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April 2019 Volume 34 Number 4

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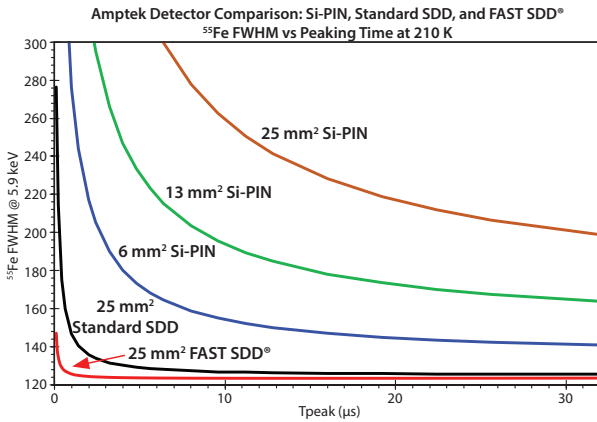
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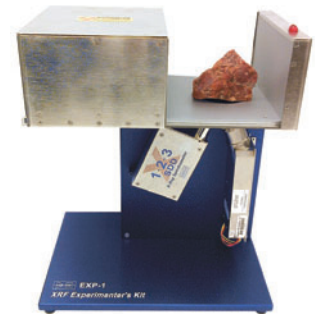
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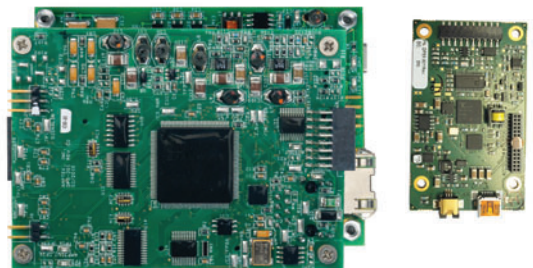


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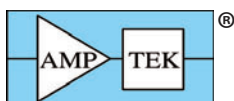
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C.A.S.T. DATA AND LIST INFORMATION: Contact Melissa Stillwell, (218) 740-6831; e-mail: MStillwell@mmhgroup.com

INTERNATIONAL LICENSING: Jillyn Frommer, (732) 346-3007, fax: (732) 647-1104; e-mail: JFrommer@mmhgroup.com

485F US Highway One South, Suite 210
Iselin, NJ 08830
(732) 596-0276
Fax: (732) 647-1235

Michael J. Tesselone

Vice President/Group Publisher
MTesselone@mmhgroup.com

Stephanie Shaffer

Publisher
SShaffer@mmhgroup.com

Edward Fantuzzi

Associate Publisher
EFantuzzi@mmhgroup.com

Michael Kushner

Senior Director, Digital Media
MKushner@mmhgroup.com

Laura Bush

Editorial Director
LBush@mmhgroup.com

John Chasse

Managing Editor
JChasse@mmhgroup.com

Jerome Workman, Jr.

Senior Technical Editor
JWorkman@mmhgroup.com

Cindy Delonas

Associate Editor
CDelonas@mmhgroup.com

Kristen Moore

Webcast Operations Manager
KMoore@mmhgroup.com

Vania Oliveira

Project Manager
VOliveira@mmhgroup.com

Sabina Advani

Digital Production Manager
SAdvani@mmhgroup.com

Kaylynn Chiarello-Ebner

Managing Editor, Special Projects
KEbner@mmhgroup.com

Dan Ward

Art Director
dward@hcl.com

Brianne Pangaro

Marketing Associate
BPangaro@mmhgroup.com

Melissa Stillwell

C.A.S.T. Data and List Information
MStillwell@mmhgroup.com

Thomas W. Ehardt

President, MultiMedia Healthcare LLC
TEhardt@mmhgroup.com

Dave Esola

VP & General Manager,
MultiMedia Healthcare LLC
DEsola@mmhgroup.com

Jillyn Frommer

Permissions
JFrommer@mmhgroup.com

Jesse Singer

Production Manager
jsinger@hcl.com

Wendy Bong

Audience Development Manager
WBong@mmhgroup.com

Morgan Hight

Audience Support Analyst
MHight@mmhgroup.com



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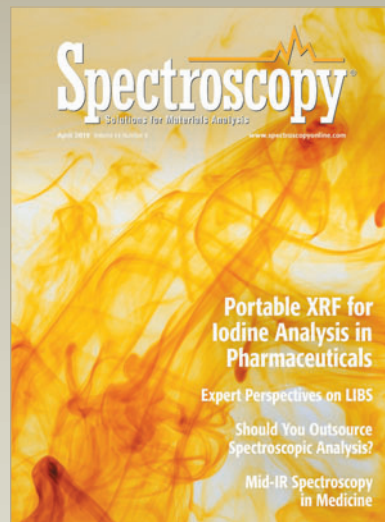
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Spectroscopy (ISSN 0887-6703 [print], ISSN 1939-1900 [digital]) is published monthly by MultiMedia Healthcare LLC 325 W 1st St STE 300 Duluth MN 55802. *Spectroscopy* is distributed free of charge to users and specifiers of spectroscopic equipment in the United States. *Spectroscopy* is available on a paid subscription basis to nonqualified readers at the rate of: U.S. and possessions: 1 year (12 issues), \$74.95; 2 years (24 issues), \$134.50. Canada/Mexico: 1 year, \$95; 2 years, \$150. International: 1 year (12 issues), \$140; 2 years (24 issues), \$250. Periodicals postage paid at Duluth, MN 55806 and at additional mailing offices. POSTMASTER: Send address changes to *Spectroscopy*, P.O. Box 6196, Duluth, MN 55806-6196. PUBLICATIONS MAIL AGREEMENT NO. 40612608, Return Undeliverable Canadian Addresses to: IMEX Global Solutions, P. O. Box 25542, London, ON N6C 6B2, CANADA. Canadian GST number: R-124213133RT001. Printed in the U.S.A.

Spectroscopy

April 2019

Volume 34 Number 4



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News Spectrum

Pittsburgh Spectroscopy Award Presented to Yukihiro Ozaki at Pittcon 2019

Yukihiro Ozaki, a former professor in the Department of Chemistry at the School of Science and Technology at Kwansai Gakuin University in Japan, received the Pittsburgh Spectroscopy Award during a symposium at Pittcon 2019 in Philadelphia, Pennsylvania. He has been active in molecular spectroscopy for the last four decades.

Ozaki received his BS, MS, and PhD degrees from Osaka University. He has published more than 1000 manuscripts and publications, and his papers have been cited more than 26,000 times. His research has focused on basic studies and applications of Raman, infrared (IR), near-infrared (NIR), far-ultraviolet (FUV), and terahertz (THz) spectroscopy.

After the award presentation, Ozaki gave a talk on attenuated total reflection (ATR), titled "Frontiers of ATR-Far-Ultraviolet Spectroscopy." He described the development of an ATR-FUV spectrometer for the wavelength range from 140 to 300 nm. This spectrometer has enabled Ozaki and his team to measure the spectra of liquid and solid samples in the complete FUV region, without facing problems such as peak saturation.

Wolfgang Petrich Receives Coblenz Society Williams-Wright Award

The Coblenz Society Williams-Wright Award was presented to Wolfgang Petrich of Roche Diabetes Care GmbH and Heidelberg University, at Pittcon 2019, in Philadelphia, Pennsylvania. The award recognized Petrich for his work in the development and application of biomedical applications of infrared-based clinical laboratory instrumentation in the fields of metabolism, rheumatology, cardiology, and veterinary medicine, as well as pioneering applications of quantum cascade laser technology to biology and medicine.

Petrich, who studied physics at Heidelberg University in Germany, and at ETH Zurich in Switzerland, received his PhD from the Max Planck Institute for Nuclear Physics (Heidelberg, Germany) for research in atomic physics and quantum optics. He joined the medical diagnostics industry in 1996, and made substantial contributions to applied biomedical optics, principally infrared, Raman, and fluorescence spectroscopy.

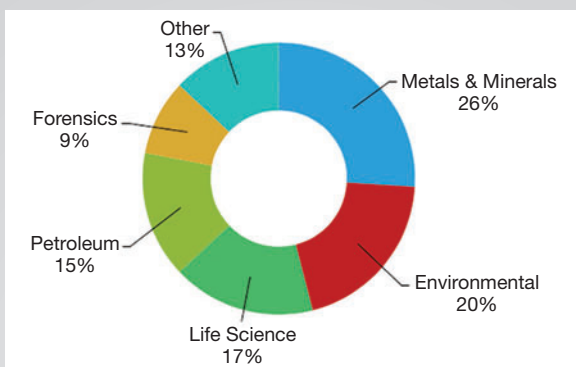
Following the presentation of the award, Petrich gave a talk on biomedical vibrational spectroscopy that provided an overview of recent advances and achievements. ■

MARKET PROFILE: LASER-INDUCED BREAKDOWN SPECTROSCOPY

Because of the manner in which samples are analyzed in laser-induced breakdown spectroscopy (LIBS), the applications for this technique seem endless. For the life sciences, some applications include noninvasive analysis of human hair and teeth, cancer tissue diagnosis, bacterial or viral identification, and detection of bio-aerosols and biohazards. For environmental purposes, LIBS can be used to analyze soils

and minerals in geology, mining and construction, and air and water quality. Additionally, it can be used to control industrial sewage and exhaust gas emissions. LIBS can also be used in industry for in situ metal melting control, control of steel quality, and 2D mapping of metal alloys.

Much development has occurred during the past decade to take LIBS technology to new heights, if not the next level. In 2012, applications for aerospace became successful when the Mars Science Laboratory mission bought ChemCam, a LIBS instrument with contributions from JPL and Ocean Optics, to the surface of Mars. In a recent development, LIBS has seen the introduction of double-pulsed laser systems to distinguish between orthogonal and perpendicular configurations. This advancement is useful in conducting analysis in liquids, as the initial laser pulse forms a cavity bubble in which



LIBS demand by end-market for 2018.

the second pulse acts on the evaporated material.

The total market for LIBS spectrometers was measured at about \$40 million in 2018. In the past few years, the market has shifted to handheld devices for metal and environmental analysis requirements, with contenders such as B&W Tek, Bruker, Hitachi, Rigaku, SciAps, and StellarNet producing only handheld analyzers. Meanwhile, seasoned LIBS companies such as TSI and Applied

Spectra exclusively offer their desktop LIBS spectrometers. Overall, demand for LIBS for metal applications accounted for over a quarter of the market share in 2018 and is expected continue being a major application for metal analysis for years to come.

Market size and growth estimates were adopted from TDA's *Industry Data*, a database of technology market profiles and benchmarks covering laboratory and process analytical instrumentation that are updated quarterly. It also includes data from the *2019 Instrument Industry Outlook* report from independent market research firm Top-Down Analytics (TDA). For more information, contact Glenn Cudiamat, general manager, at (310) 871-3768 or glenn.cudiamat@tdaresearch.com. Glenn is a market research expert who has been covering the analytical instrumentation industry for more than two decades.

Focus on Quality

Outsourcing Spectroscopic Analysis?

If a laboratory does not have the workload, spectrometer, or the resources for a spectroscopic analysis, then contract analysis is the way to go. But is outsourcing a universal panacea in the current data integrity-centric world? Are you feeling lucky?

R D McDowall

The topic of this month's column is outsourcing analytical work to a third party, typically a contract organization such as a Contract Research Organization (CRO). It can also encompass outsourcing the whole analytical workload to a bioanalytical laboratory (non-clinical and clinical development studies) or quality control laboratory (when the whole manufacturing process is outsourced to a Contract Manufacturing Organization [CMO]). Managing contract analysis was one of my roles when I had a real job working in the pharmaceutical industry, rather than as a consultant to this industry. Outsourcing work sounds great. You send off the samples, put your feet up, relax, and wait for the report to come back, but let's look at the reality. All organizations outsource analytical work, so we can't be wrong? Right? Well, let's see....

There may be several reasons for outsourcing analytical work to a CRO:

- Lack of capacity or expertise in your laboratory
- Insufficient workload to justify purchase of a specific instrument
- Company policy when a development project reaches a particular phase
- Your organization is virtual, and therefore all work is performed by third parties

But is outsourcing a universal panacea, as it is pervasive throughout the pharmaceutical industry? Let us explore what is the situation in the data integrity environment that many of us now have to work in:

- Is the CRO or CMO's scientific and regulatory knowledge adequate?

- Do you know how the laboratory works?
- Do you have the confidence in the staff?
- Do the staff have the culture, training, and ethics to ensure the integrity of your data?
- Who owns the data from the work?
- Where will the records be stored?
- Who is responsible, and who is accountable, for the work?

It all boils down to whether a pharmaceutical company should just accept a summary report, or if due diligence requires that the electronic records be reviewed by them as well. It all depends on the sponsor's approach to the management of regulatory and business risk.

Regulatory Perspective

The US Good Laboratory Regulations (GLR) (21 CFR 58) (1) and Good Manufacturing Practice (GMP) (21 CFR 211) (2) originated in the 1970s, when outsourcing was in its infancy, and therefore there is no mention of the concept in either regulation, the implication being that all work is covered, regardless of where and who performs it. In contrast, the proposed Good Laboratory Practice (GLP) update (3) has more mentions of contract analysis, and proposes a clear definition of roles and responsibilities between the sponsor and the CRO(s) involved in the study.

European GMP has updated Chapter 7, and retitled it "Outsourcing," to reflect the greater increase in extended supply chains (4), where there are two roles:

- Contract giver (sponsor)
- Contract acceptor (CRO)

It is clear from Chapter 7, and also the proposed GLP Up-

Table I: Outsourcing Clauses from the WHO Guidance on Good Data Management Practices (5)

Clause	Topic of Clause (First Sentence Only)
7.1	The increasing outsourcing of GXP work to contracted organizations (contract research organizations, suppliers and other service providers), emphasizes the need to establish and robustly maintain defined roles and responsibilities to assure complete and accurate data and records throughout these relationships
7.2	The organization that outsources work has the responsibility for the integrity of all results reported, including those furnished by any subcontracting organization or service provider. These responsibilities extend to any providers of relevant computing services
7.3	To fulfil this responsibility, in addition to having their own governance systems, outsourcing organizations should verify the adequacy of the governance systems of the contract acceptor, through an audit or other suitable means.
7.4	The personnel who evaluate and periodically assess the competence of a contracted organization or service provider should have the appropriate background, qualifications, experience and training to assess data integrity governance systems and to detect validity issues.
7.5	The expected data integrity control strategies should be included in quality agreements and in written contract and technical arrangements, as appropriate and applicable, between the contract giver and the contract acceptor.
7.6	Where data and document retention is contracted to a third party, particular attention should be paid to understanding the ownership and retrieval of data held under this arrangement.

date, that the contract giver or sponsor is responsible, and accountable, for the work performed by any third party, as we shall soon see.

Data Integrity Guidance on Outsourcing

There has been a tsunami of regulatory guidance documents issued by regulatory authorities and industry bodies since 2015. This WHO guidance document has Section 7 focused on the data integrity issues when outsourcing work (5), and Table I gives the first sentence from each clause. Please note that there is much more information than presented in the table, and readers are encouraged to read the whole section. There is also more detailed advice from the Pharmaceutical Inspection Cooperation Scheme (PIC/S) in Section 10 of PI-041 Good Practices for Data Management and Integrity in Regulated GMP/GDP Environments (6).

Let's see when the wrong CRO is chosen, either because price was a major factor, or lack of due diligence in the selection process.

Do You Feel Lucky?

There have been two major data integrity cases involving CROs: Cetero and

Semler. Imagine your company has submitted a New Drug Application (NDA) to the FDA containing bioequivalence data generated by Cetero Research. The study compares the absorption and distribution of your generic version of a drug and the ethical version. Your application has been accepted by the FDA, but later you receive a letter (7), stating:

“The pervasiveness and egregious nature of the violative practices by Cetero has led FDA to have significant concerns that the bioanalytical data generated at Cetero from April 1, 2005 to June 15, 2010, as part of studies submitted to FDA in New Drug Applications (NDA) and Supplemental New Drug Applications (sNDA) are unreliable.

FDA has reached this conclusion for three reasons:

- (1) the widespread falsification of dates and times in laboratory records for subject sample extractions,
- (2) the apparent manipulation of equilibration or “prep” run samples to meet pre-determined acceptance criteria, and
- (3) lack of documentation regarding equilibration or “prep” runs that

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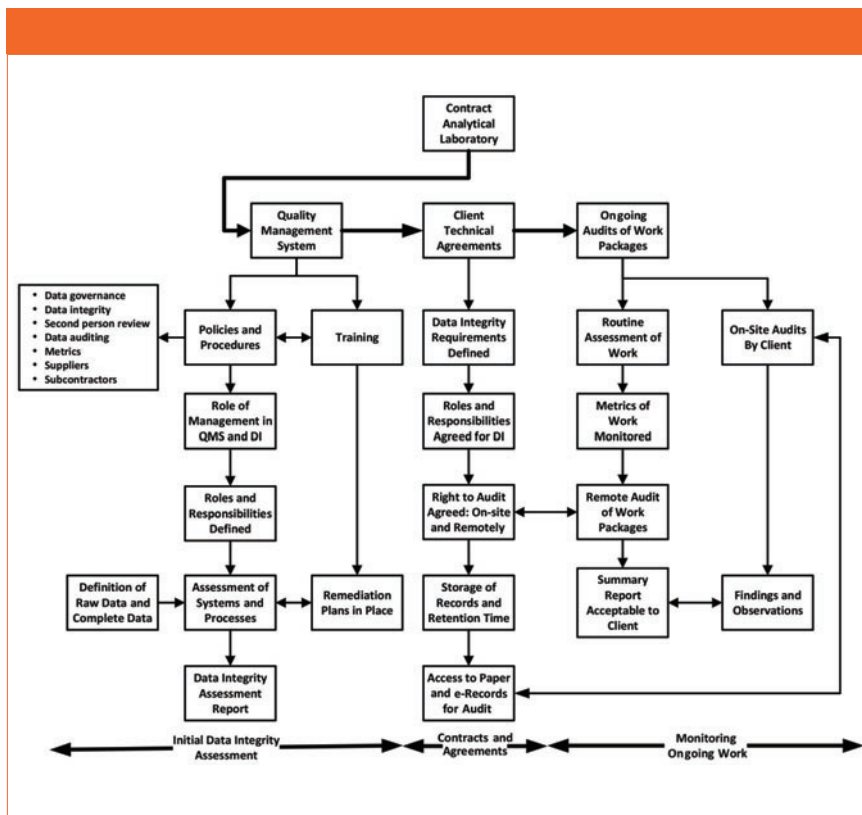


Figure 1: Overview of data integrity assessment, agreement, and monitoring work at a contract laboratory (11). Note: QMS = quality management system; DI = data integrity.

prevented Cetero and the Agency from determining the extent and impact of these violations.”

Whoa! Outsourcing is supposed to save money. What was a financial panacea has now turned into a financial disaster. The impacted studies had to be repeated in a different CRO; for more information on the falsification, see the untitled FDA letter (8).

Studies carried out at Semler Research Private Limited were also found to contain falsified bioequivalence data that resulted an untitled letter, and sponsor companies having to repeat bioanalytical studies (9,10).

The issue here is clear that the selection of a contract facility is not a mere box ticking exercise, but one where adequate due diligence must be performed. This is to ensure that a selected facility has an equivalent approach to data integrity as a sponsor. Outsourcing analytical work is not a passive “contract and forget” approach, but requires, according to that well-known data integrity expert Ronald Reagan, a “trust, but

verify” approach. Yes, we trust you, but we will verify what you are doing.

Assessment of a CRO

Supplier assessment is a key requirement of GXP regulations and regulatory expectations for outsourcing (5,12). It is important that due diligence is exercised to ensure that the work will be carried out to your specifications and analytical procedures, and that this results in an acceptable analytical report. Some typical areas for assessment are facilities, Quality Management System (QMS), scientific and regulatory knowledge, staff training, qualification and validation of instruments and software, together with record keeping practices.

Initial Selection of a Contract Facility

The primary selection criteria are the ability to deploy your validated analytical procedure (or for CRO to use their method), the spectroscopic technique to be used, and the skills required to perform the work. To be fair to CROs, the sponsor definition of validation may

vary from a minimal effort and a one page report to a full validation following ICH Q2(R1) (13). Secondary criteria may also be applicable, such as location, facilities, analysis price, recommendations from colleagues, or placement of previous work with the facility. From these criteria, a list of candidates can be reduced to one or two.

Assessment of the Laboratory

As you may be time limited on site, it is essential to prepare for the assessment. You will need to get information about the following items to read:

- Quality management system overview including any certifications, such as GXP certificate, ISO 17025 certification with scope of certification and methods within the scope
- List of policies and procedures
- Data governance covering areas such as management leadership in data integrity and review of the QMS, company ethics, progress towards an open culture, and assessment of processes and systems with current remediation approaches
- Data integrity procedures and training
- Site Master File (if a GMP facility)
- Data integrity initiatives at the CRO

Initial Assessment of Data Integrity

On-site, you need to follow an agenda to ensure you cover all areas, and to make the best use of time. Remember that audits sample, and you will not have time to go into too much detail, unless a problem is found that needs to be understood in more detail. We will now look at some of the topics we need to cover from a data integrity perspective. Ask specifically about what approaches there are for these topics, such as ensuring data integrity.

- What is the role of senior management in data integrity?
- How can an analyst admit a mistake or raise a compliance concern?
- How have computerized systems been assessed for data integrity and how are any issues being remediated (by procedural, or technical means)?
- Has assessment of paper processes been undertaken?
- How are blank forms controlled and reconciled?

- How are second person reviews conducted, and how are audit trails reviewed?
- If work is subcontracted, how are the third parties controlled, and how does the CRO ensure that their approach to data integrity is acceptable?
- What quality oversight is there to ensure data integrity (for example, audits and investigations)?

Control of Electronic Records

One important area to look at is controls for the generation, interpretation, reporting, and storage of electronic records. This will not be a static audit, but will require the auditor to walk around the laboratory, and view the systems themselves. In particular:

- What are the controls to prevent users going around the back of the application to delete records and time travel to repeat any analysis? These must be investigated and verified by seeing them in operation.
- Procedural controls are not unreliable and inconsistent. What the auditor needs to see is that the available technical controls are specified, implemented, and validated with whatever procedural controls are required, such as separation of roles in the system, electronic records protection, and audit trails being turned on.
- There should also be an on-going plan to replace older data systems with networked data systems, together with progress against the plan.

If client confidentiality may be cited as refusing to show these systems, this is a clear indicator that the CRO does not have an effective system for handling confidential information and hiding the sponsor's identity. If the CRO does not show you the technical controls for ensuring data integrity, what are the options?

Walk away.

Walking away is cheap, compared with the cost of non-compliance if there are data integrity issues that are found later. What is the cost of finding a new supplier compared with repeating a bioequivalence study, or a product recall, due to data integrity issues? Minimal.

Reporting the Assessment

At the end, there will be a written report, with the overall assessment of the facilities, infrastructure, scientific, and technical competence, and the data integrity status of the organization. Together with the report, there may be a list of findings (against regulations or procedures) and recommendations (opportunities for improvement).

