS substrate was found to be highly sensitive, robust, and amiable to several different target analytes. A SERS detection limit of 41.75 and 0.54 fmol for Rhodamine 6G and benzadine, respectively, was demonstrated. The substrate also showed high stability and reproducibility, as well as molecular sieving effect thanks to the protective shell of the metal–organic framework. Subsequently, the potential practical application of the novel SERS substrate was evaluated by quantitative analysis of organic pollutant *p*-phenylenediamine in environmental water

and tumor marker alpha-fetoprotein in human serum. The method showed good linearity between 1.0 and 100.0 ng/mL for *p*-phenylenediamine and 1.0–130.0 ng/mL for alpha-fetoprotein with the correlation coefficients of 0.9950 and −0.9938, respectively. The recoveries ranged from 80.5% to 114.7% for *p*-phenylenediamine in environmental water and 79.3% to 107.3% for alpha-fetoprotein in human serum. These results foresee promising application of the novel metal–organic framework based composites as sensitive SERS-active substrates in both environmental and clinical samples. ■

## Raman Spectroscopy Enables Noninvasive Biochemical Characterization and Identification of the Stage of Healing of a Wound

**Rishabh Jain †, Diego Calderon ‡, Patricia R. Kierski ‡, Michael J. Schurr §, Charles J. Czuprynski ƒ, Christopher J. Murphy ⊥¶, Jonathan F. McAnulty ‡, and Nicholas L. Abbott †**

† Department of Chemical and Biological Engineering, University of Wisconsin-Madison, Madison, Wisconsin 53706, United States

‡ Department of Surgical Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, Wisconsin 53706, United States

§ Department of Surgery, School of Medicine and Public Health, University of Colorado-Denver, Denver, Colorado 80217 United States

ƒ Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, Wisconsin 53706, United States

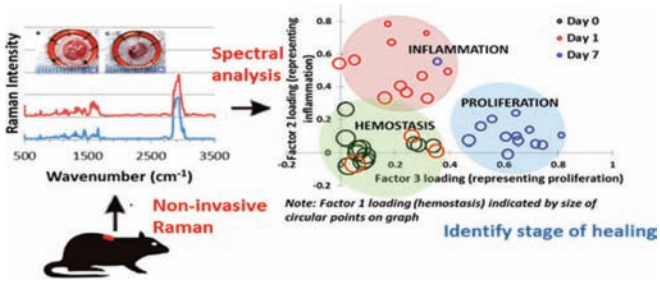
⊥ Department of Surgical and Radiological Sciences, School of Veterinary Medicine, University of California-Davis, Davis, California 95616, United States

¶ Department of Ophthalmology & Vision Science, School of Medicine, University of California-Davis, Davis, California 95616, United States

*Anal. Chem.*, **2014,** *86* (8), 3764–3772

**DOI:** 10.1021/ac500513t

Accurate and rapid assessment of the healing status of a wound in a simple and noninvasive manner would enable clinicians to diagnose wounds in real time and promptly adjust treatments to hasten the resolution of nonhealing wounds. Histologic and biochemical characterization of biopsied wound tissue, which is currently the only reliable method for wound assessment, is invasive, complex to interpret, and slow. Here we demonstrate the use of Raman microspectroscopy coupled with multivariate spectral analysis as a simple, noninvasive method to biochemically characterize healing wounds in mice and to accurately identify different phases of healing of wounds at different time-points. Raman spectra were collected from "splinted" full thickness dermal wounds in mice at 4 time-points (0, 1, 5, and 7 days) corresponding to different phases of wound healing, as




verified by histopathology. Spectra were deconvolved using multivariate factor analysis (MFA) into 3 "factor score spectra" (that act as spectral signatures for different stages of healing) that were successfully correlated with spectra of prominent pure wound bed constituents (i.e., collagen, lipids, fibrin, fibronectin, etc.) using non-negative least squares (NNLS) fitting. We show that the factor loadings (weights) of spectra that belonged to wounds at different time-points provide a quantitative measure of wound healing progress in terms of key parameters such as inflammation and granulation. Wounds at similar stages of healing were characterized by clusters of loading values and slowly healing wounds among them were successfully identified as "outliers". Overall, our results demonstrate that Raman spectroscopy can be used as a noninvasive technique to provide insight into the status of normally healing and slow-to-heal wounds and that it may find use as a complementary tool for real-time, in situ biochemical characterization in wound healing studies and clinical diagnosis. ■

## Toward Biocompatible Nuclear Hyperpolarization Using Signal Amplification by Reversible Exchange: Quantitative in Situ Spectroscopy and High-Field Imaging

**Jan-Bernd Hövener †‡§, Niels Schwaderlapp ‡, Robert Borowiak †‡§, Thomas Lickert ‡, Simon B. Duckett ||, Ryan E. Mewis ||, Ralph W. Adams ||, Michael J. Burns ||, Louise A. R. Highton ||, Gary G. R. Green ||, Alexandra Olaru ||, Jürgen Hennig ‡, and Dominik von Elverfeldt ‡**

† German Consortium for Cancer Research (DKTK), Heidelberg, Germany

‡ Medical Physics, Department of Radiology, University Medical Center Freiburg, 79098 Freiburg, Germany

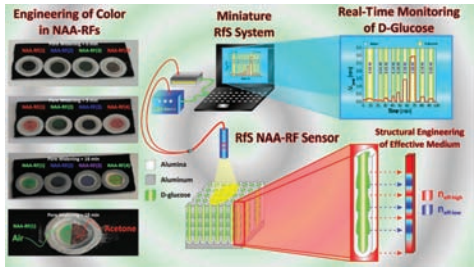
§ German Cancer Research Center (DKFZ), Heidelberg, Germany

