Tap Into

The official magazine of the Virginia Section of the American Water Works Association

VIRGINIA

Winter 2015

A Navy Approach to Water Quality Management

INSIDE:

- Paper-Based Sensors for Detection of Cyanobacteria in Water Samples
- Emergency Tank Repair Prevention and Preparation

American Water Works Association
VirginiaSection

P.O. Box 11992, Lynchburg, VA 24506-1992
Address service requested

Paper-Based Sensors for Detection of Cyanobacteria and Their Toxins in Water Samples

Ishan Jain
Master of Science, Environmental Engineering,
Virginia Tech, Blacksburg.
Email: *ishanj13@vt.edu* Phone: 540-613-0895

Abstract

Occurrence of cyanobacteria and their toxins is a worldwide phenomenon. Cyanotoxins such as mirocystins and nouldrin are deadly to animals and humans at low levels of exposure and need to be monitored effectively to ensure safety of consumers.

In this article, use of surface enhanced Raman spectroscopy (SERS) is proposed for detection and characterization of cyanobacteria and their toxins. SERS has emerged as one of the most promising analytical technique in the past decade; and is particularly well suited for environmental analyses due to high sensitivity, specificity, ease of operation and rapidity. Detection and characterization of environmental contaminants, through SERS highly depends on uniformity, SERS activity and reproducibility of its substrates. Therefore, in this article, use and development of paper based substrates (also called paper based microfluidic devices) will be discussed.

Introduction

Consumers today demand drinking water that looks, smells and tastes good, as the presence of unpleasant taste and odor in water create a perception of unsafe drinking water [1]. The major sources of taste and order problems in municipal water supplies are certain type of blue-green algae (cyanobacteria) and actinomycetes [1].

Cyanobacteria are ubiquitous and found worldwide, especially in calm, high nutrient water sources, especially in summer [2, 3]. According to a study in 2009, water bodies of at least 50 countries were infested by cyanotoxins poisoning [4]. Even in the United States, at least 36 states were implicated in illness and death of animals and human associated with cyanobacteria[4]. Furthermore, according to a World Health Organization (WHO) report in 1999, cyanobacteria and their toxins are responsible for animal poisoning and adverse human health impacts in Klamath River, Eel River, Clear Lake, Pinto Lake, and various parts of northern California's water sources [5].

People may be exposed to cyanobacterial toxins by drinking or bathing in contaminated water. The most frequent and serious health effects are caused by drinking water containing the toxins (from cyanobacteria), or by ingestion during recreational water contact [6]. Based on the type of cynaobacteria and level of exposure, their toxins may cause nerve and liver damage [7]. Microcystis aeruginosa releases toxins called Microcystin which may cause liver failure and promote tumor growth at low level of exposure [6].

According to the WHO guidelines, the acceptable limit for the toxin Microcystin-LR (a natural occurring toxin produced by cyanobacteria, MC-LR) in drinking water is 1 μg/L [6]. Although due to excessive human demand on water resources, amount of toxins usually exceed this limit. The US EPA considered these cyanotoxins in contaminant candidate list 3 (CCL3) and has prepared draft documentation on toxicological review and guidelines for many cyanobacterial toxins such as anatoxin-a, cylindrospermin, and microcystins (MC-LR, MC-LA, MC-YR and MC-RR). Hence, there is a dire demand to monitor these algae and their toxins in drinking water recourses.

Current methods for detection of cyano-bacteria and their toxins

The existing methods for cyanobacterial detection are chlorophyll counts, microscopic identification, fluorescence monitoring, mass spectroscopy, and gas chromatography [8].

US EPA proposed a multistep method to monitor cyanobacteria and their toxins in water samples (Figure 1). This proposed method has multiple stages and requires expertise. Also, these methods are time-consuming, complex, and require expert trainings. Furthermore, these methods can't distinguish among nontoxic algae to toxic cyanobacteria [9, 10].