Depending on the overall findings and CAPA responses to the findings by the CRO, a decision will be made to use

the laboratory or not. If you go ahead, we can go to the next stage of the process outlined in Figure 1, which is negotiating the agreement that will include how data integrity must be included.

Agreements Must Include Data Integrity

The next stage of the process is to ensure that work is agreed jointly in a technical or quality agreement or contract. Apart from the usual terms and conditions of such documents, which are out of the



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scope of this column, there is the need to ensure that data integrity is included in these documents, and that roles and responsibilities are defined adequately between the two parties.

The sponsor must:

- Provide to the laboratory robust and accurately written analytical procedures and technical information, with records and examples
- Provide troubleshooting advice, if required, when transferring, establishing or running a procedure
- For routine analytical work, the sponsor needs access to all records to verify the work was performed correctly
- The right to audit remotely and on-site (including for cause where necessary), to assess the analytical work and the integrity of any data

The CRO must:

- Define original records and raw data as electronic where a computerized system is involved, and link any paper printouts with the underlying records
- Provide accurate, complete, and reliable documentation throughout the analysis that meet attributable, legible, contemporaneous, original, and accurate (ALCOA)+ principles (5,6)
- Perform effective second person review of all work, from sampling to reportable result (as applicable to the work)
- Provide adequate QA oversight of the analysis
- Generate monitoring and trending results for communication to the sponsor
- Notify the sponsor when out of expectation (OOE), out of trend (OOT), and out of specification (OOS) results are found. The sponsor should be informed, or involved (depending on the agreement terms), in any laboratory investigations.
- Permit remote and on-site audits of the work performed, including review of electronic records

These needs and responsibilities to be included in agreements and contracts between the two parties.

Who Owns the Data?

In the past, when paper records were generated by a CRO, it was easy to determine where the records would be stored either short-term or long-term. This was

a simple matter of moving piles of cellulose from one location to another. Now there is more of a problem, as there are both paper and electronic records. Paper from the manual stages of the analytical process is simple, but far more problematic are the electronic records, as typically these need the application that generated them to open and view them.

When there are different systems at the two sites, then it would make sense to keep the records with the laboratory where they were generated for the record retention period. However, depending on the time of retention, there may be one or two upgrades of software involved. What does the agreement say about this? Probably nothing. What needs to be included in the agreement is the due diligence of the CRO to inform the sponsor that software will be upgraded, and that the results from a standard data set come to the same decision before and after the upgrade (4). Software validation and data migration are essential components in ensuring that the data are still readable, and maintain the same content and meaning.

In all cases, storage of the records must be part of any agreement or contract, and include what happens in case of inspection at the contract giver's site to determine how access to the electronic records will be achieved.

On-Going Monitoring of Work and Audits

It is important to monitor the work being carried out at a CRO, including data integrity aspects to assure themselves that the agreement is being followed. If this is not undertaken, it may be because of a sponsor's attitude that they have confidence in the CRO. However, realize that you cannot outsource analysis and forget about it; the work must be monitored. This takes time and effort within the sponsor's organization, but is an essential corollary of outsourcing. Alternatively, consider the alternative. Would you prefer a Cetero or Semler scenario?

Monitoring can be one of three types:

- Remote monitoring of results and associated data
- Remote audit

- On-site audit including for cause audits

Monitoring the Results

The type of monitoring undertaken by the sponsor will depend on the nature of analytical work undertaken by the contract laboratory. For bioanalytical work, it may be checks of the standard curves and back calculated concentration values, monitoring the results from the three concentrations of quality control samples, viewing blank injections, plasma concentration versus time profiles, and incurred sample reanalysis results. In contrast, quality control monitoring may be looking at the chromatograms and individual values from aliquots, as well as the reportable results and trending these over time and batch, and impurity profiles between batches, among other factors.

Actively monitoring outsourced analytical work means that trends or issues could be identified, discussed with the contract laboratory, and rectified before they become major problems. Equally so, the contract facility should also be monitoring and discussing with the sponsor the same issues if they are monitoring the work correctly.

Remote Assessment of Work Packages

Following on from the monitoring of results is the remote assessment of work packages, such as the review of the whole of the analytical batch or study records. This can be a combination of video conferencing and direct access via a secure internet link between the CRO and sponsor. Electronic records, including the metadata and audit trails, can be reviewed remotely. Access can be via a link where a member of the contract laboratory staff operates the data system following the requests of the sponsor's representative. Paper records can be reviewed remotely via video link, or scanned, with the scans then verified as true copies by electronic signature, and viewed at the sponsor site. However, it is the approach to sponsor's due diligence that is the basis for ensuring work is carried out correctly. Any findings identified remotely can be documented, and CAPAs generated by the contract laboratory.

The main issue is that the sponsor needs confirmation that the data integrity of the analytical results is acceptable, and that they can rely on the results to take decisions. There would typically be more audits early in a contractual relationship between a sponsor and CRO, with the number and frequency reduced over time as the two organizations become confident with working together.

On-Site Audits

On site audits come in two forms: *regular scheduled audits* or *for cause* audits. The latter is the audit of a specific batch or batches due to a problem, such as an increase in serious adverse events associated with a product batch. In either case, the approach is usually the same:

- Access to the electronic records and paper records for all systems to check the work has been done correctly, and data integrity is assured
- Ensuring procedures have been followed and there have been no attempts at short cuts, copying data from one batch to another, or other poor data

management practices. An area of focus is where procedural controls have been used to remediate data vulnerabilities in processes or computerized systems.

- Audit of one or more work packages
- Checking the effectiveness of previous CAPAs. This may not be applicable to a for cause audit.
- Look for work that is too perfect, not enough exceptions, or containing documentation errors
- Look at the time that data was collected or processed. Late at night or on the weekends can lead to increased opportunity to manipulate data in negative ways.

Out of these audits may come findings and recommendations for the contract laboratory to act upon and generate CAPAs.

Trusted Partner or Scum of the Earth?

From my experience, the attitude of people who use CROs fall into one of two camps. Either contractors are a necessary evil and are the scum of the earth, or those that take a more reasonable

approach that a CRO is an extension of their own laboratory and are a trusted partner. Personally, I have always taken the latter approach, as the work they are doing is on my behalf. Why would I treat the laboratory and their staff any different to my own analysts? In my experience, you will always get more out of people if they are treated right, rather than have a poor sponsor attitude and reputation that precedes you before you walk through the door.

Aspects of being a trusted partner are honesty and openness. A CRO should be able to discuss data integrity issues with prospective and current sponsors. Discussion of poor data management practices found, and how they are working to resolve them, is a major plus from the author's perspective. Finding out that a CRO has hidden data integrity violations is a case for running away.

Summary

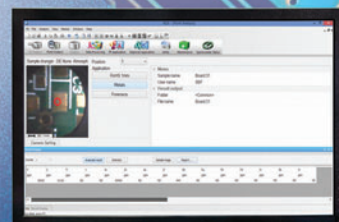
You may be thinking that contracting analytical work is a major problem. Far from it; if handled correctly, the process should be considered as an exten-

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sion of your own laboratory, and a collaborative process. If handled wrongly, it can be a millstone hanging around an organization's neck for a decade or so. Approach using contract facilities with your eyes open with a "trust, but verify" attitude, especially when it comes to data integrity.

Acknowledgment

I would like to thank Kevin Roberson for his helpful review comments in preparation of this column.

References

- (1) *21 CFR 58 Good Laboratory Practice for Non-Clinical Laboratory Studies*. (Food and Drug Administration: Washington, DC, 1978).
- (2) *21 CFR 211 Current Good Manufacturing Practice for Finished Pharmaceutical Products*. (Food and Drug Administration: Silver Spring, MD, 2008).
- (3) *21 CFR Parts 16 and 58 Good Laboratory Practice for Nonclinical laboratory Studies; Proposed Rule*. Federal Register **81**(164), pp. 58342-58380 (2016).
- (4) *Compliance Policy Guide CPG Sec. 130.300*

- (5) *WHO Technical Report Series No.996 Annex 5 Guidance on Good Data and Records Management Practices*. (World Health Organization: Geneva, Switzerland, 2016).
- (6) *PIC/S PI-041-3 Good Practices for Data Management and Integrity in Regulated GMP / GDP Environments Draft*. (Pharmaceutical Inspection Cooperation Scheme, Geneva, Switzerland, 2018).
- (7) *FDA Letter to ANDA Sponsors Conducting Bioequivalence Studies at Cetero Research*. 2011; Available from: <http://www.fda.gov/downloads/drugs/drugsafety/ucm267907.pdf>.
- (8) *Cetero Research Untitled Letter* (11-HFD-45-07-02). (Food and Drug Administration: Silver Springs, MD, 2011).
- (9) *Notification to Pharmaceutical Companies: Clinical and Bioanalytical Studies Conducted by Semler Research Are Unacceptable*. 2016; Available from: <https://www.fda.gov/Drugs/DrugSafety/ucm495778.htm>.
- (10) *FDA Untitled Letter: Semler Research Center Private Limited*. (Food and Drug Administration: Silver Spring, MD, 2016).
- (11) R.D. McDowall, *Data Integrity and Data Governance: Practical Implementation in Regulated Laboratories*. (Royal Society of Chemistry, Cambridge, United Kingdom, 2019).
- (12) *EudraLex - Volume 4 Good Manufacturing Practice (GMP) Guidelines, Chapter 7 Outsourced Activities*. (European Commission: Brussels, Belgium, 2013).
- (13) *ICH Q2(R1) Validation of Analytical Procedures: Text and Methodology*. (International Conference on Harmonization: Geneva, Switzerland, 2005).



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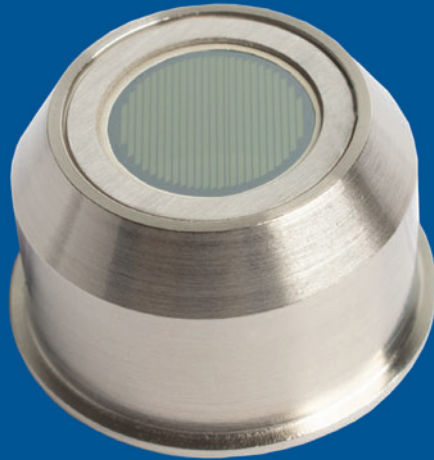


R.D. McDowall is the director of R.D. McDowall Limited and the editor of the "Questions of Quality" column for *LCGC Europe*, *Spectroscopy's* sister magazine. Direct correspondence to:

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Lasers and Optics Interface

Expert Perspectives on Laser-Induced Breakdown Spectroscopy (LIBS)

Laser-Induced Breakdown Spectroscopy (LIBS) has transitioned from a method found only in research laboratories, to a technique in wide use in commercial settings. Handheld LIBS systems are used to make measurements of metal scrap and other items in the field, while online LIBS systems are widely used in industrial high-speed sorting. This month's column polls several leading LIBS experts to give readers a sense of some of the history of, and most exciting upcoming research problems in, LIBS.

Steve Buckley

Over the past several years, I've had the pleasure of writing several columns on laser-induced breakdown spectroscopy (LIBS) for *Spectroscopy*. During that time, LIBS has matured immensely. It has transitioned from an experimental and lab-based method, to a method that is increasingly found in commercial and industrial use. Methods of quantification have improved, and the suite of lasers and spectrometers that are employed in LIBS systems are increasingly fit for purpose. With these changes in mind, it seems appropriate to cast a wide net and poll a panel of LIBS experts with a variety of backgrounds about their thoughts on recent developments in LIBS, and where this exciting field of spectroscopy is headed.

Our expert panel includes: Dr. Matthieu Baudelet, assistant professor in the Department of Chemistry in the National Center for Forensic Science at the University of Central Florida (Winter Park, FL), Dr. Amy Bauer, associate editor for *Applied Spectroscopy*, and principal scientist in the Chemlogix Division at TSI, Inc. (Shoreview, MN), Dr. David Hahn, professor and chairperson of the Department of Mechanical and Aerospace

Engineering at the University of Florida (Gainesville, FL), and Dr. Steven Rehse, associate professor in the Department of Physics, University of Windsor (Ontario, Canada). We thank each of them for their time and thoughtfulness in responding to these questions.

LIBS is now a mature technique. Compared with other analytical methods, what do you think its relative strengths are, and how does this translate into applications?

Baudelet: LIBS is the best technique when the need includes the combination of speed and sensitivity. This makes it the most attractive elemental technique for chemical mapping at the moment. Its latest demonstrations of 10 ppm at kHz, or even at 100 Hz, rate make it very interesting for biomedical studies, where a fast multi-elemental image can help in diagnosis. LIBS can also overcome spectral interferences much more easily than mass spectrometry, which can make it the "go-to" technique for complex samples (when provided the right tools for data analysis).

Hahn: The long-standing strength of LIBS remains the lack of a need for rigorous sample preparation, such as acid digestion. LIBS allows for direct analysis of solids, liquids, and gaseous or aerosol samples, which is unmatched by other analytical schemes. Calibration does require care, as matrix effects are present with LIBS as with many other analytical schemes.

Bauer: Herbert Laitinen, in his original *Analytical Chemistry* editorial, "The Seven Ages of an Analytical Method," declared a method "mature" when it moved into the hands of the non-specialist. In the case of LIBS, this would seem, at first blush, to apply to the increasingly common use of handheld LIBS instruments to perform alloy identification (ID) in the metals recycling industry, and the use of handheld instruments to perform analyses on geological samples in situ. Additionally, LIBS is beginning to be applied to process control applications in various industries, but has not yet become common and is not yet seen as a "gold standard" analytical technique in any industry.

A thing that distinguishes LIBS from other analytical methods is the fact that it can perform noncontact measurements. However, this is not always as much of an advantage as it seems. LIBS is touted traditionally as requiring very little sample preparation, compared to atomic absorption (AA), or inductively coupled plasma (ICP), for example, but it is becoming more visible that care in sample preparation results in less uncertainty and greater measurement precision. There is an interesting balance that is present in LIBS analyses, one that pits easy and noncontact analysis against the need for good correlation to standard reference results and reduction of measurement-to-measurement variance.

Rehse: I believe it is the generality of the technique, and the ease with which it can be applied in so many different situations, that is its primary strength. What I mean by this is that it is not a "niche" technique; it can be applied in so very many different applications. And I mean this, not in the abstract, but in the specific. While my own specialty and area of concentration has been on

bacterial analysis, over the years I have been involved in one way or another with experiments involved in such disparate applications as monitoring lead contamination in dirt from the sides of highways, analysis of ores for mineral prospecting, analysis of vegetables skins for signs of health or disease, analysis of gases from steel blast furnaces, analysis of fish bones to deduce invasiveness patterns, and several other applications. LIBS can and has made fruitful and significant contributions in many fields like this. There are such a wide range of applications, the significance is compounded by being "spread around" all over the analytical world.

Do you have a favorite "Aha" moment of your own, from your lab or data analysis that made you more excited about LIBS? Share a result that you are particularly proud of, if you don't mind.

Bauer: I was pretty excited when I first fiber-coupled a yttrium aluminum garnet (YAG) laser, in 1995 or so, and used

the resulting plasma to decide whether there was lead in paint or not. In those early days, I was mostly involved with spark induced breakdown spectroscopy (SIBS), the electrical analog of LIBS, and my group was involved in attempts to use it to monitor bioaerosols. At some point, we started to image the plasma with a filter and an intensified charged coupled device (iCCD), and discovered that the actual plasma was much smaller than we had been predicting based on visible evidence, like a tiny lightning bolt. It was at that point that I began to understand that these plasmas were much more complicated than I previously gave them credit for. As people continue to try to understand the physics of plasma formation and evolution, each paper demonstrates this fact again and again. These physics remain my favorite part of the whole LIBS equation.

Rehse: I do. It is when I first got back "into" LIBS after a hiatus. I was lucky enough to be working at Los Alamos National Laboratory as a student in the

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early 1990s, when David Cremers was performing some of the seminal experiments on LIBS in a laboratory across the hall from mine. I always thought it was a fascinating technique, but my studies led me elsewhere. Fast forward to 2004, when I was performing a postdoctoral fellowship at the University of Western Ontario, and my lab was approached by a local company who wanted to talk with someone who knew LIBS to consult with them on its suitability for analyzing used engine oil for the presence of wear metals as an indicator of engine performance. Having not thought about LIBS for almost 10 years, I did a survey of the current status of the field and realized, "Hey, these people have got something here." I decided then to jump into the field on my own.

Hahn: My "aha" moment came more than 20 years ago, when I was using LIBS for emissions analysis, focusing on toxic metals. Recognizing that our targeted metals were condensed as solids, I did some simple math to model the sampling rates, and realized that only a small percentage of LIBS events actually sampled a target particle. Our signal to noise was greatly diminished by averaging many "zeros", but a careful analysis of each LIBS spectrum could greatly change the outcome. The ultimate result was an approach that is now referred to as conditional analysis.

Baudelet: Lately I've been very excited by our approach for quantifying spectral interferences in LIBS, which was recently published (1). This is an important brick in the edifice of analytical LIBS towards its recognition by the forensic practitioners, who need to be able to evaluate error rates of their measurements. Much more needs to be done to make it a staple of LIBS by including molecular emission, for example.

What do you think that the most important technical implementation or advance has been in LIBS over the past 3-5 years? How will this influence LIBS practice in the future?

Rehse: I really think the advances in high-resolution 2D mapping, as dem-

onstrated in the papers of L. Sancey, V. Motto-Ros, and associates (2), and K. Rifai, F. Doucet, and associates (3), are incredible advances. The ability to rapidly create an elemental map on quite large two-dimensional surfaces with high resolution is a significant and important technical implementation of an idea that we all knew could be done, but no one had actually turned into a practical device. When that data is then interpreted with chemometric algorithms for autonomous classification of the surfaces, this gives true utility and power to the technique.

Baudelet: Laser ablation molecular emission spectroscopy (LAMIS) and the revival of using molecular emission directly in plasma measurements started in 2010, and is still triggering enthusiasm within the LIBS community to create an optical emission technique for isotopic analysis and even halogens analysis. However, my feeling is that nanoparticle-enhanced LIBS (NELIBS) has been the most important new addition to the LIBS toolkit for better analytical performance in the last five years. It has enhanced many applications from the analysis of transparent materials to even explosive detection and protein analysis. We can see the analogy with Raman and surface-enhanced Raman spectroscopy (SERS), and should learn from that community how we can propose the best NELIBS tool for each application.

Hahn: I will offer two advances. The continuous improvement in data processing (such as chemometrics, machine learning, and artificial intelligence (AI)) schemes will continue to open up application spaces for sorting, in situ analysis and other applications. This, I believe, remains a significant advance that the LIBS community must continue to utilize. A second advancement is lower cost, high repetition rate laser systems. As noted above, sample inhomogeneity remains a challenge, notably when working in field applications. Using many thousands of laser shots can potentially help address such issues.

Bauer: The development of a whole new host of laser sources, including

miniaturized millijoule and microjoule devices, have opened up an amazing new world of in situ measurements with LIBS. The most impressive technical developments, though, opened up the possibility for the development of ChemCam, the LIBS device aboard the Mars Science Laboratory rover. This device permits geological analysis and identification of hydrated minerals at a distance of 7 meters on Mars. Every time I think about the dramatic accomplishments of the ChemCam, I feel thrilled and optimistic at the same time.

What paper or papers have influenced your thinking the most in terms of your basic scientific understanding? Why have they been influential to you personally?

Bauer: Like I said earlier, I'm really very interested in understanding the basic phenomenology of sample ablation and plasma formation. My favorite papers currently are the ones that dare to try to explain the production of particles and splashing during nanosecond laser or surface encounters.

Baudelet: Many scientific contributions have influenced my basic scientific understanding of LIBS and its use for analytical chemistry. Three books in particular:

- "Flame Spectroscopy" by Radu Mavrodineanu and Henri Boiteux, for the best course on elemental and molecular emission spectroscopy;
- "Theory of Spectrochemical Excitation" by Paul Boumans, for the very fundamental understanding of matter excitation; and
- "Laser Processing and Chemistry" by Dieter Bauerle, for the complete and detailed overview of laser-matter interaction and plasma formation.

These have been in my desk for research and teaching since the beginning.

Hahn: I am a big fan of the early LIBS papers by David Cremers and Leon Radziemski. They addressed many of the fundamental issues while presenting very nice experimental data and analysis. I wore those early papers out with notations and constant reading.

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Rehse: In my particular area of investigation, I am always drawn back to the work of Dr. Jennifer Gottfried that she described in her papers (4,5). I think they were important for a reason. Firstly, they showed just how far one can push the LIBS technique to discriminate between highly similar targets. In many of these cases (such as explosives), the targets are elementally identical, only their stoichiometry is different! These papers showed that, with careful construction of a classification library and suitable algorithms, the targets can be reliably discriminated. Secondly, no one before or since has so exhaustively studied the effect that the mounting surface (material upon which a trace target is scattered) and similar interferent materials can have on degrading classification accuracy. They tested dozens of combinations of similar materials, scattered on a wide variety of surfaces, and clearly and accurately reported the results in a way that allowed some sensible conclusions to be drawn. I think they showed that LIBS has an incredible ability to differentiate similar targets, but that a lot of work has to first go into understanding the ways in which a spectrum can become “contaminated,” or ways in which an algorithm may be “fooled.” I find myself looking at these papers a lot.

What areas of LIBS research would you advise new students or new practitioners to work on? What is your favorite “frontier” problem in LIBS?

Hahn: As an engineer that has enjoyed fruitful collaborations with analytical chemists and physicists for nearly two decades, I encourage students to relate their underlying fundamental training and knowledge to the LIBS arena. So as a mechanical engineer, that was heat and mass transport, allowing me to explore these underlying processes within the laser-induced plasmas, and ultimately to help translate new understanding to improved analytical approaches. My favorite frontier problems remains the local effects of analyte mass (such as small particulates) within a larger LIBS plasma as related to quantitative analyte response.

Rehse: I am still a believer that LIBS-based in vivo surgery is a possibility. I would like to see surgeons or dentists ablating away tumors or caries tissue, for example, while the ablation events are analyzed and categorized spectroscopically shot-by-shot to convey real-time information about the nature of the tissue being removed. This information could be relayed to the surgeon by means of a wearable head’s up display. Rather than relying solely on the surgeon’s experience and intuition, I would like to see true diagnostic information being made available during the procedure to guide the surgeon’s actions and decisions. I think this type of information could ultimately improve patient outcomes, while reducing pain and suffering by minimizing the removal of healthy tissue from surgical margins and ensuring the removal of all the tissue that needs to be removed.

Baudelet: LIBS, as a laser-ablation based technique, still relies on matrix-matched standards, especially for biological materials. Compacted powders are often not the answer for good quantitative practice. Finding better calibration standards and methods is a field of research that my group is focusing on so LIBS can be an even more robust analytical technique. This requires the full cooperation of material scientists, analytical chemists, specialists of laser-matter interaction and the support of certification institutions.

Bauer: I would love to be guiding a student at this exciting time. I would have them focus on ablation processes and plasma evolution processes involving new laser sources. From a more practical standpoint, the community is realizing that sample processing before analysis is very helpful to yield better results. LIBS folks are starting to remember traditional analytical tools, like internal standards and standard additions, and pairing them with very sophisticated data analysis tools, like PCA and support vector machines (SVM). The intentional pairing of careful sample handling and crafty data analysis will result in more robust and accurate LIBS analysis.

