34

Laser-Induced Breakdown Spectroscopy for the Analysis of Chemical and Biological Hazards

Lianbo Guo

Wuhan National Laboratory for Optoelectronics, Huazhong University of Science and Technology, Wuhan, China

34.1 Introduction

In the past decades, the danger of chemical and biological hazards (agents or substances that could harm health and the ecosystem) has attracted enormous attention to researchers. How to detect chemical hazards (e.g. heavy metals and explosives) and biological hazards (e.g. bacteria and virus) has become an impressive challenge? Many analytical techniques (such as Raman spectroscopy and inductively coupled plasma mass spectrometry [ICP-MS]) are applied for the analysis of chemical and biological hazards. Among others, laser-induced breakdown spectroscopy (LIBS), as a rapid, facile, and powerful laser-based technique, has been utilized to detect chemical and biological hazards in many reported works (e.g. environment and biomedicine).

34.2 LIBS for Chemical Hazards Analysis

34.2.1 Introduction to Chemical Hazards

Chemical hazards are types of chemical agents (in the form of gas, liquid, or solid) that can cause acute or long-term detrimental health effects. The chemical hazards can be simply classified into two kinds including inorganic hazards (such as metals and non metals) and organic hazards (such as macromolecules). The danger of both kinds of chemical hazards could be enormous due to improper operations. For example, the flammable and explosive macromolecules (organic hazards) are fatally hazardous if mixed or stored with incompatible chemicals. Moreover, some of the chemical hazards (e.g. heavy metals) could cause adverse damage to the ecosystem if they leaked during transportation and storage. Therefore, in view of these dangers, it is crucial to detect those chemical hazards using LIBS.

34.2.2 Detection of Inorganic Chemical Hazards by LIBS

Inorganic chemical hazards include metallic or non metallic elements, strong acids and alkalis, and a few toxic compounds. Among those hazards, LIBS is mainly applied to detect metallic and non metallic elements.

Laser Induced Breakdown Spectroscopy (LIBS): Concepts, Instrumentation, Data Analysis and Applications, *Volume 2*, First Edition. Vivek K. Singh, Durgesh K. Tripathi, Yoshihiro Deguchi and Zhenzhen Wang.

34.2.2.1 Detection of Metallic Elements

Overexposure to metallic elements, especially heavy metals, could cause great damage to the human body. For example, cadmium (Cd) is a toxic element, which can result in bone demineralization and renal dysfunction. Excessive exposure to Cd would damage lung function and increase the risks of lung cancer [1]. Lead (Pb) is harmful to the human body; it can damage the brain, kidneys, and nervous system [2]. Moreover, Table 34.1 indicates the common heavy metals and their health effects [3]. Because of the high toxicity of heavy metals, many LIBS studies have been conducted to detect heavy metals [4, 5]. The analysis of metallic elements by LIBS has been introduced in Chapter 13.

34.2.2.2 Detection of Nonmetallic Elements

Similar to metallic elements, non metallic ones also can threaten health following over-absorption. For example, chlorine (Cl), with exceptional oxidizing properties, is commercially used for treating drinking water by killing pathogenic bacteria and viruses. However, highly concentrated Cl could cause vomiting, coma, and even death. Therefore, it is essential to detect the concentration of nonmetallic elements in many areas (such as the environment).

In fact, the detection of nonmetallic elements by LIBS is a challenge, as those nonmetallic elements have high excited energy and thus are hard to ablate using a pulse laser [6]. To realize qualitative and quantitative determination of nonmetallic elements by LIBS, many facile and novel approaches were proposed to enhance the emission-line intensities. For example, the research group of Prof. Li Xiang You, from Huazhong University of Science and Technology, has made lots of progress in detecting nonmetallic elements [6–8]. To realize sensitive analysis to F and Cl, they synthesized CaF and CaCl by combining calcium in calcite and F and Cl in the sample [6]. Figure 34.1 indicates the LIBS emission spectra of NaF (a) and NaCl (b) aqueous solution depositing on the calcite substrate, depositing on the glass slide substrate and only the calcite substrate. The CaF and CaCl molecular emission lines by LIBS were analyzed, which demonstrated that the molecular emission of CaF and CaCl has a longer lifetime and high spectral intensity than that of their atomic emissions. The results indicated that the limits of detections (LOD) were 0.38 and 1.03 mg/l for F and Cl elements, respectively, in which the detection limits could meet the World Health Organization's detection requirements for F and Cl elements in the water. This proposed method could obtain high sensitivity for F and Cl in water by using LIBS assisted with molecular emission.

Table 34.1 Heavy metals and relative health effects.