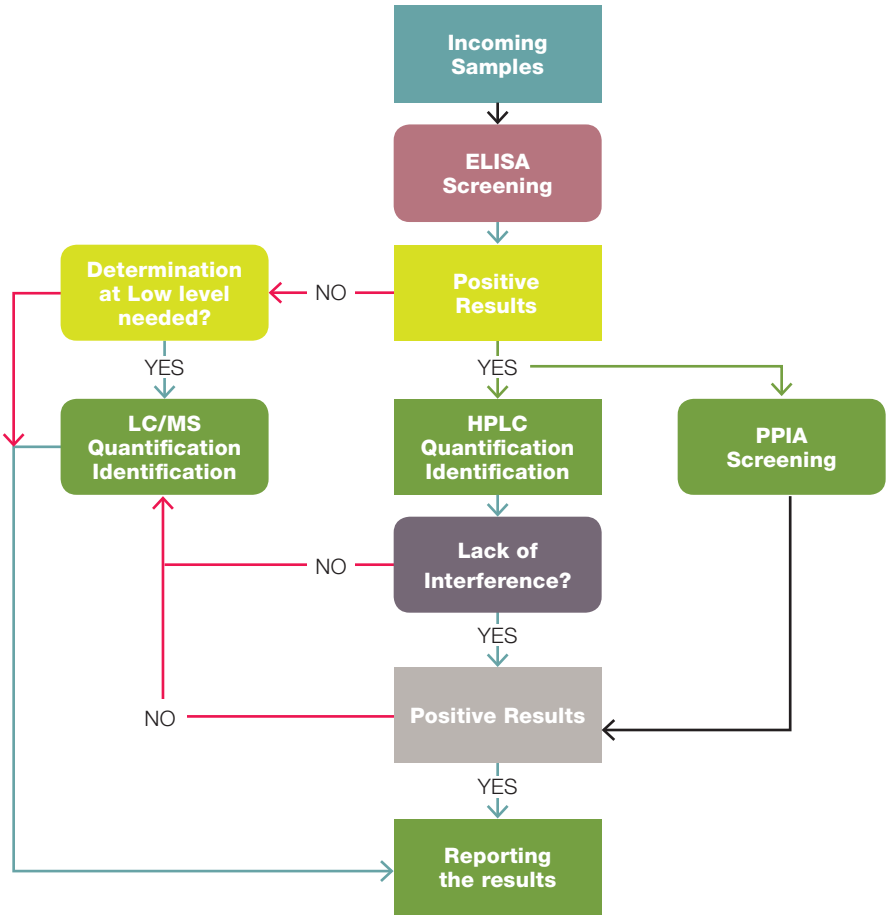

Figure 1 EPA proposed monitoring method of cyanobacteria and its toxins using Enzyme-linked immuno- sorbent assay (ELISA), Protein Phosphates inhibition assay (PPIA) and High Pressure Liquid Chromatography (HPLC) (from EPA factsheet, 2012 [8]).

Raman spectroscopy for cyanobacteria detection

In an effort to distinguish toxic from non-toxic *Microcystis*, Halvorson R.A. et al., collected normal Raman spectra of several strains of *Microcystis aeruginosa* and the common green algae *Pseudokirchneriella subcapitata* (Figure 2) [11]. The preliminary data shows distinct differences in the Raman spectra (as noted by the black arrows) of the green algae and the cyanobacteria. Most importantly, it shows the readily apparent differences in the spectra of the toxic and non-toxic strains. This observation indicates that a Raman spectroscopy based approach to detect and differentiate cyanobacteria is potentially feasible and merits further study.

Theoretically, normal Raman spectroscopy can detect and characterize every polarizable molecules. However, its applications to detect cyanobacteria or environmental analytes are mostly limited by its ability to produce very low signals intensity, which make it extremely difficult to detect target analytes. Therefore, researchers have been exploring microfluidics, fluorescence methods of probing, biosensors, and surface enhanced Raman spectroscopy for environmental analyses [12-14]. Among these novel techniques, surface enhanced Raman spectroscopy (SERS) is most favorable method for environmental analyses mainly due to its ability to provide unique fingerprints for specific analytes with high selectivity, sensitivity, and rapidity [12, 15].