Summary

This is an exciting point in the scientific understanding and practical application of LIBS. We hope that this combination of scientific, retrospective, and forward-looking thoughts from our panel of experts increases your interest, and your excitement, about the possibilities for LIBS technology. Thanks to all of our panelists for their energy expended and wisdom imparted in contributing to this column! Please send us your questions and requests for future column topics at the address below.

References

- (1) J. Chappell, M. Martinez, and M. Baudelet, *Spectrochimica Acta B* **149**, 167–175 (2018).
- (2) L. Sancey, V. Motto-Ros, B. Busser, S. Kotb, J.M. Benoit, A. Piednoir, F. Lux, O. Tillement, G. Panczer, and J. Yu, *Scientific Reports* **4**, 1–7 (2014).
- (3) K. Rifai, F. Doucet, L. Özcan, and F. Vidal, *Spectrochimica Acta B* **150**, 43–48 (2018).
- (4) J.L. Gottfried, *Anal. Bioanal. Chem* **400**(10), 3289–3301 (2011).
- (5) J.L. Gottfried, F.C. De Lucia, Jr., C.A. Munson, and A.W. Miziolek, *Applied Spectroscopy* **62**(4), 353–363 (2008).



Steven G. Buckley, PhD, is the Vice President

of Product Development and Engineering at Ocean Optics, an affiliate associate professor at the University of Washington, and has started

and advised numerous companies in spectroscopy and in applications of machine learning. He has approximately 40 peer-reviewed publications and 6 patents. His work in practical optical spectroscopy, such as LIBS, Raman, and TDL spectroscopy, dovetails with the coverage in this column, which reviews methods (new and old) in laser-based spectroscopy and optical sensing. Direct correspondence to: SpectroscopyEdit@mmhgroup.com.

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Vibrational Spectroscopy of Individual Aerosol Droplets by Optical Tweezers

Aerosols are key components in a wide range of environmental systems and industrial contexts, spanning air pollution and clouds, to engineered nanomaterials, to drug delivery to the lungs. However, the basic properties of aerosols are often elusive, due to their small sizes and capacity to rapidly respond to changing ambient conditions. In this article, we discuss Raman spectroscopy of individual picoliter volume droplets using optical tweezers. Single droplet spectroscopy enables highly accurate and precise measurements of droplet size and refractive index, as well as measurement of droplet chemical composition. Time-resolved measurements permit investigation of fundamental droplet properties, including hygroscopic response, vapor pressure, and surface and bulk characteristics. Such information improves our molecular level understanding of chemical processes within single droplets, and facilitates broader understanding of complex systems.

Bryan R. Bzdek and Jim S. Walker

Aerosols, which are solid particles or liquid droplets suspended in air, are ubiquitous. Aerosols negatively affect human health through air pollution (1), but also improve health by serving as delivery agents for pharmaceuticals (2). With respect to the atmosphere, aerosols are generally thought to cool climate by scattering solar radiation and by serving as the seeds for cloud droplets (3). Aerosols also have a wide range of industrial applications, including spray drying, inkjet printing, personal care products, combustion, and agricultural chemicals. To resolve aerosol impacts on health and climate, or to engineer effective industrial products, knowledge of parameters like particle size, composition, interaction with light, and surface and bulk properties is required. Moreover, it is beneficial to resolve these properties on a single particle basis, as even a nominally homogeneous and monodisperse particle ensemble has inherent distributions in key properties like particle size.

Aerosols are intrinsically interesting, because they exhibit unique properties relative to bulk systems (4). Figure 1 highlights some of these properties, with a hygroscopic growth curve for a sodium chloride (NaCl) particle, plotting a change in droplet mass (relative to a dry particle) against ambient relative humidity (RH). At low RH, a NaCl aerosol contains no water, and is

therefore a solid particle. With increasing RH, water adsorbs to the solid particle surface until reaching the deliquescence RH (~75% for NaCl), which is the RH at which the solid particle absorbs enough water to undergo spontaneously a phase transition to a liquid droplet, and is equivalent to the water activity at the solubility limit of a bulk solution of NaCl. Beyond this point, the liquid droplet size increases with increasing RH. Starting from a liquid droplet at high RH, lowering RH decreases droplet size due to water evaporation. However, rather than undergo a phase change at the deliquescence RH, the droplet will continue to shrink until reaching the efflorescence RH (~43% RH for NaCl), which is the RH where the liquid droplet spontaneously undergoes a phase change to form a solid particle, and highlights a hysteresis in phase that depends on the direction of the RH change. This observation is remarkable; liquid droplets between the deliquescence and efflorescence RH can exist in metastable, supersaturated solute states. In the case of NaCl, the aqueous concentration at the deliquescence RH (that is, the bulk solubility limit) is 6.2 molal, whereas, at the efflorescence RH, it is 13.2 molal. For a soluble organic, the solute concentration can range from ~5 molal at the deliquescence RH to >30 molal at dry conditions. Such compositions can exhibit highly non-ideal behav-

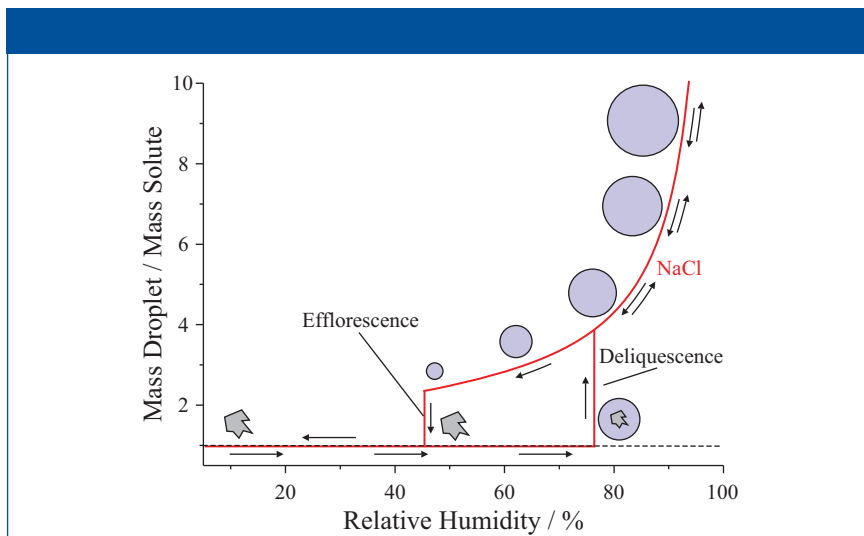


Figure 1: Illustration of a hygroscopic growth curve for a sodium chloride (NaCl) particle. Note the hysteresis in particle phase between 43% and 75% relative humidity (RH), where, depending on the RH pathway, NaCl is either a solid particle or a liquid droplet.

ior, and may inhibit nucleation of a solid particle, instead forming an amorphous glass. The high surface-to-volume ratio of aerosols also means that particles can respond rapidly (<1 ms) to changes in the gas phase, potentially resulting in different product distributions after a reaction due to size-dependent competition among gas-diffusion, surface accommodation, and particle bulk transport.

To measure individual droplet properties by spectroscopic approaches, the droplet location must first be controllable. Optical tweezers are one method to capture and manipulate 3–10 μm radius droplets over long time periods. The approach, recently recognized by the 2018 Nobel Prize awarded to Arthur Ashkin, employs a tightly focussed laser beam to create a gradient force optical trap that immobilizes a particle by exploiting the refractive index (RI) difference between the particle and surrounding medium. Optical tweezers are perhaps better known for applications to condensed systems, but in fact some of the early optical trapping work captured liquid droplets in air (5,6). Figure 2 illustrates a typical aerosol optical tweezers (AOT) setup, similar to that sold commercially by Biral, configured to allow production of multiple, steerable optical traps for the study of droplet coalescence (7,8). A spatial light modulator dynamically shapes the phase front of a continuous

wave 532 nm laser to form multiple, steerable optical traps. When the trap separation is sufficiently small, droplet coalescence is induced. Brightfield imaging is accomplished by a blue LED. Backscattered Raman light is imaged onto the entrance slit of a spectrograph to perform single droplet spectroscopy. Elastic backscattered light, the intensity of which varies during droplet coalescence, is directed to a photodiode, and recorded with an oscilloscope. Droplets are typically nebulized from solution, and captured in an isolated RH-controlled trapping chamber. This approach enables measurement of a range of physical and chemical properties of an optically trapped droplet.

In this article, we describe the utility of the AOT approach to characterize spectroscopically individual picoliter-volume droplets. We first discuss the principles of cavity-enhanced Raman spectroscopy, and then highlight the precise and time-resolved measurements of chemical and physical properties enabled by these measurements.

Raman Spectroscopy of Individual Droplets

Cavity enhanced Raman spectroscopy is the primary tool for analyzing optically tweezed droplets, because it allows high precision measurements of size and RI, as well as information



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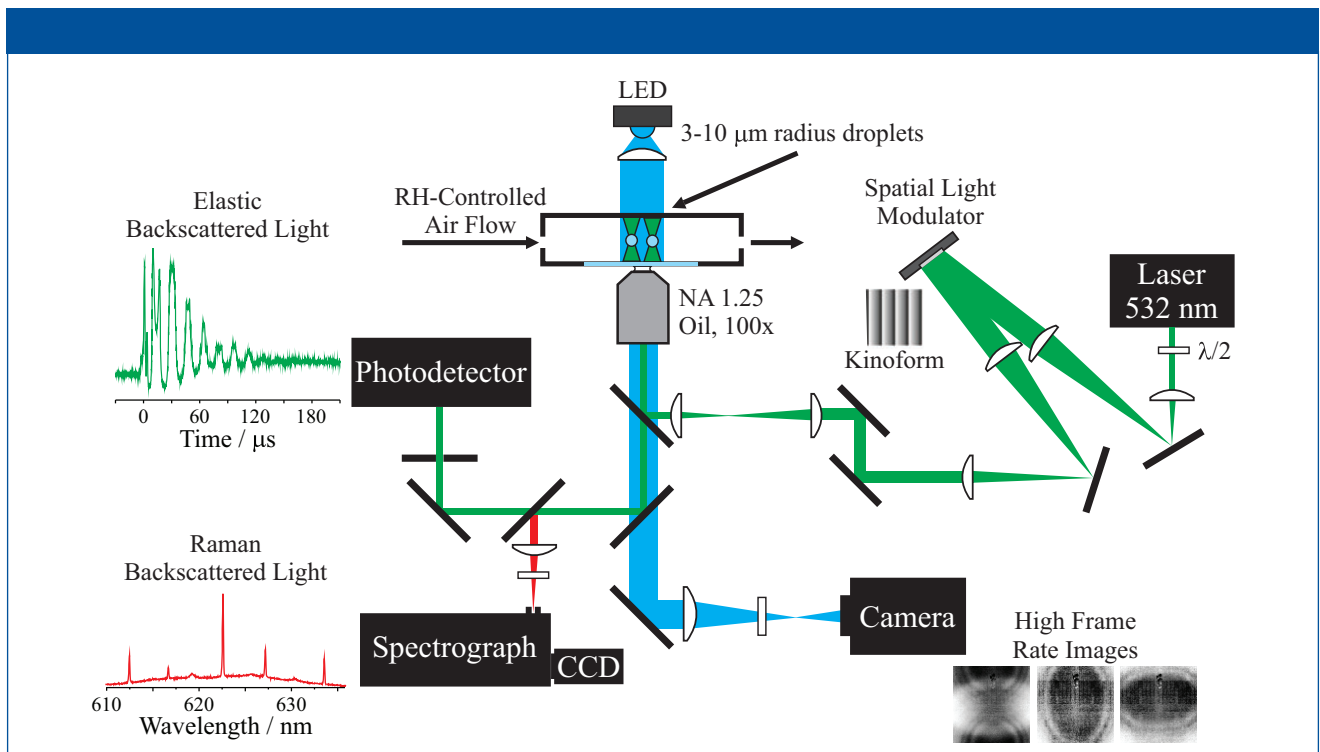


Figure 2: Schematic of a holographic aerosol optical tweezers (AOT) setup capable of collecting Raman spectra on individual droplets as well as elastic backscattered light to study droplet coalescence (7,8).

about droplet chemical composition. The AOT setup permits efficient acquisition of Raman scattered light because the trapping laser beam and microscope objective are also used as a Raman excitation source and high efficiency collection optic, respectively, allowing a time resolution <1 s.

Figure 3(a) shows a Raman spectrum from an optically tweezed aqueous sucrose droplet. The Raman signal consists of two components: the spontaneous signal and the stimulated signal (9–14). The spontaneous signal is the broad, underlying Stokes band that provides information about the droplet's chemical composition. The stimulated signal presents as the superimposed structure, and arises because the spherical droplet behaves as a low loss optical cavity at wavelengths commensurate with whispering gallery modes (WGMs). When spontaneous Raman emission overlaps with a WGM, a standing wave forms around the circumference of the droplet, and stimulates further emission at the same frequency, resulting in sharp peaks at discrete wavelengths. Each stimulated peak is described by a mode order n

(defined by the number of standing waves around the droplet circumference) and mode number l (defined by the number of radial maxima in the distribution of the mode intensity). For each mode number and mode order, there exists a transverse electric (TE) mode (having no radial electric field component) and a transverse magnetic (TM) mode (having no radial magnetic field).

WGM wavelengths are highly sensitive to droplet size and composition. Figure 3(b) illustrates the magnitude of the shifts in WGM wavelengths as an aqueous sucrose droplet changes radius in response to a change in ambient RH. WGM wavelengths can be calculated using Mie theory (15), if the size and RI are known. The wavelengths at which stimulated Raman signals are observed experimentally and compared to a library of Mie theory simulations calculated for various size and RI combinations, with the best match giving the correct size and RI. The comparison can be performed in <1 s using custom built software, allowing the physical properties of the

droplet to be tracked in real time, with accuracy of ± 2 nm in radius and 0.0005 in RI (16). The next sections illustrate how such accurate and precise measurements permit elucidation of a wide range of fundamental droplet properties.

Hygroscopicity and Vapor Pressure

Figure 4 shows a change in the properties of a glycerol droplet as RH is systematically decreased. These data were collected using the commercially available instrument from Biral. Small changes in WGM wavelengths can be related to precise changes in droplet radius as in Figure 4(a), and RI as in Figure 4(b). As RH is stepped from 80% to 30% in 10% intervals, the droplet responds by losing water, resulting in a step change in droplet size. This loss of water also increases the glycerol concentration, leading to a corresponding increase in droplet RI, as the RI of glycerol is larger than that of water. Note the high precision in the measurement, indicating very little second-to-second variation in droplet parameters. The observed changes in droplet size and RI describe the hy-

grosopic response of the droplet. Hygroscopicity impacts the number and size distribution of atmospheric cloud droplets, as well as the optical properties of atmospheric aerosol. Because particle deposition in the respiratory tract is size-dependent, hygroscopicity also influences deposition and ultimately health effects.

Figure 5 shows how hygroscopic response can be used to identify changes in particle composition (17). Figures 5(a) and 5(b) show droplet radius and RI (retrieved from the stimulated Raman signal), whereas Figure 5(c) shows the ratio of the intensities of the C-H to O-H stretching regions in the spontaneous Raman signal ($I_{\nu_{C-H}}/I_{\nu_{O-H}}$). Initially, a NaCl droplet is captured and equilibrated to a constant gas flow at 80% RH. During 1000–1400 s, a flow of aqueous NaCl aerosol is introduced into the trapping chamber without altering the ambient RH. The introduced aqueous droplets coalesce with the trapped droplet, increasing the trapped droplet's radius, but maintaining the same RI, as the droplet chemical composition remains unchanged. Then, during 3000–3400 s, a flow of aqueous sucrose aerosol is introduced into the trapping chamber, again maintaining an RH around 80%. In this case, droplet size changes due to the accretion of sucrose aerosol. The accretion of sucrose also increases the droplet RI, and the resulting change in droplet composition is reflected in the observed increase in the $I_{\nu_{C-H}}/I_{\nu_{O-H}}$ ratio.

The changes in radius and RI can be visualized in a plot of radial growth factor (GF_d) against droplet RI or RH. Any droplet will have a well-defined relationship between real RI and RH, owing to the relationship between RI and solute concentration, provided the droplet chemical composition does not change beyond water uptake or loss in response to changes in ambient RH. This relationship leads to a defined trajectory between droplet RI and droplet wet radius (r_{wet}). However, dry particle radius, r_{dry} , will vary from measurement to measurement. A plot of radial growth factor [$GF_d(RH)$] accounts for variations in particle dry

size by defining the change in r_{wet} relative to r_{dry} :

$$GF_d(RH) = \frac{r_{wet}}{r_{dry}} \quad [1]$$

Figure 5(d) shows a plot of GF_d against RI for three NaCl droplets (black, blue, and red symbols) studied across a wide range in RH (96% to 49% RH, corresponding to refractive indices of 1.345 to 1.410, respectively). All these data collapse onto each other (visually represented by

the line), illustrating the reproducibility in hygroscopic growth afforded by the AOT approach across multiple measurements. The green symbols show the hygroscopic growth of the NaCl droplet doped with sucrose aerosol ($t > \sim 3400$ s in Figures 5(a)–5(c)). The offset is due to the change in the relationship between droplet composition and equilibrium size, as RH is varied and highlights how compositional changes that result in only a few percent change in droplet radius can be resolved due to the high

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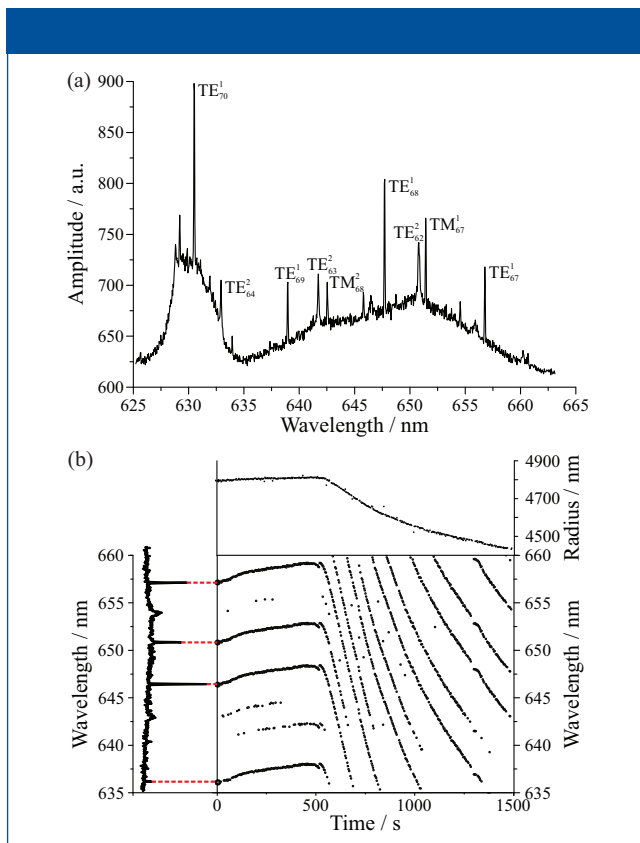


Figure 3: (a) A cavity-enhanced Raman spectrum of an optically tweezed aqueous sucrose droplet. The spontaneous Raman signal centred around 650 nm is the O-H stretching vibration of water, whereas the signal around 630 nm is the C-H stretching vibration. The stimulated Raman signal is the superimposed structure on the spontaneous band; (b) whispering gallery mode (WGM) position is closely related to droplet size.

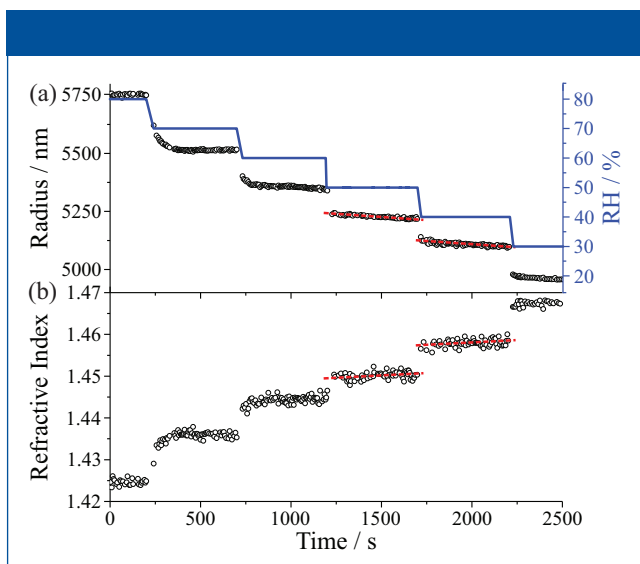


Figure 4: Response of a glycerol droplet to changes in ambient relative humidity (RH). Changes in whispering gallery mode (WGM) position are used to quantify changes in (a) droplet radius, and (b) refractive index (RI). The red dotted lines indicate the loss of semivolatile glycerol during periods of constant RH.

accuracy of droplet size and RI obtained from the Raman signal.

Although droplet size may change substantially, due to changes in the RH around the droplet, size may also change due to evaporation of semivolatile molecules from the droplet (18). This evaporation is evident in Figure 4, as in between the step changes in droplet size due to RH steps, the glycerol droplet size decreases linearly and its RI increases linearly (dotted lines) owing to its vapor pressure ($p_G^0 \approx 10^{-2}$ Pa). A molecule's vapor pressure is fundamentally important, because it determines partitioning between the gas and condensed phases. The slower change in droplet size and RI is due to the evaporation of glycerol (as well as commensurate water to maintain the droplet water activity equivalent to the RH). In the experiment, a humidified N_2 flow is used, resulting in a concentration gradient from the droplet surface to the gas phase, leading to slow volatilization of glycerol at a rate governed by its vapor pressure at the solution composition, p_G , which in turn depends on the glycerol mole fraction, x_G , and the activity coefficient, γ_G :

$$p_G = x_G \gamma_G p_G^0 \quad [2]$$

Measurement of the rate of change in droplet size (dr^2/dt) is related to p^0 for a given molecule through the Maxwell equation:

$$\frac{dr^2}{dt} = \frac{2M_i D_{i,air}}{RT\rho F_i} (p_{i,r} - p_{i,\infty}) \quad [3]$$

where M_i is the molecular mass of semivolatile compound i , D_i is its diffusion constant in the surrounding gas, R is the ideal gas constant, T is temperature, ρ is the droplet density, F_i is the mass fraction of compound i , $p_{i,r}$ is the vapor pressure in equilibrium at the droplet surface, and $p_{i,\infty}$ is partial pressure of the molecule at infinite distance from the droplet surface (assumed zero).

Aerosol Surfaces and Bulk Properties

We next discuss droplet surface and bulk properties, which are key to a range of processes. For example, surface tension helps determine what fraction of atmospheric particles grow into cloud droplets by governing the critical supersaturation in RH that must be surpassed (19). Moreover, the surface composition of droplets can affect the transport of molecules like water into and out of the droplet (20), as well as promote very different reactions from those occurring in the droplet bulk (21). Knowledge of bulk properties allows a better understanding of transport processes within the droplet, which can help explain droplet heterogeneity, reactivity, and composition (22). For example, a highly viscous droplet can inhibit diffusion of reactant molecules, resulting in slower apparent reaction rates (23). Understanding the interplay between surface and bulk properties enables control over droplet structure, which is important in many industrial contexts (24).

