PITTCON® CONFERENCE & EXPO 2016

JAIMA Japan Analytical Instruments Manufacturers' Association

EXTENDED ABSTRACT

JAPAN SYMPOSIUM

March8(Tue.)2016

The State-of-the-Art Technologies from Japan: Analytical Instruments with / for Nano-Chemistry Technology and Advanced Diagnostics

Pittcon 2016 Symposium

Japan Symposium 2016

“The State-of-the-Art Technologies from Japan: Analytical Instruments with/for Nano-Chemistry Technology and Advanced Diagnostics”

Date: March 8, 2016
Place: Georgia World Congress Center,
Atlanta, GA USA

Organizer:

Japan Analytical Instruments Manufacturers’ Association (JAIMA)
&
The Japan Society for Analytical Chemistry (JSAC)

A Message from the President of JAIMA

The Japan Analytical Instruments Manufacturers' Association(JAIMA) is honored to hold the Japan Symposium at PITTCON 2016 also this year. This is the eighth symposium that we have organized at the Pittsburg Conference in collaboration with the Pittcon Committee. Globalization is one of the most important objectives of JAIMA nowadays, and the importance of this program is becoming greater for JAIMA.
I believe this symposium surely provides the opportunity to exchange information of the state-of-the-art technologies related to analytical instruments at an international level.
This year's symposium will focus on "Nano-chemistry and Advanced Diagnosis". I am sure that the analytical instruments will play important roles also in this field. A lot is expected with Advanced Diagnosis with/for various new analytical technologies as it will help maintain "health" of human society. I hope this symposium will point towards new possibilities of analytical instruments and technology, and I would like to sincerely thank all of the researchers and people responsible for organizing it.

Gon-emon Kurihara
President of
Japan Analytical Instruments Manufacturers' Association

A Message from the President of JSAC

It is a great pleasure for me to write a message to the Japan Symposium 2016 held in PITTCON 2016, Atlanta. I would like to praise tribute to the preceding successful Japan Symposia organized under the cooperation of JSAC and JAIMA, and this year both organizations are hosting the symposium "The
State-of-the-Art Technologies from Japan: Analytical Instruments with/for Nano-Chemistry Technology and Advanced Diagnosis (I) and (II)". The symposium will attract much interest, because recent rapidly accelerating progress in science and technology has evoked developments of more and more sophisticated analytical methodologies in various frontiers of environmental science, materials science, pharmaceuticals, foods, clinics, forensic science, in addition to physics, chemistry, biology, and geology.

The Japan Society for Analytical Chemistry has made continuous fruitful relationship with JSAC and JAIMA and I hope the Japan Symposium will make continuous and significant worldwide contributions to analytical chemistry and its related sciences and technologies.

Koji Suzuki, Ph.D.
President of
The Japan Society for Analytical Chemistry (JSAC)

A Message from the Pittcon President

The Pittsburgh Conference is most pleased to continue its ongoing collaboration with JAIMA and JSAC and was proud to present the USA Symposium “Chemical Spectroscopic Imaging: New Ways to Understand Our World” at JASIS 2015.

Pittcon looks forward to hosting the Japanese Society for Analytical Chemistry and the Japan Analytical Instrument Manufacturers’ Association for the presentation of the Japanese Symposium “The State-of-the-Art Technologies from Japan: Analytical Instruments with/for Nano-Chemistry Technology and Advanced Diagnosis” at Pittcon 2016 in March, 2016 in Atlanta, Georgia.

Our thanks to Gon-emon Kurihara, President of JAIMA, Koji Suzuki, President of JSAC, and Koichiro Matsuda, Chairman of the Joint Committee for the Pittcon 2016 JAIMA, JSAC Symposium for bringing this symposium to Pittcon 2016.

This close interaction between our two groups provides a very valuable synergy for the international exchange of scientific information and technology and the ability to network with experts from around the globe.

Dr. William R. Sharpe,
President of Pittcon 2016

A Message from the Pittcon 2016 Program Chairman

We are most honored that Pittcon is host to the 8th JAIMA/ JSAC (Japan Analytical Instruments Manufacturers' Association/ The Japan Society for Analytical Chemistry) Symposium: “The State-of-the-Art Technologies from Japan: Analytical Instruments with/for Nano-Chemistry Technology and Advanced Diagnosis (I & II).” The presentations in this all-day session highlight the cutting-edge work of some of the leading researchers from both Japan and from several of the major Japanese analytical instrument companies. All of the presentations promise to inform and inspire the attendees and hopefully open new avenues to advance their own research. Pittcon 2016 is proud to be the venue for this Symposium and is looking forward to its ongoing relationship with JAIMA and JSAC as our three organizations continue to provide world-class venues for the dissemination of the results of analytical research and scientific endeavor.

Chuck Gardner, Ph.D., PMP
Technical Program Chairman, Pittcon 2016

Contents

Creation of Bio/Chemical Sensing Probes

Koji Suzuki, PhD, Professor

Department of Applied Chemistry,
Fac. of Sci. & Tech., Keio University

Abstract

The creation of original chemical sensors is one of the main concerns in our research group. In biological research fields, chemical sensors such as synthetic biochemical probes play an important role in the investigation of biological functions, and in cellular or in vivo imaging.

One of our recent research highlights was the creation of a magnesium-imaging probe (KMG series fluorescent molecules) for living cells. The KMG-series molecules for magnesium (Mg) imaging were designed and synthesized based on our prior knowledge of Mg ionophore design [1]. While the first example (KMG-20) was based on a coumarin fluorophore [2], we later developed the fluorescein derivative KMG-104, which is the best magnesium fluorescent probe for imaging applications in the cytoplasm of living cells [3] and also developed KCM-1 for simultaneous imaging of Mg and Ca ions in living cells [4]. More recently, a flash-type Mg fluorescent probe was developed for specific protein labeling [5].

To obtain bright fluorescent probes, we have developed a set of fluorescent dyes (BODIPY-based KFL series dyes), which have excellent optical properties like sharp fluorescence spectra with high quantum yields, and moreover, the wavelength is finely tunable over a wide spectral range including the NIR region by introducing proper electron-donating groups into the furan moieties of the chromophore [6-8]. By linking an ion recognizing ionophore with a fluorescent dye as a transducer, a chemical probe (fluorescent probe) is obtained, transforming a simple molecular recognition ligand into a sensor ligand. For instance, a KFL fluorescent dye combined with a BAPTA chelating group resulted in a bright fluorescent probe for calcium ions [9]. We also have developed a set of chemiluminescent (CL) dyes (BODIPY-based KCL and KBI series dyes) with excellent CL properties. The luciferin-based CL probe KBI is a useful probe for highly sensitive detection of ROS such as $O^{2-\cdot}$ [10].

Several bioluminescent (BL) systems have been investigating based on synthetic coelenterazine (CTZ) derivatives as substrates in combination with Renilla luciferase (Rluc) variants or artificial luciferases (Aluc) as the enzyme. It was found that extending the conjugated system at the 6-carbon position of CTZ is more effective compared to extensions at the 2-, 5-, or 8-carbon positions. The 6-position carbon variants of CTZ combined with the known Rluc mutant Rluc8.6 resulted in the most intense bioluminescence in the blue spectral region [11]. In addition, with the system consisting of a CTZ derivative and the artificial luciferase Aluc, we have succeeded in the development of the most high-intensity artificial bioluminescence system [12].

References

[1] ***Anal. Chem.***, **67**, 324-334 (1995), [2] ***Anal. Chem.***, **74**, 1423-1428 (2002), [3] ***J. Am. Chem. Soc.***, **126**, 16353-16360 (2004), [4] ***J. Am. Chem. Soc.***, **127**, 10798-10799 (2005), [5] ***J. Am. Chem. Soc.***, **136**, 2374–2381 (2014), [6] ***J. Am. Chem. Soc.***, **130**, 1550–1551 (2008), [7] ***Chem. Eur. J.***, **15**, 1096-1106 (2009), [8] ***Anal. Sci.***, **30**, 327-349 (2014) (Special Review), [9] ***Chem. Commun.***, **47**, 10407-10409 (2011), [10] ***Chem. Commun.***, **21**, 3047-3049 (2009), [11] ***Chem. Commun.***, **51**, 391-394 (2015), [12] ***Bioconjugate Chem***. Article ASAP, DOI: 10.1021/acs.bioconjchem.5b00421.

Biographical Sketch

Koji Suzuki, Professor of Department of Applied Chemistry, Faculty of Science and Technology, Keio University, was born in 1954 in Tokyo. He received Bachelor (1977), Master (1979) and Ph. D. (1982) degrees of Engineering from Keio University. He became a faculty member of Keio University in 1982, Associate Professor in 1993, and full Professor in 1998. From 1990 − 1992, he was a Guest Professor at the Swiss Federal Institute of Technology (ETH), Zurich. He is now the President of the Japan Society for Analytical Chemistry (JSAC), the Chair of Analytical Division of the 116th Committee for Chemistry of Functional Organic Chemicals at The Japan Society for the Promotion of Science (JSPS), and the Cooperative Member of the Science Council of Japan (SCJ, Cabinet Office) and the Chair of Analytical Chemistry Division of SCJ. He received many awards including The Chemical Society of Japan Award for Creative Work in 1997, The Hitachi Environment Foundation Environment Prize in 2005, The JSAC Award in 2007, and The JASC-JAIMA Advanced Analytical Technology Award in 2009. His research focuses on chemical and biochemical sensors based on functional molecule creation. He is also interested in seeing, listening to, or eating what is impressing.

Ab initio powder structure determination opening up new research fields

Masaki Kawano, PhD, Professor
Department of Chemistry, Tokyo Institute of Technology

Abstract

Powder diffraction technique has been widely used for qualitative analysis over half a century. In sharp contrast, the history of ab initio powder structure analysis is quite short especially in soft materials fields. I will introduce the applications of ab initio powder structure analysis for exploring a new class of materials and science. One of representative methods for structure analysis is single crystal structure analysis. However, in reality there are a number of crystalline powder materials rather than single crystals in industrial fields. Recently we developed the preparation method of interactive porous materials using kinetic/thermodynamic control. Construction of interactive pores is a key to production of functional porous materials. In this talk, I will introduce interactive porous materials, the applications, and new science unveiled by crystallography.

Biographical Sketch

Dr. Masaki Kawano has been Professor of Department of Chemistry, Graduate School of Science and Engineering, Tokyo Institute of Technology since Sept. 2015. He received his Ph.D. in Coordination Chemistry at Waseda University in 1993. He became Assistant Professor at the same school (1992-1994). He joined as a postdoctoral fellow at the department of Chemistry, University of Wisconsin-Madison (1994-97) and moved to Tokyo Institute of Technology (1997-2003) to join as a postdoctoral fellow in CREST. Then he moved to the University of Tokyo as a Lecturer (2003-04) where he was promoted to Associate Professor. He moved to Pohang University of Science and Technology as Professor of Division of Advanced Materials Science (2009-2015). He was awarded The CSJ Award for Creative Work in Inorganic Chemistry (2009), Chemistry Innovation UT GCOE Lectureship (2008), The UK-JAPAN researchers exchange program (2005), The Lectureship Award of Young Generation Special Forum from the chemical society of Japan (2002). His research interests include Coordination chemistry, Supramolecular chemistry, In situ chemical crystallography, Crystalline-state photochemistry, ab initio powder X-ray crystallography.

Introduction

Coordination polymer has been actively investigated in the past quarter century because of programmability of architecture based on self-assembly of metal ions with bridging ligands. The predictability of self-assembled structures is attributed to directionality of coordination bonds. During self-assembly, a global minimum can be achieved by repetition of coordination bond formation and the cleavage. Using thermodynamic conditions, a number of thermally stable coordination polymers were prepared. However, unpredictable products were often obtained as kinetic products. Because of thermal stability of thermodynamic structures and the predictability,

most of researchers studied thermodynamic products for various applications. Another reason is difficulty of structure determination of kinetic products which are often obtained as microcrystalline powder. The powder structure analysis can be a high hurdle for kinetic network study, because the materials are relatively fragile and the unit cell volume often can be over 10,000 $Å^3$. The fragile nature of materials prevents one from grinding sample to prepare uniform powder sample. The large unit cell causes severe overlapping of diffraction peaks. We overcame these difficulties by developing the sample preparation method, *instant synthesis*, and using synchrotron radiation.[1] Kinetic products have a potential for opening up new fields in coordination polymer science, because kinetic products have more interactive pores than thermodynamic ones.

Discussion

The energy landscape of self-assembly of metal ions and bridging ligands shows many local minima of potential wells before the global minimum is achieved. The local minimum state can be stabilized by various weak intermolecular interactions. Our first finding of selective kinetic network formation was the reaction of $ZnBr_2$ with TPT (2,4,6-tris(4-pyridyl)triazine) in nitrobenzene/methanol.[1] Even though using the same starting materials and solvent, depending on the reaction time (one week vs. 30 sec), we prepared two types of porous network structures selectively in >50 % yields (Fig. 1) which have the same chemical formula, $[(ZnBr_2)_3(TPT)_2]\cdot$(solvent). Although these products are coordination polymer, they can be considered as polymorph. Because of the infinite structure, the local minimum potential wells during self-assembly are much deeper than those of molecular crystals. Therefore, we could readily trap the kinetic network.

We made a strategy for preparing kinetic networks. The structural comparison of the two porous networks described above indicates the following features of a kinetic network: 1) it has fewer intermolecular interactions within a framework; 2) it is apt to have a larger void and 3) more interactive sites in a pore, as compared with a thermally more stable network. The feature of 3) is a natural consequence of 1), which is important in that it can be a basic strategy for forming an interactive pore without using elaborative methods. Although a kinetic process can be intractable, we attempted to trap kinetic networks using multi-interactive ligands.