‖ Centre for Hyperpolarisation in Magnetic Resonance, University of York, York, YO10 5DD, U.K.
*Anal. Chem.*, **2014,** *86* (3), 1767–1774
**DOI:** 10.1021/ac403653q

Signal amplification by reversible exchange (SABRE) of a substrate and *parahydrogen* at a catalytic center promises to overcome the inherent insensitivity of magnetic resonance. In order to apply the new approach to biomedical applications, there is a need to develop experimental equipment, *in situ* quantification methods, and a biocompatible solvent. We present results detailing a low-field SABRE polarizer which provides well-controlled experimental conditions, defined spins manipulations, and which allows *in situ* detection of thermally polarized and hyperpolarized samples. We introduce a method for absolute quantification of hyperpolarization yield in situ by means of a thermally polarized reference. A maximum signal-to-noise ratio of ~$10^3$ for 148 $\mu$mol of substance, a signal enhancement of $10^6$ with respect to polarization transfer field of SABRE, or an absolute $^1$H-polarization level of ≈$10^{-2}$ is achieved. In an important step toward biomedical application, we demonstrate $^1$H *in situ* NMR as well as $^1$H and $^{13}$C high-field MRI using hyperpolarized pyridine ($d_3$) and $^{13}$C nicotinamide in pure and 11% ethanol in aqueous solution. Further increase of hyperpolarization yield, implications of *in situ* detection, and *in vivo* application are discussed. ■

## Structural and Optical Nanoengineering of Nanoporous Anodic Alumina Rugate Filters for Real-Time and Label-Free Biosensing Applications

**Tushar Kumeria †, Mohammad Mahbubur Rahman †‡, Abel Santos †, Josep Ferré-Borrull ‡, Lluís F. Marsal ‡, and Dusan Losic †**
† School of Chemical Engineering, The University of Adelaide, Engineering North Building, 5005 Adelaide, Australia
‡ Departament d'Enginyeria Electrònica, Elèctrica i Automàtica, Universitat Rovira i Virgili, Avda Països Catalans 26, 43007 Tarragona, Spain
*Anal. Chem.,* **2014,** *86* (3), 1837–1844
**DOI:** 10.1021/ac500069f
In this study, we report about the structural engineering and optical optimization of nanoporous anodic alumina rugate filters (NAA-RFs) for real-time and label-free biosensing applications. Structurally engineered NAA-RFs are combined with reflection spectroscopy (RfS) in order to develop a biosensing system based on the position shift of the characteristic peak in the reflection spectrum of NAA-RFs (Δλpeak). This system is optimized and assessed by measuring shifts in the characteristic peak position produced by small changes in the effective medium (i.e., refractive index). To this end, NAA-RFs are filled with different solutions of D-glucose, and the Δλpeak is measured in real time by RfS. These results are validated by a theoretical model (i.e., the Looyenga–Landau–Lifshitz model), demonstrating that the control over the nanoporous structure makes it possible to optimize optical signals in RfS for sensing purposes. The linear range of these optical sensors ranges from 0.01 to 1.00 M, with a low detection limit of 0.01 M of D-glucose (i.e., 1.80 ppm), a sensitivity of 4.93 nm $M^{-1}$ (i.e., 164 nm per refractive index units), and a linearity of 0.998. This proof-of-concept study demonstrates that the proposed system combining NAA-RFs with RfS has outstanding capabilities to develop ultrasensitive, portable, and cost-competitive optical sensors. ■




## *In Vivo* Proton–Electron Double-Resonance Imaging of Extracellular Tumor pH Using an Advanced Nitroxide Probe

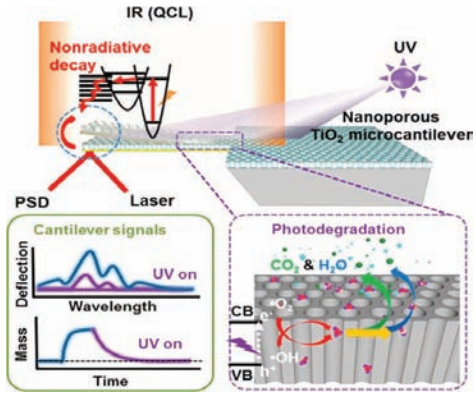
**Alexandre Samouilov †§, Olga V. Efimova †, Andrey A. Bobko †‡, Ziqi Sun †, Sergey Petryakov †, Timothy D. Eubank †‡, Dmitrii G. Trofimov ‖, Igor A. Kirilyuk ‖, Igor A. Grigor'ev ‖, Wataru Takahashi †⊗, Jay L. Zweier †§, and Valery V. Khramtsov †‡**
†The Dorothy M. Davis Heart and Lung Research Institute: ‡Division of Pulmonary, Allergy, Critical Care, and Sleep Medicine, and §Division of Cardiovascular Medicine, Department of Internal Medicine, The Ohio State University, Columbus, Ohio 43210, United States
‖ **Vorozhtsov Institute of Organic Chemistry, Novosibirsk 630090, Russia**
⊗ Hokkaido University, Sapporo, Hokkaido 060-0814, Japan
*Anal. Chem.,* **2014,** *86* (2), 1045–1052
**DOI:** 10.1021/ac402230h