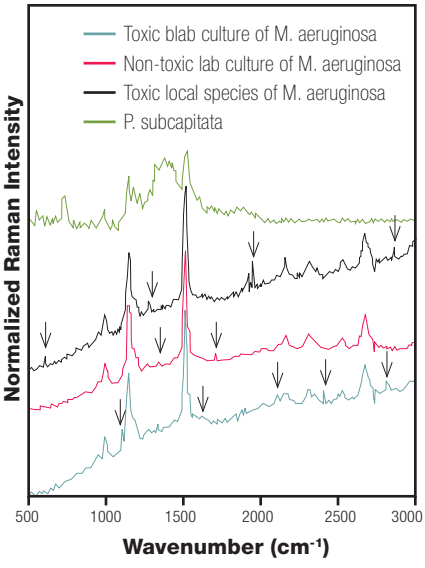

Figure 2 Differentiation of toxic and non-toxic M. Aeruginosa by normal Raman spectroscopy [11].

Properties	Advantages
Sensitivity	Highly sensitive at very low concentration of analytes (even at molecular level)
Unique fingerprint of analytes	SERS spectra provides information about inherent chemical structure of analytes
Rapid detection	Requires less than one min for each measurements
Detection in water	As water is a weak Raman scatter, it can be applied to water samples directly
Cost effective detection	Good laboratory and field detection
Variable detection	Detects chemical and biological variability in analytes

Table 1 Properties and advantages of SERS (developed from [12]).

Surface Enhanced Raman spectroscopy (SERS)

SERS is a powerful vibrational spectroscopy technique that overcomes limitations of traditional Raman spectroscopy and allows for highly sensitive structural detection of low concentration analytes through the amplification of electromagnetic fields generated by the excitation of localized surface plasmons [15-19].

In comparison with other analytical techniques, SERS has outstanding advantages. These advantages are summarized in Table 1.

Because of the properties listed in Table 1, SERS field has dramatically progressed from the originally observed enhancement on roughened silver electrode to the current fields of sensing and image applications, single and simultaneous molecules detection [15-18]. It is a novel, reliable, economical, highly surface selective and ultrasensitive method to qualitatively identify and quantitatively analyze aqueous and airborne contaminants, cyanobacteria and other pathogens [15].

However, whether, SERS can achieve above these applications really depends on its substrates activity and reproducibility of substrates [20]. SERS substrates can be made by following four methods: (1) electrochemical oxidation and reduction cycles (EC-ORC) or Vacuum deposition, (2) nanoparticles sols by wet chemical synthesis, (3) nanoparticles with controlled size, and (4) large-area surface nanostructures with defined size, shape and interparticle distance by self-assembly or lithography methods [20].

SERS substrates made of roughened noble metal (Au, Ag) surfaces are used to get signal enhancement factors ranging from 1×10^4 to 1×10^{10} [15, 21]. SERS substrates fabricated from top-down and bottom-up approaches such as e-beam lithography and colloidal lithography can achieve high enhancement factors ($>10^9$) [20]. With high signal

enhancements from SERS substrates, it will be possible to detect and identify various environmental contaminants including cyanobacteria and their toxins.

An ideal SERS substrate should have high SERS activity, suitable reproducibility (less than 20%), good stability and uniformity (deviation less than 20%). Thus providing high sensitivity which can be controlled by varying the size (more than 50 nm) and interparticle spacing (less than 10 nm) of nanoparticles [20, 22].

Unfortunately, at present, it is still difficult to obtain ideal SERS substrates which can simultaneously meet all of the above requirements [20, 22]. Conventional SERS substrates based on silicon, glass, and porous alumina are not compatible for analysis of trace amount of analytes because of their nonconformal, rigid and brittle nature [18, 23, 24].

Therefore, researchers are fabricating paper based microfluidic substrates for potential detection of cyanobacteria and other environmental contaminants [23, 25-28].