Design of a multi-interactive ligand

Introduction of interactive sites into ligands can deepen a local minimum potential well by intermolecular interactions in self-assembly. Therefore, we designed a tridentate ligand with radially-extended mode based on the hexaazaphenalene skeleton, TPHAP (2,5,8-tri(4-pyridyl)-1,3,4,6,7,9-hexaazaphenalene).[2] The potassium salt of TPHAP ($K^+\cdot TPHAP^-$) can be synthesized on gram scale by a simple one-pot reaction. The TPHAP anion has the following features: a) a large aromatic plane for π-π interaction, b) nine nitrogen atoms for H-bonds or coordination bond formation, c) a delocalized negative charge over the central skeleton for a charge transfer interaction with guest molecules, d) remarkable thermal stability of a single crystal of $K^+\cdot TPHAP^-$ up to 500 ºC under a nitrogen atmosphere. As expected, TPHAP formed various metastable networks from the same starting materials (Fig. 2).[3a] Furthermore, unexpectedly, we found ion conductive property of $K^+\cdot TPHAP^-$ solid under hydration. Depending on relative humidity, the ion conductivity drastically changed. Powder structure analysis revealed that the ion conductive phase formed 1.5 nm water layer structure. The multi-interactivity of TPHAP plays a crucial role in forming the water layer component.[3b]

Fig. 1. thermodynamic/kinetic assembly

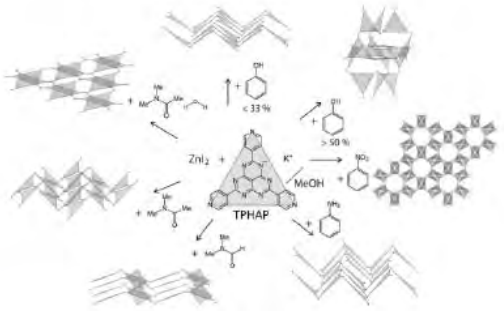


Fig. 2. Various network composed of TPHAP and ZnI_2

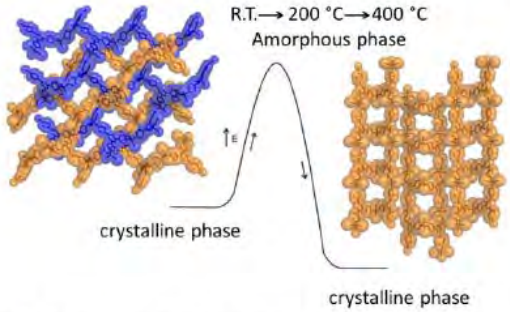


Fig. 3. From kinetic to thermodynamic network

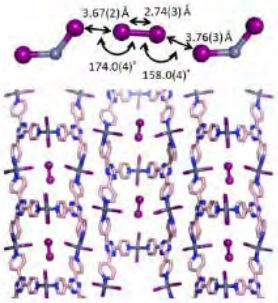


Fig. 4. Iodine encapsulating network

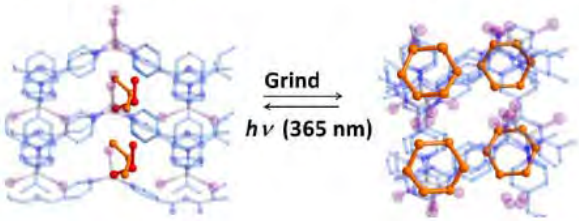


Fig. 5. Sulfur encapsulating network

Thermally stable network formation from kinetic network

Some thermally stable materials can be prepared through only kinetic intermediate by controlling the reaction conditions.[4] Interpenetrating network, $[(ZnI_2)_3(TPT)_2]\cdot$(solvent),[5] is metastable which can be prepared as fine powder by instant synthesis from ZnI_2 and TPT in nitrobenzene/methanol. When the powder of the interpenetrating network was heated, amorphous phase was generated at 200 °C. Further heating at 300 °C generated a new crystalline phase which is a saddle type of porous network (Fig. 3). The powder structure was solved by ab initio XRPD analysis. The saddle structure is remarkably stable up to 400 °C. Noteworthy is that the saddle structure cannot be prepared by grinding and heating the mixed powder of ZnI_2 and TPT. Only through the interpenetrating structure, it can be prepared, indicating that preorganization of ZnI_2 and TPT is essential to obtain the saddle structure in the solid state reaction.

The saddle structure has a pore which can encapsulate small molecules, *e.g.* nitrobenzene, cyclohexane, and I_2. All guest encapsulating network structures were solved by ab initio XRPD analysis.[6] Particularly, the I_2 encapsulation is intriguing in that I···I interactions between ZnI_2 and I_2 play a crucial role in the I_2 encapsulation (I···I_2 distance and angle: 3.67(2) Å, 178.0(4)° and 3.76(3) Å, 158.0(4)°; I-I bond length, 2.74(3) Å, Fig. 4). The geometry indicates the typical halogen-halogen interaction between positive (σ-hole) and negative sites (unshared electron).

Recently we revealed that the iodide of the saddle structure can be replaced with bromide by bromine oxidation. It should be noted that the brominated saddle structure can be prepared only through the oxidation of the iodinated saddle structure but not through the starting reagents of $ZnBr_2$ and TPT.[7]

Molecular crystalline flask: direct observation of a reactive sulfur allotrope in a pore

The advantage of crystalline porous networks can be nano-scale molecular flasks[8,9] which allow in situ crystallographic monitoring of guest encapsulation,[10] reactive species,[11] chemical reactions,[12] and so on in a pore. We attempted to trap reactive small sulfur allotropes into a pore of the saddle structure crystal via vapor diffusion at 260 °C for 6 h under vacuum. The ab initio XRPD analysis revealed that S_3 is selectively trapped in a pore (Fig. 5). This is the first crystal structure determination of a reactive sulfur allotrope.

Although S_3 is ozone analog, S_3 in a pore can be stabilized by intermolecular interactions with the iodide in a pore. S_3 can stay in a pore until around 230 °C. From the above I_2 encapsulation study, the iodide of ZnI_2 in the saddle structure can be considered as an interactive site. Furthermore, S_3 can be converted into S_6 by grinding at room temperature or heating in the presence of NH_4X (X = Cl, Br). S_6 can be reversibly converted into S_3 by UV irradiation.

Conclusions

Kinetic assembly can generate more interactive and larger pores than can thermodynamic assembly. Using a rigid T_d symmetry ligand TPPM (Tetra(4-(4-pyridyl)phenyl)methane) and kinetically labile CuI unit, recently we succeeded in selective preparation of thermodynamic and kinetic porous networks having an interactive pore which show unique physi- and chemisorption of I_2, respectively.[13] Our findings promise that interactive crystalline pores can provide unprecedented opportunity to adsorb unique guest molecules and observe chemical reactions stepwise by crystallographic technique.

Acknowledgements

The author acknowledges funding from the Veteran researcher grant (No. 2014R1A2A1A11049978), the framework of international cooperation program (No. 2014K2A2A4001500) and the BK21-Plus Grant to Pohang University of Science and Technology managed by National Research Foundation of Korea (NRF) funded by the Ministry of Education of Korea.

References

(1) Kawano, M. *et al. Angew. Chem. Int. Ed.* **2008**, 47, 1269.
(2) Yakiyama, Y. *et al. Chem. Commun.* **2012**, 48, 10651.
(3) a) Kojima, T. *et al. CrystEngComm.* **2014**, 16, 6335.
b) Yakiyama, Y. *et al. Chem. Commun.* **2015**, 51, 6828.
(4) Ohara, K. *et al. J. Am. Chem. Soc.* **2009**, 131, 3860.
(5) Biradha, K. & Fujita, M. *Angew. Chem. Int. Ed.* **2002**, 41, 3392.
(6) Martí-Rujas, J. *et al. J. Am. Chem. Soc.* **2011**, 133, 5853.
(7) Ohtsu, H & Kawano, M. *Dalton Trans.* in press.
(8) Kawamichi, T. *et al. Angew. Chem. Int. Ed.* **2008**, 47, 8030.
(9) Inokuma, Y. *et al. Nature Chem.* **2011**, 3, 349.
(10) Kawano, M. & Fujita, M. *Coord. Chem. Rev.* **2007**, 251, 2592.
(11) Ohtsu, H. *et al. J. Am. Chem. Soc.* **2013**, 135, 11449.
(12) Kawamichi, T. *et al. Nature* **2009**, 461, 633.
(13) Kitagawa, H. *et al. Angew. Chem. Int. Ed.* **2013**, 52, 12395.

Nanodroplet Formation and Chemical Analysis in Microfluidic Devices

Akihide Hibara, PhD, Associate Professor
Department of Chemistry, Graduate School of Science and Engineering, Tokyo Institute of Technology

Abstract

Simple and easy selective preconcentration method for microdroplet analysis is presented. In the method, spontaneous emulsification is utilized. In this presentation, the conception, principle and applications of the method will be discussed.

Biographical Sketch

Dr. Akihide Hibara is currently Associate Professor in Department of Chemistry, Graduate School of Science and Engineering, Tokyo Institute of Technology, Japan. Dr. Hibara was awarded Doctor of Engineering (Applied Chemistry) in 2003 from the University of Tokyo. He joined as Research Associate in Department of Applied Chemistry, School of Engineering, The University of Tokyo, in 1999 and promoted to Lecturer in 2003. He moved to Institete of Industrial Science (IIS), The University of Tokyo as Assocate Professor in 2007. Then, he moved to the current position in 2013. He was concurrently Nonpermanent Reserchер in Kanagawa Academy of Science and Technology (KAST) (1999-2009) and in Sakigake Project, Japan Science and Technolgoy Agency (2004-2008).

He has been an Organizing Committee Member (Secretariate) of International Symposiums held in JASIS (2011 JAIMA Discussion on Analytical Science and Technology, 2012-2015 RSC Tokyo International Conference). He is a Program Committee Member of the Intenational Conference on Miniaturization of Chemistry of Life Sciences (MicroTAS) (2010-2013 Technical Program Committee, 2014-present Executive Technical Program Committee).

His research interest is in developing optical analysis methods for small domain and micro/nanofluidic chemical analysis systems. Dr. Hibara has published over 70 peer reviewed articles, over 70 peer-reviewed conference proceedings, 16 book chapters (16 Japanese), and 39 reviews (8 English / 31 Japanese).

Researcher ID : B-9294-2015

Introduction

Recent advancements of micro/nanofluidic technology enable integration of analytical procedures into a microchip [1]. By utilizing integrated micro/nanofluidic technologies, numerous applications in bioanalysis, environmental analysis, process analytical chemistry, and food analysis have been demonstrated. In this presentation, novel microdroplet operation based on interface chemistry and its applications to bioassay and protein crystallization will be presented.

1. Conception of selective enrichment utilizing spontaneous emulsification

Microfluidc droplet flows are often utilized to encapsulate sample / reagent(s) / reaction product(s) into a single droplet. For bioanlysis, aqueous droplets in organic continuous phase are used and various applications have been demonstrated. However, selective enrichment of analyte, which in one of the most important sample pretreatment methods in biochemical analysis, has not been established. Recently, we have investigated the selective enrichment method in the microfluidic droplets, where spontaneous emulsification on an aqueous droplet is utilized [2]. When excess amount of surfactant is added to the organic continuous phase, surfactants adsorbe to the aqueous droplet interface and the surfactant micelles are formed in the organic phase. When the micelles approach to the aqueous droplet interface, hydrophilic space inside the micelles is hydrated by water transferred from the microdroplet through organic membrane. After acceptance of water, the micelle becomes 100-nm-sized aqueous droplet (nanodroplet) as shown in Figure 1a. Then, the microdroplets shrink. During the water transfer, solutes inside the microdroplets remain inside it, or be partitioned to the nanodroplets with the water transfer depending on its hydrophobicity and/or size of the species (Figure 1b).

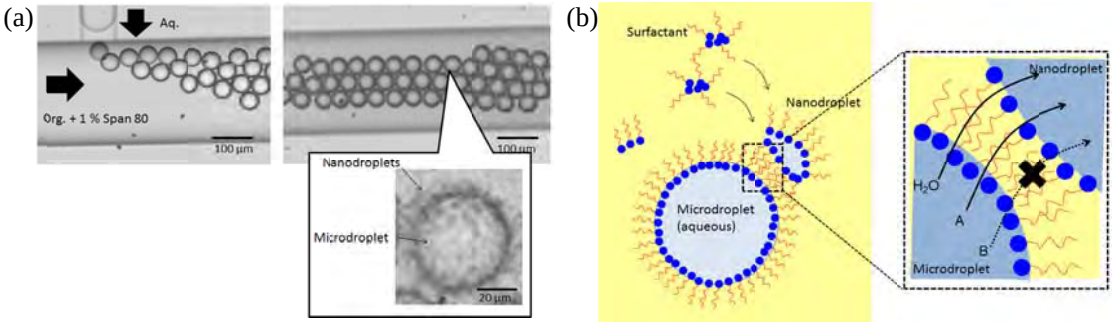


Figure 1 (a) Aqueous microdroplet formation in organic phase with surfactant (Span 80) (left), and spontaneous emulsification on the microdroplets (right). (b) Conception of selective enrichment of solute during the spontaneous emulsification. [2]

2. Demonstration of selective enrichment

Figure 2a shows enrichment factor dependence on encapsulated species. Aqueous droples were formed with a diameter of 42 μm, and their diameter shrank to 7 μm. During the shrinkage, $[Ru(bpy)_3]Cl$ was not transferred to the nanodroplet side, and its concentration inside the microdroplet was enriched 200 times. On the otherhands, fluorescein-tagged PEG was partitioned to the nanodroplet side, and significant enrichment was not observed. Figure 2b shows the micrograph of the microdroplet/nanodroplet after spontaneous emulsification. Initially, $[Ru(bpy)_3]Cl$ and fluorescein-tagged PEG were encapsulated in the microdroplet, and only $[Ru(bpy)_3]Cl$ was selectively enriched.

The selectivity was invesitaged for various species, including dyes, proteins, and nanoparticles [3]. Then, we found that hydrophobic and/or small molecule tends to be participated to the nanodroplet side and hydrophilic and/or large molecule (including nanoparticles) remains in the microdroplet.

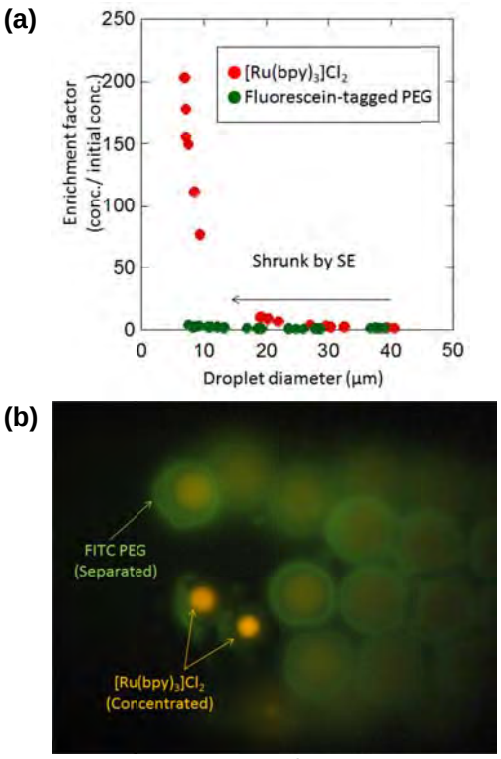


Figure 2 (a) Enrichment factor during spontaneous emulsification. (b) Micrograph of selective enrichment. [2]

By utilizing the selectivity, bound/free (B/F) separation was demonstrated. Figure 3a shows the concept of B/F separatoin during the microdroplet spontaneous emulsificaiton [2]. While free fluorescein-tagged biotin (small molecule) tends to be partitioned to the nanodroplet side, that bound to avidin remaines inside the

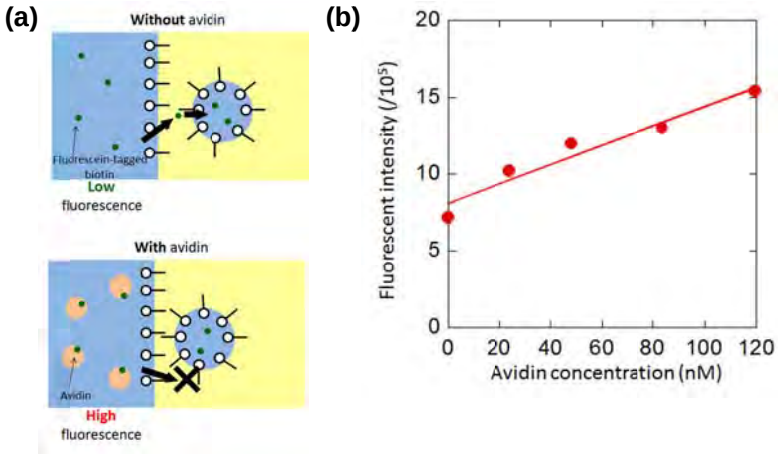


Figure 3 (a) Enrichment factor during spontaneous emulsification. (b) Micrograph of selective enrichment. [2]

microdroplet. Therefore, higher fluorescence is expected with higher avidin (analyte) concentration. Figure 3b shows the microdroplet fluorescnet intensity after emulsificatoin. As expected, the fluorescence increases with the avidin concentration.