A variable radio frequency proton–electron double-resonance imaging (VRF PEDRI) approach for pH mapping of aqueous samples has been recently developed (Efimova et al. *J. Magn. Reson.* **2011,** *209,* 227−232). A pH map is extracted from two PEDRI acquisitions performed at electron paramagnetic resonance (EPR) frequencies of protonated and unprotonated

forms of a pH-sensitive probe. To translate VRF PEDRI to an *in vivo* setting, an advanced pH probe was synthesized. Probe deuteration resulted in a narrow spectral line of 1.2 G compared to a nondeuterated analogue line width of 2.1 G allowing for an increase of Overhauser enhancements and reduction in rf power deposition. Binding of the probe to the cell-impermeable tripeptide, glutathione (GSH), allows for targeting to extracellular tissue space for monitoring extracellular tumor acidosis, a prognostic factor in tumor pathophysiology. The probe demonstrated pH sensitivity in the 5.8–7.8 range, optimum for measurement of acidic extracellular tumor pH (pHe). *In vivo* VRF PEDRI was performed on Met-1 tumor-bearing mice. Compared to normal mammary glands with a neutral mean pHe (7.1 ± 0.1), we observed broader pH distribution with acidic mean pHe (6.8 ± 0.1) in tumor tissue. In summary, VRF PEDRI in combination with a newly developed pH probe provides an analytical approach for spatially resolved noninvasive pHe monitoring, *in vivo*. ■

## Direct Detection and Speciation of Trace Explosives Using a Nanoporous Multifunctional Microcantilever

**Dongkyu Lee †, Seonghwan Kim †‡, Sangmin Jeon §, and Thomas Thundat †**
† Department of Chemical and Materials Engineering, University of Alberta, Edmonton, Alberta T6G 2 V4, Canada
‡ Department of Mechanical and Manufacturing Engineering, University of Calgary, Calgary, Alberta T2N 1N4, Canada
§ Department of Chemical Engineering, Pohang University of Science and Technology, Pohang, South Korea
|| Vorozhtsov Institute of Organic Chemistry, Novosibirsk 630090, Russia
⊗ Hokkaido University, Sapporo, Hokkaido 060-0814, Japan
*Anal. Chem.,* **2014**, 86 (10), 5077–5082
**DOI:** 10.1021/ac500745g

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## Detection and Quantification of Early-Stage Malaria Parasites in Laboratory Infected Erythrocytes by Attenuated Total Reflectance Infrared Spectroscopy and Multivariate Analysis

**Aazam Khoshmanesh †, Matthew W. A. Dixon ‡, Shannon Kenny ‡, Leann Tilley ‡, Don McNaughton †, and Bayden R. Wood †**
† Centre for Biospectroscopy and School of Chemistry, Monash University, Clayton, Victoria 3800, Australia
‡ Department of Biochemistry and Molecular Biology and Bio21 Molecular, Science and Biotechnology Institute, The University of Melbourne, Melbourne, Victoria 3010 Australia
*Anal. Chem.*, **2014**, *86* (9), 4379–4386
**DOI:** 10.1021/ac500199x

New diagnostic modalities for malaria must have high sensitivity and be affordable to the developing world. We report on a method to rapidly detect and quantify different stages of malaria parasites, including ring and gametocyte forms, using attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FT-IR) and partial least-squares regression (PLS). The absolute detection limit was found to be 0.00001% parasitemia (<1 parasite/μL of blood; $p < 0.008$) for cultured early ring stage parasites in a suspension of normal erythrocytes. Future development of universal and robust calibration models can significantly improve malaria diagnoses, leading to earlier detection and treatment of this devastating disease. ■

# DETECTION OF METHYLMALONIC ACID (MMA) IN PLASMA USING HYDROPHILIC INTERACTION LIQUID CHROMATOGRAPHY (HILIC) COUPLED WITH MASS SPECTROMETRY (MS) OR TANDEM MASS SPECTROMETRY (MS/MS)

**Maricar Dube and Patrik Appelblad** EMD Millipore

## Abstract

Methylmalonic acid (MMA) is a biomarker for vitamin B12 deficiency. This application note describes a fast, simple, and sensitive method to detect MMA in plasma that uses a zwitterionic hydrophilic interaction chromatography (ZIC®-HILIC) column with LC-MS or LC-MS/MS.

## Introduction

Methylmalonic acid (MMA) levels in serum, plasma and urine are used to monitor cobalamin (vitamin B12) deficiency[1] and methylmalonic acidemia. Different methods have been developed to quantify MMA in biological samples, including GC-MS, LC-MS/MS, HPLC, and capillary electrophoresis (CE). The main challenges that must be overcome for accurate measurement are the low physiological concentrations of MMA in human serum (100-500 nM), and the fact that MMA is a hydrophilic non-volatile compound. Retention and separation of MMA on reversed phase liquid chromatographic columns is difficult since MMA is poorly retained, and the structural isomer, succinic acid (SA), causes ion suppression because the concentration SA in serum is usually considerably higher than MMA.

Many laboratories have adopted protocols that require extraction of MMA plus steps to yield MMA-derivatives that are compatible with GC-MS or LC-MS/MS techniques using reversed phase columns[2]. This way, derivatives of MMA and SA may be differentiated due to their different fragmentation patterns. As a consequence however, the cost per MMA-test is usually considerably higher than standard immunological assays for B12.

HILIC columns efficiently separate polar hydrophilic compounds, which are not retained on reversed phase columns. The base material of HILIC columns can be either silica or polymer, and may be modified with different types of polar functionalities such as zwitterionic sulfoalkylbetaine (ZIC®-HILIC column). Because of its highly polar nature, MMA is retained on a ZIC®-HILIC column without the need to generate MMA-derivatives, making the workflow simpler, easier and faster[3]. This report describes a sensitive LC-MS/MS method to measure MMA using a ZIC®-HILIC column.