Surface and bulk droplet properties can be investigated by coalescence of two droplets. Droplets are captured in

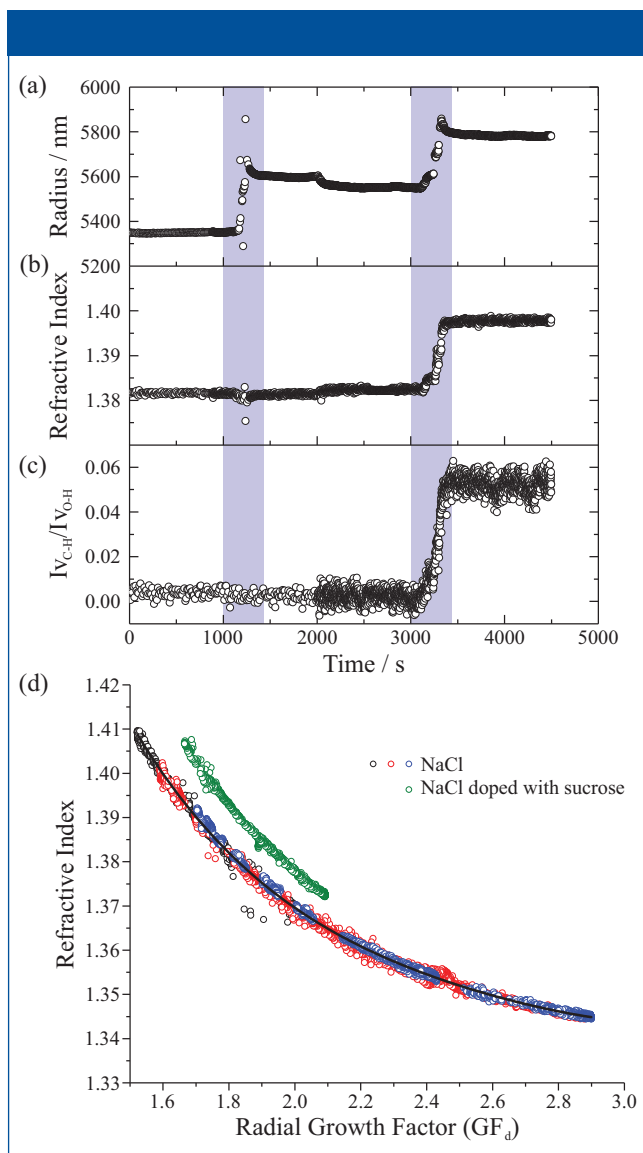


Figure 5: Droplet (a) radius, (b) refractive index (RI), and (c) chemical composition for a coalescence sampling measurement. In the first coalescence window (first purple box), a flow of aqueous sodium chloride (NaCl) droplets was introduced. In the second coalescence window, a flow of aqueous sucrose droplets was introduced; (d) Comparison of the RI-growth factor (GF_d) relationship for NaCl droplets (black, red, and blue) and a NaCl droplet after a period of coalescence sampling of sucrose aerosol (green) (17).

individual traps, equilibrated to a desired ambient condition, and then brought into coalescence at a user-defined time. For low viscosity droplets, coalescence proceeds through damped oscillations in droplet shape. Figure 6(a) shows high frame rate images of a dilute NaCl droplet coated with surfactant immediately after coalescence (8). Although high frame rate imaging visualizes the coalescence dynamics, it is more efficient to resolve the coalescence by collection of elastic backscattered light (see Figure 2). Figure 6(b) shows the elastic backscattered light from the coalescence event in Figure 6(a), along with the aspect ratios from the high frame rate images. Surface

tension is determined by the equation (25,26):

$$\sigma = \frac{\omega_1^2 a^3 \rho}{l(l-1)(l+2)} \quad [4]$$

where σ is surface tension, ω_1 is the oscillation frequency for mode l (corresponding to a characteristic deformation in droplet shape), a is the droplet radius, and ρ is the droplet density. Note that accurate measurement of surface tension depends on a precise measurement of droplet radius (a^3 term), highlighting the benefits afforded by size characterisation using the stimulated Raman spectrum. The fast Fourier transform (FFT) of the elastic backscattered light signal allows retrieval of ω_1 in Figure 6(c). It is then straightforward to calculate the droplet surface tension. Figure 6(d) shows surface tensions retrieved using a holographic AOT setup for droplets containing NaCl, glutaric acid, and a 1:1 mass mixture of both solutes. These measurements compare favorably to a statistical thermodynamic model (27). Moreover, a key benefit of performing the surface tension measurement on droplets is the ability to access supersaturated solute states that are inaccessible to bulk approaches. The measured values to the right of the vertical lines in Figure 6(d) are in the supersaturated solute regime and demonstrate the benefit of aerosol measurements to test models in previously untestable regimes. In addition, measurements have highlighted that trace contaminants in air rapidly lower the surface tension of droplets to values consistent

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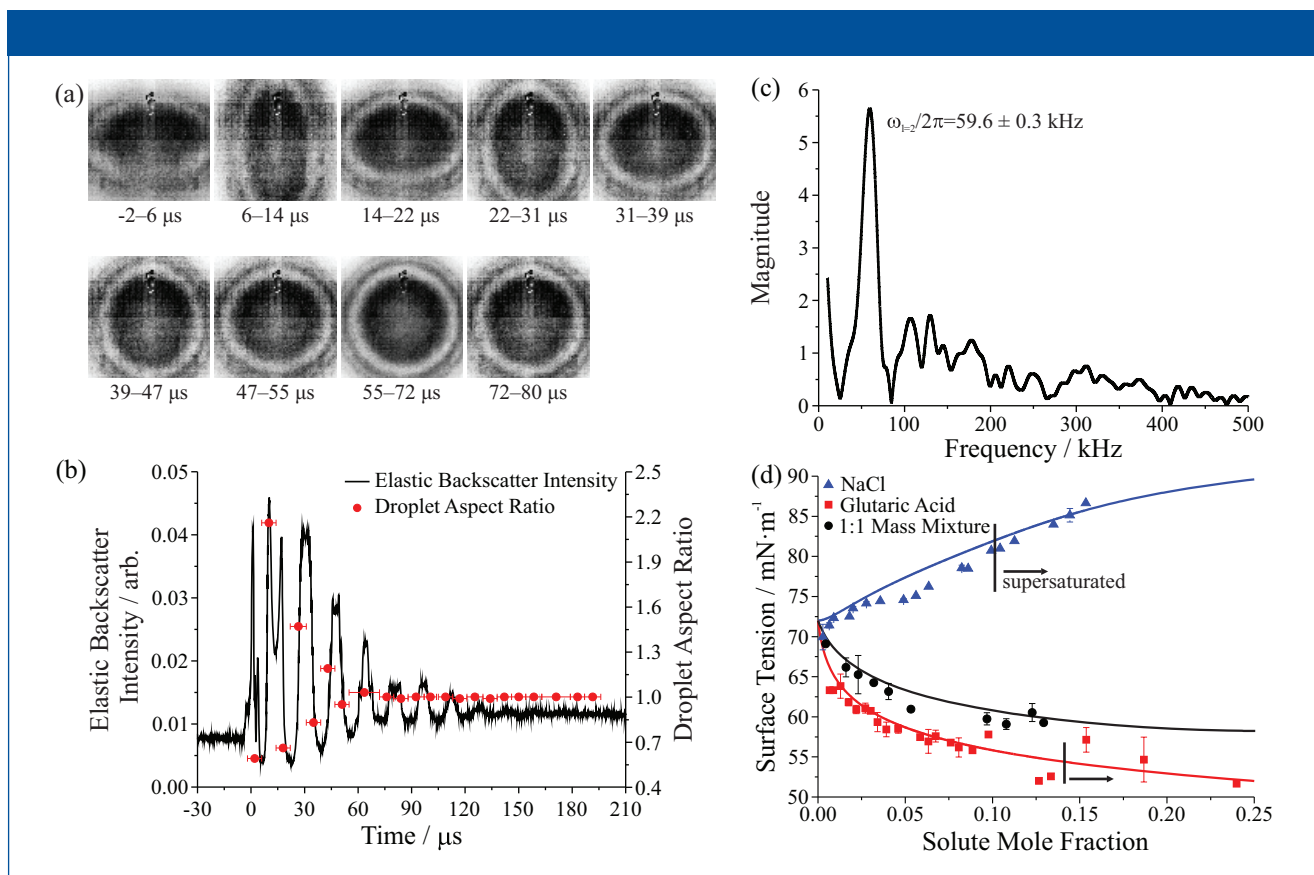


Figure 6: (a) High frame rate images after coalescence of two aqueous, surfactant-doped sodium chloride (NaCl) droplets. (b) Elastic backscattered light intensity collected after coalescence (left axis) and droplet aspect ratios from the images in part (a) (right axis); (c) A fast Fourier transform (FFT) of the signal in part (b) gives the frequency of the shape oscillations (8). In (d) measurements of picoliter droplet surface tension for NaCl, glutaric acid, and a 1:1 mass mixture of the two compounds (symbols) are compared to a statistical thermodynamic model of surface tension (lines) (27).

with those of surfactant solutions (8).

If the droplet viscosity is above a critical value, the surface oscillations are instantaneously damped, and coalescence proceeds through a slow merging of two droplets (7,28). This concept is demonstrated in Figure 7(a) with images of coalescing sucrose droplets that have been equilibrated to different RH values (29). Figure 7(b) shows that aspect ratios of the slowly coalescing droplets follow an exponential decay, and that depending on the droplet viscosity (governed by RH), the relaxation timescale (τ_l) can span from microseconds to days. The relaxation timescale is related to droplet viscosity by the equation:

$$\eta = \frac{l(l+2)(2l+1)}{2(2l^2+4l+3)} \frac{\sigma\tau_l}{a} \approx \frac{\sigma\tau_{l=2}}{a} \quad [5]$$

where η is the droplet viscosity.

Figure 7(c) shows viscosity measurements of citric acid by both bulk and AOT approaches, comparing to

1,4-butanetriol and sucrose over the entire range of RH. This figure highlights the utility of the AOT approach to measuring viscosity. First, measurements can be made over a wide range of viscosities, from values similar to that of pure water (1 mPa·s) to values beyond the glass transition (10^{10} Pa·s), spanning >13 orders of magnitude. Moreover, because it is straightforward to produce droplets containing highly viscous, supersaturated solute states, this approach can measure material properties under conditions inaccessible in bulk systems. For example, bulk solutions of citric acid can only reach concentrations corresponding to water activities ~ 0.8 (80% RH), the bulk solubility limit. However, in the aerosol phase, the solubility limit is easily exceeded, and measurements of viscous citric acid droplets are possible across the entire range of water activity, with viscosity values extending more than six orders of magni-

tude larger than those accessible in bulk solution, permitting comparison to model predictions and allowing estimation of the viscosity of atmospheric particles (28).

Putting It All Together

The previous sections highlight fundamental measurements possible due to single droplet Raman spectroscopy. We now present an example where spectroscopy was used to evaluate how changes in viscosity impact the droplet's reactivity with ozone, a common atmospheric oxidant (30). In the experiment, a droplet containing the semivolatile compound maleic acid (vapor pressure $\sim 10^{-3}$ Pa), nonvolatile sucrose, and water was equilibrated to different RH values. As discussed earlier, when a droplet containing a semivolatile compound is held at constant RH, its size will slowly decrease, due to evaporation of the semivolatile compound and an appropriate amount of solvating water. From the

size change (inferred from the changes to stimulated Raman band positions), the compound's pure component vapor pressure is inferred from Equation 3. Figure 8(a) illustrates the time-dependent fractional change in size for a droplet containing 5:1 maleic acid:sucrose held at different RH values. At higher RH (such as 70%) a steeper gradient in the radius change is observed as opposed to when the droplet is held at a much lower RH (such as 10%). However, based purely on vapor pressure considerations, the mass flux of maleic acid is expected to increase with decreasing RH, as the maleic acid mole fraction is increased at low RH. In fact, when the effective vapor pressure is calculated using Equation 3, the retrieved values span from $<10^{-5}$ Pa at 10% to 10^{-3} Pa (the true value) at 70% RH. The reason for this observation is kinetic suppression of the evaporation rate. The nonvolatile sucrose component increases droplet viscosity, consequently inhibiting diffusion of maleic acid within the particle. The decreased diffusion constant makes it harder for maleic acid to reach the droplet surface and evaporate.

Figure 8(b) shows relative changes in the intensity of the vinylic C-H stretch in the spontaneous Raman spectrum when maleic acid–sucrose droplets held at different RH values are exposed to the oxidant ozone. Ozone reacts with maleic acid (which contains a carbon-carbon double bond) but not with sucrose (which lacks a C=C bond). Ozonolysis fragments maleic acid at the double bond, forming lower molecular weight molecules with a range of vapor pressures. Monitoring the vinylic C-H stretch provides a direct measure of the reaction rate of maleic acid through cleavage of the carbon-carbon double bond. There is a clear RH dependence for reactivity as seen in Figure 8(b), with droplets at higher RH exhibiting faster reaction kinetics. For comparison, the purple triangles show the change in $I_{\nu_{C-H}}$ for a maleic acid droplet held at 73% RH in the absence of ozone (that is, the change in signal intensity entirely due to evaporation of semivolatile maleic acid). The golden triangles show reaction of a 10:1 sucrose: maleic acid mixture at 40% RH, demonstrating that ozonolysis

is effectively shut down. From the spontaneous Raman data, reaction probabilities for ozone uptake can be estimated. For the 5:1 sucrose: maleic acid droplet at 75% RH, the reaction probability is similar to that retrieved from an experiment in the bulk ($\sim 8 \times 10^{-6}$). However, for the 10:1 sucrose: maleic acid droplet at 40% RH, the reaction probability decreases by more than two orders of magnitude to $< 3 \times 10^{-8}$. The explanation for the lowering of the uptake coefficient is the change in diffusivity

for water and ozone that results from the matrix formed by interactions of sucrose and maleic acid.

This example demonstrates how precise measurement of changes to droplet size can provide information about the phase state of a droplet, permitting insight on diffusion within the droplet. Coupling those observations with spontaneous Raman spectra allows rationalization of the reactivity of droplets as a function of their viscosity.

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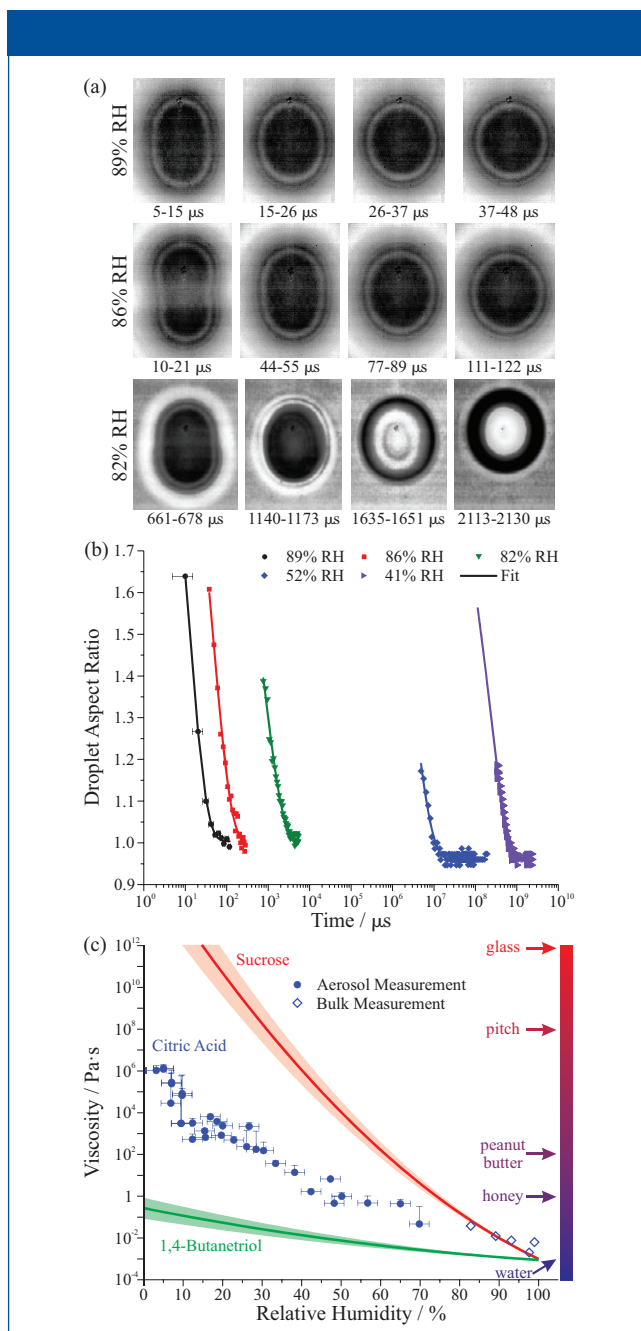


Figure 7: (a) High frame rate images of coalescing sucrose droplets equilibrated to different relative humidity (RH) values (29); (b) Droplet aspect ratios during coalescence for several droplets, each with different viscosities (28); (c) Plot of viscosity against RH for 1,4-butanetriol, citric acid, and sucrose.

Conclusions

Aerosols are complex, highly nonideal systems that exhibit properties that often cannot easily be represented by bulk studies. Optical tweezers are a versatile approach to study problems in aerosol science on a single droplet level, with a wide range of potential applications spanning atmospheric science to materials science and pharmaceuticals. We showed that single droplet Raman spectroscopy provides a spontaneous Raman signal

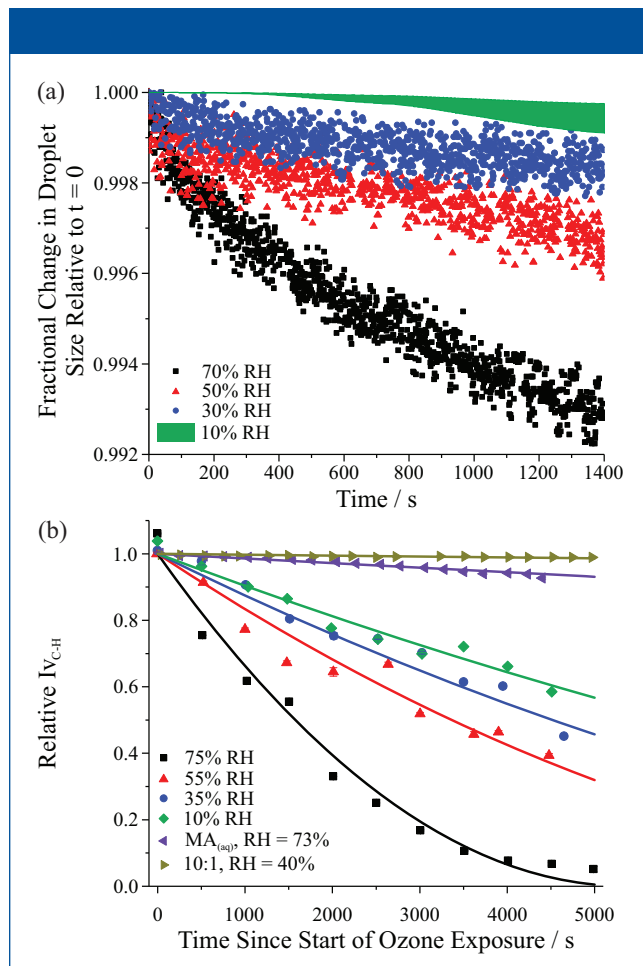


Figure 8: (a) Fractional change in droplet size over 1400 s for a maleic acid–sucrose droplet at four different relative humidity (RH) values, showing the gradual retardation in maleic acid evaporation as RH decreases. The green envelope shows the range of the data for the mixture at 10% RH (data not shown for clarity). (b) Time-dependence of the normalized spontaneous Raman signal intensity of the maleic acid vinylic C–H stretch during oxidation experiments at different RH values for a 5:1 mass ratio sucrose–maleic acid droplet. Purple triangles show change in signal intensity for an aqueous droplet evaporating at 73% RH without reaction. Gold triangles show the change in Raman intensity during reaction of a 10:1 mass ratio droplet at 40% RH (30).

that gives information on chemical composition and a stimulated Raman signal when the spherical droplet behaves as a low loss optical cavity at wavelengths commensurate with WGMs. Because WGM wavelengths are highly sensitive to droplet size and composition, comparison of experimental resonances to those predicted by Mie theory permits accurate and precise retrieval of the droplet's size and RI (to ± 2 nm and 0.0005, respectively). Consequently, dynamic changes to droplet physical, chemical, and optical properties can be monitored with high time resolution. These changes to droplet parameters enable determination of fundamental droplet properties including hygroscopic response, vapor pressure, surface tension, and bulk viscosity. Such informa-

tion allows insight into molecular processes occurring within picoliter volumes, including how parameters like viscosity can affect the volatilization and reactivity of semivolatile compounds in the droplet. Because aerosol science is fundamental to many research areas, the AOT approach has broad utility.

Acknowledgments

B.R.B. acknowledges support from the Natural Environment Research Council (NERC) through grant NE/P018459/1. No new data were created in this study.