3. Protein crystallization

Lysozyme tends to remain in the microdroplet. When supersaturation was induced by the microdroplet spontaneous emulsification, protain crystalization was observed as Figure 4 [4].

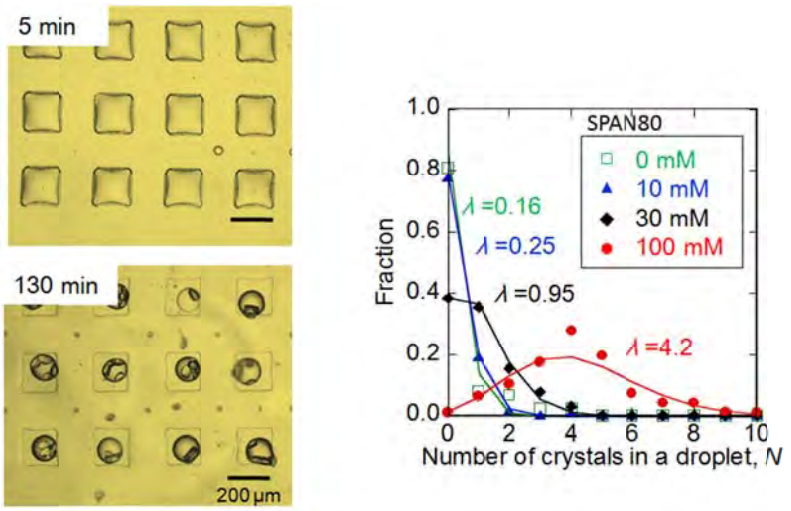


Figure 4 Microwell-based droplet formation and protein (lysozyme) crystallization utilizing spontaneous emulsification. The number of crystals in a droplet depends on the condensation rate, which can be controlled by the surfactant concentration. [4]

Acknowledgments

I acknowledge Dr. Mao Fukuyama of Kyoto Institute of Technology for her contribution to the investigation. A part of this work has been supported by KAKENHI (Grant-in-Aid for Challenging Exploratory Research Grant 26620116, and Kiban b 15H03825).

References

[1] A. Hibara, M. Fukuyama, M. Chung, C. Priest, M.A. Proskurnin, Interfacial Phenomena and Fluid Control in Micro/Nanofluidics, Anal Sci, 32(2016) in press.

[2] M. Fukuyama, A. Hibara, Microfluidic Selective Concentration of Microdroplet Contents by Spontaneous Emulsification, Anal Chem, 87(2015) 3562-5.

[3] M. Fukuyama, A. Hibara, Enrichemnet and separation of microdroplet contents, Solid-State Sensors, Actuators and Microsystems (TRANSDUCERS), 2015 Transducers - 2015 18th International Conference on2015, pp. 1842-5.

[4] M. Fukuyama, A. Akiyama, M. Harada, T. Okada, A. Hibara, Microfluidic protein crystallisation controlled using spontaneous emulsification, Anal Methods, 7(2015) 7128-31.

Reviews in Japanese

福山真央，火原彰秀，自然乳化を利用したマイクロ水滴内包物の選択的濃縮法，分析化学, **65**(2) (2016) in press (2 月).

福山真央，火原彰秀，マイクロ液滴操作と分析，ぶんせき, **2015**(1) (2015) 8-12.

火原彰秀，微小スケールの液体界面と分析操作，海洋化学研究, **27**(2) (2014) 124-7.

Development of mass microscope and applications in clinical pharmacology

Shuichi Shimma, PhD, Associate professor
Department of Biotechnology, Graduate School of Engineering, Osaka University

Abstract

Conventional mass spectrometric analysis provides quantitative information on pharmaceuticals in blood or normal/diseased tissues such as cancer. However, tissue extraction precludes determining information on their spatial distribution. To bring precise spatial distribution of molecules in specimens, imaging mass spectrometry (IMS) based on Mass Microscope was developed. Especially, cancer tissues have complicated morphology due to heterogeneity, therefore to perform IMS under the microscopic view with high sensitivity is beneficial to evaluate the pharmacokinetics. This feature attracts interest in early phase drug developments, especially in oncology.

On the other hand, there are a lot of challenges to apply IMS for drug developments, for example, avoidance of artifacts, sensitivity, reproducibility, throughput and quantification. Therefore, I consider methodological optimizations from tissue sampling to data analysis are absolutely essential for practical applications even though IMS is cutting-edge technology. In this presentation, I will introduce the IMS specific instrument "Mass Microscope" and provide the three effective sample preparation methods for high-sensitive and high-spatial resolution analysis.

Biographical Sketch

Shuichi Shimma received his B.S. degree in 2001 and M.S. degree in 2003 from University of Tsukuba, Japan. He completed the doctoral course at the graduate university for advanced studies, and received his Ph.D in 2007. He served as JSPS post-doctoral fellow and assistant professor at Osaka University between 2007 and 2012. From 2012, he entered National Cancer Center Research Institute in Tokyo and started to apply imaging mass spectrometry for clinical pharmacology. From 2015, he started an independent position of associate professor at Osaka University, Japan. His research interest is to develop instruments and applications for imaging mass spectrometry of many biomolecules (biological metabolites) as well as anti-cancer agents.

Introduction

Matrix-assisted laser desorption/ionization (MALDI) imaging mass spectrometry (IMS) has recently been applied to the analysis of localized molecules, such as biomolecules and drugs on tissue surfaces. During drug development, evaluating the intra-tissue distribution of the drug in pre-clinical and clinical studies might be useful for assessing its drug delivery. Though MALDI-IMS can provide visualization results immediately, there are many experimental steps that should be considered to obtain correct ion distribution information. For example, if the sample preparation is insufficient, the target ion signals are not detected or artifacts derived from matrix crystal aggregation are induced in the resulting images.

I have already published the experimental steps and potential parameters in a previous study

(1). Among the steps involved, the matrix application process was crucial for successful imaging. Additionally, I reported a two-step matrix application procedure (hereafter “the two-step method”) that combined the vacuum deposition of matrix crystals and the spraying of a matrix solution (2). In principle, the two-step method included *ab extra* crystal seeding and crystal development. According to the results, the ion yield was dramatically improved and tissue shrinkage was completely avoided. Furthermore, the two-step method can overcome incomplete matrix crystal formation and an inhomogeneous matrix layer, which can result in low ionization efficiency and incorrect distribution. Therefore, the two-step method is a promising break-through for achieving highly sensitive, reproducible and highly spatial resolution IMS experiments. In this talk, I would like to present the basics of two-step method and other effective sample preparation methods to visualize not only drugs but biomolecules precisely in iMScope.

Results and Discussion

Two-step method. Frozen 8-μm sections were sliced at −20°C with a cryomicrotome (Leica CM 3050S, Leica Microsystems, GmbH, Nussloch, Germany). The frozen sections were thaw-mounted on an indium-tin-oxide coated glass slide (SI0100N, Matsunami, Osaka, Japan) and allowed to dehydrate in a 50-mL conical tube containing silica gel. The glass slides placed in the conical tube were stored at −20°C until matrix application.

The two-step matrix application is described in detail below (Fig. 1). Tissue specimens in tubes containing silica gel were placed at room temperature for 10 min just before matrix deposition. A sample holder was installed in a vacuum deposition system (iMLayer, Shimadzu, Kyoto, Japan). The sample holder and matrix holder were positioned 8 cm apart. The matrix holder was filled with approximately 400 mg of α–CHCA powder (this matrix powder can use repeatedly). The vacuum pressure in the chamber was below 10^{-4} Pa during deposition. Subsequently, the α-CHCA was heated to 250°C, the boiling point of the matrix crystals, and the vapor deposition time settings were 5 min.

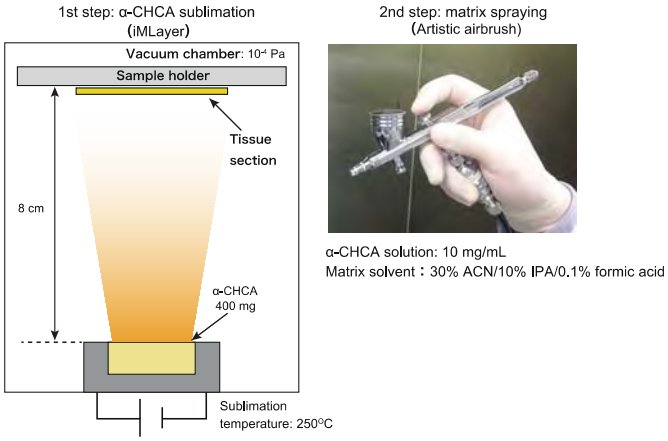


Figure 1 Workflow of two step matrix application method.

α–CHCA (10 mg/mL) was dissolved in acetonitrile, isopropanol and distilled water (all containing 0.1% formic acid; FA) at a ratio of 3:1:6. The matrix solution (100 μL) was sprayed using an artist’s airbrush (PS-270, GSI Creos, Tokyo, Japan). The inside of the airbrush was fluorinated (CYTOP CTL-107MK, AGC, Ibaraki, Japan) to minimize metal contamination from the airbrush. The distance between the tip of the airbrush and the tissue surface was 8 cm. For the first 3 cycles, matrix was sprayed for 2 sec at 60 sec intervals, and for the subsequent 7 cycles the matrix was sprayed for 1.0 sec at 30 sec intervals. Due to the prepared tiny crystal seeds on the tissue surface, immediate tiny formation was observed at the first spraying. This immediate crystallization avoided tissue shrinkage. Furthermore, these pulsed spraying and placement cycles resulted in the formation of a homogeneous matrix layer on the tissue surface using the air-brush. After this process, the morphology of obtained matrix crystals was observed using

scanning electron microscope, and dramatic change was observed between direct spraying and two-step method (Fig. 2) (3). The obtained fine structure matrix crystal provided higher ion yield for drug detection (Fig. 3).

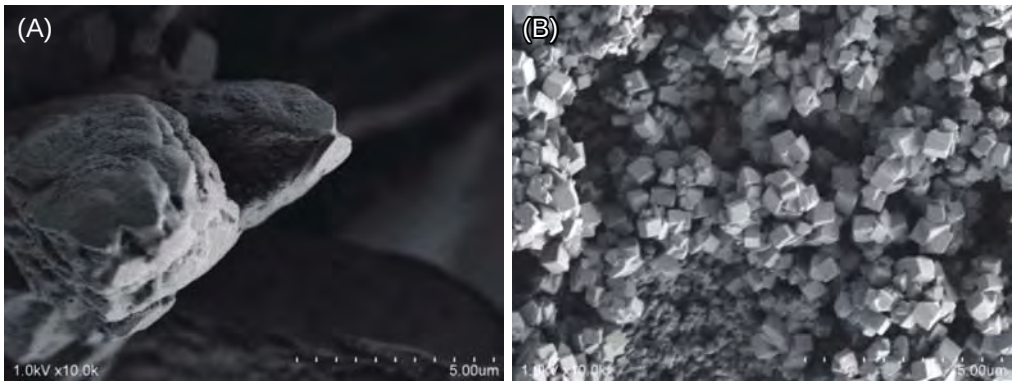


Figure 2 Difference of α-CHCA crystal morphology between conventional spraying and two-step method. (A) Conventional and (B) two-step method. (Magnification of 10k).

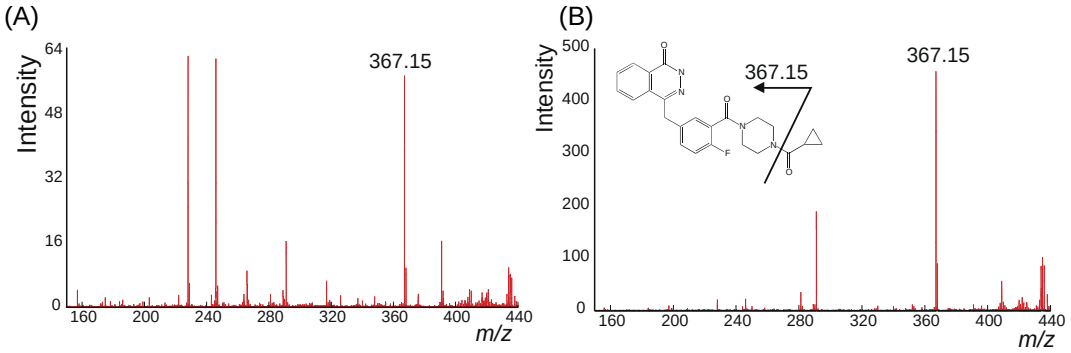


Figure 3 Improvement of ion intensity on the mouse liver section to detect anti-cancer agent (olaparib). (A) Conventional and (B) two-step method. Peak intensity was improved by 10 times in two-step method.

Conclusion

In MALDI-IMS, adequate sample preparation procedure is essential to visualize drugs and other biological molecules. However, to achieve this process effectively there are a lot of know-hows and we should select effective matrix application procedures to visualize “what you want to see”. This means not only to improve ionization efficiency but to produce tiny matrix crystals without aggregation on the tissue surface for high-spatial resolution imaging. These techniques are reported from Japanese IMS scientists and I wish those methodologies will become standard procedures in the near future.

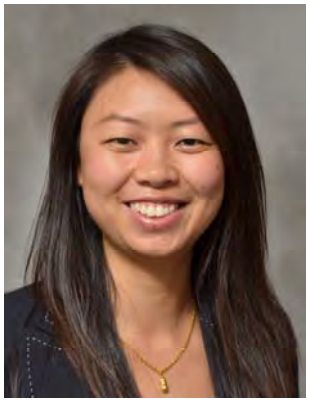
Acknowledgments

This work is supported by Grant-in-Aids from the research foundation for pharmaceutical sciences and Grant-in-Aids for regulatory science from Japan Agency for Medical Research and Development (AMED).

References

(1) Effective Sample Preparations in Imaging Mass Spectrometry. S. Shimma, *Mass Spectrometry*, **3**, S0029 (2014).
(2) Alternative two-step matrix application method for imaging mass spectrometry to avoid tissue shrinkage and improve ionization efficiency, S. Shimma et al. *J. Mass Spectrom.*, 48, 1285-90 (2013).
(3) Characterizations of Two-step Matrix Application Procedures for Imaging Mass Spectrometry. S. Shimma, *Mass Spectrometry Letters*, **6**, 21-25 (2015).

Plasma phospholipids and prevalence of mild cognitive impairment/dementia



Danni Li, PhD, Assistant Professor
Department of Lab Medicine and Pathology, University of Minnesota, Minneapolis, MN 55444, USA

Abstract

Phospholipids are altered in brains of patients with dementia and some studies suggest their plasma levels may be useful in the prediction of mild cognitive impairment (MCI) and dementia. We measured 188 plasma metabolites in participants who underwent a detailed neuropsychological assessment and classified as normal (n=153), MCI (n=145) or dementia (n=143) by expert adjudication. Among 10 phospholipids recently implicated as altered in dementia, higher concentrations of PC aa 36:6 were significantly associated with decreased prevalence of dementia (odds ratio 0.71, 95% confidence interval 0.50-1.00 per 1-standard deviation increase). Adding these phospholipids to a model including multiple predictors of dementia led to only minimal improvement in prediction (C-statistic changed from 0.702 to 0.713, $p>0.05$). Some phospholipids and metabolites were altered in MCI and dementia but in this study, cross-sectional association was relatively weak and prediction of MCI and dementia did not improve on clinical variables.