## Experimental Conditions

## Chromatography Conditions

**Table 1**

| | |
|---|---|
| Column | SeQuant® ZIC®-HILIC (3µm, 100Å) PEEK 100× 2.1 mm |
| Injection | 7 µL |
| Mobile phase | 80:20 Acetonitrile/ 100 mM ammonium acetate pH 4.5* |
| Flow rate | 400 µL/min |
| Temperature | 40ºC |
| Detection | (a) ESI-MS<br>(b) MS/MS, ESI(-),<br>MRM (m/z 117.1→73.0, 55.1) |

*** There is a gradient wash process between injections**

## Sample Preparation

800 µL acidified acetonitrile containing 170 nM of internal standard (D3-MMA) was used to precipitate proteins in 200 µL serum/plasma samples. Supernatant was directly injected into the column after centrifugation[3].

## Results and Discussion

Isocratic separation of MMA in plasma on a ZIC®-HILIC column was achieved in less than 3 minutes. The void volume was 0.5 min, while the retention times for MMA and D3-MMA were 2.14 min and 2.13 min, respectively.

For the single stage MS detection, the limit of detection (3 x SD) and limit of quantitation (10 x SD) were 30 nM and 90 nM MMA, respectively, in plasma/serum. The method is linear up to 200 µM. Day-to-day and intra-day CVs are lower than 5%. The recovery is between 90% and 93%.

For the MS/MS detection, the limit of detection (3 × SD) was 5 nM and the limit of quantification (10 × SD) for MMA was 15 nM. Figure 1 (on next page) shows the MS/MS chromatogram of MMA in a plasma sample.

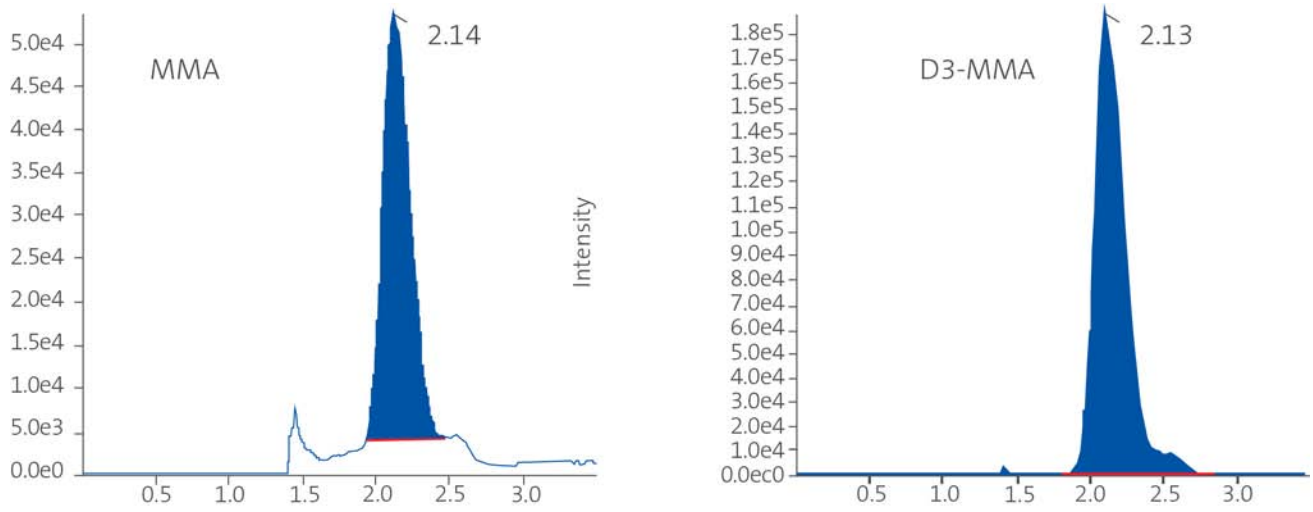


**Figure 1.**
*ZIC®-HILIC-MS/MS chromatogram of MMA (m/z 117.1 73.0) and D3-MMA (m/z 119.9 75.9) in a plasma sample*

## Conclusion

A fast, simple, and sensitive means to determine MMA levels in serum/plasma was developed that combined ZIC®-HILIC separation with single stage negative ESI-MS or tandem MS. As neither MMA sample extraction nor derivatization were required, the ZIC®-HILIC-MS/MS method reported here may be an attractive alternative to existing means for measuring MMA in clinical laboratories where the existing GC-MS or reversed phase LC-MS/MS methods are tedious and laborious.

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# DUAL-FLOW REFRACTIVE INDEX DETECTOR FOR DETERMINATION OF MOLAR MASS AVERAGES IN GEL PERMEATION CHROMATOGRAPHY

**Amandaa K. Brewer, Ph.D.** Tosoh Bioscience LLC

## Introduction

Gel permeation chromatography (GPC) is the most widely accepted and used analytical method for obtaining molar mass averages and distributions of both synthetic and biological polymers. Traditionally, molar mass averages and distributions are obtained via a peak position calibration using a series of standards of known molar mass and chemistry, analyzed by GPC coupled to a differential refractive index (RI) detector. In the context of GPC, GPC/RI continues to be heavily employed as it provides excellent day-to-day reproducibility, and is ideal for quality control procedures.