References

- (1) J. Lelieveld, J.S. Evans, M. Fnais, D. Giannadaki, and A. Pozzer, *Nature* **525**, 367–371 (2015).
- (2) B. Forbes, B. Asgharian, L.A. Dailey, D. Ferguson, P. Gerde, M. Gumbleton, L. Gustavsson, C. Hardy, D. Hassall, R. Jones, R. Lock, J. Maas, T. McGovern, G.R. Pitcairn, G. Somers, and R.K. Wolff, *Adv. Drug Deliv. Rev.* **63**, 69–87 (2011).
- (3) T.F. Stocker, D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex, P.M. Midgley, Eds. IPCC, *Climate Change 2013: The Physical Science Basis: Working Group I Contribution to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (Cambridge University Press: Cambridge, 2013).
- (4) B.R. Bzdek and J.P. Reid, *J. Chem. Phys.* **147**, 220901 (2017).
- (5) A. Ashkin, *Phys. Rev. Lett.* **24**, 156–159 (1970).
- (6) A. Ashkin and J.M. Dziedzic, *Science* **187**, 1073–1075 (1975).
- (7) R.M. Power, S.H. Simpson, J.P. Reid, and A.J. Hudson, *Chem. Sci.* **4**, 2597–2604 (2013).
- (8) B.R. Bzdek, R.M. Power, S.H. Simpson, J.P. Reid, and C.P. Royall, *Chem. Sci.* **7**, 274–285 (2016).
- (9) R. Symes, R.M. Sayer, and J.P. Reid, *Phys. Chem. Chem. Phys.* **6**, 474–487 (2004).
- (10) A. Ashkin and J.M. Dziedzic, *Phys. Rev. Lett.* **38**, 1351–1354 (1977).
- (11) S.-X. Qian and R.K. Chang, *Phys. Rev. Lett.* **56**, 926–929 (1986).
- (12) R. Thurn and W. Kiefer, *Appl. Opt.* **24**, 1515 (1985).
- (13) K.J. Vahala, *Nature* **424**, 839–846 (2003).
- (14) A.J. Trevitt, P.J. Wearne, E.J. Bieske, and M.D. Schuder, *Opt. Lett.* **31**, 2211 (2006).
- (15) G. Mie, *Ann. Phys.* **25**, 377–445 (1908).
- (16) T.C. Preston and J.P. Reid, *Opt. Soc. Am. B* **30**, 2113–2122 (2013).
- (17) A.E. Haddrell, R.E.H. Miles, B.R. Bzdek, J.P. Reid, R.J. Hopkins, and J.S. Walker, *Anal. Chem.* **89**, 2345–2352 (2017).
- (18) F.D. Pope, H.J. Tong, B.J. Dennis-Smith, P.T. Griffiths, S.L. Clegg, J.P. Reid, and R.A. Cox, *J. Phys. Chem. A* **114**, 10156–10165 (2010).
- (19) J. Ovadnevaite, A. Zuend, A. Laaksonen, K.J. Sanchez, G. Roberts, D. Ceburnis, S. Decesari, M. Rinaldi, N. Hodas, M.C. Facchini, J.H. Seinfeld, and C. O'Dowd, *Nature* **546**, 637–641 (2017).
- (20) J.F. Davies, R.E.H. Miles, A.E. Haddrell, and J.P. Reid, *Proc. Natl. Acad. Sci.* **110**, 8807–8812 (2013).
- (21) E.M. Knipping, M.J. Lakin, K.L. Foster, P. Jungwirth, D.J. Tobias, R.B. Gerber, D. Dabdub, and B.J. Finlayson-Pitts, *Science* **288**, 301–306 (2000).
- (22) J.P. Reid, A.K. Bertram, D.O. Topping, A. Laskin, S.T. Martin, M.D. Petters, F.D. Pope, and G. Rovelli, *Nat. Commun.* **9**, 956 (2018).
- (23) M. Kuwata and S.T. Martin, *Proc. Natl. Acad. Sci.* **109**, 17354–17359 (2012).
- (24) A. Baldelli and R. Vehring, *Aerosol Sci. Technol.* **50**, 1130–1142 (2016).
- (25) L. Rayleigh, *Proc. R. Soc. London* **29**, 71–97 (1879).
- (26) S. Chandrasekhar, *Proc. London Math. Soc.* **s3-9**, 141–149 (1959).
- (27) H.C. Boyer, B.R. Bzdek, J.P. Reid, and C.S. Dutcher, *J. Phys. Chem. A* **121**, 198–205 (2017).
- (28) Y.C. Song, A.E. Haddrell, B.R. Bzdek, J.P. Reid, T. Bannan, D.O. Topping, C. Percival, and C. Cai, *J. Phys. Chem. A* **120**, 8123–8137 (2016).
- (29) B.R. Bzdek, L. Collard, J.E. Sprittles, A.J. Hudson, and J.P. Reid, *J. Chem. Phys.* **145**, 054502 (2016).
- (30) F.H. Marshall, R.E.H. Miles, Y.C. Song, P.B. Ohm, R.M. Power, J.P. Reid, and C.S. Dutcher, *Chem. Sci.* **7**, 1298–1308 (2016).

Bryan R. Bzdek is an NERC Independent Research Fellow in the School of Chemistry at the University of Bristol in the United Kingdom. **Jim S. Walker** is the Aerosol Products Division Manager at Biral in Bristol, United Kingdom. Direct correspondence to: b.bzdek@bristol.ac.uk ■

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Study of the Content of Inorganic Arsenic and Heavy Metals in Samples of Rice Cakes in the City of Madrid

This study analyzes the presence of inorganic arsenic and heavy metals in different varieties of rice cakes, both so-called “bio” (organic) and “non-bio,” as well as those made with white rice and brown rice. Determination of cadmium (Cd), mercury (Hg), nickel (Ni), and lead (Pb) has been carried out by inductively coupled plasma mass spectrometry (ICP–MS) for samples previously digested in a microwave oven. The analysis of inorganic arsenic has been carried out by liquid chromatography (HPLC) combined with inductively coupled plasma–mass spectrometry (HPLC–ICP–MS) prior to acid extraction. All samples analyzed are below the limits established by local regulations. However, the content found for inorganic arsenic, cadmium, and nickel may be a risk to health for children, who are the marketing target for some of the rice cake products.

Javier Peinador Asensio, Silvia Borobia Caminos, and Pilar Jiménez Navarro

Today, societal concerns about maintaining a healthy and balanced diet is causing an increase in the consumption of certain foodstuffs that are apparently healthier. It should be noted there is a growing interest among consumers about rice cakes, eaten as an appetizer or a snack replacement for more caloric foods, such as sweet cakes or snack chips. It is possible, however, that these rice products are not as healthy as they seem, because there are studies that demonstrate the presence of certain contaminants. In spite of this, a variety of rice cakes are marketed for consumption by the entire population, and in some cases intended specifically for infants and children.

As a result of this trend toward consumption of healthy products, there is also a boom in the production and consumption of products categorized as organic, or *bio*, as opposed to normal, or *non-bio* products. For consumers, organic products are more popular, because they are considered to be of higher quality and believed to be free of possible contaminants that normal products may have.

Numerous studies (1–5) have been published showing disturbing amounts of inorganic arsenic (iAs) in these products. In this regard, the national food agency of Sweden (National Food Agency, NFA) conducted a study (6) on the content of inorganic arsenic in rice products in 2015 and recommended that children under six years old should not be fed rice cakes, due to their high content of iAs.

Arsenic is a metal that is found naturally in the Earth’s crust; it is present in the air, water, and soil. In the case of food, it can occur in several different chemical forms, both organic and inorganic. Nowadays, there is scientific evidence demonstrating that inorganic arsenic, both As (III) and As (V), is the most toxic and harmful to health, because it has been proven that it can cause problems related to fetal development, neurotoxicity, diabetes, and cardiovascular diseases. In addition, the International Agency for Research on Cancer has classified it as “carcinogenic to humans” (7), because it can cause skin cancer, bladder cancer, and lung cancer.

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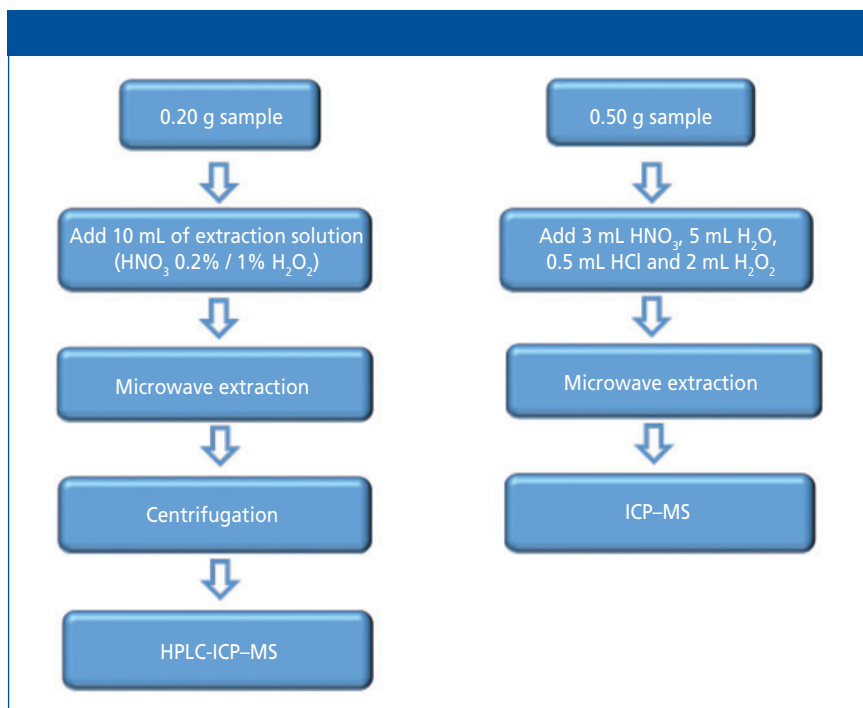


Figure 1: The steps in the analytical method used in this study.

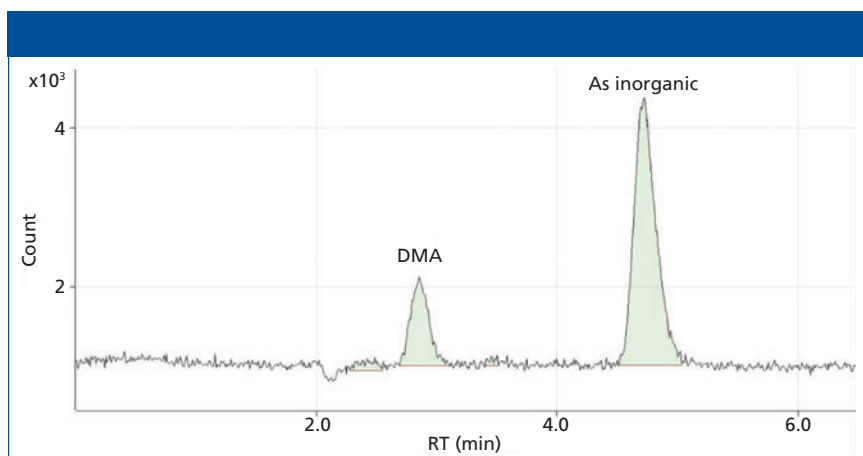


Figure 2: Arsenic speciation chromatogram showing a peak at 4.75 min for arsenate (As V).

The scientific technical committee on contaminants in the food chain (Contam) of the European food safety authority (EFSA) adopted an opinion (8) on arsenic in food. It modifies the provisional tolerable weekly intake (ISTP), established by the Joint Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) (FAO/WHO) Expert Committee on Food Additives (JECFA), based on the existence of studies demonstrating the toxicity of the iAs with exposure levels lower than those reviewed by JECFA. Thus, Contam set a limit of confidence

between 0.3 and 8 $\mu\text{g}/\text{kg}$ of body weight per day.

Scientific opinion (8) established that children under three years of age were those most exposed to foods with iAs, because rice is a predominant ingredient in a wide range of foods for this group. In general, it is estimated that dietary exposure to iAs of infants and young children is between double and triple that of adults.

In this context, this study has been carried out for the determination of iAs and expanded to included the analysis of various

heavy metals, in order to conduct a more exhaustive study of this type of food. The specific heavy metals analyzed were cadmium, mercury, nickel, and lead.

The concentration of these heavy metals can be considered especially relevant when dealing with toxic elements in doses that are harmful to the health of consumers. Except for Ni, these heavy metals are regulated, and in numerous scientific studies, their presence has been demonstrated in food and the consequences of their consumption have been studied.

The European Union (EU) has approved Commission Regulation (EU) 2015/1006 (9), which modifies the Commission Regulation (CE) 1881/2006, in which the maximum content of iAs for rice and rice products is established. In a similar way, measures at the global level (CODEX Alimentarius) have also been adopted, so it is possible to carry out control measures of iAs in rice and products derived from rice.

Commission Regulation (EU) 488/2014 (10), Commission Regulation (EU) 2015/1005 (11), and Commission Regulation (EU) 2018/73 (12) establish maximum limits for Cd, Pb, and Hg in foods, respectively. However, there are no limits in the case of Ni. Currently, European authorities are conducting a study (13) to establish maximum limits for this element in foodstuffs, as indicated in the Commission Recommendation (EU) 2016/1111 of 6 July 2016 on the monitoring of nickel in food (14).

The samples analyzed in this study correspond to different varieties of rice cakes, both with the “bio” (organic) designation and without it, and rice cakes made with white and brown rice, all sold in the city of Madrid. In total, 29 samples of rice cakes were analyzed, of which 11 were labelled as “bio,” while the remaining 18 did not have this designation. Note that 10 of the analyzed samples were made with white rice, and 19 with brown rice, because different studies have shown different amounts of iAs in white rice and brown rice.

Experimental

Instrumentation and Operating Conditions

The samples were digested in a microwave oven (Milestone model Ethos One) equipped with output power regulation and automatic control systems for temperature and pressure of all the vessels, which allows for the monitoring of both parameters during the process. The containers used for samples are PTFE tubes with lids, with a capacity of 100 mL; they were previously subjected to a rigorous cleaning process, according to the manufacturer's instructions.

The temperature program used for digestion is detailed in Table I (for the determination of heavy metals) and Table II (for the determination of iAs).

For the analysis of heavy metals, an inductively coupled plasma–mass spectrometry (ICP–MS) instrument (Agilent Technologies model 7900), equipped with an 89 position integrated autosampler (IAS), was used. The operating parameters of the ICP–MS instrument are outlined in Table III.

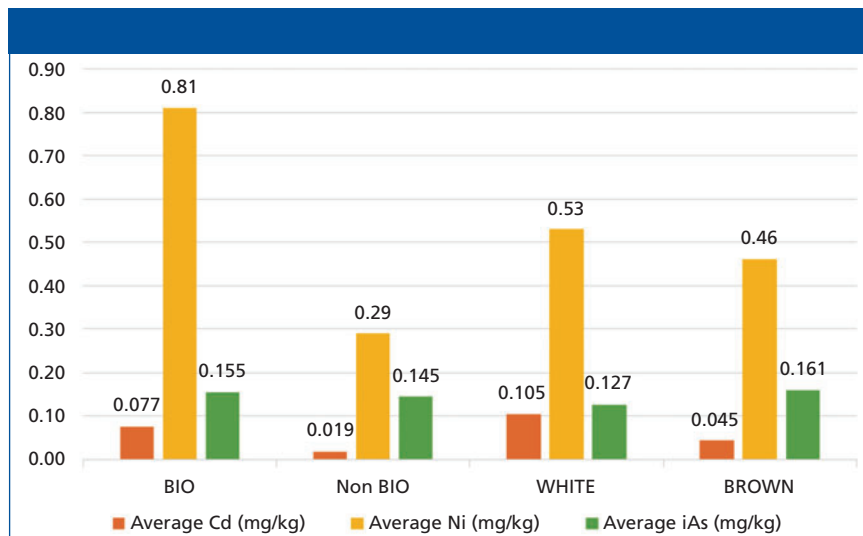


Figure 3: Results obtained in the study for cadmium, nickel, and inorganic arsenic (iAs). BIO: Total of analysed samples of rice cakes "BIO" (Brown + White); Non BIO: Total of analysed samples of rice cakes "Non BIO" (Brown + White); WHITE: Total of analysed samples of rice cakes "White rice" (BIO + Non BIO); BROWN: Total of analysed samples of rice cakes "Brown rice" (BIO + Non BIO).

Inorganic arsenic (iAs) determinations were performed using a high performance liquid chromatography (HPLC) instrument (Agilent Technologies model 1200 Infinity),

equipped with a model 1260 Quat Pump VL quaternary pump, an injector (model 1260 ALS), and a column compartment (model 1260 TCC). This HPLC system was used



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Table I: Temperature program for digestion in the determination of heavy metals

N°	Time	P (Watts)	T1 (°C)*	T2 (°C)†
1	00:04:00	800	90	50
2	00:02:00	800	90	55
3	00:03:00	1000	120	70
4	00:03:00	1000	120	70
5	00:03:00	1000	150	85
6	00:03:00	1000	150	85
7	00:04:00	1300	190	125
8	00:25:00	1300	190	125

* T1 is the temperature inside the tube guide measured with a probe

† T2 is the exterior temperature of the tubes measured by IR

Table II: Temperature program for extraction in the determination of inorganic arsenic (iAs)

N°	Time	P (Watts)	T1 (°C)*	T2 (°C)†
1	00:05:00	800	55	35
2	00:10:00	800	55	35
3	00:05:00	800	75	45
4	00:10:00	800	75	45
5	00:05:00	1000	95	60
6	00:20:00	1000	95	60

* T1 is the temperature inside the tube guide measured with a probe

† T2 is the exterior temperature of the tubes measured by IR

Table III: ICP-MS operating parameters for the analysis of heavy metals

Forward power	1550 Watts
Carrier gas	0.99 L/min
Kinetic energy discrimination	5.0 Volts
He collision gas	4.5 mL/min
Spray chamber temperature	2 °C
Data acquisition mode	Time resolved
Measured isotopes	⁶⁰ Ni, ¹¹¹ Cd, ²⁰² Hg, ²⁰⁸ Pb*
Internal standard isotopes	¹⁹³ Ir (for Pb and Hg) ¹⁰³ Rh (for Ni and Cd)
Integration time/point	1 sec

*Lead isotope 208 corresponds to the sum of the isotopes 206, 207, and 208.

in conjunction with the ICP-MS instrument referred to above. The operating parameters of this system are listed in Table IV.

The volumetric materials used for all solutions were calibrated polypropylene single-use tubes (DigiTubes SCP Science), except for the analysis of iAs, for which centrifuge tubes, also single-use, were used (VWR Metal Free, 15 mL volume). Eppendorf brand micropipettes were used for sampling corresponding aliquots.

Reagents and Standards

For the preparation of the reagents and solutions for washing and rinsing of the work material, deionized water (H₂O) class type I (resistivity: 18.2 MΩ cm) was used, purified using Milli-Q Integral system. The nitric acid (HNO₃, 65%) and hydrochloric acid (HCl, 37%) utilized are quality pro analysis (p.a.), supplied by Scharlau, and distilled in the laboratory by a Savillex distiller model DST-1000. The hydrogen peroxide (H₂O₂) was from Merck, 30%, Suprapur. Glacial acetic acid was from

Scharlau, 99.8%, ultratrace and ppb-trace analysis grade. The tuning solution for the ICP-MS instrument (Agilent Technologies) contained 1 µg/L Ce, Co, Li, Mg, Tl and Y. Finally, the argon and helium (99.999%) used in plasma generation and interference elimination were supplied by CONTSE.

In the case of the determination of heavy metals, all standard solutions were prepared with a mixture of 2% HNO₃ and 1% HCl, based on the following standards, having concentrations of 1000 mg/L: Cd (Sigma-Aldrich), and Hg, Ni and Pb (VHG-Labs).

Intermediate solutions of elements of interest were prepared as follows: Cd (1 mg/L), Hg (1 mg/L), Ni (10 mg/L), and Pb (1 mg/L) were used to prepare a multielemental standard (composed of 4 µg/L Cd, 5 µg/L Hg, 50 µg/L Ni, and 10 µg/L Pb) which is later used to prepare the calibration standards. To do this, the standard is taken and diluted 100 times, 25 times, 10 times, and 2.5 times, to obtain a calibration function with 5 levels.

For validation of Cd, Ni, and Pb, a multi-element standard of 1 µg/L was prepared from a commercial standard of 100 mg/L, provided by Scharlau, and a standard of 1.016 µg/L Hg was prepared from a 0.1016 mg/L NIST reference material, used for verification of the Hg control. Finally, 400 µg/L of Ir and Rh, prepared in a solution with 2% HNO₃, 1% HCl, and 5% acetic acid, was used as the internal standard solution. This internal standard was prepared from solutions of 1000 mg/L of Ir (Sigma-Aldrich) and Rh (Panreac).

For the analysis of inorganic arsenic, the solution used for extraction is 0.2% HNO₃ and 1% H₂O₂, noting that the solution used for the preparation of all standards was 0.2% HNO₃.

For inorganic As, the calibration standards were prepared from a standard of As (V) (from As₂O₅) of 100 mg/L in H₂O (VHG Labs). Using an intermediate solution of 1 mg/L standard with 10 µg/L prepared, this solution was diluted 2.5 times, 10 times, 25 times and 50 times, to obtain a calibration function with 5 levels.

An analysis of another As (V) reference material of was included, with the same specifications as the previous one, but in a different batch. Furthermore, a control of the process of oxidation of As (III) to As (V), using a standard of As (III), supplied by VHG Labs (from As₂O₃); 100 µg/mL in 2% HCl, was included.

The mobile phase composition was 20 mM PBS / 2 mM EDTA / 3% MeOH, in deionized water class type I, pH = 6, using Panreac methanol (CH₃OH) with a minimum assay of 99.9%, pure Panreac disodium ethylenediaminetetraacetate dihydrate (EDTA, with an assay of 99.0–101.0%, and pure Panreac sodium dihydrogen phosphate dihydrated (assay 98.0–100.5%).

Sample Treatment and Extraction Method

The steps of the analytical process are shown in Figure 1.

The homogenization of all samples was achieved by following the method

Table IV: HPLC and ICP–MS operating parameters for arsenic speciation

HPLC Operating Parameters		ICP–MS Operating Parameters	
Column	Hamilton PRP-X 100 anion exchange	Forward power	1550 Watts
Mobile phase	PBS 20 mM, EDTA 2 mM, methanol 3%, pH = 6	Carrier gas	0.99 L/min
Injection volume	50 µL	Kinetic energy discrimination	5.0 Volts
Column temperature	Ambient	Helium collision gas	4.3 mL/min
Flow rate	1.0 mL/min	Acquisition mode	Time resolved
		Measured isotopes	75
		Integration time point	0.5 sec
		Run time	6:30 min

described in the standard UNE-EN 13804:2013 (15), using a grinder to obtain a representative sample of the product to be tested.

Determination of Heavy Metals

To begin, the homogenized samples were weighed to 0.50 ± 0.05 g. For the digestion process, 3 mL HNO₃, 5 mL H₂O, 0.5

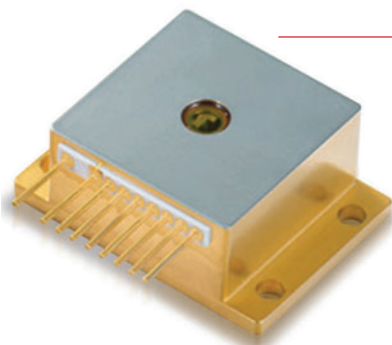
mL HCl, and 2 mL of H₂O₂ were added and digested in the microwave (conditions described in Table I), and, once the process was complete, samples were left to cool down to room temperature. The solutions were then transferred to the DigiTubes filled with up to 50 mL deionized water. These prepared samples were then ready to be analyzed by ICP–MS.

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Table V: Results obtained in the study of inorganic arsenic (iAs) and heavy metals

Samples	n	Max. iAs (mg/kg)	Mean iAs (mg/kg)	S_n	Quant. iAs	Max. Cd (mg/kg)	Mean Cd (mg/kg)	S_n	Quant. Cd	Max. Ni (mg/kg)	Mean Ni (mg/kg)	S_n	Quant. Ni	Max. Pb (mg/kg)	Mean Pb (mg/kg)	S_n	Quant. Pb
White Rice	7	0.18	0.12	0.038	7	0.033	0.033	-	1	0.60	0.23	0.18	7	0.075	0.029	0.026	5
White Rice, Bio	3	0.19	0.14	0.050	3	0.181	0.129	0.052	3	2.26	1.24	1.04	3	0.015	0.013	0.003	2
Brown Rice	11	0.24	0.16	0.063	11	0.013	0.012	0.002	2	0.87	0.32	0.30	11	0.019	0.015	0.004	3
Brown Rice, Bio	8	0.26	0.16	0.078	8	0.114	0.055	0.036	7	1.34	0.65	0.39	8	0.028	0.020	0.011	2

n = Number of samples analyzed in the study. S_n = the standard deviation for the average values.

Quant. = Number of samples that have been quantified in each case.