Biographical Sketch

Danni Li, PhD, DABCC, is the Director of Clinical Chemistry at the University of Minnesota Medical Center, Fairview and an Assistant Professor of Laboratory Medicine and Pathology at the University of Minnesota, Twin Cities. Dr. Li earned a BS in Chemistry from Zhejiang University in Hangzhou, China in 2002 and a PhD in Analytical Chemistry at the University of Minnesota in 2007. She completed the Clinical Chemistry Fellowship training at the Johns Hopkins University in 2009 and joined the faculty at the Johns Hopkins University in 2010. From January 2010 to August 2012, she was the Director of General Chemistry Laboratories at the Johns Hopkins Hospital and Assistant Professor of Pathology at the Johns Hopkins University. She is a member of American Association for Clinical Chemistry (AACC), and a Diplomat of

American Board of Clinical Chemistry (DABCC). She is also a member of American Society of Mass Spectrometry (ASMS). Dr. Li has authored over 20 peer-reviewed research articles, peer-reviewed review articles, editorial, case reports, and book chapters. For the last five years, Dr. Li has worked on discovery of biomarkers using proteomic technologies such as mass spectrometry and microarray and validation using multiplexed immunoassays. In last 3 years at the University of Minnesota (2012-2015), she has developed her research interest in plasma biomarkers for Alzheimer's disease. Recently, she received the 2015 All Investigator Research Grant from the Alzheimer's Association International Research Grant Program to support her research in plasma biomarkers for Alzheimer's disease.

Introduction

There is growing interest in the discovery of plasma biomarkers for the prediction and diagnosis of mild cognitive impairment (MCI) and dementia, in particular MCI and dementia due to Alzheimer's disease (AD). Currently, amnestic MCI and AD-type dementia, similar to other MCI and dementia, are generally diagnosed based on a full assessment of cognitive function, a neurological examination, and clinical examination of history in cognitive and behavioral changes. Positron emission tomography (PET) neuroimaging and cerebrospinal fluid proteins are important but invasive and expensive tools for verifying the diagnosis. Due to the complexity of AD pathology, mounting evidence shows that no single biomarker can yield enough sensitivity and specificity, and multiple biomarkers may be necessary to diagnose AD and track its progression. Plasma biomarkers are less invasive and, therefore, may be used as a first-step screen of AD. Moreover, the less invasive nature of plasma biomarkers makes them potentially more suitable for monitoring disease progression and treatment response to potential therapies in AD.

Recently, Mapstone *et al.* reported a panel of plasma phospholipids that identified cognitive normal adults who would progress to either amnestic MCI or AD within 2-3 years from those who remained cognitively normal (the area under curve [AUC] of the receiver-operating characteristic [ROC] analysis was 0.96 [95% confidence interval 0.93-0.99]) [1] . Subsequently, 3 publications suggested that plasma phospholipids and metabolites might aid in the clinical diagnosis of MCI and dementia [2-4] . With the general aim of re-evaluating the biomarkers identified by Mapstone and colleagues, in this presentation, I will show you data on the association of concentrations of selected phospholipids with the prevalence of MCI or dementia and in a subset of 441 participants in the Atherosclerosis Risk in Communities (ARIC) Neurocognitive Study (ARIC-NCS).

Table 1. Plasma phospholipids and their abilities to discriminate MCI/dementia from cognitive normal.

	ROC AUC MCI/Dementia	ROC AUC AD-type MCI/Dementia
PC aa C36:6	0.580	0.557
PC aa C38:0	0.534	0.509
PC aa C38:6	0.544	0.545
PC aa C40:1	0.522	0.508
PC aa C40:2	0.548	0.520
PC aa C40:6	0.546	0.572
PC ae C40:6	0.547	0.514
Lyso PC a C18:2	0.522	0.514
Propionyl-L-carnitine (C3)	0.532	0.496
Combined	**0.602**	**0.602**

Discussion

We performed targeted metabolomics of 188 metabolites (i.e., 40 acylcarnitines, 21 amino acids, 21 biogenic amines, 15 sphingolipids, 90 glycerophospholipids, and hexose) on plasma samples from 153 cognitively normal, 145 MCI, and 143 dementia participants in the large multicenter ARIC-NCS study. The objectives of this metabolomic study were two-fold: (a) to study the associations of concentrations of these metabolites with the prevalence of MCI or dementia, and (b) to determine the predictive ability of concentrations of these metabolites to differentiate dementia and/or MCI from cognitive normal. Recently, Mapstone *et al*. demonstrated a set of 10 phospholipids (PC aa C36:6, PC aa C38:0, PC aa C38:6, PC aa C40:1, PC aa C40:2, PC aa C40:6, and PC ae C40:6, lyso PC a C18:2, C3, and C16:1-OH) in plasma predicted phenoconversion from control to either amnestic MCI or dementia with a robust AUC of 0.96. We hypothesized that (a) lower concentrations of the 10 phospholipids identified by Mapstone e*t al*. would be associated with a higher prevalence of MCI and dementia, and (b) that these phospholipids would help classify individuals as cognitively normal or impaired (MCI and/or dementia). Our multinomial logistic regression analyses adjusted for age, race, sex, *APOE* genotype, and additional cardiovascular risk factors indicated that, of the 10 phospholipids identified by Mapstone *et al.*, only PC aa C40: 2 and PC aa C36: 6 were associated with prevalence of MCI and dementia, respectively. Being a hypothesis-driven analysis, we did not adjust for multiple testing for these phospholipids. Similarly, Klavins *et al*. showed an association of lower concentrations of PC aa C36: 6 with MCI and dementia [2] . In our analysis, the phospholipids identified by Mapstone *et al*. had weaker associations individually and in aggregate and a limited ability to differentiate those with dementia/MCI from the cognitively normal. Overall, other variables, including sociodemographic and cardiovascular risk factors, had better predictive ability than the phospholipids of interest, as evaluated with C-statistics.

Conclusion

Our study was unable to reproduce the identities of many phospholipids found to be significantly associated with prevalence of MCI and dementia in other studies. However, we found significant association of sphingomyelines with prevalence of MCI and dementia. Our results showed that, in general, some phospholipids and metabolites were altered in MCI and dementia.

Acknowledgments

We thank the staff and participants of the ARIC Study for their important contributions. The Atherosclerosis Risk in Community (ARIC) Study is carried out as a collaborative study supported by the National Heart, Lung, and Blood Institute contracts (HHSN268201100005C, HHSN268201100006C, HHSN268201100007C, HHSN268201100008C, HHSN268201100009C, HHSN2682011000010C, HHSN2682011000011C, and HHSN2682011000012C). Neurocognitive data are collected by the support of the National Heart, Lung, and Blood Institute U01 HL096812, HL096814, HL096899, HL096902, and HL096917 with previous brain MRI examinations funded by R01-HL70825.

References

[1] Mapstone M, Cheema AK, Fiandaca MS, Zhong X, Mhyre TR, MacArthur LH, Hall WJ, Fisher SG, Peterson DR, Haley JM, Nazar MD, Rich SA, Berlau DJ, Peltz CB, Tan MT, Kawas CH, Federoff HJ. Plasma phospholipids identify antecedent memory impairment in older adults. *Nature Medicine.* 2014;**20**(4):415-8.

[2] Klavins K, Koal T, Dallmann G, Marksteiner J, Kemmler G, Humpel C. The ratio of phosphatidylcholines to lysophosphatidylcholines in plasma differentiates healthy controls from patients with Alzheimer's disease and mild cognitive impairment. *Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring.* 2015;**1**(3):295-302.

[3] Olazarán J, Gil-de-Gómez L, Rodríguez-Martín A, Valentí-Soler M, Frades-Payo B, Marín-Muñoz J, Antúnez C, Frank-García A, Acedo-Jiménez C, Morlán-Gracia L. A blood-based, 7-metabolite signature for the early diagnosis of Alzheimer's disease. *Journal of Alzheimer's disease: JAD.* 2015;**45**(4):1157-73.

[4] Proitsi P, Kim M, Whiley L, Pritchard M, Leung R, Soininen H, Kloszewska I, Mecocci P, Tsolaki M, Vellas B. Plasma lipidomics analysis finds long chain cholesteryl esters to be associated with Alzheimer’s disease. *Translational Psychiatry.* 2015;**5**(1):e494-502.

Recent development on boron-doped diamond electrodes

Yasuaki Einaga
Department of Chemistry, Keio University,
and JST-ACCEL, Japan

Abstract

Boron-doped diamond (BDD) electrodes are very attractive material, because of their wide potential window, low background current, chemical inertness, and mechanical durability. In these years, we have reported several examples for electrochemical sensor applications. Here, we report some recent examples of electrochemical sensor application of BDD such as ozone, pH, *in vivo* detection of neurotransmitter in monkey brain, and *in vivo* detection of glutathione for assessment of cancerous tumors using BDD microelectrodes. Furthermore, other applications such as organic synthesis , ozone generation, and CO_2 reduction are also shown.

Biographical Sketch

Yasuaki Einaga was born in Niigata Prefecture, Japan in 1971. He received his Ph.D degree in 1999 from the University of Tokyo under the direction of Prof. Akira Fujishima. He joined the Department of Chemistry at Keio University as an Assistant Professor in 2001. He was promoted to Associate Professor in 2003, and to Professor in 2011. He was also a Research Director of JST-CREST project “Development of Innovative Technologies Using Diamond Electrodes for Improving Environment” from 2011 to 2014. Then, now he is a Research Director of JST-ACCEL project “Fundamentals and Application of Diamond Electrodes” from 2014. His research interests include photo-functional materials science and diamond electrochemistry.

Introduction

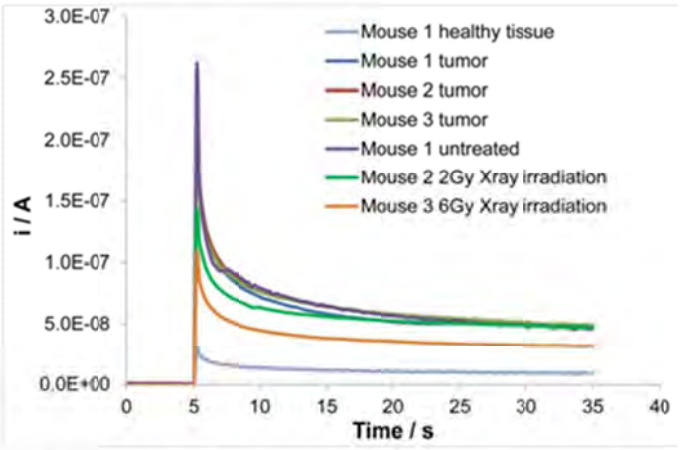
Superiority of highly boron-doped diamond (BDD) electrodes among other conventional carbon electrodes have already been reported due to their unique electrochemical properties, such as wide potential window, very low charging current, chemical inertness, and mechanical durability [1]. In the recent decades, these properties have established BDD as a superior electrode for several applications,

including sensors, biosensors, wastewater treatment, and electrosynthesis [1-2]. Moreover, since BDD is composed of carbon only, its biocompatibility is also satisfactory [3] and enables BDD for either *in vivo* detections [4-7] or applications involving the living cells [3,8]. For these purpose, micro sizes of BDD electrodes were developed [4].

This paper studies the recent developments of BDD microelectrode applications for electroanalyais. Furthermore, not only the electrochemical sensor applications but also some examples in electrosynthesis applications, including organic synthesis, [9] ozone generation [10] and CO_2 reduction [11] are also introduced.

In vivo Glutathione sensors for tumor assessments [7]

Glutathione is a tripeptide, which is found in high concentrations in many living cells. It is one of the strongest biological anti-oxidant and exists in reduced form (L-c-glutamyl-L-cysteinyl-glycine, GSH) and oxidized form (disulfide form of GSH, GSSG). Under oxidative stress, GSH will be oxidized to GSSG, which will be immediately reduced back to GSH by glutathione reductase enzyme. This fast turnover normally causes much higher GSH concentrations in living cells than GSSG. For this reason, the ratio of GSH to GSSG is often used as a sensitive indicator for stress marker of living cells. Furthermore, it was reported that GSH concentrations in cancerous cells are higher than in the healthy tissues, which is believed to relate with the high resistance of cancer stem cells against oxidative stress such as radiotherapy or chemotherapy.



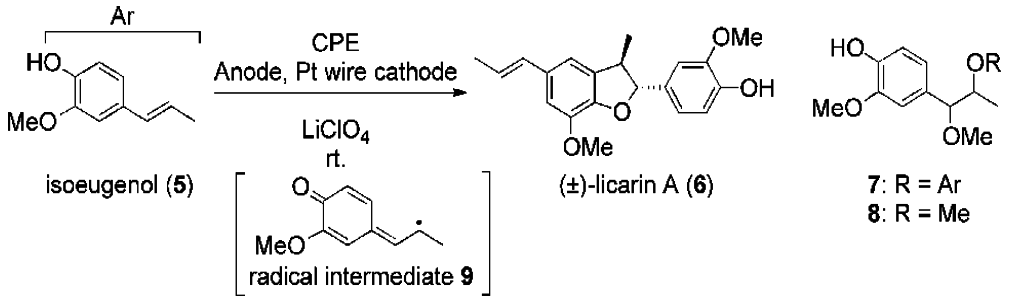
Detection of GSH using BDD microelectrodes

Our group has presented the *in vitro* and *in vivo* detection of the reduced form of GSH using BDD microelectrode for application in the assessment of cancerous tumors. An accurate calibration curve for selective detection of GSH in the presence of a constant concentration of GSSH could be obtained by amperometry at +1.3 V vs. Ag/AgCl. Additionally, *in vivo* GSH measurements in human cancer cells inoculated in immunodeficient mice have been performed. These measurements exhibit the difference of GSH level between cancerous and normal tissues. Moreover, *in vivo* GSH

detection measurements carried out before and after X-ray irradiation have proved that it is possible to assess the decrease in GSH concentration in the tumor after a specific treatment.

2. Electrosynthesis Applications [9]

BDD is known to possess a wide application range for the direct generation of hydroxyl radicals and produce inorganic peroxides such as persulfate, perphosphate, and hypochlorite. However, only a few examples hydroxyl or alkoxy radicals that electrochemically generated have been employed to promote reactions of highly functionalized organic substances. We have reported that methoxy radicals can be generated by electrochemical oxidation of methanol using BDD electrode. The results can provide the electrooxidative reaction of isoeugenol to produce licarin A in higher yields than related processes employing Pt and GC electrodes. Further studies on the applicability of electrochemical oxidation reactions taking place at the BDD electrode and in particular those reactions that lead to the formation of biologically important low molecular-weight substances are in progress.