Heavy metals	Health effects
Arsenic (As)	Internal cancer, skin lesions, and death
Cadmium (Cd)	Cancer, lung insufficiency, liver and kidney damage
Chromium (Cr)	Ulcer, skin irritation, liver and kidney damage
Mercury (Hg)	Memory problems, increased heart rate, tremors

Source: Adapted from Yahaya and Don [3].

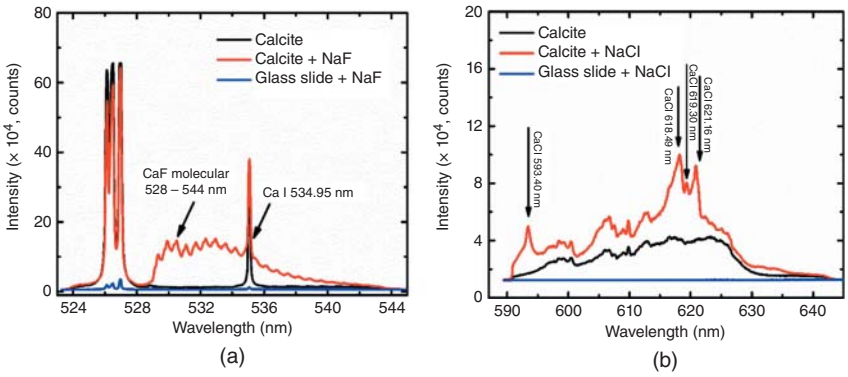

Figure 34.1 LIBS emission spectra of NaF (a) and NaCl (b) aqueous solution depositing on calcite substrate, depositing on glass slide substrate and only on calcite substrate. Source: Tang et al. [6]/ With permission of Elsevier.

34.2.3 Detection of Organic Chemical Hazards by LIBS

Organic chemical hazards are extremely dangerous to humans and the environment. In fact, most of the organic chemicals (such as pharmaceuticals, pesticides, plastics, and fuels) are important in daily life. However, hazardous outcomes may appear when the quantities and concentration of those organic chemicals are large enough. Therefore, those organic chemical hazards also need to be detected all the time. LIBS detection for chemical hazards is mainly related to the identification of explosives, which has been introduced in Chapter 18. Except for explosives, some LIBS studies focus on the identification, classification, and quantification of organic molecules (for example, pesticides) [9–11].

The following applications assist in the detection of organic chemical hazards using LIBS. Mousavi et al. [11] studied the influences of both molecular structure and ambient atmosphere on the CN and C_2 molecular emissions. The authors chose 13 organic compounds, which include polycyclic aromatic hydrocarbons, aromatic carboxylic acid, aliphatic carboxylic acid, amides, and polymers, with different structures to investigate the correlation between molecular structure and CN and C_2 molecular band emissions. Figure34.2a shows the LIBS spectra of anthracene, 1,1-biphenyl,4-nitrobenzoic acid, palmitic acid, and urea in ambient air (spectral range of 378–390 nm). Figure 34.2b indicates CN (0,0) band head intensities of LIBS spectra for various organic samples. Furthermore, to evaluate the effect of N_2 and O_2 molecules concentration on the CN and C_2 molecular band emissions, the author recorded the LIBS spectra of four different samples in Earth's atmosphere (around 80% N_2 and 20% O_2). In the LIBS spectra of urea and thiourea, the CN emissions intensity decreased from nitrogen and argon to air and oxygen atmosphere, in which the oxygen concentration was increasing.

Multari et al. [10] applied LIBS to classify and detect pesticides and dioxins in the complex matrices of tissue fats. The results showed that all samples could be differentiated from each other and the control group. Furthermore, samples with different concentrations could also be classified for all the pesticides and the dioxins involved in this study. They demonstrated that LIBS has the potential as a diagnostic technique for the rapid detection of pesticides and dioxins in complex matrices such as tissue fats and rendered oils with less or no sample