One caveat to single detector GPC performance is the baseline stability of the RI detector. A conventional RI detector is constructed in such a way that there are two sides: (1) the reference side that contains stagnant pure solvent; and (2) the sample side, which has a flowing stream of analyte in the same solvent as in the reference side. Over time, the stagnant pure solvent in the reference side slowly changes, resulting in baseline drift. For peak position calibration, a drift in the RI baseline has been shown to drastically affect the accuracy and precision of measurements of molar mass averages and distributions with an increase in the error rate of 25%.

Here, we have studied the repeatability, reproducibility, and baseline stability of a dual-flow RI detector in the EcoSEC® GPC System for the determination of molar mass averages via peak position calibration. The unique dual flow design of the RI detector is constructed in such a way that the reference side of the RI flow cell contains a flowing stream of pure solvent. The dual flow design is shown to compensate for the changes in the refractive index of the solvent over time by continuously flowing pure solvent through the reference side of the flow cell.

## Experimental Methods and Conditions

GPC analysis was performed on a system consisting of either an all-in-one EcoSEC GPC System equipped with a dual-flow refractive index detector or a modular HPLC system with an external conventional refractive index detector. Separation of polystyrene standards (PS) occurred over a column blank consisting of TSKgel® SuperMultiporeHZ-M columns, with THF as the mobile phase.

## Results and Discussion

To demonstrate the repeatability, reproducibility, and baseline stability of a dual-flow RI detector compared to a conventional RI detector a series of identical experiments was performed on the EcoSEC GPC System (Figure 1) and two conventional


**Figure 1**
*EcoSEC GPC System*

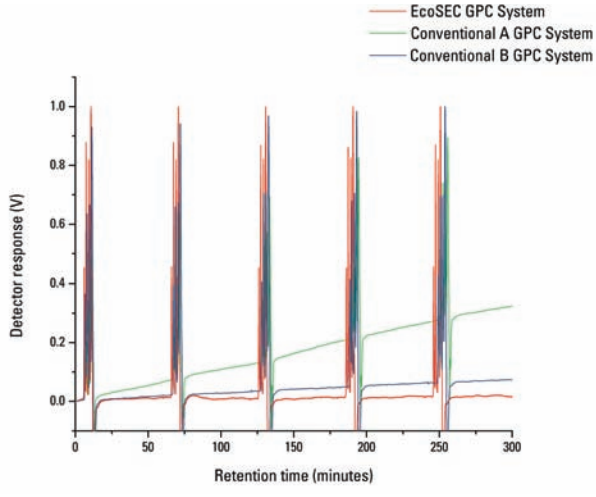



**Figure 2**
*Baseline drift comparison between a dual-flow refractive index detector and conventional systems*

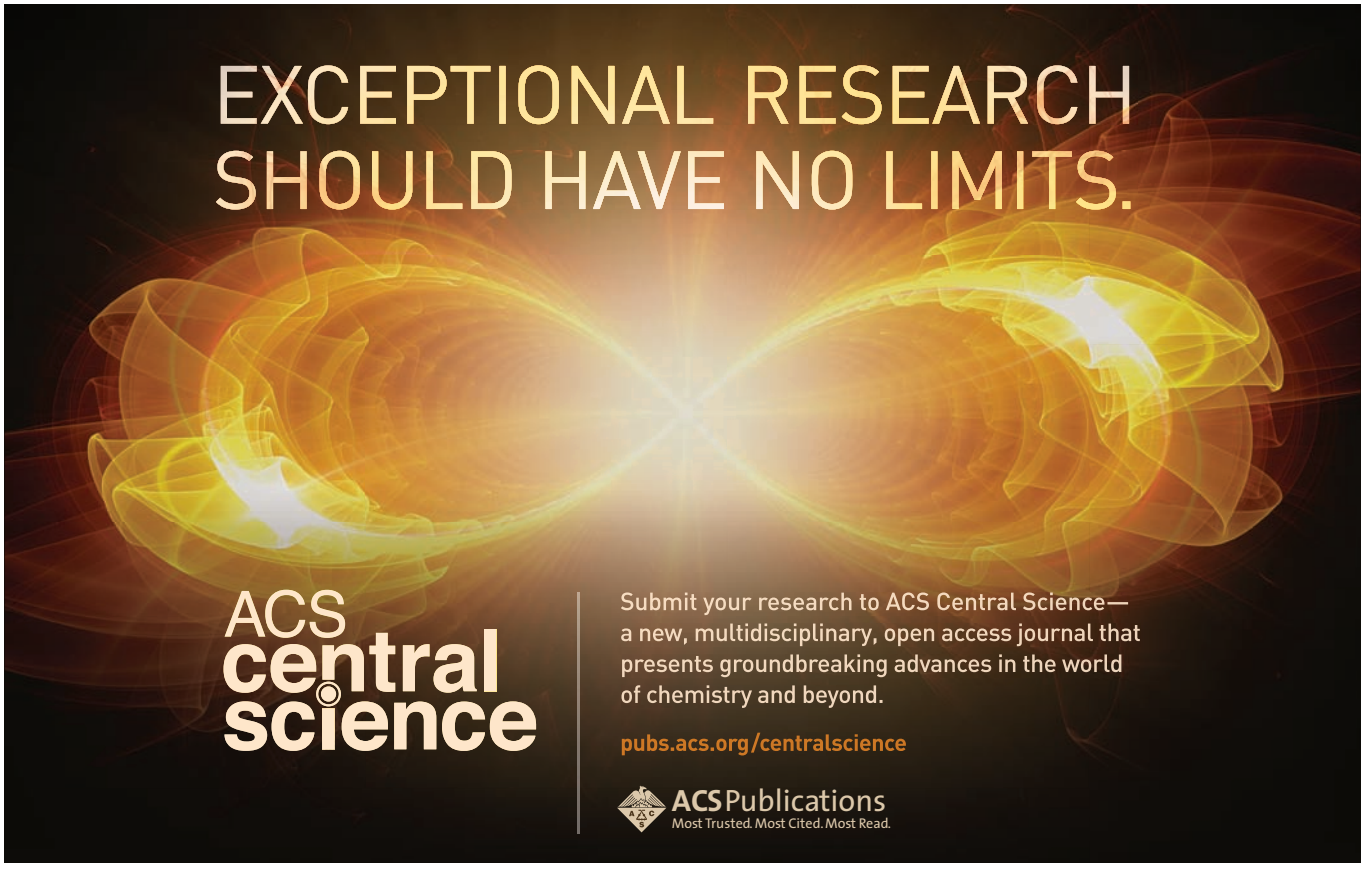
HPLC systems. As shown in Figure 2, five consecutive injections of PS with run times of one hour without auto zeroing the detector between injections for a total of five hours, resulted in an extremely stable baseline with low baseline drift for the dual-flow RI detector and a significantly drifting baseline on the two conventional RI detectors.