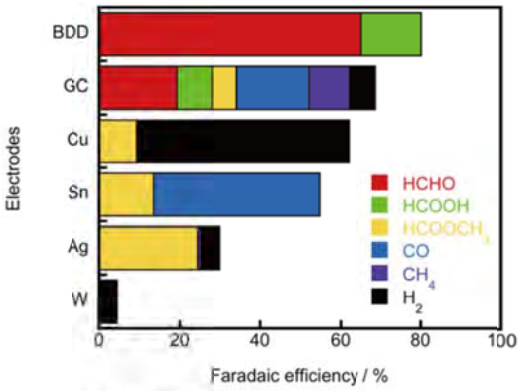
Electrosynthesis of licarin A by BDD electrodes

3. CO_2 reduction [11]

Another application of BDD in electrosynthesis is for CO_2 reduction. Methanol was selected as the electrolyte due to its five times higher solubility than in water. Tetrabutylammonium perchlorate (TBAP) was dissolved into methanol as the electrolyte. CVs at BDD electrode showed a cathodic current with an onset potential of -1.8 V vs. (Ag/AgCl) without the presence of CO_2, whereas a large peak was observed at -1.3 V after 2.5 h bubbling CO_2, suggesting the reduction potential of CO_2. Electrolysis was performed at various potentials for 1 h at room temperature and atmospheric pressure after saturation of the electrolyte with CO_2. The figure shows that the reaction products were formaldehyde, formic acid, and hydrogen. A maximum Faradaic efficiency for formaldehyde (74 %) was observed at -1.7 V vs. Ag/Ag^+. Comparison of electrolysis using other electrodes, i.e. Cu, Sn, Ag,

or W electrodes, shows no formaldehyde can be produced, whereas with a glassy carbon electrode, a low Faradaic efficiency for formaldehyde (19%) was observed. Thus, the high-yield production of formaldehyde is an inherent property of BDD electrodes.

Further, seawater was used as an electrolyte. The use of seawater as the electrolyte has generated formaldehyde (36%) at the same condition. Less Faradaic efficiency when using seawater is due to the narrow potential window in water and the inorganic and organic impurities in seawater, which lowers the electrochemical reduction of CO_2. For reference, electrolysis of CO_2 was performed in a water solution containing 0.1 M NaCl as the electrolyte. A Faradaic efficiency of 62% can be achieved, suggesting the influence of impurities in the reaction using seawater. In actual applications, the high conductivity of the BDD electrode is beneficial for the high production rate of formaldehyde.



CO_2 reduction by BDD electrodes

References

1. *Diamond Electrochemistry*, edited by A. Fujishima, Y. Einaga, T. N. Rao, D. A. Tryk, (BKC-Elsevier, Tokyo, 2005).
2. Y. Einaga, J. S. Foord, G. M. Swain, *MRS Bull.* **39**, 525 (2014).
3. T. A. Ivandini, E. Saepudin, H. Wardah, Harmesa, N. Dewangga, Y. Einaga, *Anal. Chem.* **84**, 9825 (2012).
4. A. Suzuki, T. A. Ivandini, K. Yoshimi, A. Fujishima, G. Oyama, T. Nakazato, N. Hattori, S. Kitazawa, Y. Einaga, *Anal. Chem.* **79**, 8608 (2007).
5. S. Fierro, R. Seishima, O. Nagano, H. Saya, Y. Einaga, *Sci. Rep*. **3**, 3257 (2013).
6. K. Yoshimi, Y. Naya, N. Mitani, T. Kato, M. Inoue, S. Natori, T. Takahashi, A. Weitemier, N. Nishikawa, T. McHugh, Y. Einaga, *Neurosci. Res*. **71**, 49 (2011).
7. S. Fierro, M. Yoshikawa, O. Nagano, K. Yoshimi, H. Saya, Y. Einaga, Sci. Rep. **2**, 901 (2012).
8. T. A. Ivandini, Harmesa, E. Saepudin, Y. Einaga, *Anal. Sci.* **31**, 643 (2015).
9. T. Sumi, T. Saitoh, K. Natsui, T. Yamamoto, M. Abe, Y. Einaga, S. Nishiyama, *Angew. Chem. Int. Ed.* **51**, 5443 (2012).
10. Y. Honda, T. A. Ivandini, T. Watanabe, K. Murata, Y. Einaga, *Diamond Relat. Mater.* **40**, 7 (2013).
11. K. Nakata, T. Ozaki, C. Terashima, A. Fujishima, Y. Einaga, *Angew. Chem. Int. Ed.* **53**, 871 (2014).

Plasmonic Nanomaterials

Tetsu Tatsuma, PhD, Professor
Center for Photonics Electronics Convergence
Institute of Industrial Science, University of Tokyo

Abstract

Plasmonic metal nanoparticles, which are characterized by strong light absorption, scattering, and their controllability, attract attention as new photofunctional materials. The lecture will focus on application of plasmonic nanoparticles to sensors, photovoltaic cells, photocatalysts, and advanced optical materials, as well as some basic aspects of localized surface plasmon resonance (LSPR). We found plasmon-induced charge separation (PICS) at the metal nanoparticle-semiconductor interface and applied it to photovoltaics and photocatalysis. Now many other research groups are working on photosensors and photocatalysts based on PICS. We also applied PICS to plasmonic sensors for chemical and biological applications, image display, and data storage. We also developed other plasmonic sensors and color routers.

Biographical Sketch

Dr. Tetsu Tatsuma is Professor of Center for Photonics Electronics Convergence, Institute of Industrial Science, University of Tokyo, Japan. He received his B.Sc. (1988), M.Sc. (1990) and Ph.D. (1993) degrees in electrochemistry from University of Tokyo. He held the position of Research Associate at Faculty of Technology, Tokyo University of Agriculture and Technology before joining School of Engineering, University of Tokyo as Assistant Professor in 1998. After the promotion to Associate Professor in 2000, he joined the present Institute (2001), and was promoted to Professor in 2008. He received Award for Young Scientists from the Electrochemical Society of Japan in 1998, Award for Creative Work from the Chemical Society of Japan in 2011, Award for Scientific Achievement from the Electrochemical Society in 2012, and The Japanese Photochemistry Association Award in 2014. His research interests include development of devices and functional

materials on the basis of nanoscience and photoelectrochemistry.

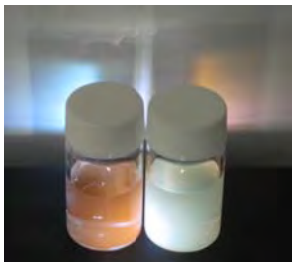
Introduction

Plasmonic nanomaterials, which exhibit localized surface plasmon resonance (LSPR), attract much attention recently. LSPR is resonance of electron oscillation in a metal nanomaterial with electric field oscillation of incident light. Plasmon has a certain lifetime, and it finally decays. There are at least four routes of plasmon decay; (i) nonradiative decay, which is observed as light absorption, (ii) radiative decay, which is observed as light scattering, (iii) decay as a result of energy transfer (in a broad sense) to a dye molecule or semiconductor via near-field excitation, far-field excitation, or resonant energy transfer, and (iv) decay as a result of uphill electron transfer to semiconductor in contact. Strong light absorption of plasmonic nanoparticles is responsible for brilliant colors of stained glasses and cut glasses. The color is easy to control because resonant wavelength of a plasmonic nanoparticle strongly depends on its size and shape. Since the resonant wavelength is also dependent on the local refractive index, plasmonic nanoparticles modified with a receptor (e.g. antibody) for an analyte (e.g. antigen) are used for LSPR sensors for biosensing and chemical sensing. Plasmonic nanoparticles that exhibit strong light scattering often serve as markers in bioanalysis. Those nanoparticles are also used for optical materials that show unique scattering properties. A typical example is ancient Lycrugus cup. The effect of energy transfer is applied to surface enhanced Raman scattering (SERS) and enhancement of fluorescence. Recently plasmonic nanoparticles are used as nanoantennae for photocurrent enhancement of photovoltaics and reaction rate enhancement of photocatalysis. The electron transfer effect, which we reported for the first time as plasmon-induced charge separation, can be applied to photovoltaics, photocatalysis, and photochromic materials, as well as electric LSPR sensors.

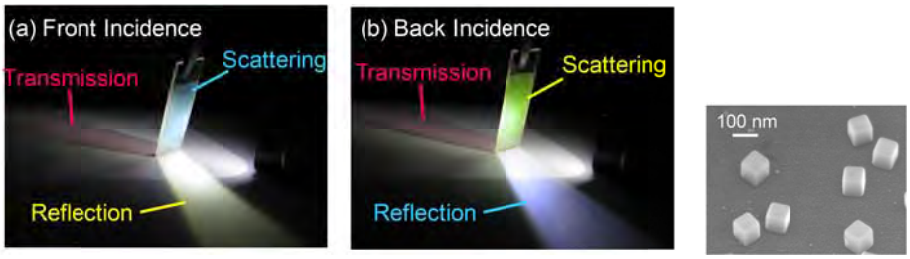
1. Light absorption

Plasmonic gold nanoparticles.

2. Light scattering[1,2]

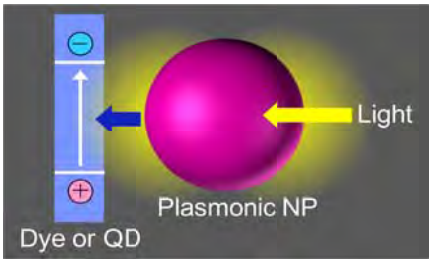


"Lycrugus cup" effect.

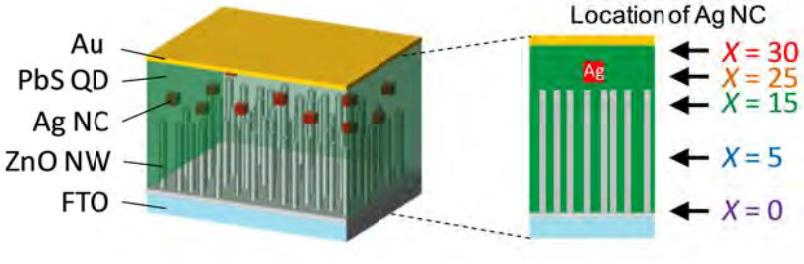


Asymmetric plasmonic color router based on Ag nanocubes.[1]

3. Plasmonic nanoantenna effect[3-5]

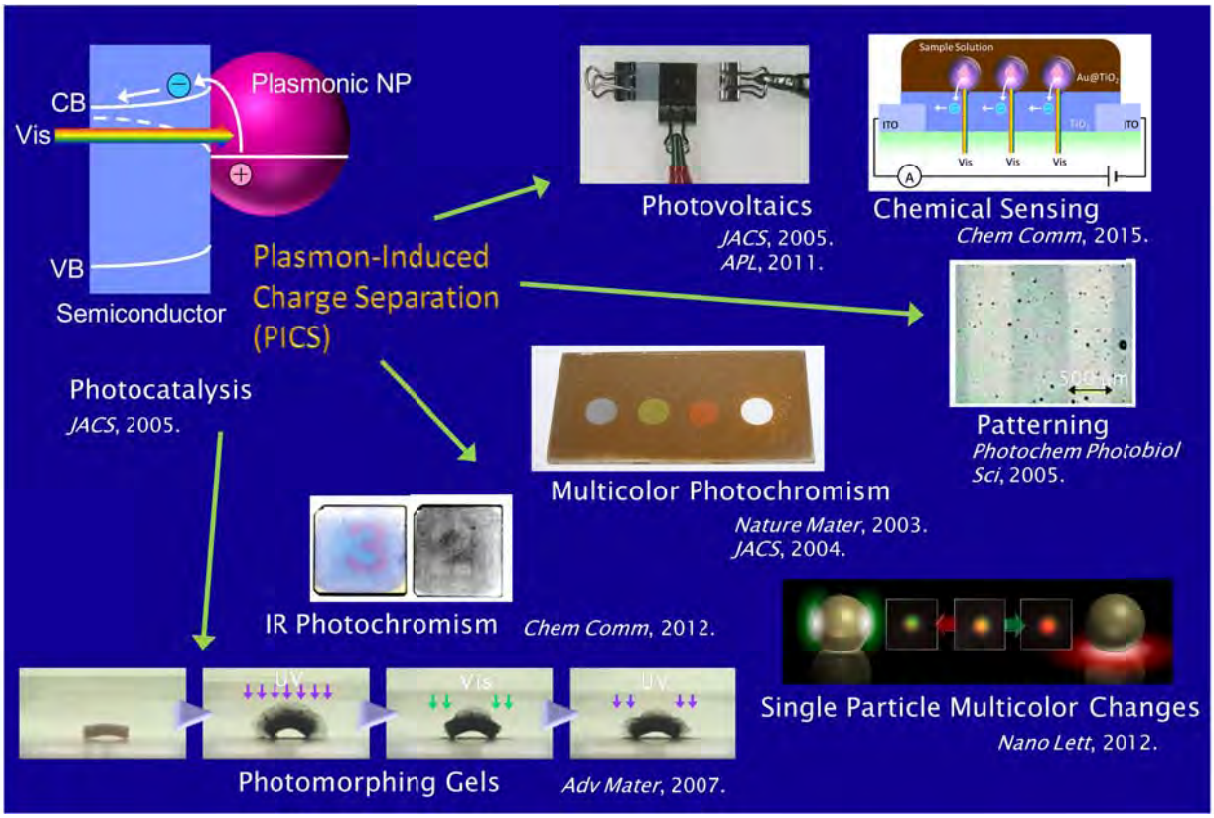


Plasmonic nanoantenna effect.



Colloidal quantum-dot solar cell with plasmonic nanoantennae.[5]

4. Plasmon-induced charge separation (PICS)[6-17]



Plasmon-induced charge separation and its applications.

Conclusion

We developed asymmetric color routers[1] and transparent scattering projection screens[2] by using plasmonic nanoparticles that exhibit strong light scattering. Plasmonic nanoantenna effects on photoelectrochemical systems were studied systematically[3,4] and applied to enhancement of power conversion efficiency of bulk-heterojunction colloidal quantum dot solar cells.[5] We found plasmon-induced charge separation (PICS)[8] and revealed that PICS is based on electron transfer from resonant plasmonic nanoparticles to semiconductor.[8,16] PICS was applied to photovoltaics,[8,12] photocatalysis,[8] image display,[6,7,9,13] data storage,[14] photoactuator,[10] and LSPR sensors.[15,17] We also developed a potential-scanning LSPR sensors, for which conventional wavelength scan is not necessary.[20]

References

1. K. Saito and T. Tatsuma, *Adv. Opt. Mater.*, 3, 883-887 (2015).
2. K. Saito and T. Tatsuma, *Nanoscale*, 7, 20365-20368 (2015).
3. T. Kawawaki, Y. Takahashi, and T. Tatsuma, *Nanoscale*, 3, 2865-2867 (2011).
4. T. Kawawaki, Y. Takahashi, and T. Tatsuma, *J. Phys. Chem. C*, 117, 5901-5907 (2013).
5. T. Kawawaki, H. Wang, T. Kubo, K. Saito, J. Nakazaki, H. Segawa, and T. Tatsuma, *ACS Nano*, 9, 4165-4172 (2015).
6. Y. Ohko, T. Tatsuma, T. Fujii, K. Naoi, C. Niwa, Y. Kubota, and A. Fujishima, *Nature Mater.*, 2, 29-31 (2003).
7. K. Naoi, Y. Ohko, and T. Tatsuma, *J. Am. Chem. Soc.*, 126, 3664-3668 (2004).
8. Y. Tian and T. Tatsuma, *J. Am. Chem. Soc.*, 127, 7632-7637 (2005).
9. K. Matsubara and T. Tatsuma, *Adv. Mater.*, 19, 2802-2806 (2007).
10. T. Tatsuma, K. Takada, and T. Miyazaki, *Adv. Mater.*, 19, 1249-1251 (2007).
11. E. Kazuma, N. Sakai, and T. Tatsuma, *Chem. Commun.*, 47, 5777-5779 (2011).
12. Y. Takahashi and T. Tatsuma, *Appl. Phys. Lett.*, 99, 182110 (2011).
13. E. Kazuma and T. Tatsuma, *Chem. Commun.*, 48, 1733-1735 (2012).
14. I. Tanabe and T. Tatsuma, *Nano Lett.*, 12, 5418-5421 (2012).
15. E. Kazuma and T. Tatsuma, *Nanoscale*, 6, 2397-2405 (2014).
16. E. Kazuma and T. Tatsuma, *Adv. Mater. Interfaces*, 1, 1400066 (2014).
17. T. Tatsuma, Y. Katagi, S. Watanabe, K. Akiyoshi, T. Kawawaki, H. Nishi, and E. Kazuma, *Chem. Commun.*, 51, 6100-6103 (2015).
18. N. Sakai and T. Tatsuma, *Adv. Mater.*, 22, 3185-3188 (2010).
19. E. Nakamura, A. Kogo, N. Sakai, and T. Tatsuma, *Appl. Phys. Lett.*, 105 , 083113 (2014).
20. H. Nishi, S. Hiroya, and T. Tatsuma, *ACS Nano*, 9, 6214-6221 (2015).