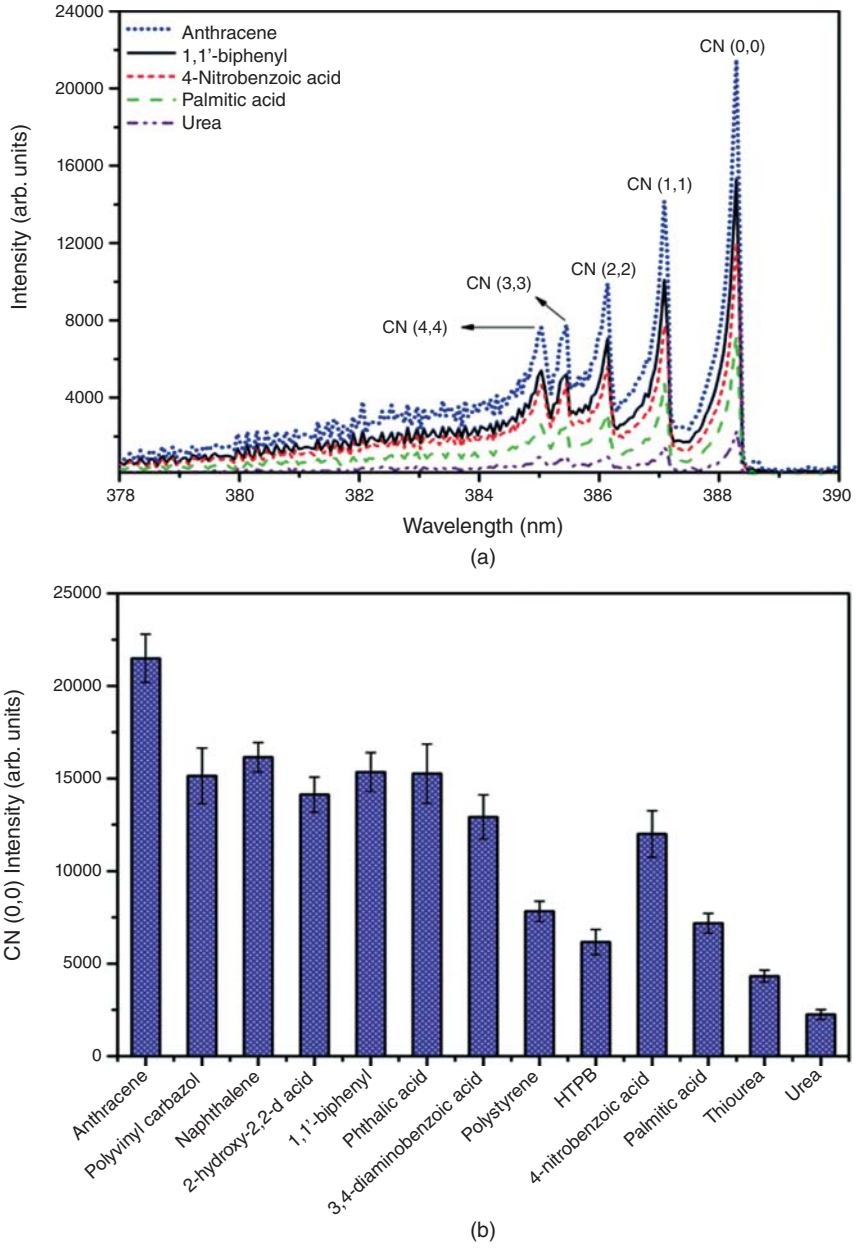

Figure 34.2 (a) CN molecular emission from LIBS spectra of anthracene, 1,1-biphenyl,4-nitrobenzoic acid, palmitic acid, and urea in ambient air. (b) CN (0,0) band head intensities from LIB spectra of various organic samples. Source: Mousavi et al. [11]/ Springer Nature.

preparation. By a series of comparative experiments under different ambient atmospheres for the different molecular structures, the formation mechanism of molecular species was analyzed and the major formation processes were presented.

34.3 LIBS for Biological Hazards Analysis

34.3.1 Introduction to Biological Hazards

Biological hazards are quite close to human daily life, such as harmful bacteria, viruses, and bioagents. Those hazards could cause various kinds of health effects, such as skin irritation, cancer, and so on. Because of the risks of biological hazards to the human body, the detection of biological hazards is critical.

Many works have been reported to analyze biological hazards by LIBS. Most of those LIBS studies focused on the detection of bacteria, and some studies were conducted for the detection of viruses.

34.3.2 Detection of Bacteria by LIBS

Bacteria are kinds of micro- and single-celled organisms that exist in diverse environments (for example, soil, the ocean, and human body). Bacteria have different shapes, which can be spheres, rods, or spirals. In fact, bacteria have a strong connection with humans. Some bacteria are vital to the human body such as those that assist with our digestive system, while some bacteria are harmful or even deadly causing diseases like pneumonia. Therefore, the detection of harmful bacteria becomes crucial. LIBS, as a powerful and versatile analytical technique, has been applied in the classification, quantification, and identification of bacteria.

34.3.2.1 Discrimination and Classification of Bacteria by LIBS

The discrimination and classification of bacteria are valuable as different bacteria have different treatment methods. Usually, many statistical methods are applied to assist the discrimination and classification of bacteria, which can be divided into unsupervised algorithms and supervised algorithms. The details are as follows.