Microfluidic paper based sensors for SERS applications

Paper is abundant, inexpensive, and its structure and porosity can be modified easily [18, 29, 30]. Microfluidic paper based devices (*μPADs*) provide a novel system for fluid handling and fluid analysis for a variety of applications including health diagnostics and environmental applications [31]. Paper based device transports liquids using capillary forces without requirement of any external forces [32]. There are various techniques to fabricate the paper based microfluidic device such as ink jet etching, wax printing, plasma treatment, screen printing and laser printing [33].

Wax printing is very promising in producing hydrophobic and probing zone in paper. *μPADs* (prepared by wax printing) employ hydrophobic barriers printed on cellulose paper to direct the flow of a sample from a sample spotting zone to a detection zone where analytes concentrations are probed.

These paper based sensors prepared (Figure 3) are used to estimate the signal enhancement with respect to normal Raman. One of the cases where detection of microcystin-RR (MC-RR) was performed, observed signals higher than normal Raman spectroscopy as shown in Figure 4. These significant enhancements produced clear and distinguish peaks of MC-RR. This analysis showed that SERS has a great potential to distinguish and detect cyanobacteria in water samples.

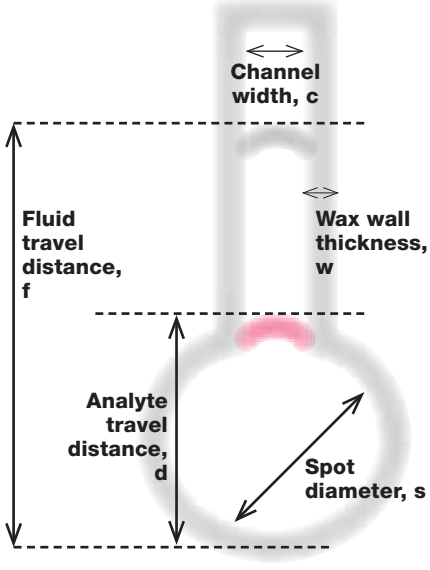

Figure 3 Wax-printed microfluidic paper-based sensors

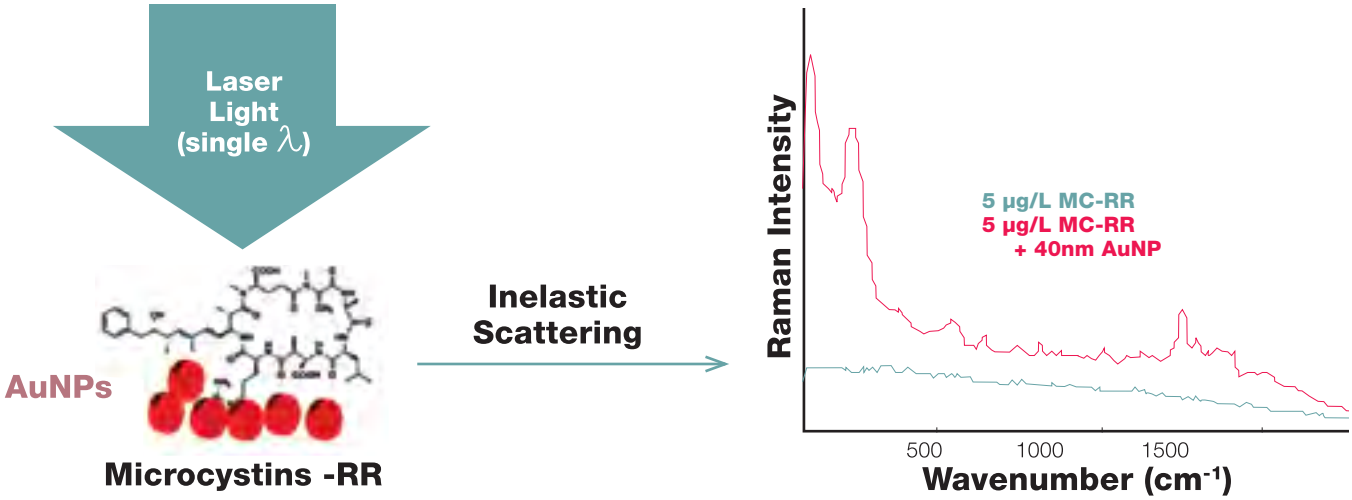

Figure 4 Use of paper-based sensors for microcystins-RR detection using SERS. Observed signal intensity of SERS signals (in red) were higher than normal Raman signal (in blue) [11].