Determination of Inorganic Arsenic

In this study, the determination of inorganic arsenic was carried out as the sum of arsenite (As [III]) and arsenate (As [V]). This quantifies the peak corresponding to the As (V) obtained with a retention time of 4.75 min (see Figure 2).

The homogenized sample has been weighed 0.2 ± 0.02 g, and 10 mL of the solution prepared for extraction has been added. Later, the sample was treated in the microwave (conditions described in Table II). In this process, all of the As (III) in the sample is oxidized to As (V). Once the process has been completed, samples are left to cool down to ambient temperature, transferred to 15 mL tubes, and centrifuged for 10 min at ≥ 4000 rotations per min (rpm). The supernatant is filtered using 0.45 μm pore size nylon filters, and is poured into 1 mL polypropylene vials to inject into the HPLC instrument.

Validation and Quality Control

The analytical method used for the determination of heavy metals is required to meet the criteria of the Commission Regulation (EC) N° 333/2007 of March 28, 2007 (16) laying down the methods for sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-monochloropropanediol (3-MCPD), and benzo(a)pyrene in foodstuffs and its modifications. Validation has been carried out according to the requirements of the standard UNE/EN-ISO 17025 (17) for testing laboratories, and it is accredited by the Entity National Accreditation (ENAC). In the case of

the determination of iAs, the analytical method is in process of accreditation. The results of the validation are shown in Table VI.

For quality control, we have participated in different intercomparison exercises and additions of known concentrations of the analyte used to assess the accuracy of the method. In the case of iAs, a standard of As (III) has been used as described in Section 2.2.

Results and Discussion

The results obtained in this study are shown in Table V, detailing the maximum concentrations and average concentrations obtained for iAs, Cd, Ni, and Pb, as well as the number of samples that have been quantified in each case. The results for Hg are not included in this table, because the concentrations obtained are <0.010 mg/kg, and they have not, therefore, been able to be accurately quantified.

All the results obtained in the determination of iAs were able to be quantified. Nevertheless, it should be noted that no sample exceeded the maximum value established by Commission Regulation (EU) 2015/1006 (9) regarding maximum levels of inorganic arsenic in foodstuffs, set at 0.30 mg/kg for rice cakes. However, taking into account a value of 0.3 $\mu\text{g/kg}$ of body weight as the threshold of tolerable risk considered in the studies carried out by the EFSA (8), in the case of a six-year-old child (with an approximate weight of 20 kg), for example, the admissible threshold would be 6 μg of iAs, and the consumption of a single rice cake (about 30 g), with the highest value of found iAs (0.256 mg/kg) would result in the intake of ap-

proximately 7.7 μg of iAs. Therefore, special attention should be given to rice cake consumption by children.

In the case of Pb, the results obtained are not considered significant, because the concentrations were below the thresholds referred to in toxicity studies published for this heavy metal (18).

In terms of the results obtained for Cd, 60% of the analyzed samples are quantified as having a concentration >0.010 mg/kg. It should be noted that all of these results are below the reference value in the Commission Regulation (EU) 488/2014 (10), where the maximum content of Cd in rice has been established as 0.20 mg/kg. However, health authorities establishes a tolerable daily intake of 0.36 $\mu\text{g/kg}$ body weight (19) of this heavy metal to ensure all consumers have sufficient protection. Referring to a previous example, a six-year-old child should not exceed 7.1 μg of Cd daily intake. Hence, based on the data obtained in this study and where the maximum found value was present (0.181 mg/kg), consumption of a single rice cake would contribute 5.4 μg of Cd.

On the other hand, the values obtained for Ni show a high concentration of this metal in rice cakes, levels that even exceeded the concentrations of other contaminants that are regulated. Taking as a reference the measured maximum value, the consumption of a single rice cake would contribute up to 67.8 μg of Ni, which, at first sight, could be considered relevant if compared to the established maximum levels for other metals.

In addition, in the case of iAs, there are no significant differences between the rice cakes considered to be "bio"

and “non bio”; on the other hand, more iAs is seen in the rice cakes made with brown rice than those made with white rice. The content of iAs is not affected by the type of rice cultivation (bio or non bio), but the processing of the rice has important influence as a factor that reduces the concentration of iAs in white rice compared to brown rice, as shown in Figure 3.

The results for Cd and Ni highlight that the “bio” rice cakes present concentrations clearly higher than the “non-bio” rice cakes, as shown in Figure 3. In the case of Cd, there are also significant differences between rice cakes made with white rice versus those made with brown rice. On the contrary, in the case of Ni, we can see that there are no significant differences between the content of these metals in either variety of cake.

In this case, based on the determined quantities of Cd and Ni, we can appreciate a large influence in the type of rice cultivation but not so much in the processing of the rice.

Conclusions

All rice cakes samples analyzed comply with the legislation in force, because they do not exceed the maximum levels permitted; therefore, these foods should be considered safe for consumption.

However, based on the results obtained in this study for iAs, Cd, and Ni, it would be advisable that consumption of rice cakes by children be regulated by health authorities and that greater control be maintained over rice cakes that have with both the designation of “bio” and are made from brown rice.

On the other hand, the test method used for the analysis of Cd, Hg, Ni, and Pb by microwave acid digestion and determination by inductively coupled plasma–mass spectrometry is considered a valid option to carry out a study as presented in this publication, obtaining results in accordance with the requirements of the UNE-EN ISO 17025 standard.

The coupling of liquid chromatography to ICP–MS is a recommended method for chromatographic separa-

tion of the different species of arsenic, for the analysis of inorganic arsenic, determined as the sum of As (III) and As (V).

References

- (1) R. Guillod-Magnin, B.J. Brüscheiler, R. Aubert and M. Haldimann, Arsenic species in rice and rice-based products consumed by toddlers in Switzerland. *Food Addit. Contam. Part A* **35**(6), 1164–1178 (2018). DOI: 10.1080/19440049.2018.1440641.
- (2) A.J. Signes-Pastor, M. Carey, and A.A. Meharg, *Food Chem.* **191**, 128–134 (2016).
- (3) M. Molin, S.M. Ulven, H. Margrete Meltzer, and J. Alexander, *J. Trace Elem. Med. Biol.* **31**, 249–259 (2015).
- (4) H.N. Lynch, G.J. Greenberg, M.C. Pollock, and A.S. Lewis, *Sci. Total Environ.* **496**, 299–313 (2014).
- (5) N. Ferré-Huguet, R. Martí-Cid, M. Schuhmacher, and J.L. Domingo, *Biol. Trace Elem. Res.* **123**(1–3), 66–79 (2008).
- (6) Livsmedelsverket National Food Agency, Sweden. 2015. Study reveals problems with arsenic in rice and rice products. <https://www.livsmedelsverket.se/en/about-us/press/study-reveals-problems-with-arsenic-in-rice-and-rice-products>.
- (7) International Agency for Research on Cancer (IARC). Monographs on the Identification of Carcinogenic Hazards to Humans. List of classifications, volumes 1–123. <https://monographs.iarc.fr/list-of-classifications-volumes/>
- (8) EFSA (European Food Safety Authority): EFSA Panel on Contaminants in the Food Chain (CONTAM): Scientific Opinion on Arsenic in Food. *EFSA Journal* **7**(10), 1351 (2009).
- (9) Commission Regulation (EU) 2015/1006 of 25 June 2015 amending Regulation (EC) No 1881/2006 as regards maximum levels of inorganic arsenic in foodstuffs.
- (10) Commission Regulation (EU) No 488/2014 of 12 May 2014 amending Regulation (EC) No 1881/2006 as regards maximum levels of cadmium in foodstuffs.
- (11) Commission Regulation (EU) 2015/1005 of 25 June 2015 amending Regulation (EC) No 1881/2006

as regards maximum levels of lead in certain foodstuffs.

- (12) Commission Regulation (EU) 2018/73 of 16 January 2018 amending Annexes II and III to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for mercury compounds in or on certain products.
- (13) EFSA (European Food Safety Authority): EFSA Panel on Contaminants in the Food Chain (CONTAM): Scientific Opinion on the risks to public health related to the presence of nickel in food and drinking water. *EFSA Journal* **13**(2), 4002 (2015).
- (14) Commission Recommendation (EU) 2016/1111 of 6 July 2016 on the monitoring of nickel in food.
- (15) UNE-EN 13804:2013. Foodstuffs - Determination of elements and their chemical species - General considerations and specific requirements.
- (16) Commission Regulation (EC) No 333/2007 of 28 March 2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs.
- (17) General requirements for the competence of testing and calibration laboratories (ISO/IEC 17025:2017).
- (18) EFSA (European Food Safety Authority): EFSA Panel on Contaminants in the Food Chain (CONTAM): Scientific Opinion on Lead in Food. *EFSA Journal* **8**(4), 1570 (2013).
- (19) EFSA (European Food Safety Authority). 2012. Scientific Report: Cadmium dietary exposure in the European population. *EFSA Journal* **10**(1), 2551 (2012).

Javier Peinador Asensio, Silvia Borobia Caminos, and Pilar Jiménez Navarro are with the Public Health Laboratory of Madrid, Spain. Direct correspondence to: peinadoraj@madrid.es

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Feasibility Study of Portable X-ray Fluorescence (pXRF) for the Determination of Iodine in Pharmaceutical Products

A quick and accurate method for the determination of iodine in pharmaceutical products is critical for manufacturers to ensure product quality and to comply with the legal requirements as determined by authorities such as the Therapeutic Goods Administration (TGA) in Australia. As a noninvasive and nondestructive method of testing that requires little to no sample preparation, pXRF has been found to be one of the most efficient and economic analytical tools for elemental analysis in a wide range of applications. In this study, an approach using pXRF offers another option for the determination of iodine in multivitamin tablets, which is proving superiority for quality control on the production line.

Michael Wu

Iodine is an essential trace element that plays an important role in human growth and development. Both iodine deficiency and iodine excess are detrimental to human health (1).

Iodine is often added in multivitamin tablet products, with concentrations ranging from 50 to 150 μg per tablet. Some special sea kelp tablets contain relatively high levels of iodine. Given that an elevated concentration of iodine is associated with various negative health effects, a quick and reliable method for the determination of iodine is necessary for the purposes of quality control and product release.

The conventional method to determine iodine for pharmaceutical manufacturers is highly time-consuming, due to the extraction process required. It has been evidenced that inductively coupled plasma–optical emission spectrometry (ICP-OES) and inductively coupled plasma–mass spectrometry (ICP-MS) are effective means of measuring iodine in pharmaceuticals (2). However, both methods require lengthy procedures for sample dissolution and extraction and the setup of the required instruments. In addition, neither method is practical as a quick on-site testing method for quality control.

This study explored the feasibility of iodine determination in pharmaceutical products using portable X-ray fluorescence (pXRF), which has been recognized as an efficient tool and as the first-line screening method of choice in many industries. This study aims to develop both a quick approach for homogeneity testing of mixed product powders, and an analytical method for the accurate determination of iodine ($> 50 \mu\text{g/g}$) in multivitamin tablets.

Materials and Methods

In this study, more than 10 multivitamin products from the local supermarket were collected. The products were manufactured with different compositions, with the iodine concentration ranging from 50 μg per tablet to 280 μg per tablet, according to their labels.

For accurate analysis of the tablets, they were blended into fine powders, and packed in an XRF sample cup sealed with Mylar TF-160-255 film. The analysis was performed on a portable XRF analyzer (SkyRay Explorer 7000). A user mode (or calibration) was developed based on the factory settings. This XRF analyzer provides an “expert” mode, in which

the user can setup various instrument conditions including the excitation source, collimator, filters, peak signal calculations, and interference corrections. A test stand was used to ensure the sample test position is consistent. All samples were analyzed with the settings of 45kV 50 uA and 180 s. It was observed that after 120 s, the signals were almost consistent.

For the homogeneity tests, tablets may be determined directly, but care must be taken to ensure the tablets are placed in the same test position in front of the test window.

Results and Discussions

In this section, the method was assessed and discussed according to the ICH Harmonized Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology Q2(R1) (3).

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present. The most powerful and principal energy signal of iodine $K\alpha$ is found at 28.612 keV, at which the possible spectral overlap may be from tin $K\beta$ at 28.485keV, antimony at $K\beta$ 29.73keV, and tellurium at $K\alpha$ 27.47keV. In this study, a pXRF analyzer demonstrated a resolution of approximately 0.15 keV. No evidence of possible overlap from these interferences was observed, thus demonstrating the specificity of the method.

Accuracy

The label values of each product and the expected values of the spiked samples were used as the acceptable values to benchmark the measured results (Figure 1). In addition, NIST SRM 3280 multivitamin tablets were analyzed, and the certified value of iodine was used as the standard value for assessing the accuracy of the method. The initial calibration curve was established using pure iodine standards made from the mixture of potassium iodide and silicon

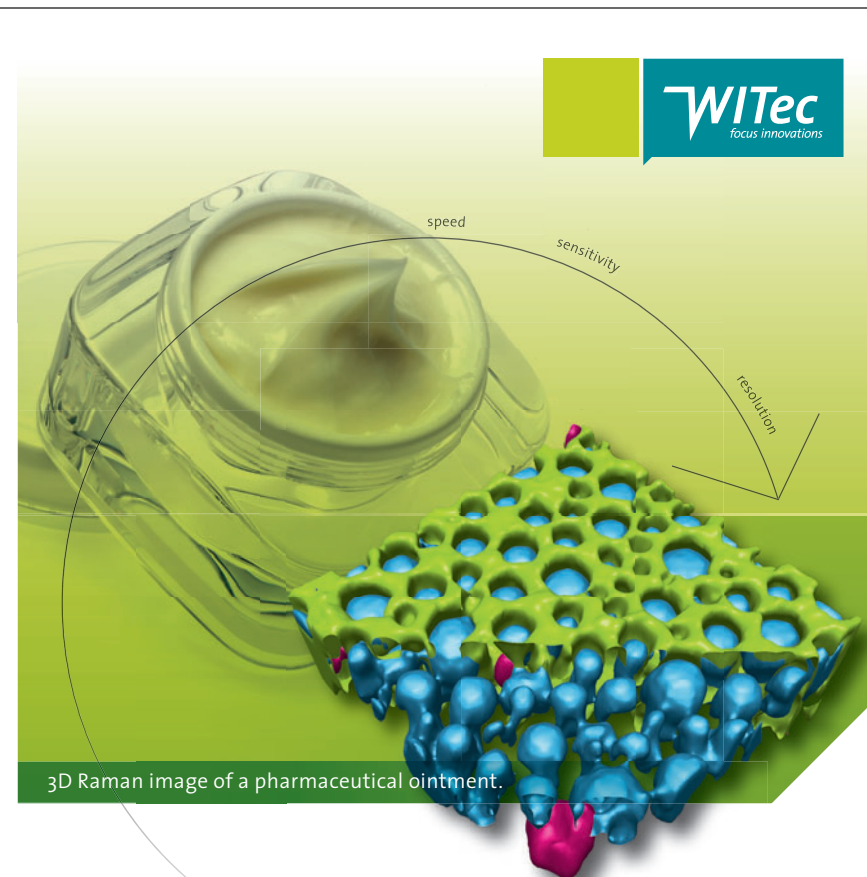
dioxide. By analyzing all different multivitamin samples, including the NIST SRM 3280, an adjustment factor was input into the calibration curve to correct for matrix effects. Then, the adjusted calibration curve was verified by analyzing the NIST SRM 3280. Once consistent results were achieved, a generic calibration for the multivitamin matrix for all products was generated.

The accuracy test results are listed in Tables I and II.

As a matter of fact, each tablet product has a different matrix. To obtain more accurate results for each individual product, the calibration curves may be fine-tuned for each specific tablet type.

Precision

In this study, short-term repeatability checks were carried out and the relative standard deviation (RSD%) in percentage was used to assess precision. Like any other in-



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Table 1: Accuracy data of various multivitamin tablets

Tablets	Obtained ($\mu\text{g/g}$)	Label or Expected Values ($\mu\text{g/g}$)	Recovery (%)
13 August 2018			
Product 1	254	231	110
Product 2	374	369	101
Product 3*	81	79	103
Product 4	59	66	90
Product 5	92	94	98
Product 6	189	203	93
Product 7	160	158	101
Product 8	151	158	96
Product 9	86	97	89
Product 10	139	133	104
Product 11	128	133	97
Product 12	237	278	85
Product 13	886	900	98
Product 14	100	106	94
16 August 2018			
Product 1	134	133	101
Product 2	155	158	98
Product 3	232	243	96
Product 4	127	108	118
Product 5	246	231	106
Product 6	197	201	98
Product 7	132	133	100
Product 8	94	102	93
Product 9	101	94	107
Product 10	121	106	114
Product 11	71	66	108
Product 12	868	900	96
Product 13	357	369	97
Product 14*	78	79	99
*Calibrated with specific matrix match standards			

strumental method, the precision varies with the analyte concentrations. The RSD% is usually higher when the concentration is close to the reported limits. The precision test results at the level of around 80 $\mu\text{g/g}$ and 100 $\mu\text{g/g}$ are illustrated in Figure 2 and Figure 3.

The same precision test at the 80 $\mu\text{g/g}$ level was performed on three

different dates, and the RSDs (as %) were 7.8%, 9.5%, and 8.7%, respectively.

The precision achieved was comparable to the precision of other methods. The National Institute of Standards and Technology (NIST) reported precisions of 7.6–8.6% for iodine (~132 $\mu\text{g/g}$) on multivitamin certified reference material using the methods, such as ICP-MS

and instrumental neutron activation analysis (INAA) (4).

For the iodine content level at 500 $\mu\text{g/g}$, the RSD% values were found consistently to be from 3 to 5%.

Detection Limit

In this study, the detection limit was reported as three times the standard deviation of a real multivitamin sample, with very low iodine concentration analyzed seven times. The typical detection limit of this method is around 20–30 $\mu\text{g/g}$.

Increasing the measurement time will improve the detection limit. The current measurement time was set as 180 s, further increasing the measurement time to 240–300 s will improve the detection limits to around 10–20 $\mu\text{g/g}$.

Linearity

The typical linearity of this method was up to 1000 $\mu\text{g/g}$ with a coefficient of determination (R^2) of greater than 0.999 (Figure 4), which covers the iodine concentration in almost any multivitamin product.

It must be noted that, for an XRF method, the calibration curve established from the pure analyte may not be suitable for the real samples. Ideally, a calibration curve should be specifically established for each product using standard addition calibrations for each product to get the best results.

Range

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy, and linearity.

With the tablet products sourced from the supermarket, the lowest concentration is 50 μg per tablet and the highest concentration is 280 μg per tablet; considering the tablet weight, the lowest concentration is 62.5 $\mu\text{g/g}$ and the highest concentration is 900 $\mu\text{g/g}$. An RSD of approximately 15% at the low concentration of 62.5 $\mu\text{g/g}$, and an RSD of

around 5% at the high concentration of above 300 $\mu\text{g/g}$, were observed.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. In this study, the following variations were deliberately introduced, and the effects of these were assessed:

Effects of the Tablet Shape, Coating, and Size

The tablet may be placed in front of the XRF window for direct iodine measurement. However, the tablet position, size, shape, and coating will affect the test results. Direct measurement may be used for comparisons between each single tablet of the same product, each time the tablet must be placed in front of the XRF measuring window in the same manner.

For quantitative analysis, it is suggested to blend the tablets into powder before measurement, so that the effects of the tablet sizes and shapes are minimized.

Effects of Powder Density and Sample Depth in the XRF Cup

Once a fine powder was obtained, the powder was packed into an XRF cup by repeated tapping. The cup should be filled to at least 1.5 cm in depth. No significant difference in analytical signal was observed when the cup was filled to more than 1.5 cm. It was observed that the XRF signal intensity changed when the sample density changed.

Effects of Measurement Time

The longer measuring time will improve the measurement precision and the detection limit and quantity limits. However, when the measuring time is longer than 180 s, less improvement was observed.

Matrix Effects

Physical matrix effects result from the variation in the physical and chemical characteristics of the samples, which include sample composition,

Table II: Accuracy data of NIST SRM 3280 multivitamin tablets

NIST SRM 3280 Sample Number	Obtained Value (Iodine, $\mu\text{g/g}$)	Recovery, %*
1	123	93
2	115	87
3	112	85
4	131	99
5	117	88
6	130	98
7	138	104
8	137	103
9	131	98
10	138	104
Average	127	96
sd	9.9	7.5
RSD (%)	7.8	7.8

*Recoveries were calculated based on the certified value of 132.7 $\mu\text{g/g}$.

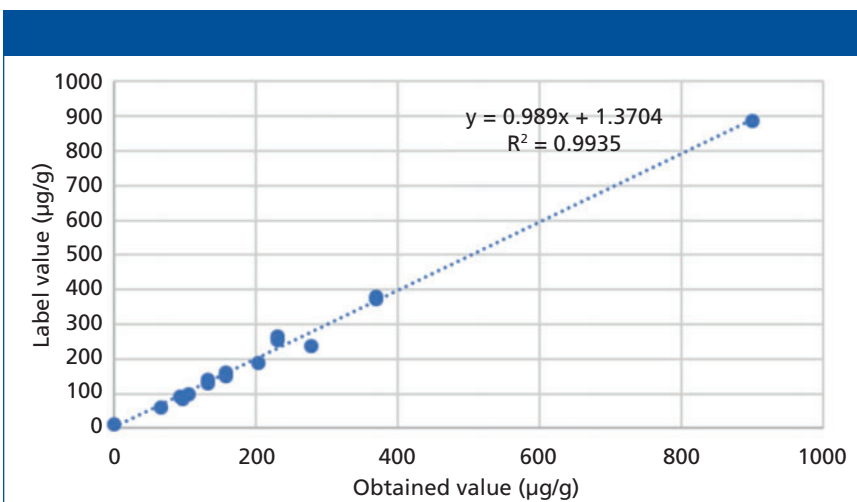


Figure 1: Correlation between the label values and the obtained values by the pXRF method.

particle size, uniformity, homogeneity, and surface conditions. It was observed that the iodine signal was suppressed in some product matrices, but was not changed in other products. In general, pXRF is a technique that requires “full match” of the calibration standards and the samples in terms of the composition and test conditions if accurate results are required.

Applications

With the method developed, it takes about 10 min to complete the analysis, including blending tablets into

powder, packing the sample powder into an XRF cup, and measuring in the pXRF analyzer.

With the feature of nondestructive and in-situ testing, pXRF is an ideal option for on-line monitoring of product quality at different stages of production.

In addition, the multivitamin tablets can be directly placed in front of the pXRF test window for iodine determination, which makes it an easy way to identify the difference or the homogeneity between tablets for the same type of product.

