

QUANTITATIVE ANALYSIS OF LEAD IN TEA SAMPLES BY LASER-INDUCED BREAKDOWN SPECTROSCOPY

J. Wang,^a M. Shi,^a P. Zheng,^{a,b*} and S. Xue^a

UDC 543.42.062:546.815/.819

Laser-induced breakdown spectroscopy (LIBS) is applied at natural atmosphere to compare the quantitative analysis performances of the toxic heavy metal element lead (Pb) in Pu'er tea leaves, determined by three calibration methods: the external standard method, the internal standard method, and the multiple linear regression method. The Pb I line at 405.78 nm is chosen as the analytical spectral line to perform the calibration. The linear correlation coefficients (R^2) of the predicted concentrations versus the standard reference concentrations determined by the three methods are 0.97916, 0.98462, and 0.99647, respectively. The multiple linear regression method gives the best performance with respect to average relative errors (ARE = 2.69%), maximum relative errors (MRE = 4.94%), average relative standard deviations (ARSD = 9.69%) and maximum relative standard deviations (MRSD = 24.44%) of the predicted concentrations of Pb in eight samples, compared to the other two methods. It is shown that the multiple linear regression method is more accurate and stable in predicting concentrations of Pb in Pu'er tea leaf samples.

Keywords: laser-induced breakdown spectroscopy, Pu'er tea leaves, quantitative measurement, external standard method, internal standard method, multiple linear regression method.

Introduction. There are several kinds of fermented dark teas that come from Yunnan province, China. Fermentation in the process of tea production includes microbial fermentation and oxidation of the tea leaves after they have been rolled and dried. The best known tea in this category is Pu'er of Yunnan Province, which is named after the most famous trading post for dark tea in ancient China. Analysis of heavy metals in agricultural products is valued due to the importance of food safety. Heavy metals are discharged into the environment by industrial pollution, vehicle exhausts, fertilizers, pesticides, domestic garbage, etc. Heavy metals are known to be carcinogenic and toxic, affecting human health directly. Thus, the detection of heavy metals in agricultural products is necessary to protect human health and the ecosystem.

Laser induced breakdown spectroscopy (LIBS) is a type of the atomic emission spectroscopy that applies highly energetic laser pulses to solid [1–5], liquid [6–10], gas [11–13], or aerosol [14–16] samples, *in situ* or remotely [17]. In recent years, many scholars have carried out LIBS research in the quantitative analysis of plant samples [1, 3–5]. The external and internal standard methods are commonly used for the quantitative analysis by LIBS. These calibration models cannot achieve the best results analyzing complex natural multi-matrix plant samples. But the multiple linear regression method is able to calibrate complex nonlinear relationships in the complex matrices [18].

Lead is the most common heavy metal element in tea, and Pu'er tea leaves have higher concentrations of metal elements than other teas because of their full fermentation [19]. In this work, concentration analyses of Pb in eight samples of Pu'er tea leaves are achieved by using the LIBS technology combined with the multiple linear regression method and compared with the external and internal standard methods.

Experiment. A schematic view of the experimental setup is presented in Fig. 1. A Q-switched Nd:YAG laser (Big Sky Laser Technology, Ultra 100), at the fundamental wavelength of 1064 nm, with pulse duration of 8 ns, repetition rate of 20 Hz and maximum pulse energy of 100 mJ, is used as the excitation laser. The laser energy can be switched by changing Q delay time, which can be measured by a laser power meter. The laser beam is focused by a plano-convex lens ($f = 100$ mm) perpendicular to the surface of the sample on a two-dimensional translation stage controlled by a controller (Zolix SC300-

*To whom correspondence should be addressed.

^aCollege of Optoelectronic Engineering, Chongqing University of Posts and Telecommunications, Chongqing 400065, China; e-mail: zhengpc@cqupt.edu.cn; ^bChongqing University, Chongqing 400065, China. Abstract of article is published in Zhurnal Prikladnoi Spektroskopii, Vol. 84, No. 1, p. 171, January–February, 2017.