The Unique Combination of Nanotechnology with Raman and SPRi Platforms Offers Innovative and Ultrasensitive Solutions for Diagnostics

Marinella G. Sandros, PhD
Business Development Manager for Life Sciences,
SPRi Product Manager,
Adjunct Assistant Professor
HORIBA Scientific
Department of Nanoscience, University of North Carolina at Greensboro

Abstract

Raman Microscopy allows for simultaneous chemical identification and imaging through the distinctive vibrational signatures of chemical bonds and SPRi provides quantitative interaction data by measuring changes in refractive index on the sensor interface. Both optical non-destructive techniques provide unique diagnostic applications. During this lecture, we will highlight how Raman Microscopy and SPRi have enjoyed tremendous gains after integration with nanotechnology. Diagnostic biomarkers for many diseases at the early stage are often in low abundance presenting many challenges for their detection. Extending the application of SPRi and Raman Microscopy systems through the unique combination with nanomaterials provides ultra signal enhancement. Research developments in detecting biomarkers at ultralow levels (10^{-15} to 10^{-18} M) will be presented. Furthermore, cutting-edge techniques like Tip Enhanced Raman Spectroscopy offering nanoscale resolution with ultrasensitivity will also be reviewed.

Biographical Sketch

Dr. Marinella Sandros currently is the business development manager for life Sciences and SPRi product manager at HORIBA Scientific. She also currently holds an adjunct assistant professor position in the department of Nanoscience at the University of North Carolina at Greensboro since August 2015. Previously, she held a full time assistant professor position in the nanoscience department from 2010-2015. Her doctoral training was in bioinorganic chemistry from Wayne State University followed by her postdoctoral fellowship in biomedical engineering at McGill University. Dr. Sandros has extensive expertise in synthesizing and characterizing various types of nanomaterials to enhance their biointerfacing properties using different methods of coatings for biological applications. Furthermore, she has over 12 yrs experience in developing novel fluorescent, Raman and Surface Plasmon Resonance imaging (SPRi)-based biosensors through the integration of nanomaterials with biological interfaces. Previously, her group focused on developing ultrasensitive SPRi based platform to detect diagnostic biomarkers present in serum.

Finally, Dr. Sandros has authored numerous high impact journals demonstrating her stong expertise in developing, characterizing and applying nanomaterials.

Introduction

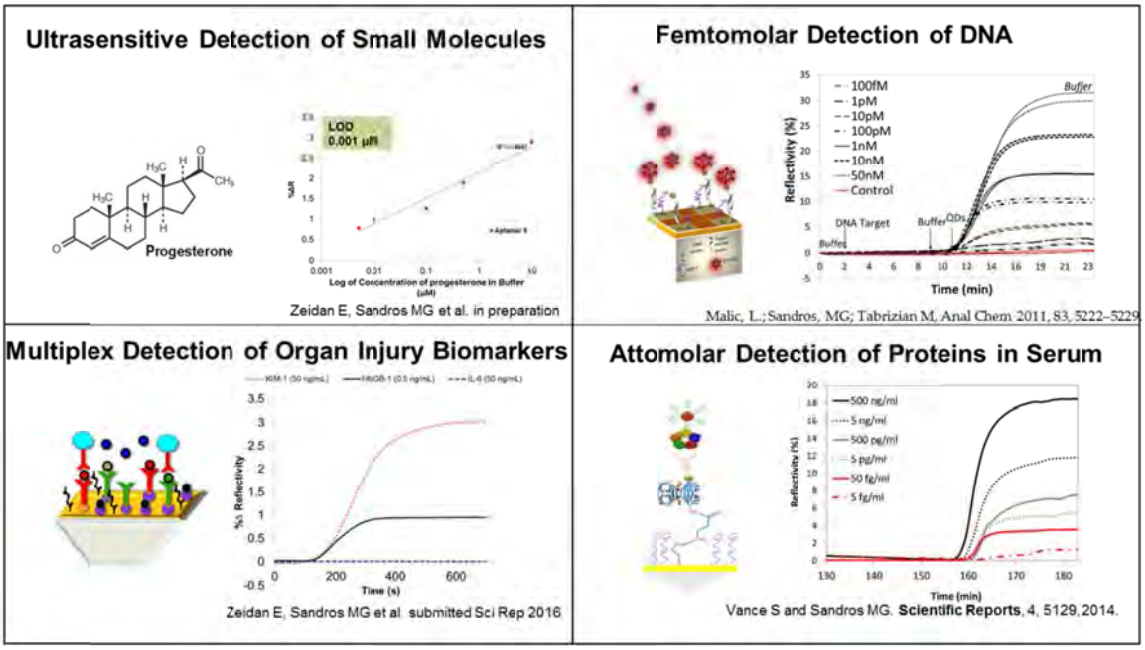
Expanding on recent progress in control of matter at the nanoscale, nanomaterials have impinged tremendous advancement on SPRi and Raman applications.

The detection principle behind SPR involves a sensing surface consisting of a prism that is coated with a thin metal layer (gold or silver); when a polarized light hits the prism “surface plasmons” are generated. The binding of an analyte to a surface receptor immobilized on the metal surface modifies the resonance conditions resulting in a shifted resonance dip, which can then be correlated to the analyte concentration. SPR-based biosensors are now commercially available that offer a real-time, label free technique to monitor biomolecular binding events and biochemical reactions and also recognize biomarkers.[1-3] More recently, SPRi was developed in response to the need for multiplexing (i.e. monitoring multiple binding events simultaneously), which was missing in classical SPR biosensors. Thus, SPRi has emerged as a tool to monitor several binding events simultaneously.

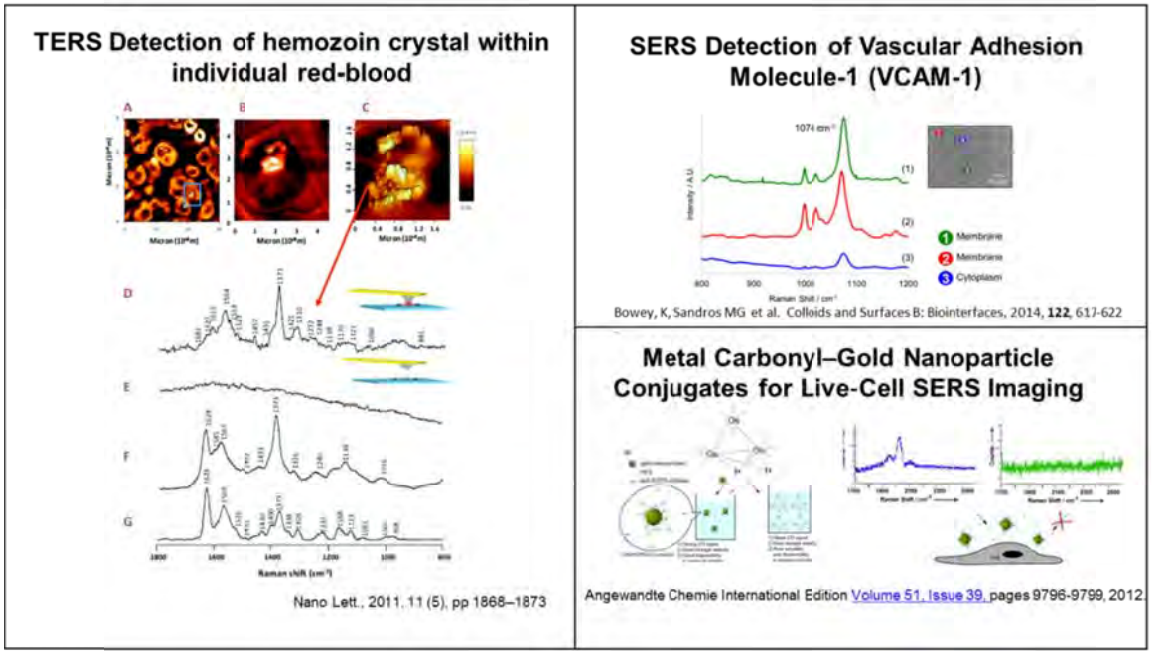
Alternatively, Raman spectroscopy provides information about molecular vibrations that can be used for sample identification and quantitation in a label free manner. The advantage of Raman compared to other characterization techniques, specifically for biological applications, is that it does not require sample preparation, it is non-destructive and little interferences from water or fluoresces as it is not the case with FT-IR. Moreover, the recent integration of Raman spectroscopy with confocal-designed optical microscope allows for simultaneous sample identification and imaging. Biomedical applications that have benefited from confocal Raman are the study of cellular processes (such as differentiation), tissue engineering (the study of cellular localization on scaffolds), drug release or degradation of nanoparticles within cells.

Recently, there has been an explosion of publications highlighting how the integration of nanoscale matters with Raman and SPRi can lead to super enhanced detection and sensitivity. In this lecture, I will review how Surface Enhanced Raman Scattering (SERS), Tip Enhanced Raman Spectroscopy (TERS) and SPRi signal amplification has advanced measurement of biological activity.

1. Quantum Dot SPRi Signal Amplification



2. SERS and TERS Biomedical Applications



Discussion

Initial reports of SPRi detection signal dramatically increasing was using near infrared (NIR) quantum dots (QDs) binding to the gold surface. A 25-fold signal enhancement was observed for a 1 nM concentration of single-stranded DNA (ssDNA) (Figure 1) and a 50-fold enhancement for a 1 µg/mL concentration of prostate-specific antigen (PSA), a cancer biomarker, thus substantiating the potential of NIR QDs to detect interactions of a diverse set of small

biomolecules. This significant enhancement is attributed to the QD's mass-loading effect and spontaneous emission coupling with propagating surface plasmons, which allowed the SPRi limit of detection to be reduced to 100 fM and 100 pg/mL for ssDNA and PSA, respectively. Furthermore, this novel method of QD signal amplification can in principle be used to study interactions of a wider selection of small biomolecules like progesterone (Figure 1), which are currently undetectable with the SPRi instrument at low concentration.

For clinical relevancy, an ultrasensitive SPRi-based nano-aptasensor for the detection of C-reactive protein (CRP) at 5 fg/ml (43 aM [10^{-18} M] and/or 7 zeptomole [10^{-21} moles, (assay volume of 150 μl)]) level in spiked human serum was developed. This ultrasensitive system was engineered through the unique integration and combination of the SPRi platform with microwave-assisted surface chemistry, aptamer technology and NIR QDs. Expanding on this work, a dual microarray SPRi based immuno-platform for the multiplex and ultrasensitive detection of two biomarkers, the High Mobility Group Box 1 (HMGB-1) and Kidney Injury Molecule-1 (KIM-1) biomarkers, was developed using a sandwich assay that implements functionalized quantum dots (NanoEnhancers) as signal amplifiers to achieve a sensitivity level of 5 pg/mL.

By using, a SERS probe Bowey et al.[4](Figure 2) showed that one can pinpoint and quantify the expression of a biomarker at the single cell level; they also showed that different types of tags can be used to detect multiple biomarkers simultaneously[5]. Furthermore, single biomolecule sensing was achieved by using a unique construct of gold nanospheres-on-gold thin film.[6]

Alternatively, TERS integrates atomic force microscopy (AFM) with Raman microscopy offering the sensitivity of SERS with ultra-high spatial resolution provided by the AFM tip. This technique has been shown to identify molecular information at the nanoscale for the detection of different virus strains[7], peptide nanotapes[8], DNA sequencing[9] and formation of hemozoin crystal within individual red-blood cells infected with the malaria parasite (Figure 2)[10], to name a few.

Conclusion

The field of medical diagnostics will have tremendous potential gains in the near future from Raman spectroscopy and SPRi. These technologies as illustrated above have the ability to detect and visualize the undetectable leading to novel breakthroughs and paving the door for personalized medicine.

Acknowledgments

This research is supported by the US Army Department of Defense, Friends for an earlier breast cancer test and US Army Medical Research and Materiel Command.

References

1. Rich, R. L.*, et al. Journal of Molecular Recognition* **2008**, *21*, 355.
2. Homola, J. *Analytical and Bioanalytical Chemistry* **2003**, *377*, 528.
3. Hoa, X. D.*, et al. Bioelectronics and Biosensors* **2007**, *23*.
4. Bowey, K., *et al. Colloids and Surfaces B: Biointerfaces* **2014**, 122, 617-622
5. Kim, I., *et al. Journal of Molecular Structure* **2012**, 1023, 197-203
6. Li, L., *et al. Analyst* **2013**, 138, 4574-4578
7. Hermann, P *et al. Analyst* **2011**, 136, 1148-1152
8. Paulite, M. *et al. ACS Nano* **2013**, 7, 911-920
9. Treffer, R., *et al. Beilstein Journal of Nanotechnology* **2011**, 2, 628-637
10. Wood, B. R., *et al. Nano Letters* **2011**, 11, 1868-1873

Medicinal Cannabinomics & Mass Spectrometry Applications to Cannabis Testing Laboratories

Scott A. Kuzdzal, PhD.
Shimadzu Scientific Instruments, Inc., Columbia MD, USA.

Abstract

Thirty-seven states have pro- medical cannabis laws, including twenty-three states (and DC) that have medical laws, and four states that have full legalization. There are now over 2 million legal medical marijuana patients in the U.S.A., and nearly half of Americans are now living in states with some form of medical marijuana or CBD legislation. Cannabis testing laboratories have emerged to accurately determine cannabinoid potencies in cannabis products as well as ensure that these products are free from contaminants.

High performance liquid chromatography and gas chromatography are important cannabis testing laboratory separation techniques. Mass spectrometry is playing an increasingly important role in cannabis testing laboratories for the analysis of cannabinoids and terpenoids in cannabis products, included extracted oils. Mass spectrometry is also being employed for the ultra-low level quantitation of contaminants including pesticides and heavy metals. Application of ultra-fast electrospray ionization LC-MS/MS with continuous polarity switching for the simultaneous analysis of pesticides in positive and negative mode and approximately 200 pesticides were measured with over 500 MRM transitions per run. Additionally, data presented illustrates how a triple quadrupole GC-MS/MS operated in the MRM mode, can be used to analyze for trace-level pesticide residues in complex plant matrices such as medical cannabis flowers and extracted oils.