The repeatability and reproducibility of the molar mass averages as obtained via the dual-flow and conventional RI detectors were also compared. The reproducibility of the weight-average molar mass, $M_w$, of the dual-flow RI detector was determined to be superior by a factor of 3 to that of a conventional RI detector. Additionally the day-to-day reproducibility and repeatability for the determination of molar mass averages was shown to vary less than 0.5% for the dual-flow RI detector, while the conventional RI detector produced day-to-day variations in molar mass averages between 1% and 3%.

## Conclusion

A stable RI detector baseline is required for successful experiments, and repeatable and reproducible molar mass averages. Extreme care must be taken when molar mass averages and distributions are determined via peak position calibration as uncertainties and instabilities in the RI baseline can result in relatively large errors, inconsistencies, and deviations in molar mass averages. The repeatability and reproducibility of the molar mass averages were shown to increase greatly when a conventional RI detector was replaced with a dual-flow RI detector.





# MODULAR SPECTROSCOPY TOOLS FOR MEASURING INTRINSIC PROTEIN FLUORESCENCE

**Yvette Mattley, Ph.D.** Ocean Optics

## Abstract

In this application note, a UV LED and a high performance modular spectrometer were used together to measure the intrinsic fluorescence of the protein lysozyme in different conformational states. The objective was to demonstrate the power of modular spectroscopy for measuring inherent protein fluorescence as a means to monitor changes in the folded state of proteins.

## Introduction

Proteins contain aromatic amino acids that fluoresce when excited with UV light. This intrinsic protein fluorescence depends on the amino acid composition and conformational state of the protein. As the protein goes from a native (folded) to a denatured (unfolded) state, the local environment surrounding the aromatic amino acids changes, affecting the fluorescence properties of the amino acids.

Proteins containing tryptophan and tyrosine (280 nm and 274 nm excitation, respectively) are best suited for conformation monitoring by UV-excited fluorescence emissions due to the relatively high quantum yield and similar excitation wavelengths of these amino acids. Phenylalanine is used less frequently as an indicator of protein conformation because it has a much lower quantum yield with a lower excitation wavelength (~257 nm excitation).

The native state of a protein can be altered in different ways including elevating temperature, adding chaotropic or other chemical agents such as guanidine hydrochloride or urea, and changing pH. As the protein unfolds, amino acids previously buried in the hydrophobic core of the protein are exposed to the solvent. Solvent exposure quenches the fluorescence of the amino acids and decreases the intensity of the intrinsic protein spectrum.

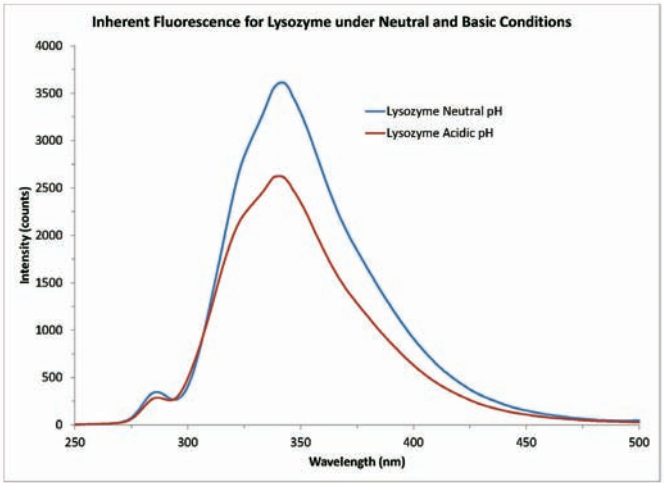



**Figure 1**
*Changes in the fluorescence spectra of lysozyme with exposure to a low-pH*

## Experimental Conditions

A 280 nm UV LED in combination with a high-sensitivity QE *Pro* spectrometer was used to measure fluorescence from samples of lysozyme diluted in neutral and acidic solutions. We prepared 3 mg/mL lysozyme (L6876 Sigma) in phosphate buffered saline (1X PBS pH 7.4), and 0.1 M HCl/KCl (pH 1) solution. Lysozyme suspended in 1X PBS was in its native (folded) state, while lysozyme suspended in the acidic 0.1 M HCl/KCl solution began to denature and expose amino acids previously contained within the core of the protein to the solvent environment.