Summary

Surface enhanced Raman spectroscopy has great potential to detect cyanobacteria and their toxins. Use of paper based sensors for this purpose will provide a rapid, inexpensive, and reliable detection protocol to allow water bodies to be regularly monitored for cyanobacteria and their toxins. Such a detection method with capabilities for detecting analytes across multiple contaminant classes would be immensely useful for protecting human health from drinking water threats. Development of these paper based sensors may hold great hope for the environmental analyses in the future.

This study could be the milestone to monitor cyanobacteria and cyanotoxins for regulating agencies such as CDC and EPA. It will be useful in development of an effective guideline for various cyanotoxins and other contaminants.

Acknowledgements

I would like to offer many thanks to Prof. Peter Vikesland (Virginia Tech, Blacksburg) for providing an opportunity to develop paper-based sensors in his lab. I also appreciate help from Dr. Weinan Leng, Dr. Rebecca Halvorson Lahr, and Marjorie Willner for providing figures and data for this article.

References

1. Gottler R., C.J., Westerhoff P., and Abbaszadegan M., *Addressing Concerns About Tastes and Odors and Cyanotoxins in Tap Water.* AWWA Research Foundation Report 91157, 2007.
2. I., C., *Cyanotoxin Occurence in Freshwater.* Springer-Verlag, 2001.
3. Carmichael, W.W., *Assessment of Blue-Green Algal Toxins in Raw and Finished Drinking water.* AWWA Research Foundation and American Water Works Association, 2001.
4. Graham JL, L.K., and Kamman N., *Monitoring Recreational Freshwaters.* Lakeline, 2009. Summer.