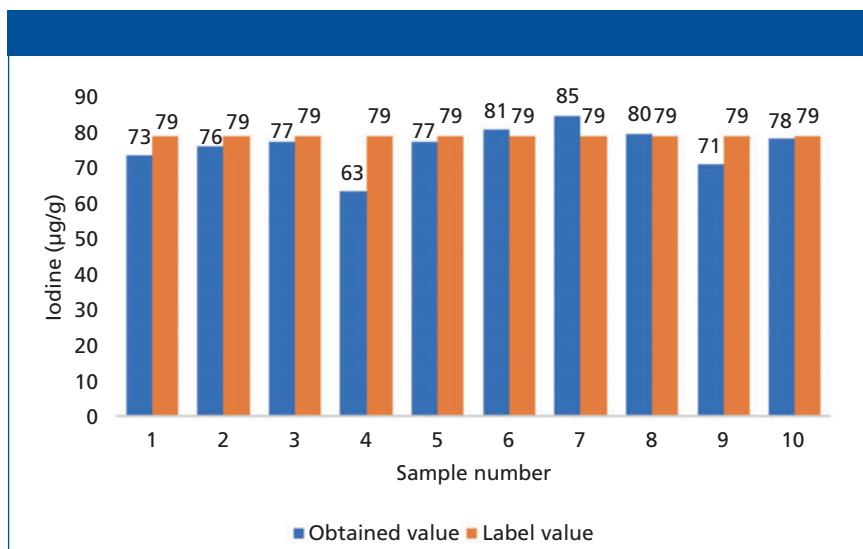


Figure 2: Iodine accuracy and precision test results at the level of ~ 80 µg/g. The average concentration of the iodine was 76 µg/kg with RSD = 7.8% (n = 10).

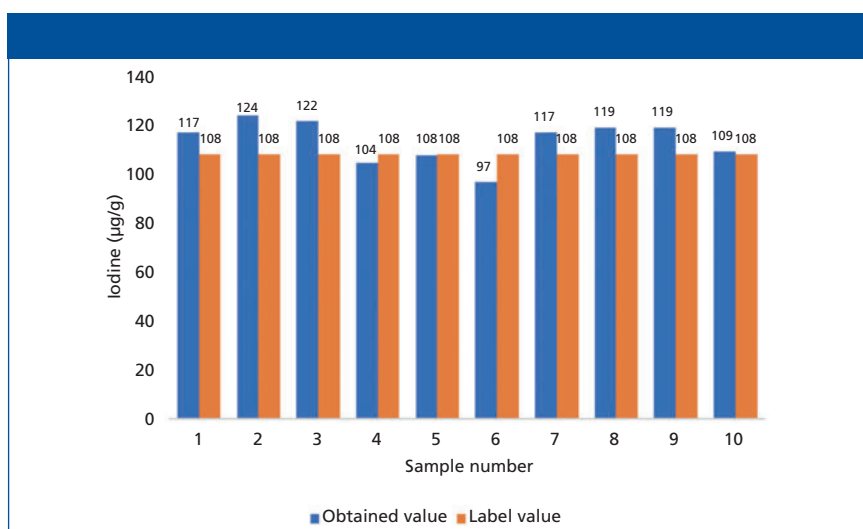


Figure 3: Iodine accuracy and precision test results at the level of ~ 100 µg/g. The average value of iodine in 10 tested tables was 113 µg/kg and the RSD% was 7.6%.

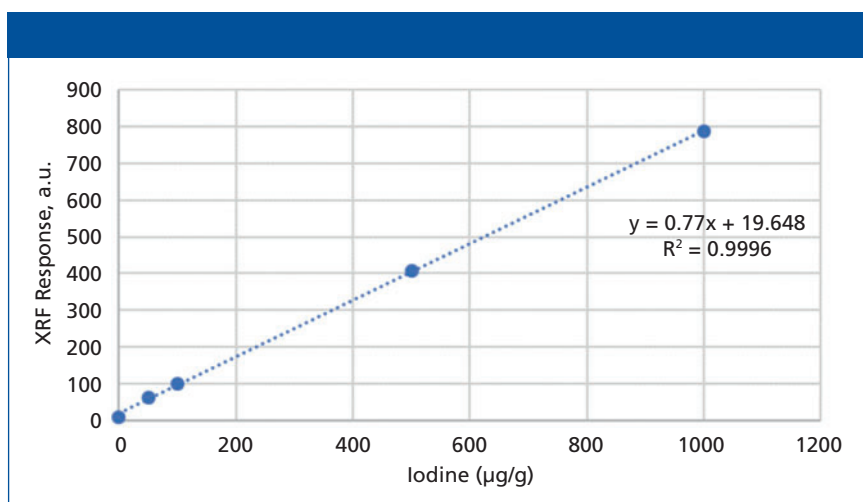


Figure 4: Iodine calibration curve.

Conclusions

The analytical performance of the method for the determination of iodine in pharmaceutical products, such as multivitamin tablets, was evaluated in terms of accuracy, precision, and other parameters. The method presents advantages such as direct determination of iodine with minimum sample preparation and fast results within minutes, making pXRF an ideal tool for quality control purposes in the pharmaceutical production line. This method may potentially be applied for other elements such as iron, zinc, and selenium in multivitamin tablets and other products.

Acknowledgments

The author acknowledges Dr. Andrew Evans, Ms. Luminita Antin, Mr. Andrew Taylor and Ms. Xiao Teng Zhou for discussions of technical questions.

References

- (1) Iodine, Fact Sheet for Health Professionals, Office of Dietary Supplements, National Institutes of Health, updated on 2nd March 2018.
- (2) C. P. Shelor and P.K. Dasgupta, *Analyt. Chim. Acta* **702**, 16–36 (2011).
- (3) ICH Harmonized tripartite guideline, validation of analytical procedures; Text and Methodology Q2(R1), Current Step 4 version Parent Guideline dated October 27, 1994 (Complementary Guideline on Methodology dated November 6 1996, incorporated in November 2005).
- (4) G. Turk, K. Sharpless, D. Cleveland, C. Jongsma, E. Mackey, A. Marlow, R. Oflaz, R. Paul, J. Siebler, R. Thompson, L. Wood, L. Yu, R. Zeisler, S. Wise, J. Yen, S. Christopher, R. Day, S. Long, E. Greene, J. Harnly, I-P Ho, and J. Betz, *J. AOAC Int.* **96**(6), 1281 (2013).

Michael Wu is with UBO Services Australia Pty Ltd. in Cherrybrook, Australia. Direct correspondence to: michael.wu@uboservices.com.au ■

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SPECTROSCOPY SPOTLIGHT

MOVING MID-IR SPECTROSCOPY FORWARD IN MEDICINE



Mid-infrared (mid-IR) spectroscopy excels in the speed of data acquisition, and this feature has led spectroscopists to pursue mid-IR spectral imaging in particular as a valuable future tool for medicine, in areas such as histopathology and cytology for disease research, and clinical diagnostics. Nick Stone of the University of Exeter, UK, spoke to *Spectroscopy* about the advantages of the technique, and the latest technological development that are advancing the capabilities of mid-IR spectroscopy for use in medicine.

Alasdair Matheson

You are well known for your work in Raman spectroscopy. Recently, however, you have collaborated on some projects using mid-IR spectroscopy for biological samples. Are there circumstances where mid-IR offers specific advantages for biological or clinical applications?

Absolutely. Raman and IR spectroscopy are complementary techniques that are able to probe the vibrational modes of biomolecules of interest, and therefore are useful to study the composition and microenvironment of tissues and cells as disease develops (1). We have worked on Raman and IR in parallel for many years to ensure that complementary information is available to us in our work developing methods for clinical diagnostics. Working with both techniques has allowed us to assess the relative pros and cons of each approach for particular clinical applications. We previously undertook a parallel study on IR and Raman for identification of secondary cancers in mediastinal lymph nodes. Both approaches provided an almost identical performance when using multivariate prediction of disease (2).

There are, of course, pros and cons for each approach, particularly around sample preparation and use in vivo and ex vivo. Raman usually allows for little sample preparation, and IR usually requires dry, thin sections. However, mid-IR imaging excels in the speed of acquisition of the data, and this advantage has led us and other groups to consider mid-IR spectral histopathology as a key

future tool for medicine (3). Current “gold standard” cancer diagnostics use formalin-fixed paraffin-embedded tissue sections for staining and histopathological analysis. These sections, when mounted on the appropriate IR transmitting slide, can be used unstained for analysis of disease-specific molecular changes.

My team in Exeter have been collaborating recently on two European projects seeking to develop IR technologies to further maximize the technique’s potential for spectral histopathology. Rohit Bhargava of the University of Illinois at Urbana-Champaign, Wolfgang Petrich of the University of Heidelberg and pharmaceutical company Roche, in Frankfurt, Germany, and others, have shown the value of discrete frequency imaging with quantum cascade lasers (QCLs) as a method to speed up the collection of images at a set of specific wavelengths of interest (4,5).

We worked as part of the MINERVA consortium, a European Commission funded project that aims to develop photonic technology in the mid-IR to improve early cancer detection, to develop mid-IR supercontinuum sources with acousto-optical tunable filters for application in discrimination of malignancies in the colon (6). We are also working, as part of the Mid-TECH consortium, a consortium of academic and industrial partners in Europe to advance mid-IR spectroscopy, on a combination of tunable light sources (QCLs, or optical parametric oscillators [OPOs]), coupled with upcon-

version technologies, to enable detection of IR-converted light with silicon-based charge-coupled device (CCD) detectors, with the objective of simplifying systems and reducing costs by removing the need for cryogenic cooling of detectors (7).

Have there been any recent technological advances that have made mid-IR spectroscopy more viable as a technique in medicine?

Certainly, there are a number of recent developments that will revolutionize the use of IR for real-time microscopic imaging of biological specimens. As I mentioned previously, tunable laser light sources—QCLs, supercontinuum, or OPO systems—allow for significantly improved intensity and optimized high-resolution imaging, compared with global, thermal IR sources.

The major clinical benefit of discrete frequency imaging is that clinicians can view molecular images in real time, much as they would now, but with the ability to dial up the molecular “stain” of interest by changing the wavelength.

Furthermore, chalcogenide fibers for transmission of mid-IR light and upconversion imaging are likely to have a major impact on this field (8).

The issue of coherence can limit image quality, and a number of strategies have been developed to overcome this. One approach is simply to measure all of the light in a single detector, and raster the illumination point; that is, to map it rather than image it. This overcomes variations

in intensities measured at each pixel caused by speckle when using coherent sources to obtain wide-field images.

One of your recent collaborations involved the use of mid-infrared multispectral tissue imaging using a supercontinuum source. What was the aim of this project, and what were the benefits of using a chalcogenide fiber supercontinuum source?

The aim of this project was to provide a bright and tunable source as an alternative to QCLs. QCLs have been demonstrated to be most dominant at long wavelengths (5–12 μm), and following recent developments in the MINERVA project, supercontinuum sources are now dominant at shorter wavelengths (2–5 μm). However, the MINERVA consortium were able to deliver a supercontinuum source able to cover much of the fingerprint region of the spectrum (1000–1800 cm^{-1} or around 5–10 μm), albeit with low power at longer wavelengths (9). Further developments in this area are ongoing. One output from the project was a shorter wavelength supercontinuum source covering the high wavenumber region of the IR spectrum, and providing an intense source able to be used for a number of biomedical applications (10). Successful development of a broadband supercontinuum light source covering the fingerprint and high wavenumber region is likely to be much cheaper than the requirement for numerous QCLs to cover the same range. That said, a tunable filter is still needed, unless the supercontinuum source is used as the source for the Fourier-transform (FT) interferometer, which then removes the benefit of rapid discrete frequency imaging.

What were the main obstacles you had to overcome?

Technology! It is difficult to make chalcogenide fibers of sufficient quality. Angela Seddon's group in Nottingham were able to do this, and, with Ole Bang's group in DTU in Denmark, they were able to deliver the world's first supercontinuum light source in the fingerprint region (9). Christian Pederson and Peter Tideman-Lichtenberg's group, also at DTU, have been driving forward the upconversion detection instrumentation and methodology (11).

Coherence in wide-field imaging is also an issue that a number of groups have been seeking solutions for. As mentioned above, the simplest solution is to merge all the photons into one detector and remove the speckle problem, but this reduces other strengths of the approach for real-time imaging.

Could the approach using a chalcogenide fiber supercontinuum source be useful in other tissue imaging applications?

Yes. This approach is applicable to all biological tissues, although, to date, really only the high wavenumber region of the IR spectrum has had sufficient photons generated with a supercontinuum source to provide data of similar quality to an FT-IR instrument. Clinical diagnostics are possible in this wavelength range (12), but more subtle changes, such as early cancers, usually need the fingerprint data to improve the accuracy of discrimination.

You also used mid-IR for hyper-spectral imaging for label-free histopathology and cytology. What specific advantages does mid-IR offer for these applications?

As mentioned before, mid-IR spectroscopy allows molecular analysis of unstained tissues, rapid analysis, and, potentially, the automated prediction of disease, and analysts only need to view a single section for numerous molecular stains of interest (13). Cytology is trickier because of Mie scattering effects, but Achim Kohler and others have worked hard to overcome this problem with post-measurement corrections (14).

What were your main findings?

Discrete frequency imaging approaches and upconversion detection can be readily used to provide molecular distributions across tissue sections. There is still some way to go to ensure that these instruments can deliver the spectral quality or reproducibility of FT-IR instruments, but this does not appear to be an impossible task (15). Ongoing work is showing the viability of generating similar IR spectral histopathological images using various instrument configurations (16).

Are you planning to explore your research in mid-IR further?

Yes. Now that we have systems that can provide the signals we need, we will begin

to explore the clinical needs where this will be most useful, and seek to translate it to the clinic, if possible, for the benefit of patients.

In what specific area of clinical use do you think mid-IR spectroscopy has the greatest potential? Are there any misconceptions that are stopping mid-IR spectroscopy from being used in this field?

Linking mid-IR imaging with digital pathology is the next key step. Many experts in machine learning have been able to provide pretty good predictions of disease based on pattern recognition and two stains in an RGB image. A few more channels, albeit at lower spatial resolution, should provide much improved performance, particularly for the pathologies that are most difficult to reliably identify, such as dysplasias and early cancers.

The real power of vibrational spectroscopic measurements lies in their ability to measure the biochemistry of the tissues of interest; this is the downstream expression of any genetic mutation, and will provide us with information relating to the future outcome of that disease. We are at the birth of this field in one sense, even though IR and Raman have been around for decades. The real breakthroughs will come when tens to hundreds of thousands of patients' samples, with particular conditions and their outcomes, are measured, and machine learning approaches are used to extract the key prognostic markers. Then, and only then, will IR really be providing the significant added value that it has promised for so long.

References

- (1) M.J. Baker, H.J. Byrne, J. Chalmers, P. Gardner, R. Goodacre, A. Henderson, S.G. Kazarian, F.L. Martin, J. Moger, N. Stone, and J. Sulé-Suso *Analyst* **143**(8), 1735–1757, 2018.
- (2) M. Isabelle, N. Stone, H. Barr, M. Vipond, N. Shepherd, and K. Rogers, *J. Spectrosc.* **22** (2–3), 97–104, 2008.
- (3) J. Nallala, G.R. Lloyd, M. Hermes, N. Shepherd, and N. Stone, *Vib. Spectrosc.* **91**, 83–91, 2017.
- (4) K. Yeh, S. Kenkel, J.N. Liu, and R. Bhargava, *Anal Chem.* **87**(1):485–493 (2015 Jan 6). doi: 10.1021/ac5027513. Epub 2014 Dec 22.
- (5) N. Kröger, A. Egl, M. Engel, N. Gretz, K. Haase, I. Herpich, B. Kränzlin, S. Neudecker, A. Pucci, A. Schönhals, J. Vogt, and W. Petrich, *J. Biomed. Opt.* **19**, 111607 (2014).
- (6) MINERVA project, <http://minerva-project.eu/>.

- (7) Mid-TECH project, <http://www.midtech-itn.eu/>
- (8) L. Sójka, Z. Tang, D. Furniss, H. Sakr, E. Beres-Pawlik, A.B. Seddon, T.M. Benson, and S. Sujecki, *Opt. Quant. Electron.* **49**(21). (2017). doi: 10.1007/s11082-016-0827-0.
- (9) C.R. Petersen, U.V. Møller, I. Kubat, B. Zhou, S. Dupont, J. Ramsay, T Benson, S. Sujecki, N. Abdel-Moneim, Z. Tang, D. Furniss, A. Seddon, and O. Bang, *Nature Photonics*, **8**, 830–834 (2014).
- (10) C.R. Petersen, N. Prtljaga, M. Farries, J. Ward, B. Napier, G.R. Lloyd, J. Nallala, N. Stone, and O. Bang, *Optics Letters* **43**(5), 999–1002 (2018).
- (11) S. Junaid, J. Tomko, M.P. Semtsiv, J. Kischkat, W.T. Masselink, C. Pedersen, and P. Tidemand-Lichtenberg, *Optics express* **26**(3), 2203–2211 (2018).
- (12) G.R. Lloyd and N. Stone, *Appl. Spectrosc.* **69**(9), 1066–1073 (2015).
- (13) F Penaranda, V Naranjo, R Verdu-Monedero, GR Lloyd, J Nallala, and N. Stone, *Digital Signal Processing* **68**, 1–15 (2017).
- (14) T. Konevskikh, R. Lukacs, R. Blümel, A. Ponosova, and A. Kohlera *Faraday Discuss.* **187**, 235–257 (2016). Doi: 10.1039/C5FD00171D.
- (15) M. Hermes, R.B. Morrish, L. Huot, L. Meng, S. Junaid, J. Tomko, G.R. Lloyd, W. T. Masse-link, P. Tidemand-Lichtenberg, C. Pedersen, F. Palombo, and N. Stone, *J. Optics* **20**(2), 023002 (2018).
- (16) Y.P. Tseng, P. Bouzy, C. Pedersen, N. Stone, and P. Tidemand-Lichtenberg, *Biomed. Optics Express* **9**(10), 4979–4987 (2018).



Nick Stone

Nick Stone holds the position of professor of Biomedical Imaging and Biosensing and NHS Consultant Clinical Scientist at the University of Exeter. He recently led the Department of Physics and Astronomy for three years after holding the role of director of research. Stone has worked to pioneer the field of novel optical diagnostics within the clinical environment, moving from the NHS (Gloucestershire Hospitals), after almost 20 years of working closely at the clinical–academic–commercial interface to pull through novel technologies to be used where they have most clinical need. He is an internationally recognized leader in bio-

medical applications of vibrational spectroscopy (Raman and infrared).

Stone graduated with a BSc (Hons) from Bath University in Applied Physics with Industrial Training in 1992. Since then he has undertaken numerous studies which include an MSc with Distinction from Heriot-Watt and St Andrews Universities in Laser Engineering with Applications; an MSc with Distinction in Applied Radiation Physics with Medical Physics at Birmingham University; a PhD in the application of Raman spectroscopy for cancer diagnostics at Cranfield University and an MBA (Health Executive) at Keele University.

Stone has received numerous awards for his research both personally and within his research group. He recently won the International Raman Award for Most Innovative Technological Development 2014 and was the runner up in the 2013 NHS Innovation Challenge Prize. He won the Chief Scientific Officer's National R&D Award for 2009. He has published over 150 papers and book chapters.

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SPECTROSCOPY SPOTLIGHT

LIBS: FUNDAMENTALS, BENEFITS, AND ADVICE TO NEW USERS



Laser-induced breakdown spectroscopy (LIBS) has developed significantly in recent years, and adoption of the technique has increased at the same time. Yet many spectroscopists are still quite unfamiliar with the technique. In this interview, Mohamad Sabsabi of the National Research Council of Canada explains the fundamentals of the technique, its recent evolution, and its benefits and limitations, and offers advice for new users.

Laura Bush

Can you describe the concept of laser-induced breakdown spectroscopy (LIBS), for those who are new to the technique?

The reader can find the concept of LIBS described in almost any LIBS paper. Basically, everyone knows when we focus a laser beam on a sample, the irradiation in the focal volume leads to local heating of the material. When the irradiance of the laser pulse exceeds the threshold of material ablation ($> \text{MW}/\text{cm}^2$), there is vaporization and a hot ionized gas (called a plasma) is formed. In this plasma, atoms and ions are in excited states that emit light by radiative decay. Quantitative and qualitative analyses can be carried out by collecting and spectrally analyzing the plasma light and monitoring the spectral line emission positions and intensities. The technique based on that approach is called laser-induced breakdown spectroscopy (LIBS).

The LIBS technique is a form of atomic emission spectroscopy of plasma generated by a laser focused on the material to be analyzed. It is similar to other optical emission spectroscopy techniques based on plasmas, such as spark ablation, glow discharge, inductively coupled plasma, or arc plasma techniques. However, these techniques use an adjacent physical device (electrodes or a coil) to produce the plasma, whereas LIBS uses the laser-generated plasma as the hot vaporization, atomization, and excitation source. This gives LIBS the advantage that it can interrogate samples at a distance and analyze the material without contact, independent of the nature of the sample, thus making it suitable for in-the-field and real-time analysis of any type of material,

whether in the solid, liquid, slurry, or gas phase. The capabilities of LIBS to effectively carry out fast, in situ, real-time, and remote spectrochemical analysis with minimal sample preparation, and its potential applications to detect traces of a wide variety of materials, make it an extremely versatile analytical technique.

Can you tell about how LIBS has evolved?

These attributes of LIBS attracted the interest of spectroscopists, analytical chemists, and physicists since the invention of the laser in the 1960s; indeed, the first work on LIBS appeared in 1962. Since then, more than 13,700 papers have been published in the field of LIBS, covering both fundamentals and applications. Any literature will reveal the significant increase in the annual number of LIBS papers in recent decades, from a few in the 1960s to an annual rate of more than 900 today. And the field is still growing.

LIBS has been the subject of several books in recent years (1–7) and numerous review papers, and some of these document the history of the technique. In particular, I refer the reader to a chart provided in the introduction of the Cremers and Radziemski book (1) that illustrates the evolution of LIBS.

When we look at the development of the technique, we need to consider that the LIBS plasma is quite simple and yet complicated at the same time. You need a laser as a source of energy to generate the plasma. The plasma formed depends on the characteristics of the laser (energy, duration, focussing condition, wavelength,

beam quality), on the characteristics of the sample (thermal conduction, melting and vaporization temperature, and so on), and on the ambient atmosphere where it is created. To extract the information from the light emitted, you need a spectrometer to diffract the light, and a detector to convert photons to an electrical signal you can work with. It involves several fields of science, such as laser–matter interaction, plasma physics, atomic physics, plasma chemistry, spectroscopy, electro-optics, and signal processing. The LIBS plasma is transient (that is, it is space- and time-dependent), unlike an inductively coupled plasma, arc plasma, or glow discharge plasma, which are all stationary. This characteristic dictates some restrictions on the ability to transfer tools used with other emission spectroscopy techniques to LIBS. Therefore, the development of LIBS over the years has been closely tied to the development of enabling tools (such as pulsed lasers, detectors, and spectrometers) and ongoing improvements in their performance.

We can distinguish four periods in the development and use of LIBS over the last five decades. During the first period, prior to the 1990s, the plasma was generated by inadequate lasers, and the emission of the plasma was observed mostly time- and space-integrated, with the limited use of single channel photomultipliers (PMT) as detectors for time-resolved spectroscopy, so only limited analytical quantification was achievable.

Then, in the 1990s, the arrival of the intensified charge-coupled device (ICCD) detector after the Cold War made it possible

to observe time-resolved emission for several lines simultaneously in a given spectral window, rather than only one line as allowed by the single channel photomultiplier tube (PMT). This ability attracted some research groups to develop the understanding of the LIBS plasma and how it can be used for spectrochemistry. This development provided new capabilities for LIBS at the end of the 1990s and beginning of the 2000s, which allowed LIBS to address new emerging applications.