34.3.2.1.1 Unsupervised Algorithms Principal component analysis (PCA) is the mainly used unsupervised classification and dimensionality reduction algorithm in LIBS analysis. PCA is the process of computing the principal components and applying them to perform the change of basis of data, sometimes only the first few principal components are used and the rest are ignored. Impressive benefits have been obtained after using PCA to process LIBS data, for example, significant data reduction, noise reduction, and visualization of spectral position.

The issue of the best way to apply PCA was examined by researchers. Gamble et al. [12] studied the effect of sample preparation on the discrimination of bacterial isolates cultured in liquid nutrient media by LIBS. The LIBS-based discrimination relies on the proportions of the inorganic compounds in cells, such as Na, K, Mg, and Ca. They studied the effects

of the potential of hydrogen (PH) and trace mineral contents in the water sources, which was applied to isolate the bacteria, according to the reliability of the resulting discriminant analysis. Four genera were cultured in the same environmental conditions and the only difference was the source water. The PCA was applied to extract the related variations in the LIBS spectra acquired from the four bacteria genera and six sources of water types. Mahalanobis discriminant analysis was applied for classification. The results indicated that not only the four genera could be discriminated from each other in each source water type, but also that each genus could be discriminated by the source water types. They demonstrated that the water source, applied for the purification of the culture, needs to be controlled precisely with regard to pH, ionic strength, and proportionate amounts of mineral cations.

Some researchers have tried to combine LIBS with other techniques (such as Raman spectroscopy) to enhance the feasibility of PCA in bacteria. While some researchers apply nano-enhanced LIBS (NELIBS) to enhance LIBS signal for PCA analysis [13, 14]. For example, Liao et al. [14] proposed a novel strategy by combining three-dimensional surface-enhanced Raman scattering (3D SERS) and LIBS to detect bacteria qualitatively. They prepared SERS-active Ag nanoparticles (AgNPs) with an improved in situ synthesis method. SERS spectral were obtained by the dried droplet within bacteria. Four types of bacteria were classified via the PCA and hierarchy cluster analysis (HCA) was applied to classify four bacterial types. LIBS was performed for the quantitative detection of bacterial detection based on intracellular mineral cations. Figure 34.3a indicates the SERS spectra

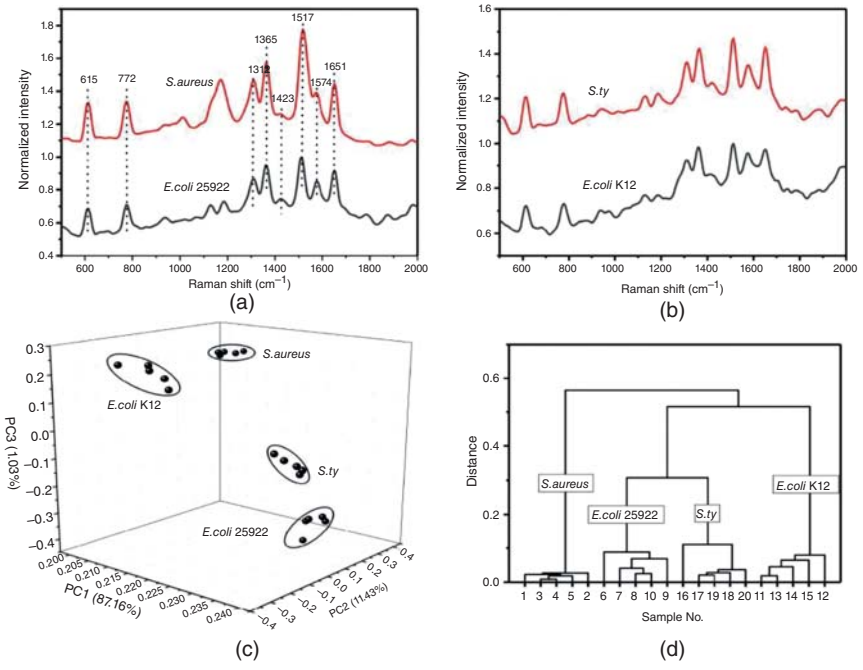

Figure 34.3 (a) SERS spectra of the *S.aureus* (Gram-positive) and *E.coli* 25922 (Gram-negative); (b) SERS spectra of *S.ty* (pathogenic) and *E.coli* K12 (nonpathogenic); (c) the 3D PCA scores plot (PC1, PC2, and PC3); (d) the dendrogram of HCA for four types of bacteria. Source: Liao et al. [14] / With permission of Elsevier.

of the *Staphylococcusaureus* (Gram-positive) and *Escherichia coli* 25922 (Gram-negative). Figure 34.3b shows the SERS spectra of *S.ty* (pathogenic) and *E. coli* K12 (nonpathogenic). Figure 34.3c, d indicate the 3D PCA scores plot (PC1, PC2, and PC3) and the dendrogram of HCA for four types of bacteria. The whole experimental process was verified around 30 minutes. They declared that this strategy has great potential for bacteria analysis.