5. (CDPH), C.D.o.P.H., *Blue-Green Algae (Cyanobacteria) Blooms*. 2013.
6. WHO, *Cyanobacterial toxins: Microcystin-LR in drinking-water. Background document for preparation of WHO Guidelines for drinking-water quality.* Geneva, World Health Organization (WHO/SDE/WSH/03.04/57), 2003.
7. Chorus I, B.J., eds., *Toxic cyanobacteria in water: A guide to their public health consequences, monitoring and management.* Published by E & FN Spon, London, on behalf of the World Health Organization, Geneva, 1999.
8. EPA, U., *Cyanobacteria and Cyanotoxins: Information for Drinking Water Systems.* Office of water 4304T, 2012.
9. Halvorson, R.A., W. Leng, and P.J. Vikesland, *Differentiation of Microcystin, Nodularin, and Their Component Amino Acids by Drop-Coating Deposition Raman Spectroscopy.* Analytical Chemistry, 2011. 83(24): p. 9273-9280.
10. Halvorson, R.A. and P.J. Vikesland, *Drop Coating Deposition Raman (DCDR) for Microcystin-LR Identification and Quantitation.* Environmental Science & Technology, 2011. 45(13): p. 5644-5651.
11. Halvorson R.A., *Personal communication*. 2011-2013.
12. Li, D.-W., et al., *Recent progress in surface enhanced Raman spectroscopy for the detection of environmental pollutants.* Microchimica Acta, 2014. 181(1-2): p. 23-43.
13. Jokerst, J.C., J.M. Emory, and C.S. Henry, *Advances in microfluidics for environmental analysis.* Analyst, 2012. 137(1): p. 24-34.
14. Li, C.-z., et al., *Paper based point-of-care testing disc for multiplex whole cell bacteria analysis.* Biosensors & Bioelectronics, 2011. 26(11): p. 4342-4348.
15. Halvorson, R.A. and P.J. Vikesland, *Surface-Enhanced Raman Spectroscopy (SERS) for Environmental Analyses.* Environmental Science & Technology, 2010. 44(20): p. 7749-7755.
16. Spencer, K.M., et al. *Surface-enhanced Raman for monitoring toxins in water.* 2004.
17. Jarvis, R.M., A. Brooker, and R. Goodacre, *Surface-enhanced Raman scattering for the rapid discrimination of bacteria.* Faraday Discussions, 2006. 132(0): p. 281-292.
18. Lee, C.H., L. Tian, and S. Singamaneni, *Paper-Based SERS Swab for Rapid Trace Detection on Real-World Surfaces.* Acs Applied Materials & Interfaces, 2010. 2(12): p. 3429-3435.
19. Cialla, D., et al., *Surface-enhanced Raman spectroscopy (SERS): progress and trends*. Analytical and Bioanalytical Chemistry, 2012. 403(1): p. 27-54.
20. Lin, X.-M., et al., *Surface-enhanced Raman spectroscopy: substrate-related issues.* Analytical and Bioanalytical Chemistry, 2009. 394(7): p. 1729-1745.
21. Ji, X., et al., *Size control of gold nanocrystals in citrate reduction: The third role of citrate.* Journal of the American Chemical Society, 2007. 129(45): p. 13939-13948.
22. Yeh, Y.-C., B. Creran, and V.M. Rotello, *Gold nanoparticles: preparation, properties, and applications in bionanotechnology.* Nanoscale, 2012. 4(6): p. 1871-1880.
23. Lee, C.H., et al., *Highly Sensitive Surface Enhanced Raman Scattering Substrates Based on Filter Paper Loaded with Plasmonic Nanostructures.* Analytical Chemistry, 2011. 83(23): p. 8953-8958.
24. Lee, C.H., et al., *Plasmonic paper as a highly efficient SERS substrate.* Chemical, Biological, Radiological, Nuclear, and Explosives (Cbrne) Sensing Xiii, 2012. 8358.
25. Fan, M., G.F.S. Andrade, and A.G. Brolo, *A review on the fabrication of substrates for surface enhanced Raman spectroscopy and their applications in analytical chemistry.* Analytica Chimica Acta, 2011. 693(1-2): p. 7-25.
26. Qian, C., et al., *Comparison of Gold and Silver Nanoparticles as Active Substrates for SERS Spectra Measurements on Liver Cancer Cells.* 2012 7th International Conference on System of Systems Engineering (Sose), 2012: p. 92-95.
27. Khlebtsov, B.N., et al., *Surface-Enhanced Raman Scattering Substrates Based on Self-Assembled PEGylated Gold and Gold-Silver Core-Shell Nanorods.* Journal of Physical Chemistry C, 2013. 117(44): p. 23162-23171.
28. Chen, H.-C., et al., *Surfactant-assisted preparation of surface-enhanced Raman scattering-active substrates.* Rsc Advances, 2014. 4(21): p. 10553-10559.
29. Fu, A., et al., *Paper-based microfluidic immune sensor chip useful for detecting antigen, comprises paper base, microfluidic channel and labeled antibody marker carrier cluster.* Chengdu Junya Technology Co Ltd.
30. Li, X., J. Tian, and W. Shen, *Progress in patterned paper sizing for fabrication of paper-based microfluidic sensors.* Cellulose, 2010. 17(3): p. 649-659.
31. Ngo, Y.H., et al., *Gold nanoparticles-SERS for paper biodiagnostics applications.* Abstracts of Papers of the American Chemical Society, 2012. 244.
32. Martinez, A.W., et al., *Diagnostics for the Developing World: Microfluidic Paper-Based Analytical Devices.* Analytical Chemistry, 2010. 82(1): p. 3-10.
33. Dungchai, W., O. Chailapakul, and C.S. Henry, *A low-cost, simple, and rapid fabrication method for paper-based microfluidics using wax screen-printing.* Analyst, 2011. 136(1): p. 77-82.