As mass spectrometry adoption continues to grow and its utility expands, it is critical that mass spectrometry knowledge gained from more established scientific disciplines, including food safety, clinical, pharmaceutical and environmental markets, be applied to cannabis testing laboratories. This presentation will also discuss future MS-based opportunities for techniques like MALDI-TOF MS, SFE/SFC-MS, direct ionization methods and integrated “cannabis analyzers”.

Biographical Sketch

Dr. Scott Kuzdzal graduated from SUNY Fredonia in 1992. He received his Ph.D. in Analytical Chemistry in 1997 from the University of California at Riverside. He served as a postdoctoral fellow at the Johns Hopkins University School of Medicine, where he co-founded and directed the Johns Hopkins Center for Biomarker Discovery with Dr. Daniel Chan. He helped complete the Human Genome Project working as Serum and Membrane Proteomics Group Leader at Celera Genomics. Scott has a strong clinical chemistry background and has directed Toxicology and Therapeutic Drug Monitoring Labs at Johns Hopkins Medical Institutions. He currently serves on the editorial review board of Proteomics and hosts the United States Human Proteome Organization (USHUPO) website (USHUPO.org). He has published protein and peptide diagnostic biomarkers for pancreatic cancer (HIPAP1), Alzheimer's disease, Duchenne's Muscular Dystrophy and ovarian cancer using a wide variety of separation and detection methods. Scott has lectured at continuing medical education courses at Johns Hopkins Hospital, George Mason University/INOVA Fairfax Hospital and the NIH, as well as conferences worldwide. He has co-authored several proteomics book chapters and has multiple patents. Scott currently serves as General Manager of Marketing for Shimadzu Scientific Instruments where he continues to innovate and commercialize exciting new, award-winning analytical platforms such as the MegaTOF Ultra High Mass Matrix Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF) mass spectrometer, the Perfinity Workstation and LabSite Informatics Service.

Introduction

Cannabis contains more than 500 unique compounds; we have a long way to go before understanding the health benefits of all of them or appreciating the synergies between cannabinoids, terpenes and flavonoids. For more than 80 years, cannabis chemistry has been suppressed, and the industry itself needs to recover. In the USA, there are currently 24 states (and the District of Columbia) that have legalized medical marijuana and another four states that have legalized recreational marijuana. The analytical upshot? Cannabis sold for either medicinal or recreational use needs testing for potency and to reduce the risk of contamination. Indeed, cannabis-testing laboratories are sprouting up across the USA; they are vital to accurate cannabis product labeling, as well as cannabis quality control. Cannabis testing also serves to determine peak harvesting times for growing operations.

The important psychoactive component in marijuana is tetrahydrocannabinol (THC), making it the primary focus of potency testing. Natural Cannabis plant material contains THC-A, the

non-psychoactive, carboxylic acid form of THC. Since THC-A is converted to THC upon heating, high performance liquid chromatography (HPLC) is the method of choice for testing because it can distinguish THC from THC-A. Gas chromatography (GC) can also determine total THC.

A big challenge in potency testing is the complexity of the cannabinome; there are at least 80 cannabinoids present in cannabis, each with unique therapeutic properties [1]. Most cannabis analytical testing, however, includes values for less than a dozen cannabinoids. Cannabidiol (CBD) – another major component of cannabis [2]– is a phytocannabinoid that accounts for 35 to 40 percent of cannabis extracts. THC-A, THC and CBD, cannabidiolic acid (CBDA), cannabigerolic acid (CBGA), cannabigerol (CBG), cannabinol (CBN), Δ9-tethrahydrocannibinol (Δ9-THC), Δ9-tethrahydrocannibinolic acid (Δ9-THCA) and cannabichromene (CBC) are commonly measured cannabinoid (CBx) profiles. These cannabinoids are all separated and analyzed using conventional HPLC (not UHPLC) in under two minutes [3]. More work is needed to develop analytical conditions for rapid and accurate separation and detection of the 70-plus other cannabinoids.

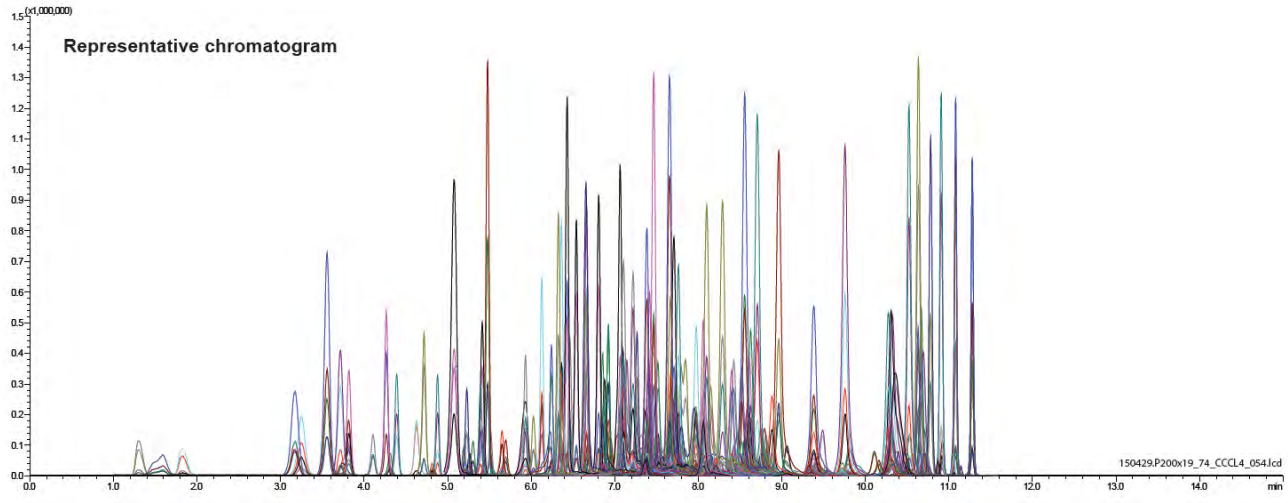
Health disorders with reported cannabinoid impact.

See www.Leafly.com for more information regarding cannabis reported health benefits.

1. Analytical measurement of cannabis products.

Application of HPLC technology to cannabinoid separation at Rose City Laboratories (www.RoseCityLabs.com), left, and measured Blueberry Kush potency of 20.41% THC, right.

2. Measurement of Pesticides Residues in Cannabis.



Analysis of over 200 pesticides residues in cannabis using triple quadrupole LC-MS/MS.

Discussion

Medicinal cannabis refers to the use of cannabis and its corresponding cannabinoids, as a therapy to treat diseases and/or alleviate symptoms. While less than 6% of today's studies on marijuana analyze its medical properties, publications to date indicate that cannabis shows great promise for the treatment of many diseases and symptoms. The cannabis industry is projected to be an $8B industry by 2018. With this growth has come an explosion in cannabis testing labs, which perform testing that ensures patients receive safe products free from pesticides, contaminants, residual solvents and microorganisms.

This presentation will provide an overview of medicinal cannabis, including grow operations and dispensaries. This will be followed by an overview of lab testing, highlighting the analytical instrumentations used for cannabinoid profiling and potency testing. Knowledge of both the cannabinoid profile and potency is essential in informing users as well as learning more about potential health benefits.

Quantitation of the cannabinoids d9-THC-A, d9-THC, d8-THC, CBD-A, CBD, CBG, CBN and CBC were carried out using HPLC, LC-MS/MS and GC-MS on both dry products and concentrated oils. Extraction of cannabinoids using different solvents was investigated and yields for these extractions were compared, with a focus on cannabinoids with reported health benefits. Typical cannabis THC-A potencies ranged from 5 to 25 % in plant materials and edibles, but were much higher for concentrated oils. One "Melly Nova G.I." strain provided an extremely high THC-A level of 31.8 %. Multiple butane hash oil samples showed THC-A concentrations over 91 %. Fifteen terpenes, including linalool, camphene and humulene were also quantitated using GC-MS. Cannabis breeding designed to create new strains with maximum CBx levels was performed and end product potencies were determined.

While the premium products in medicinal cannabis dispensaries are typically those with high THC concentrations, other non-psychoactive "CBx" cannabinoids like cannabidiol (CBD) have been reported to reduce convulsions, inflammation, nausea and anxiety, and even eradicate tumors in some patients.

The analytical detection of pesticides in cannabis remains a challenge. Pesticides are used in commercial cannabis grow operations to kill mites that thrive on cannabis plants. Female mites lay over 2,000,000 eggs per day at 90• Fahrenheit. Also, they are mutating throughout the cannabis

industry with resistance to some pesticides. Thrips (tiny, slender insects with fringed wings), aphids, and root gnats are common indoor pests. Spider mites, caterpillars, grasshoppers threaten greenhouse grows. *Halyomorpha halys*, also known as the brown marmorated stink bug, is a voracious eater and has an affinity for cannabis plants.

An enormous number of pesticides are available in the commercial marketplace, and no lab can test for all of them. GCMS is the preferred instrumentation platform for such testing. While there are currently no guidelines for residual pesticide screening in cannabis, most labs test for the most common pesticides employed during cannabis cultivation: organophosphates, carbamates, pyrethroids and avermectins.

Prevention of fungal infection is an essential aspect of cannabis production. Although proper growing and harvesting practices should be followed to prevent spoilage, in some cases antifungal agents have been applied to crops suffering from severe infection. Residues from these agents pose a risk to consumers, and testing by a sensitive and selective technique such as LC-MS-MS is needed to determine whether a specimen is contaminated. In the present work, samples from a cannabis plant treated with the antifungal myclobutanil were prepared with a QuEChERS-style extraction and measured by liquid chromatography-triple quadrupole mass spectrometry.

Conclusion

Analytical testing plays a critical role in delivering safe products to the marketplace. Instrumentation companies with quality control testing and analysis experience in pharmaceuticals, clinical, environmental and food safety are helping the cannabis industry meet the challenging demands of cannabis product testing. Much research is needed to better understand the full impact of cannabinoids on human health. This presentation will conclude with opportunities and needs for future mass spectrometry based cannabis testing.

Acknowledgments

This research was carried out plant samples provided by a licensed grower and samples were analyzed in a licensed laboratory in full compliance with Washington and Oregon state laws. G.I. Grow is a licensed biomedical farm in Oregon that strictly abides by Oregon Medical Marijuana Program guidelines. We also acknowledge the help from several testing laboratories, including Trace Analytics, Rose City Laboratories and MRX Labs. Lastly, we thank Josh Crossney at CANNCON, Inc., a

non-profit organization devoted to cannabis science research and testing (see www.canncon.org for more information). To learn more about LC, GC, LCMS, GCMS, ICP, and other analytical instruments used in natural products research, please visit www.GrowYourLab.com.

Disclaimer

Shimadzu products mentioned in this article are for Research Use Only and are not for use in diagnostic procedures. Shimadzu Scientific Instruments is not condoning the use of recreational nor medical marijuana, we are merely being solicited by customers for cannabis testing at their facilities and are herein providing a market summary of the cannabis industry.

References

1. L Hanus, “Biogenesis of cannabinoid substances in the plant”, Acta Universitatis Palackianae Olomucenesis Facultatis Medicae 116: 47-53 (1987).

2. LM Borgely, et al., “The pharmacologic and clinical effects of medical cannabis”, Pharmacoteherapy (Review) 33 (2):195-209 (February 2013).

3. K Kovash, et al., “Medicinal cannabis and the need for enhanced cannabinoid profiling”, poster presentation at MSACL 2015.

4. E Rosenthal and D Downs, “Beyond Buds”, ISBN:978-1-936807-23-9 (2014).

5. MJBiz Daily article, “FDA says CBD products don’t qualify as dietary supplements”, May 15, (2015).

6. M Ferner, “U.S. legal pot sales to hit $8 Billion a year in 2018: Report”, Huffington Post (04/11/2014 15:02 EDT).

Application of Laser/Desorption Ionization Mass Spectrometry as a Novel Surface Analytical Tool

Takaya SATOH, PhD
JEOL Ltd.

Abstract

Imaging mass spectrometry(IMS) for matrix-assisted laser desorption/ionization(MALDI) has been expanded during the last decade in biological applications. The MALDI-IMS also has an application potentiality to material science field; however, the studies in this field were limited. In this presentation, application of LDI-IMS as a novel surface analytical tool is discussed in following three items. (I) Development of a high-mass resolution time-of-flight mass spectrometer for IMS. (II) Improvement in a lateral resolution of IMS and investigation of detection depth using silver nanoparticles surface assisted laser desorption/ionization. (III) Cooperative analyses with depth profiling of x-ray photon microscopy and non-destructive Raman microscopy.

Biographical Sketch

Dr. Takaya Satoh was received the Ph. D degree in physics from Osaka University, Osaka Japan, in 2002. He has started working in JEOL from 2002. He has developed a unique time-of-flight mass spectrometer with spiral ion optical system, so called “SpiralTOF”. Recently, his research interest includes imaging mass spectrometry as a surface analytical tool and cooperative analysis with existing surface analytical tools.

Introduction

Imaging mass spectrometry (IMS) [1] for matrix-assisted laser desorption/ionization (MALDI) has been expanded during the last decade in biological applications, to assess the distribution of proteins, peptides, lipids, drugs, and metabolites in a tissue specimen. MALDI-MS is a powerful tool for the characterization of synthetic polymers and additives. Although MALDI-IMS could also be applied in materials science, the number of reports is still limited. The laser desorption/ionization (LDI) IMS, including MALDI-IMS, has an advantage in organic compounds analysis due to an ability of

analyzing molecular structure, where other spectroscopic methods can obtain only elemental information. In this presentation, the potentiality of LDI-IMS as a new surface analytical tool is discussed in next 3 points: (I) development of high mass resolution time-of-flight mass spectrometer for non-target LDI-IMS, (II) solvent-free silver nanoparticle surface assisted laser desorption/ionization (Ag-NP SALDI-IMS) for improvement of spatial resolution and investigation of detection depth, and (III) cooperative analysis with x-ray photoelectron spectroscopy(XPS) and Raman spectroscopy.

Instrumentation

The time-of-flight mass spectrometer with a spiral ion trajectory [2], SpiralTOF (JEOL Ltd., Tokyo, Japan), has been developed for improvement of mass resolution. The spiral ion optical system made by four toroidal electrostatic sectors is shown in Fig. 1. Each toroidal electrostatic sector has eight stories made by nine Matsuda plates piled up inside a cylindrical electrostatic sector. The ions pass the four toroidal electrostatic sectors sequentially and revolve along a figure-eight-shaped orbit on a certain projection plane. During multiple revolutions, the ion trajectory shifts perpendicular to the projection plane every revolution cycle, thus generating a spiral trajectory. The total flight path of SpiralTOF was 17 m, which is 5-10 times longer than a reflectron TOF mass spectrometer.