34.3.2.1.2 Supervised Algorithms Four kinds of supervised algorithms are commonly applied in LIBS analysis, including neural network (NN), support vector machines (SVM), discriminant function analysis (DFA), and partial least squares (PLS).

Neural network is a series of algorithms that helps to recognize the basic relationships in a set of data by a process that imitates the way the human brain works. There are various strengths of NN, including the ability to work with insufficient knowledge, good fault tolerance, and the ability of parallel processing. SVM is a classification algorithm based on statistical learning methods. SVM has many exceptional advantages such as being effective in high-dimensional spaces. DFA is a statistical process that is applied to classify unknown individuals and predict the probability of their classification into a certain group. The principle of DFA includes extracting a linear combination of variables, which maximizes the differences between natural group means and allowing identification of the variables, which predict group membership from a set of predictors [15]. PLS [16] could reduce the predictors to a smaller set of uncorrelated components and perform least squares regression on these components, rather not perform on the original data. PLS regression is extremely powerful when the predictors are highly collinear, or when more predictors than observations. Unlike multiple regression, PLS does not assume that the predictors are fixed, which means that predictors could be measured with error, making PLS more suitable for measuring uncertainty.

The research group of Prof Caceres, from Universidad Complutense de Madrid, provided several methods by combining LIBS with statistical methods [17–19]. For example, they [17] proposed a method based on LIBS and NN for the identification and discrimination of specific bacteria strains (*Pseudomonas aeroginosa*, *Escherichia coli*, and *Salmonella typhimurium*). The results demonstrated 100% reliable identification for the known and unknown bacterial samples with extra similar spectral characteristics. In their work, the only difference was the type of bacteria, which means that the identification only takes the bacterial type into account and ignores the effect of air and culture medium. They declared that it could help reduce analysis time without affecting the model's discrimination capacity. However, they admitted that the production of robust classification models needs introducing advanced statistical models and better reproducibility data. All in all, they declared that their work has medical diagnosis potential, which can be extended to characterize different bacteria and to differentiate pathogenic bacterial strains.

Some researchers have applied DFA and PLS to process the LIBS data for discrimination and classification [20, 21]. For example, Rehse et al. [20] investigated the effect of mixed cultures and sample dilution on bacterial identification by LIBS. The sample mixture and dilution are the challenges of LIBS utilization in clinical diagnoses. The authors characterized the effect of the presence of a second bacterial species upon identification of the majority species. Figure 34.4 indicates a DFA plot showing the first two DF scores for LIBS spectra from two species of Staphylococcus (*aureus* and *saprophyticus*), two species

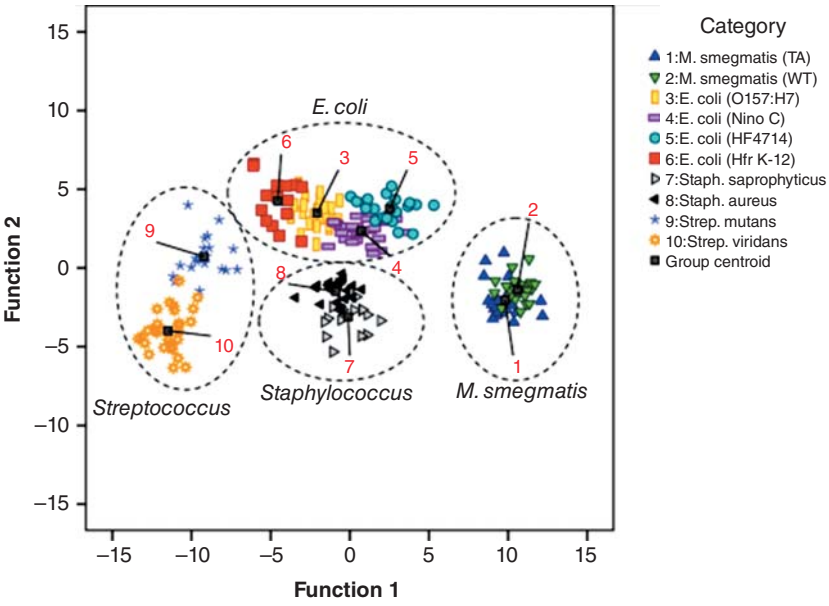

Figure 34.4 (Color online) DFA plot showing the first two DF scores for LIBS spectra from two species of Staphylococcus (*aureus* and *saprophyticus*), two species of Streptococcus (*viridans* and *mutans*), two conditional mutants of *Mycobacterium smegmatis* (WT and TE), and four strains of *E. coli* (enterohemorrhagic *E. coli* O157:H7, Nino C, HF4714, and HfrK12). Source: Rehse et al. [20] / With permission from The Optical Society.