In addition, the echelle spectrometer coupled with an intensified charge-coupled device (ICCD) camera allowed time-resolved broadband spectra, and opened new ways to extract more information from the LIBS plasma. This capability was strengthened by the arrival of the Sony linear CCD array chip, which enabled the use of a low cost gated CCD camera. The combination of a gated CCD with low cost compact Czerny Turner spectrometers enabled a growth in the number of laboratories working on LIBS along with newcomers, and an increase of new applications that became feasible with the new capabilities. More importantly, it encouraged some LIBS spin off companies to enter the market.

In the third period, from 2000 to 2010, the first conference devoted to LIBS was held. It was organized in Pisa in 2000 by Vincenzo Palleschi's group. Since then, the series of LIBS International conferences has been organized every two years, alternating with the Euro-Mediterranean symposium conference (EMSLIBS), which was started in Cairo by Mohamed Abdel Harith's group in 2001. A similar LIBS symposium began in North America in 2007 and was organized by Jagdish Singh and Andrzej Miziolek. During that period, LIBS found its way across a variety of applications and disciplines in geology, metallurgy, planetary science, defense, food, environment, industry, mining, biology, automotive, materials science, aerospace, forensics, pharmaceuticals, security, and more. Also, more companies entered the market to commercialize LIBS systems.

In the last 10 years, the miniaturization of LIBS equipment has opened new opportunities to perform real time measurements and respond to emerging needs under conditions in which other spectroscopic techniques cannot be applied. In addition, the progress of laser technologies, such as the diode pumped laser and the fiber laser, with the improvement of the beam quality, led to better conditions for plasma generation and better analytical performance. Furthermore, the high repetition rate and the low cost of ownership of these devices have met the requirements of acceptance for several industrial applications in terms of speed of analysis and cost. Big players entered the market, and now offer handheld LIBS systems. Nowadays, as an example, the operating lifetime of a fiber laser is around 100,000 hours, or 11 years, of 24/7 use without any consumables, which is better than the TV in our houses. I remember, 26 years ago, in the first LIBS work we carried out in our laboratory at the National Research Council (NRC) of Canada, we were using an excimer laser to generate the plasma. During operation, you started with a certain energy per pulse in the morning and ended with half that energy at noon. At the same time, the solid YAG laser was bulky and very fragile, even for laboratory use.

To summarize, during the last three decades, extensive research has been carried out on the influence of the parameters affecting the analytical signal, to improve LIBS performance. Meanwhile, dynamic technological development in the field of solid state lasers, electro-optical detectors, and signal processing was successfully

harnessed for LIBS. The analytical performance of LIBS for a multi-element analysis now achieves a level that is equal to, or even better than, that of classical methods. LIBS is currently considered one of the most active research areas in the field of analytical spectroscopy.

Furthermore, in the last decade, we have been witnessing more newcomers in the LIBS field from different regions of the globe, in particular from China. An analysis of the literature during this period shows clearly that China has surpassed the United States as the largest contributor to the LIBS field in terms of the number of papers.

What benefits does LIBS offer over alternative spectroscopic techniques?

To answer that fairly, the right question would be: What are the attributes of LIBS that can offer an advantage over other spectroscopic techniques for a given application? In my opinion, all techniques are useful for what they are able to do and for their ability to achieve the expected analytical performances for their appropriate use. For instance, if you have a broken leg, you go to the hospital to see an orthopedist, not an eye doctor. Both types of doctors are needed, but for different types of problems. Following the same logic, the benefits of LIBS over alternative spectroscopic techniques stem from the fields of use. The analytical community should understand that logic. In my opinion, the time has come to accept LIBS as a technique among other spectroscopic techniques.

What are the features of LIBS that make it beneficial?

I already mentioned the key features of LIBS in a previous answer.



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In summary, LIBS can be applied on any type of material (conductive or not), independently of the nature of the sample or its phase (solid, liquid, or gas). It has the ability to interrogate a sample *in situ* and remotely. It needs a minimal to no sample preparation. It can analyze any element in the periodic table, regardless of its Z number (low or high). It ablates a minute mass of materials (in the ng to μg range). It is suitable for fast, online analysis.

The literature shows clearly that LIBS has emerged as a new member of the family of analytical methods, with strengths and weaknesses like other techniques. To make this clearer for the reader, I will highlight some general features by citing a few examples (not an exhaustive list), comparing the LIBS to conventional techniques.

Solid analysis: When you analyze non-conducting samples such as mineral ore, soil, drug, ash, plastic, or wood, LIBS has the advantage of analyzing light elements ($Z < 20$) that cannot be analyzed by X-ray fluorescence (XRF), whether with a portable system or in the laboratory. Furthermore, the sensitivity of LIBS is better than XRF using a handheld system, not only for light elements, but also for other elements such as gold and precious elements.

Analysis of metals: For the analysis of most solid metals, arc/spark spectroscopy is mostly used as the standard technique. LIBS and arc/spark spectroscopy have similar detection limits. However, LIBS is more suitable for fast analysis, whether for online or at line measurement, compared to arc/spark spectroscopy. In addition, LIBS can analyze a sample without preparation like cutting or polishing, and the laser can clean oxide or dust from the surface prior to analysis. LIBS can be used for depth profilometry with very high spatial depth resolution in the nanometer range, and also for microanalysis.

Analysis of liquids: Atomic absorption (AA) and ICP are standard techniques for the analysis of liquids. Both techniques have better relative detection limit than LIBS. However, if the need is for at line or online fast analysis, or for analysis in a harsh environment, LIBS has a clear advantage. Again, it has the advantage of eliminating the sampling step and avoids contamination.

Microanalysis: LIBS has a clear advantage for microanalysis because it operates in air at atmospheric conditions, so there is no

need for a vacuum. In addition, it provides fast analysis, thanks to the high repetition rate of the laser (which can be in the range of MHz for a fiber laser).

The analysis of molten metals: ICP and arc/spark techniques cannot be applied to the analysis of molten metals because of their offline character and their need for physical contact with the sample. LIBS offers unique capabilities for that purpose, and has come to be considered a standard technique for this application. Our laboratory developed and patented an approach for LIBS analysis of molten metals (8) that is being implemented worldwide.

For the analysis of particles or inclusions, LIBS has the advantages in terms of sensitivity and speed of analysis. ICP, XRF and glow discharge–optical emission spectroscopy are not suitable for particle detection, because of difficulties related to the handling and more importantly the small mass of the particles, which is well below their minimal absolute detection limits. LIBS is a very appropriate tool for this application because of its very low absolute limit of detection (LOD) (which is element-dependent but in the range of attograms to femtograms), and also the minute mass needed for analysis.

What are the limitations of LIBS?

For some analytical applications, LIBS has some limitations and inconveniences. Here are a few examples, not to be considered an exhaustive list.

Laboratory analysis of liquids for environmental applications: Because of the small bulk of the LIBS plasma (a few mm^3) and its transient nature, LIBS suffers from a relatively higher LOD compared to ICP or AA (which have stationary plasmas, with few cm^3 of volume). The LOD of LIBS is element dependent, but mostly in the ppm range, or even higher in the case of a portable system. This makes it ill suited to the analysis of liquids when a lower LOD is required, such as in environmental applications. Inductively coupled plasma optical emission spectroscopy (ICP-OES) and atomic absorption spectroscopy enjoy LODs that are three orders of magnitude better than that of LIBS for the analysis of liquids.

Laboratory analysis of solid samples: The probed bulk from a sample to be analyzed by XRF is much larger than the volume

ablated by LIBS during the same measurement time, which gives better sampling in XRF than in LIBS for some applications. This means that XRF is less dependent on sample homogeneity than LIBS. This statement should be taken with precaution, however. Someone would argue that this issue can be solved to some extent by using a laser with a high repetition rate, or by using a better sampling strategy. This is true for some cases, but not always feasible.

Analysis of mineral ore samples: For the analysis of precious metals in mineral ore, the analysis that can be done by LIBS is of the surface, which is not always representative of the bulk material. AA, in turn, requires samples to be digested; although digestion is a lengthy and time-consuming process, it helps ensure representative sampling. The surface analysis issue is not limited to LIBS, however. Other analytical techniques, such as XRF, infrared and Raman spectroscopy, also analyze only the surface of a sample rather than the bulk material.

Laser safety for standoff analysis: Given the ability of LIBS to interrogate a sample remotely, there are also concerns related to laser safety in an open path beam. The pulsed laser used for LIBS is considered a Class IV device, and special care should be taken in some applications to avoid exposure of the eyes to the laser beam or even its reflection or diffracted light. Sometimes, this can be done by restricting access to the area, by wearing goggles that do not transmit the laser beam, or by containing the laser beam, thus shifting the device classification from IV to I.

What are the most common application areas for this technique?

Clearly, there has been much progress in the application of LIBS. Based on the literature, we can confirm that LIBS is gaining acceptance in many industrial applications, and continues to receive significant research emphasis around the world. And we increasingly see LIBS units in corporate laboratories, and even controlling industrial processes.

LIBS has been used in many areas such as geology, ecology, geochemistry, forensics, pharmaceuticals, semiconductors, consumer electronics, metallurgy, mining, photovoltaics, metallurgy, planetary science, defense, food, the environment, industry,

mining, biology, automotive, materials science, aerospace, forensics, pharmaceuticals, security, and battery applications, where consistent payback has been found using this technology. In addition, there are new areas for standoff measurements and real-time continuous process monitoring in industry, such as in raw-material or product screening for impurities and contamination, where LIBS has been implemented.

Do you have any advice for analysts using LIBS for the first time?

There is still room for newcomers to improve the analytical performance of LIBS in resolving new challenges and to explore new applications. In particular, the advent of new enabling tools (laser, detector and spectrometer) will promise bright future for reaching new areas not studied yet. Here are some recommendations for newcomers to LIBS:

1) Attend tutorial courses given at LIBS conferences to know the state of the art.

2) Remember that it is very easy to generate the plasma, but it is very complicated to make it useful for spectrochemistry.

3) For the analytical chemist and the use of chemometrics: the LIBS spectrum is very rich, and there is a lot of useful information hidden in the spectrum. Prior to any chemometrics work, check the accuracy of your experimental data, and understand their experimental conditions. Remember that we correlate the spectrum taken from the laser-generated plasmas to the sample by assuming the plasma composition is representative of the sample. This composition varies with the conditions of creating the plasmas. That condition is valid only if you control the plasma generation in a reproducible way from shot to shot. This assumes the same ablation process of creating reproducible plasma and excitation. If we put garbage data in, then the model will provide garbage data out.

4) Newcomers should be careful in using the phrase "It has been done for the first time." They should look not only at recent publications but also at the old literature. There is no excuse for newcomers, and also not for editors, for insufficient literature searches. In the patent domain, a complete search is a normal practice used by a patent agent or examiner to determine for patentability. Why can it not be done properly for papers?

5) LIBS companies should be working on solutions tailored toward the needs of users, and make it easy for them to implement the technology and use it in their day-to-day work. Not all users can afford to hire a PhD to develop a method and run a system. Users need a fast, practical solution and not a system that is so difficult to use that it ends up being shelved, thus hindering further adoption of the technique.

8) LIBS R&D institutions should focus on programs responding to both the short and long term questions where the technology can have a real impact, and avoid reinventing the wheel.

9) As LIBS continues to mature, manufacturers will go through similar learning curves to what we saw with XRF and OES technologies. Mobile OES and handheld XRF analyzers have been around for decades, and the core components have vastly improved in performance and accuracy.

What is your outlook for LIBS?

The principle of operation of LIBS is quite simple, although the physical processes involved in the laser-matter interaction are complex and still not completely understood. LIBS remains an evolving technology, as optics and photonics researchers seek new ways to take advantage of its strength and to overcome some of its challenges. In my opinion, the LIBS field is becoming crowded but not saturated yet.

In particular, as the need for quick and on-the-spot analysis is increasing, the adoption of portable and handheld instruments is gaining momentum. That importance of such instruments is due to their ability to support online analysis of samples where it is difficult to carry benchtop instruments. Their key application areas include drug identification, food inspection, environmental applications, metallurgy, and the defense sector. Portable instruments are gaining more importance especially in the food and healthcare industries. Higher growth is expected in many regions of the world, where the need for safety in drugs, food, and environmental health is increasing. Portable instruments do not require the use of reagents, do not produce analytical waste, are fast and allow on-the-spot analysis, and have increasing features and functionality. Thus, portable instruments are good candidates to respond to the growing needs for in-situ analysis and they also con-

tribute to keeping the environment green. The portability aspect of the LIBS devices constitutes a major asset of this evolving technology. However, the level of portability needed for some applications imposes some restrictions on the choice of many of the core components used in a low cost LIBS handheld sensor unit.

References

- (1) D.A. Cremers and L.J. Radziemski, *Handbook of Laser-induced Breakdown Spectroscopy* (Wiley, Chichester, West Sussex, UK, 2006).
- (2) A.W. Miziolek, V. Palleschi, and I. Schechter, Eds., *Laser Induced Breakdown Spectroscopy (LIBS): Fundamentals and Applications* (Cambridge University Press, Cambridge, UK, 2006).
- (3) J.P. Singh and S.N. Thakur, Eds., *Laser-Induced Breakdown Spectroscopy* (Elsevier, Oxford, UK, 2007).
- (4) Y.-I. Lee and J. Sneddon, *Laser Induced Breakdown Spectroscopy: A Practical and Tutorial Approach* (Taylor & Francis, Abingdon, Oxfordshire, UK, 2016).
- (5) R. Noll, *Laser Induced Breakdown Spectroscopy: Fundamentals and Applications* (Springer, Heidelberg, Germany, 2012).
- (6) M. Baudelet, *Laser-Induced Breakdown Spectroscopy: A Fundamental Approach for Quantitative Analysis* (Momentum Press, Buchanan, NY, 2014).
- (7) S. Musazzi, U. Perini, Eds., *Laser-Induced Breakdown Spectroscopy: Theory and Applications* (Springer, Heidelberg, Germany, 2012).
- (8) J.M. Lucas, M. Sabsabi, and R. Heon, Method and apparatus for molten material analysis by laser induced breakdown spectroscopy. Filed in 2002, US patent 6909505B2, 2005.

Mohamad Sabsabi is a principal research officer at the National Research Council (NRC) of Canada, which he joined in 1992. Sabsabi and his team have pioneered and implemented LIBS technology for many applications. He initiated and led, for four years, the NRC High Efficiency Mining (HEM) program to improve the mining value chain by developing advanced sensors, process technologies, and advanced materials. He holds 18 patents and has more than 500 publications (papers and conference presentations) covering fundamental aspects and industrial applications of laser-induced plasmas.



Mohamad Sabsabi

Calendar of Events

May 2019

28–30 North American Workshop on Laser Ablation 2019

Austin, TX
www.jsg.utexas.edu/nawla2019/

30–June 1 American Chemical Society Middle Atlantic Regional Meeting

Baltimore, MD
<https://www.acs.org/content/acs/en/meetings/regional/middle-atlantic>

June 2019

2–6 ASMS 2019

Atlanta, GA
<https://www.asms.org>

4–7 American Chemical Society Central Regional Meeting

Midland, MI
<https://www.acs.org/content/acs/en/meetings/regional/central>

11–13 American Chemical Society Green Chemistry & Engineering Conference & International Conference on Green and Sustainable Chemistry

Reston, VA
<http://www.gcande.org>

23–27 SPIE European Conference on Biomedical Optics

Munich, Germany
<http://www.spie.org/conferences-and-exhibitions/european-conferences-on-biomedical-optics?SSO=1>

24–27 Laser World of Photonics

Munich, Germany
<https://world-of-photonics.com/index-2.html>

July 2019

21–24 2019 NACRW

Naples, FL
www.nacrw.org

August 2019

4–8 71st American Association for Clinical Chemistry (AACC) Annual Scientific Meeting & Clinical Lab Expo

Anaheim, CA
<http://www.2019aacc.org/>

5–9 2019 National Environmental Monitoring Conference (NEMC)

Jacksonville, FL
<http://www.nemc.us>

5–9 Denver X-ray Conference

Lombard, IL
www.dxcicdd.com

11–15 SPIE Optics + Photonics

San Diego, CA
<http://spie.org/conferences-and-exhibitions/optics-and-photonics>

25–29 American Chemical Society Fall 2019 National Meeting & Expo

San Diego, CA
<https://www.acs.org/content/acs/en/meetings/national-meeting>

September 2019

8–13 COLA 2019: The 15th International Conference on Laser Ablation

Maui, HI
<https://cola2019.org/>

24–26 Spectro Expo

Amsterdam, The Netherlands
www.spectroexpo.com

October 2019

8–11 ACIL 2019 Annual Meeting

Nashville, TN
www.acil.org

13–18 SciX Conference

Palm Springs, CA
www.scixconference.org

15–16 Gulf Coast Conference

Galveston, TX
www.gulfcoastconference.com

16–19 American Chemical Society Midwest Regional Meeting

Wichita, KS
<https://www.acs.org/content/acs/en/meetings/regional/midwest.html>

November 2019

3–6 American Association of Pharmaceutical Scientists 2019 PHARMSCI 360

San Antonio, TX
<https://www.aaps.org/pharmsci/annual-meeting>

8–20 Eastern Analytical Symposium and Exhibition

Princeton, NJ
eas.org

December 2019

1–6 2019 Materials Research Society Fall Meeting and Exhibit

Boston, MA
www.mrs.org/fall2019

March 2020

1–5 Pittcon Conference & Expo 2020

Chicago, IL
<https://pittcon.org>

PRODUCTS & RESOURCES

ICP-OES analyzer

Spectro's SPECTROGREEN inductively-coupled plasma-optical emission spectrometry (ICP-OES) analyzer is designed with Dual Side-On Interface technology that uses a vertical plasma torch, observed via a new variety of direct radial-view technology. According to the company, two optical interfaces capture emitted light from both sides of the plasma, using a single extra reflection for added sensitivity.

Spectro Analytical Instruments GmbH, Kleve, Germany.
www.spectro.com



Handheld Raman analyzer

The NanoRam-1064 handheld Raman analyzer from B&W Tek is designed for nondestructive identification of raw materials. According to the company, the analyzer is paired with 21 CFR Part 11 compliant software, and can identify samples as it minimizes fluorescence, making it a suitable tool for the analysis of colored samples, natural products, and to differentiate between different grades of cellulose, polysorbate, and Opadyr.

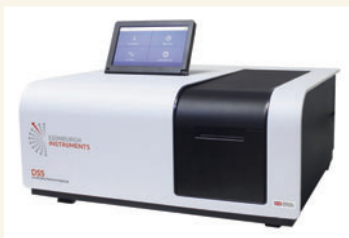
B&W Tek, Newark, DE.
www.bwtek.com



Dual-Beam UV-vis spectrophotometer

The DS5 UV-vis spectrophotometer from Edinburgh Instruments is designed with a dual lamp and Czerny-turner configuration monochromator. According to the company, the spectrophotometer features a compact high-throughput optical system, low stray light, and baseline flatness, with wavelength and photometric accuracy and reproducibility.

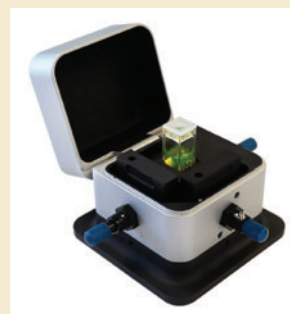
Edinburgh Instruments, Livingston, UK.
www.edinst.com



Cuvette holder

The Square One cuvette holder from Ocean Optics is designed for accurate, repeatable absorbance and fluorescence measurements. According to the company, the cuvette holder has three collimating lenses with fiber optic connectors, and an integrated cover to reduce ambient light.

Ocean Optics, Largo, FL.
www.oceanoptics.com/product/square-one-cuvette-holder



Pharmaceutical analyzer application note

An application note from Renishaw describes how its RA802 pharmaceutical analyzer can be used to screen a range of pharmaceutical samples for the presence and crystalline form of active pharmaceutical ingredients (APIs). According to the company, the note explains how determining the API forms present at various stages in the manufacturing process can be used to understand polymorphic conversion under different storage conditions.

Renishaw Inc., West Dundee, IL.
www.renishaw.com/raman



Handheld Raman analyzer

Rigaku's ResQ CQL 1064-nm handheld Raman analyzer is designed for chemical threat identification. According to the company, the unit's ergonomics, analytical performance, and sample presentation enable identification of suspicious powders, liquids, gels, and mixtures.

Rigaku Corporation, Austin, TX.
www.rigaku.com



X-ray window

The BX-1 X-ray window from Moxtek is designed to withstand temperatures up to 200 °C. According to the company, the window is suitable for applications requiring high transmission of low energy X-rays, is constructed entirely out of low-Z materials, and has improved helium permeability performance and temperature tolerance.

Moxtek, Inc., Orem, UT.
www.moxtek.com



Temperature-controlled spectroscopy accessories

The temperature controller from PIKE technologies is designed to provide temperature control and responsive ramping for the company's heated and cooled accessories. According to the company, the controller is NRTL, CE, and RoHS certified, and features a small footprint and user-settable ramp rate or PID control.

PIKE Technologies, Madison, WI.
www.piketech.com



Sample imaging tool

ParticleScout from WITec is an imaging tool designed to identify, quantify, classify, and analyze particles in a sample. According to the company, the tool enables measurements that move from an overview survey through targeted investigations of particles grouped by attributes to the precise chemical characterization of individual particles.



WITec GmbH,
Ulm, Germany.
www.witec.de

Raman spectrometer

The Mira P Raman spectrometer from Metrohm is designed for material variation in regulated industries. According to the company, the spectrometer is barely larger than a smartphone, and provides results in seconds.



Metrohm USA,
Riverview, FL.
www.metrohmusa.com

Certified reference material

Certified reference material from Starna reportedly provides qualification data, even below 200 nm. According to the company, its TS8 reference material was developed with suitable spectral characteristics in the region of 190–230 nm, and the TS8 solution and a solvent blank are supplied in an identically matched pair of 10 mm far UV quartz cells.



Starna Cells, Inc.,
Atascadero, CA.
www.starnacells.com

Portable Raman spectroscopy system

The portable StellarCASE-Raman system from StellarNet is designed for material identification and composition analysis using Raman spectroscopy, and is suitable for applications in forensics, anticounterfeiting, and enhanced Raman spectroscopy. According to the company, the system uses free SpectraWiz ID software to build and search a library for any application.



StellarNet, Inc.,
Tampa, FL.
www.StellarNet.us

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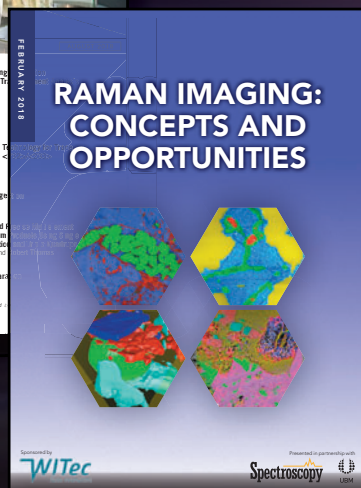
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