## Results and Discussion

The intrinsic protein fluorescence spectra for lysozyme diluted in 1X PBS (neutral pH) and 0.1 M HCl/KCl (acidic pH) are shown in Figure 1. When lysozyme was exposed to low pH, the protein conformation changed exposing the tryptophan and tyrosine amino acids to a different environment. As a result, the fluorescence spectrum decreased in intensity as the protein changed from a folded conformation to an unfolded state.

## Conclusion

Intrinsic fluorescence is a powerful indicator of protein structure and function. Inherent protein fluorescence can give researchers insight into the protein's conformational state, and corresponding biological activity under different conditions, including changes in temperature, pH and ion concentration. These changes in intrinsic protein fluorescence can be used to monitor protein unfolding for medical diagnostics applications where researchers are investigating neurodegenerative and other diseases associated with improper protein unfolding.

Ocean Optics, Inc.
830 Douglas Avenue,
Dunedin, FL 34698
Tel: (727) 733-2447
Email: info@oceanoptics.com
Website: www.oceanoptics.com

# ON-LINE QUALITY CONTROL MEASUREMENTS IN VARYING CONDITIONS

**Yvette Mattley, Ph.D.** Ocean Optics

## Abstract

With the use of a new generation of robust, repeatable and stable instrumentation, manufacturers can more easily assess sample quality under rigorous conditions. In this application note, we investigate the thermal stability of a spectrometer system for process line transmission measurements at different temperatures.

## Introduction

Even as advances in engineering technologies and manufacturing processes have lowered the cost of making and distributing products, the demand for continued improvement is as strong as ever. In an environment where small improvements in characterization of raw materials or subtle changes in process parameters can result in significant production savings, the ability to design faster, smarter and more robust instrumentation is paramount.

When the emergence of miniature spectrometers coincided with development of modular fiber optics, spectroscopy was no longer limited to the lab. Now you can bring the instrument to the sample, which allows industrial users to integrate the measurement into the process. Small-footprint modular systems can be rapidly configured for a variety of absorbance, reflectance and emission measurements, with a number of potential applications.

The Flame spectrometer addresses some of the limitations associated with miniature spectroscopy systems in dynamic process environments.

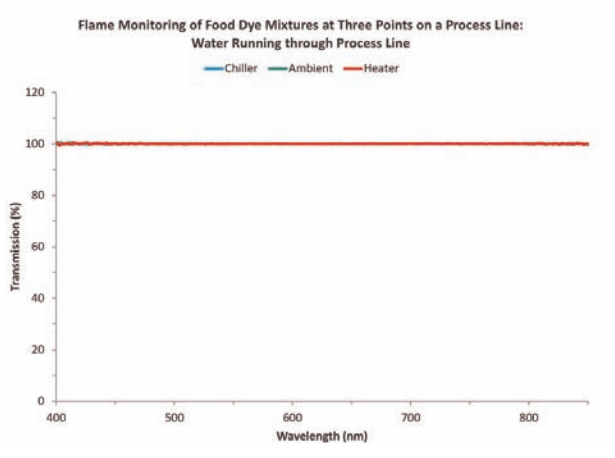



**Figure 1**

*Water was tested as the reference for each condition – chilled (24 °C), ambient (27 °C) and heated (30 °C).*

## Experimental Conditions

To evaluate the effectiveness of the Flame spectrometer at different temperatures, we measured transmission of several

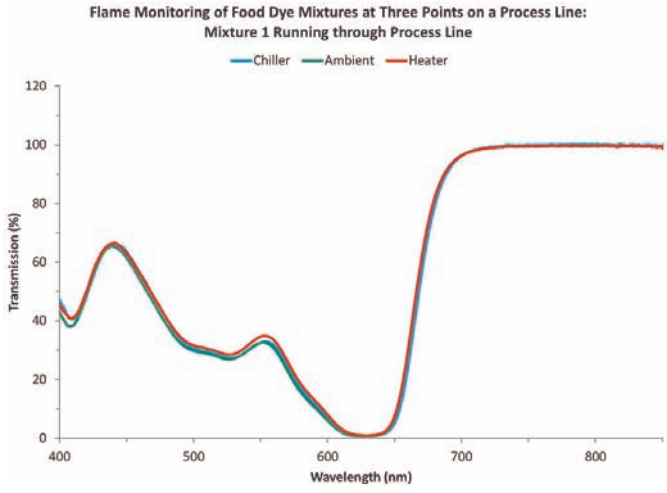



**Figure 2**

*Mixture 1: spectra measured across each temperature condition – chilled (24 °C), ambient (27 °C) and heated (30 °C) – were consistent.*

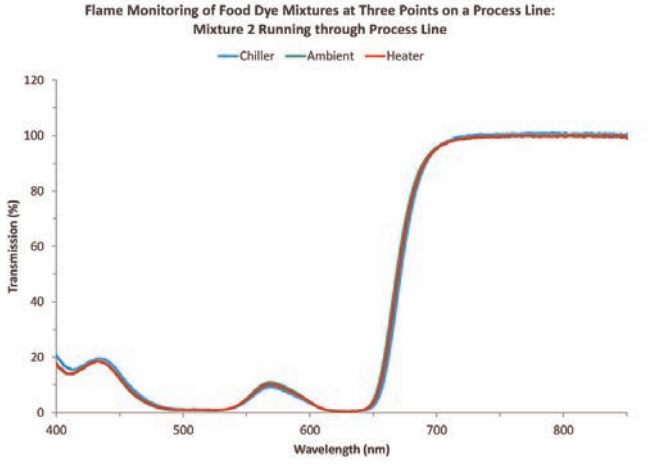



**Figure 3**

*The Flame spectrometer produced consistent results across different temperature conditions, as these transmission spectra reveal.*

concentration levels of food dye mixtures on a simulated process line with typical conditions encountered in a process environment. Then we isolated each Flame spectrometer in a different temperature environment – cool (using a chiller), ambient and hot (using a lab heater). Several sample mixtures were prepared for testing, using the Z-type flow cell to move each sample through the system. Water moving through the flow cell was measured as a reference (Figure 1).