of Streptococcus (*viridans* and *mutans*), two conditional mutants of *M. smegmatis* (WT and TE), and four strains of *E. coli* (enterohemorrhagic *E. coli* O157:H7, Nino C, HF4714, and HfrK12). The results indicated that the specimens with a number of bacterial cells (around 2500) were identified with 100% accuracy, compared with undiluted specimens. Moreover, a linear dependent total spectral power was determined as a function of cell number. In this study, high-selective results were obtained for the LIBS-based analysis of nine separate bacterial strains from four genera.

34.3.2.2 Quantification of Bacteria by LIBS

In fact, the toxicity of the bacteria is related to the quantity and concentration. Therefore, the quantification of bacteria is also crucial. Fewer works have been conducted to quantify various strains of bacteria by LIBS for the moment.

First of all, to get better results, some researchers propose a facile sample pretreatment by centrifugation and filtration of bacteria for enrichment. For example, the research group of Prof. Rehse, from the University of Windsor [22, 23], developed a simple and efficient centrifugation filtration method to concentrate and isolate liquid bacterial specimens for LIBS analysis. In this study, a custom-built centrifuge tube was prepared to hold the filter media during centrifugation. The insert design and assembly are shown both disassembled and assembled in Figure 34.5. By this method, much larger volumes of the bacterial liquid could be processed in a shorter period of time. The results demonstrated that this work could isolate the cells and contrate the cells from a dilute suspension rapidly and

Figure 34.5 The centrifuge insert design and prototype. Source: Malenfant et al. [22], ELSEVIER.

conveniently. Moreover, they improved this centrifugation filtration method by designing and fabricating a metal cone device [23]. The bacterial cells would be centrifugated and concentrated into a circular area (about 1 mm diameter). Two-dimensional LIBS mapping was performed to demonstrate the enrichment of bacterial cells. Furthermore, calibration curves were built to qualitatively analyze the sensitivity of this method. The LOD indicated that this proposed enrichment method could represent a factor of 50 reductions compared with the common method without enrichment.

However, as micro agents, the bacteria has weak LIBS signal, making it hard to quantify and get better sensitivity. Therefore, some researchers propose nano-enhanced LIBS method such as modifying substrate with nanostructure [24, 25]. For example, Liao et al. [24] proposed a novel biointerface, based onsilicon nanowires (SiNWs) array, for bacterial capture and sensing by LIBS and SERS. The SiNWs were fabricated by metal-assisted chemical etching and decorated with uniform Au@Ag core-shell nanoparticles (Au@Ag NPs), which formed multi-scale topographic structures with nanowires, providing effective attachment sites for bacterial adhesins. Figure 34.6 indicates the flow diagrams of SiNWs-Au@Ag and Apt-SERS tag preparation, bacterial capture, and sensing. They indicated that the Au@Ag NPs decorated SiNWs (SiNWs-Au@Ag) substrate had high capacity for capturing bacteria in drinking water (8.6 and 5.5×10^6 cells/cm^2 for *E. coli* and *S. aureus* in 40 minutes, respectively) via physical and chemical effects. The results showed that the bacteria in drinking water could be sensitively analyzed by combining