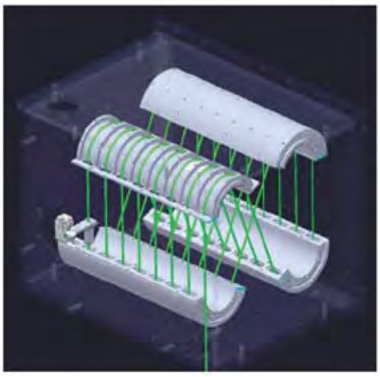


Fig.1 Spiral ion optical system

Experiment

Two-layered grid patterned thin films, which consisted of upper Irganox 1010 layer on the bottom Polystyrene (PS) layer, were made on a silicon wafer. At first, PS solution was spin-coated onto a clean Si wafer. This step produced a 180-nm-thick PS film. A 55-line-per-inch grid was layered, and then Irganox 1010 was vapor-deposited on it to a thickness of 160 nm. After removing the grid, a thin film of Irganox 1010 and PS with a grid pattern remained, and the sample was labeled Irganox-grid/PS. To prepare the samples for Ag-NP SALDI-IMS, their surfaces were coated with a layer of Ag-NPs (10-nm thick) by vacuum vapor

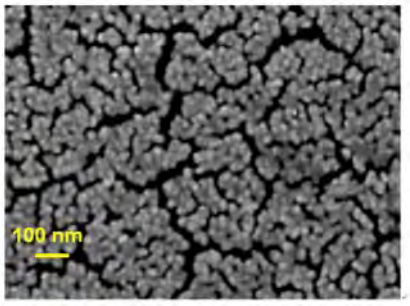


Fig.2 SEM image of silver nanoparticles on sample surface.

deposition. These coated samples were labeled as Ag/Irganox-grid/PS. A scanning electron microscope image of Ag-NPs on the sample surface (Fig. 2) was recorded using a JSM-7610F (JEOL Ltd., Tokyo, Japan).

Discussion

High mass resolution imaging mass spectrometry

The MALDI-IMS for lipids directly on a tissue specimen are shown in Fig. 3. The averaged mass spectrum for all of the image pixels is shown in Fig. 3a. A number of peaks observed in *m/z* 700 – 1000 were originated in different type of lipids. The enlarged mass spectrum for *m/z* 820 – 823, which showed several doublet and triplet peaks for minor components, are shown in Fig.3b. The Phosphatidylcholine (PC), two types of phosphatidylethanolamines (PE), galactosylceramide (GarCer) and their corresponding isotopic peaks were observed within 3 u. The mass images of the four peaks are also shown in Fig. 3b. Each compounds showed a distinctly different distribution across the surface of the mouse brain. The result shows isobaric separation is important for MALDI-Imaging, because the inherent distribution of the compounds of doublet or triplet peaks will be lost using reflectron TOF mass spectrometer with insufficient mass resolution.

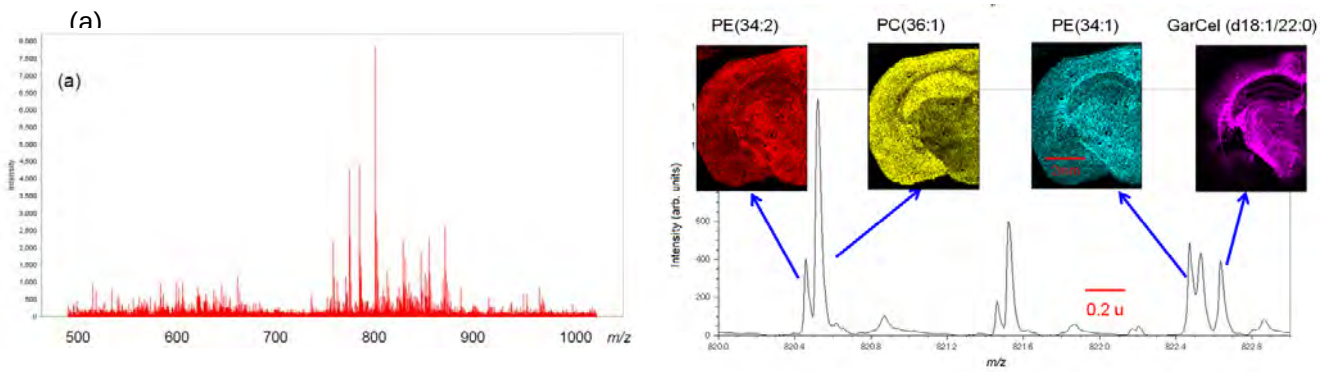


Fig.3 High mass resolution IMS for lipids in brain tissue section.

Solvent-free Ag-NP SALDI-IMS

Solvent-free Ag-NP SALDI-IMS are expected to improve the lateral resolution of an additive on a synthetic polymer surface. Ag-NP SALDI-IMS was performed using Ag/Irganox-grid/PS. The extracted mass images of $[\text{Irganox-Ag}]^+$ and $[\text{PS-Ag}]^+$ series are shown in Figs. 4a and b, respectively. The line intensity profiles taken along the red solid line in Fig. 4a are shown in Fig. 4c. The intensities of the $[\text{Irganox-Ag}]^+$ and $[\text{PS-Ag}]^+$ series alternated depending on the grid shape. The ions from the PS layer under the Irganox 1010 layer were not observed. This result indicated that ionization only

occurred in the Irganox 1010 layer, which is 150-nm thick. The detection depth was estimated to 50 nm by changing the thickness of Irganox 1010 layer.

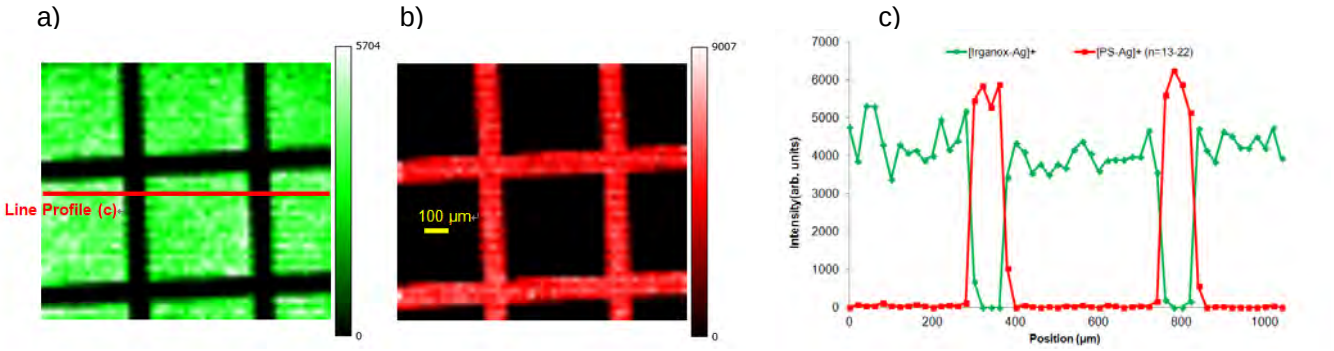


Fig.4 Ag-NP SALDI IMS for Irganox 1010 on PS layer.

Cooperative analyses with XPS and Raman Spectroscopy

It is important to perform cooperative analysis with existing surface analytical instrument for a complete analysis of complex material system, because every method has disadvantages. One of the disadvantage of LDI-IMS is it cannot be applied to depth profiling. The cooperative research was performed with XPS depth profiling. The other disadvantage of LDI-IMS is it damages the sample surface by laser irradiation. The sequentially analysis of the thin-film was performed: At first with Raman microscopy, which is known as a non-destructive analysis, and then with SALDI-IMS.

Conclusion

The LDI-IMS can be a powerful tool for analyzing localization of organic compounds in sample surface. It is expected to use in material science field as a novel surface analytical tool.

Acknowledgments

The IMS data for lipids in mouse brain tissue specimen were acquired in a joint research project with the Graduate School of Science, Osaka University. We thank Mr. N. Moriguchi, Assistant Professor Dr. H. Hazama and Professor Dr. K. Awazu for providing the mouse brain tissue specimens. We also thank Associate Professor J. Matsuo and his group at Quantum Science and Engineering Center, Kyoto University for providing their organic thin-films samples.

References

1. J. H. Jungmann et al., *J. Proteomics.*, 75, 5077 (2012)
2. T. Satoh et al., *J. Am. Soc. Mass Spectrom.*, 18, 1318, (2007)

Closing Remarks

It is a great pleasure for the Japan Society for Analytical Chemistry (JSAC) and Japan Analytical Instruments Manufacturers' Association(JAIMA) to co-host the eighth Japan Symposium 2016. I would like to take this opportunity to express our gratitude for the continued support of the Pittcon Committee members especially William R. Sharpe, Pittcon 2016 President and Dr. Chuck Gardner, Technical Program Chair. of Pittcon 2016. The seventh Japan Symposium was held in 2015 and a great success with more than 200 attendees. I would like to take this opportunity to express our gratitude for the continued support of the Pittcon Committee members especially Charlie Holifield, President of Pittcon 2015 and Dr. Hub MacDonald, Technical Program Chair. of Pittcon 2015.

Mr. Gon-emon Kurihara was appointed as Chairman of JAIMA in May last year, and he has stressed the importance of implementing measures for the globalization of JAIMA. We will promote symposiums for exchange with other countries even more vigorously than we did last year, in particular with the United States and countries in Asia. Please continue to give us your support.

Finally, in addition to hoping for a successful Pittcon 2016 and JAISIS 2016. I hope that the Japan Symposium at Pittcon will be continued for many years to come as a demonstration of the fruit of the collaboration between Pittcon and JSAC & JAIMA. I also look forward to Pittcon holding the seventh U. S. Symposium at the International Conference Session of JAIMA Conference 2015, where the JAISIS 2015 will also take place.

March 8, 2016

Chairman of Joint Committee for Pittcon-2016 JAIMA & JSAC Symposium,
Chairman of Special Committee for JAIMA International Conference
Koichiro Matsuda, Ph. D.

Vice-Chairman of Joint Committee for Pittcon-2016 JAIMA & JSAC Symposium,
Professor, Department of Applied Chemistry, Faculty of Science and Technology,
Keio University, President of The Japan Society for Analytical Chemistry (JSAC)
Koji Suzuki, Ph. D.

Members of Steering Committee

＊Joint Committee for Pittcon-2016 JAIMA & JSAC Symposium

Chairman	Koichiro Matsuda, JAIMA, HORIBA. Ltd.
Vice-Chairman:	Koji Suzuki, JSAC, Keio University
	Takaho Watanabe, JSAC, Food and Drug Safety Center
	Osamu Niwa, JSAC, Advanced Industrial Science and Technology
	Nobuo Uehara, JSAC, Utsunomiya University
	Hideaki Hisamoto, JSAC, Osaka Prefecture University
	Akihide Hibara, JSAC, The University of Tokyo
	Daniel Citterio, JSAC, Keio University
	Kosaburo Kawanishi, Hitachi High-Technologies Corporation
	Takeshi Kawamoto, JAIMA, SHIMADZU Corporation
	Izumi Oi, JAIMA, JEOL Ltd.
	Masao Nomoto, JAIMA, JEOL Ltd.
	Kyoichi Komori, JAIMA
	Nobuyoshi Kataoka, JAIMA

＊Special Committee for JAIMA International Conference

Chairman	Koichiro Matsuda, JAIMA, HORIBA. Ltd.
Vice-Chairman	Takeshi Kawamoto, JAIMA, SHIMADZU Corporation
	Kosaburo Kawanishi, Hitachi High-Technologies Corporation
	Izumi Oi, JAIMA, JEOL Ltd.
	Masashi Taguchi, JAIMA, Kyoto Electronics Manufacturing Co., LTD.
	Masao Nomoto,JAIMA, JEOL Ltd.
	Yoshitoki Iijima, JEOL Ltd.
	Makoto Nishino, SHIMADZU Corporation
	Satoshi Nomura, HORIBA. Ltd.
	Katsuihiro Nakagawa, SHIMADZU Corporation
	Yasuo Horiguchi, JASCO International Co., Ltd.
	Go Wakao, SHIMADZU Corporation
	Kenichi Yaoita, Rigaku Corporation
	Kentaro Hayashi, JAIMA
	Nobuyoshi Kataoka, JAIMA
	Kyoichi Komori, JAIMA
	Fusao Tonotsuka, JAIMA
	Yoko Tabayashi, JAIMA

Japan Symposium

Pittcon 2016 Symposium

March 8, 2016

Japan Analytical Instruments Manufacturers' Association (JAIMA)

1-12-3 Kanda Nishiki-cho, Chiyoda-ku,

Tokyo, 101-0054 Japan

TEL : +81-3-3292-0642

URL : http://www.jaima.or.jp

8th JAPAN SYMPOSIUM 2016

PITTCON 2016 SYMPOSIUM

March 8(Tue),2016 Place: Georgia World Congress Center, Atlanta, GA U.S.A.

Morning Session

The State-of-the-ArtTechnologies from Japan: Analytical Instruments with / for Nano-Chemistry Technology and Advanced Diagnostics (I)

-arranged by Koichiro Matsuda, Japan Analytical Instruments Manufacturers' Association (JAIMA)

Koichiro Matsuda, JAIMA, Presideing
Takeshi Kawamoto, JAIMA, Co-Presiding

8:30 - 8:35	**Introductry Rermarks** Gon-emon Kurihara, President of JAIMA
8:35 - 9:10	**Creation of Bio/Chemical Sensing Probes** Koji Suzuki , Keio University
9:10 - 9:45	**Ab Initio Powder Structure Determination Opening up New Research Fields** Masaki Kawano , Tokyo Institute of Technology
9:45 - 10:20	**Laser Spectroscopy for Micro/Nano Scale Liquid Interfaces** Akihide Hibara , Tokyo Institute of Technology
10:20 - 10:55	**Development of Mass Microscope and Applications in Clinical Pharmacology** Shuichi Shimma , Graduate School of Engineering, Osaka University
10:55 - 11:30	**Plasma Phospholipids and Prevalence of Mild Cognitive Impairment/dementia** Danni Li , University of Minnesota

Afternoon Session

The State-of-the-ArtTechnologies from Japan: Analytical Instruments with / for Nano-Chemistry Technology and Advanced Diagnostics (II)

-arranged by Koichiro Matsuda, Japan Analytical Instruments Manufacturers' Association (JAIMA)

Koichiro Matsuda, JAIMA, Presideing
Koji Suzuki, Keio University. Professor

13:30 - 13:35	**Introductry Rermarks** Koji Suzuki, President of JSAC
13:35 - 14:10	**Recent development on boron-doped diamond electrodes** Yasuaki Einaga , Keio University
14:10 - 14:45	**Plasmonic Nanomaterials** Tetsu Tatsuma , The University of Tokyo
14:45 - 15:20	**The Unique Combination of Nanotechnology with Raman and SPRi Platforms Offers Innovative and Ultrasensitive Solutions for Diagnostics** Marinella G. Sandros , University of North Carolina (Horiba Jobin Yvon Inc.)
15:20 - 15:55	**Medicinal Cannabinomics & Mass Spectrometry Applications to Cannabis Testing Laboratories** Scott Kuzdzal , Shimadzu Scientific Instruments Inc.
15:55 - 16:30	**Application of Laser/Desorption Ionization Mass Spectrometry as a Novel Surface Analytical Tool** Takaya Sato , JEOL Ltd.

Japan Analytical Instruments Manufacturers' Association
1-12-3, Kanda Nishiki-cho, Chiyoda-ku, Tokyo, 101-0054, Japan
Phone : +81-3-3292-0642 Fax : +81-3-3292-7157 E-mail : webmaster@jaima.or.jp
http://www.jaima.or.jp

The Japan Society for Analytical Chemistry
1-26-2 Nishigotanda, Shinagawa-ku, Tokyo, 141-0031 Japan
Phone : +81-3-3490-3351 Fax : +81-3-3490-3572 E-mail : analytsci@jsac.or.jp
http://www.jsac.or.jp

Printed in Japan 2016.02