## Results and Discussion

Although the Flame spectrometers measured the transmission of the mixtures flowing through the system at different temperature conditions, the resulting spectra—and sample composition information derived from the spectra—were nearly identical (Figure 2 and Figure 3). This result is significant for process line applications, where temperatures can vary from zone to zone within the stream. For quality control professionals, getting the correct answers under all sorts of conditions—including temperature extremes—is critical.

## Conclusion

Process environments can be harsh, with extremes in temperature and humidity, and the harmful effects of dust and vibration. That's why process-ready spectroscopic instrumentation such as Flame has been designed with few moving parts, has a high degree of thermal stability, and is easily adapted for different setups. The availability of such robust, repeatable, thermally stable instrumentation allows manufacturers to assess sample quality online at multiple points in processes, helping to improve yields, eliminate waste and reduce costs.

Ocean Optics, Inc.
830 Douglas Avenue,
Dunedin, FL 34698
Tel: (727) 733-2447
Email: info@oceanoptics.com
Website: www.oceanoptics.com

# THERMOPLASTIC ELASTOMER FLOW PROPERTY DETERMINATION BY DYNAMIC RHEOLOGY

TA Instruments

## Abstract

Rate-dependent viscosity data of thermoplastic elastomers is important for effective material property prediction and manufacturing process design. Rheological data also reveals compositional differences and the presence of a percolation threshold of the dispersed phase.

## Introduction

Thermoplastic elastomers have gained considerable interest for their appealing combination of rubber-like final properties and convenient thermoplastic processing. Thermoplastic vulcanizates (TPV) are among the most prevalent thermoplastic elastomers for the replacement of cured rubber parts. TPV can be processed like thermoplastics, however characterization of their flow behavior is critical to effective manufacturing design. Many TPV producers offer material grades based on both variable hardness and tailor-made processing types such as injection molding, blow molding or extrusion.

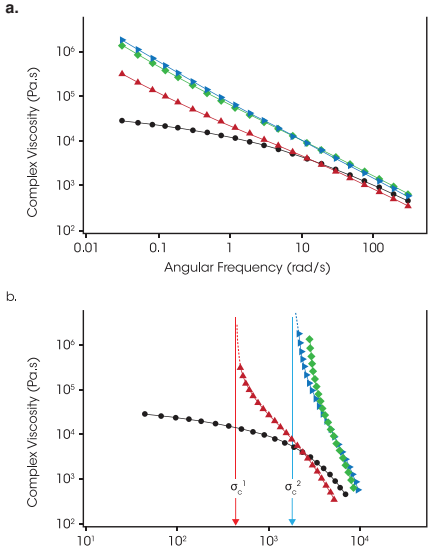



**Figure 1**

*(a) Frequency dependent complex viscosity of four TPV materials of varying composition. (b) Rubber content of samples and corresponding yield stress*

## Experimental Conditions

Samples of TPV of varying composition were tested with the RPA elite oscillatory shear rheometer (TA Instruments, New Castle, DE USA). The RPA elite is a closed-cavity dynamic shear rheometer with grooved biconical dies designed for rubber and elastomer characterization. Pre-molded sample discs were used to improve homogeneity. Samples were loaded at 180°C and conditioned with a low strain and frequency (0.5%, 10 rad/s) for 10 minutes prior to data collection. Frequency sweep experiments were performed at 1% strain.

## Results

Four samples were tested, each with a different TPV content, to demonstrate shear-thinning behavior, which is common for polymers. At moderate to high frequencies (shear rates), the complex viscosity values are similar for each specimen (Figure 1a). Larger differences become evident at low frequencies, which correlate with low shear rate behavior. The fourth sample (Figure 1, black curve) exhibits a Newtonian viscosity plateau at low frequencies.

## Discussion

TPV are multiphase materials with a discontinuous cured rubber phase dispersed in a continuous polyolefin phase. The ratio of cured rubber to polyolefin is used to adjust the final hardness value; greater polyolefin content leads to higher hardness values. In Figure 1, the specimen indicated by the black curve has a hardness value of 50 Shore D, indicative of a polyolefin-rich material. All other materials (red, blue, and green curves) have hardness values from 50 to 75 Shore A, and are rubber-rich materials.

The viscous behavior of the low-hardness materials (high rubber-phase content) can be appropriately described by the Herschel-Bulkley model: $\sigma = \sigma_c + K(\dot{\gamma})^n$

This model is specifically used for compounded materials such as rubbers and plastics with filler content above the percolation threshold. This model highlights a critical stress ($\sigma c$) at which the viscosity is infinite and under which the material does not flow, information that is particularly important for injection mold and extruder die design. The critical stress for rubber-rich TPV is clearly illustrated in Figure 1b. This representation of the data also reinforces the earlier conclusion that the polyolefin-rich material (black curve) does not exhibit a critical stress.

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