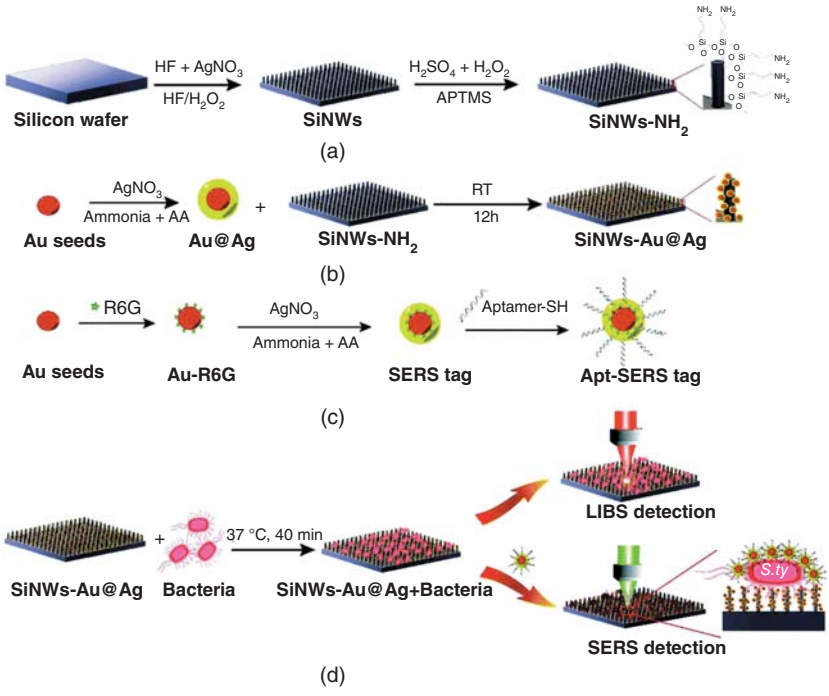

Figure 34.6 Flow diagrams of SiNWs-Au@Ag and Apt-SERS tag preparation, bacterial capture, and sensing. Source: Liao et al. [24] / The Royal Society of Chemistry.

LIBS and SERS. Therefore, it can be demonstrated that this flexible sensing platform has high potential in prevention and control of microbial hazards in water.

34.3.3 Detection of Virus by LIBS

Viruses are another kind of biological hazards, which are made of genetic materials (DNA or RNA) and a protein or membranous coat. The viruses are quite different from the bacteria although both need to grow and replicate inside the living cells and organism. The invasion of viruses into cells could cause great damage to the host (such as animals and plants). Therefore, it is crucial to detect viruses and avoid the bad effect of viruses. As an exceptional analytical technique, LIBS has been utilized to detect viruses.

At present, few LIBS works focused on detecting viruses in crops. The invasion of viruses (for example, tobacco mosaic virus [TMV]) inside crops could cause production loss and low-quality products. Peng et al. [26] applied LIBS to the detection of TMV-infected tobacco. The partial least squares discrimination analysis (PLS-DA) was performed to build the classification models according to the full spectrum and observed emission lines of two different samples (fresh leaves and dried leaf pellets). According to the research, the moisture content in fresh leaves would lower the stability of detection and has a bad influence on the classification results. Furthermore, by detecting dried leaves, good classification results were obtained of the data from the full spectrum (97.2%) and observed emission line (88.9%). Also, the research verified that the SVM could increase the precision

of classification results and reduce the effect of moisture content. The preliminary results indicated that LIBS assisted with chemometrics could provide a rapid, low-cost, and efficient platform for TMV-infected disease determination in tobacco leaves.

34.4 Conclusion

This chapter introduced the common chemical and biological hazards (the species and the danger of those hazards). Moreover, the recent LIBS applications related to chemical and biological hazards detection have been summarized. According to those reported studies, it can be concluded that impressive progress has been made in LIBS analysis with regard to chemical and biological hazards. All those studies demonstrated that the LIBS spectral data could provide enormous elemental and molecular information to support the determination, discrimination, and classification of chemical (e.g. molecular) and biological (e.g. bacterial) hazards. All in all, as a nascent and rising spectroscopic technique, LIBS is expected to become a versatile and universally applicable platform for chemical and biological analyses.

References

1 Bernard, A. (2008). Cadmium & its adverse effects on human health. *The Indian Journal of Medical Research* 128 (4): 557–564.

2 Al-Saleh, I., Al-Enazi, S., and Shinwari, N. (2009). Assessment of lead in cosmetic products. *Regulatory Toxicology and Pharmacology* 54 (2): 105–113.

3 Yahaya, Y.A. and Don, M.M. (2014). Pycnoporus sanguineus as potential biosorbent for heavy metal removal from aqueous solution: a review. *Journal of Physical Science* 25 (1): 1–32.

4 Ma, S., Tang, Y., Ma, Y. et al. (2019). Determination of trace heavy metal elements in aqueous solution using surface-enhanced laser-induced breakdown spectroscopy. *Optics Express* 27 (10): 15091.

5 Yang, P., Zhou, R., Zhang, W. et al. (2019). High-sensitivity determination of cadmium and lead in rice using laser-induced breakdown spectroscopy. *Food Chemistry* 272: 323–328.

6 Tang, Z., Hao, Z., Zhou, R. et al. (2021). Sensitive analysis of fluorine and chlorine elements in water solution using laser-induced breakdown spectroscopy assisted with molecular synthesis. *Talanta* 224: 121784.

7 Tang, Z., Liu, K., Hao, Z. et al. (2021). Validity of nanoparticle enhanced molecular laser-induced breakdown spectroscopy. *Journal of Analytical Atomic Spectrometry* 51429501: 0–7.

8 Zhang, W., Zhou, R., Liu, K. et al. (2020). Sulfur determination in laser-induced breakdown spectroscopy combined with resonance Raman scattering. *Talanta* 216 (April): 120968.

9 De Lucia, F.C. and Gottfried, J.L. (2010). Characterization of a series of nitrogen-rich molecules using laser induced breakdown spectroscopy. *Propellants, Explosives, Pyrotechnics* 35 (3): 268–277.

10 Multari, R.A., Cremers, D.A., Scott, T., and Kendrick, P. (2013). Detection of pesticides and dioxins in tissue fats and rendering oils using laser-induced breakdown spectroscopy (LIBS). *Journal of Agricultural and Food Chemistry* 61 (10): 2348–2357.

11 Mousavi, S.J., Hemati Farsani, M., Darbani, S.M.R. et al. (2016). CN and C_2 vibrational spectra analysis in molecular LIBS of organic materials. *Applied Physics B: Lasers and Optics* 122 (5): 1–16.

12 Gamble, G.R., Park, B., Yoon, S.C., and Lawrence, K.C. (2016). Effect of sample preparation on the discrimination of bacterial isolates cultured in liquid nutrient media using laser-induced breakdown spectroscopy (LIBS). *Applied Spectroscopy* 70 (3): 494–504.

13 Prochazka, D., Mazura, M., Samek, O. et al. (2018). Combination of laser-induced breakdown spectroscopy and Raman spectroscopy for multivariate classification of bacteria. *Spectrochimica Acta Part B: Atomic Spectroscopy* 139: 6–12.

14 Liao, W., Lin, Q., Xie, S. et al. (2018). A novel strategy for rapid detection of bacteria in water by the combination of three-dimensional surface-enhanced Raman scattering (3D SERS) and laser induced breakdown spectroscopy (LIBS). *Analytica Chimica Acta* 1043: 64–71.

15 Ramos, S.D.S. and Rickard Liow, S.J. (2012). Discriminant function analysis. In: *The Encyclopedia of Applied Linguistics* (ed. C.A. Chapelle). Wiley.

16 Wold, S., Eriksson, L., and Kettaneh, N. (2010). *PLS in Data Mining and Data IntegrationHandbook of Partial Least Squares. Springer Handbooks of Computational Statistics* (ed. V. Esposito Vinzi, W. Chin, J. Henseler and H. Wang). Berlin, Heidelberg: Springer.

17 Marcos-Martinez, D., Ayala, J.A., Izquierdo-Hornillos, R.C. et al. (2011). Identification and discrimination of bacterial strains by laser induced breakdown spectroscopy and neural networks. *Talanta* 84 (3): 730–737.

18 Manzoor, S. et al. (2014). Rapid identification and discrimination of bacterial strains by laser induced breakdown spectroscopy and neural networks. *Talanta* 121: 65–70.

19 Manzoor, S., Ugena, L., Tornero-Lopéz, J. et al. (2016). Laser induced breakdown spectroscopy for the discrimination of Candida strains. *Talanta* 155: 101–106.

20 Rehse, S.J., Mohaidat, Q.I., and Palchaudhuri, S. (2010). Towards the clinical application of laser-induced breakdown spectroscopy for rapid pathogen diagnosis: the effect of mixed cultures and sample dilution on bacterial identification. *Applied Optics* 49 (13): C27–C35.

21 Gottfried, J.L. (2011). Discrimination of biological and chemical threat simulants in residue mixtures on multiple substrates. *Analytical and Bioanalytical Chemistry* 400 (10): 3289–3301.

22 Malenfant, D.J., Paulick, A.E., and Rehse, S.J. (2019). A simple and efficient centrifugation filtration method for bacterial concentration and isolation prior to testing liquid specimens with laser-induced breakdown spectroscopy. *Spectrochimica Acta. Part B: Atomic Spectroscopy.* 158 (January): 105629.

23 Paulick, A.E., Malenfant, D.J., and Rehse, S.J. (2019). Concentration of bacterial specimens during centrifugation prior to laser-induced breakdown spectroscopy analysis. *Spectrochimica Acta. Part B: Atomic Spectroscopy* 157 (January): 68–75.

24 Liao, W., Lin, Q., Xu, Y. et al. (2019). Preparation of Au@Ag core-shell nanoparticle decorated silicon nanowires for bacterial capture and sensing combined with laser induced

breakdown spectroscopy and surface-enhanced Raman spectroscopy. *Nanoscale* 11 (12): 5346–5354.

25 Yang, E., Liao, W., Lin, Q. et al. (2020). Quantitative analysis of *Salmonella typhimurium*based on elemental-tags laser-induced breakdown spectroscopy. *Analytical Chemistry* 92 (12): 8090–8096.

26 Peng, J., Song, K., Zhu, H. et al. (2017). Fast detection of tobacco mosaic virus infected tobacco using laser-induced breakdown spectroscopy. *Scientific Reports* 7 (March): 1–9.