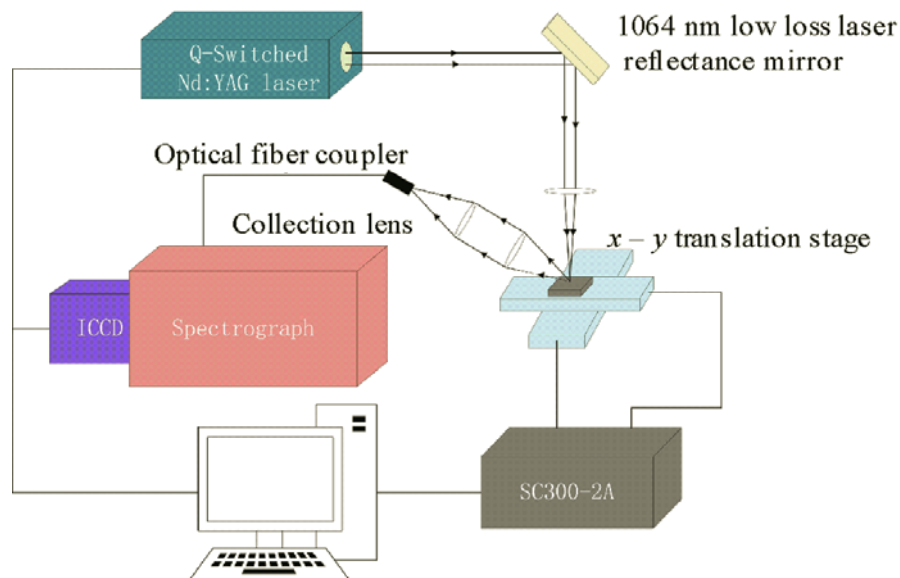


Fig. 1. Schematic diagram of the LIBS experimental setup.

2A) to produce intense, transient plasma. The light emitted from the plasma is focused by a microscope objective lens system and collected by a multimode silica fiber of 2-m length. The light is then transmitted through the fiber to the entrance of a computerized Czerny-Turner spectrograph (Andor Model SR-750A). The spectrograph is equipped with three ruled gratings of 2400, 1200, 300 grooves/mm, which are interchangeable under computer control and provide high-resolution spectra in the wavelength range of 200–900 nm. An intensified gated CCD camera (Andor DH340T-18U-03) is coupled to the spectrograph output. The ICCD camera has 2048×512 pixels and is cooled to -15°C by a Peltier cooler to reduce noise. To get more precise spectra and reduce the bremsstrahlung effect, the laser energy used is of 30 mJ and the detection delay is of 1.5 μs , while the gate width and laser repetition rate are of 2 μs and 4 Hz. Twenty laser pulses are integrated to obtain each spectrum, and 10 measured spectra are averaged to increase the stability and reduce the standard deviation of the spectral intensities.

Sample preparation. Eight Pu'er tea leaf samples (S1, S2, S3, S4, S5, S6, S7, and S8) are analyzed, which are laboratory-made standard reference samples. A proper amount of $\text{Pb}(\text{NO}_3)_2$ ($\geq 99.9\%$) is mixed uniformly with the powdered Pu'er tea leaf sample in eight beakers with deionized water. The standard reference concentrations of Pb in the eight samples are 3000, 4500, 8000, 10,000, 15,000, 20,000, 25,000, and 30,000 mg/kg, respectively. The mixtures in the beakers are dried in a drying oven at 30°C to make the standard reference samples. Since the dried mixtures of Pu'er tea leaves and $\text{Pb}(\text{NO}_3)_2$ are irregular granular solid samples, each mixture sample in the beakers is ground into powder and stirred uniformly for 2 h. Each powder sample is pressed into tablets (0.5 g, 13-mm diameter, and 2-mm thickness) with a hydraulic press (2 min under pressure of 10 t for each tablet), in order to reduce experimental errors and achieve better ablation efficiency, as well as higher repeatability of the LIBS measurements.

External standard method and internal standard method. In order to achieve quantitative analysis of LIBS, calibration models need to be established to determine the concentrations of the objective element. The Lomakin–Scheibe equation is the most commonly used equation for spectral quantitative analysis:

$$I = aC^b,$$

where I is the measured intensity of the spectral line, a is the experiment constant, C is the concentration of the objective element, and b is the self-absorption coefficient, which is a function of C , $b = b(C) \leq 1$.

When self-absorption is ignored, $b = 1$, the intensity of the element spectral line is proportional to its concentration:

$$I = aC.$$

The equation above is used for the external standard method. If there is a reference element, the equation above can be changed to

$$I/I_C = aC/a_C C_C,$$

where I_C is the spectral line intensity for the reference element, a_C is the experimental constant of the reference element, and C_C is the reference element concentration. If the concentration of the reference element is the same for all the samples, the equation above can be changed to

$$I' = AC,$$

where I' is the relative spectral line intensity of the objective element as compared to the reference element, A is a changed experimental constant, and C is still the concentration of the objective element. This equation is used for the internal standard method.

The internal standard method can eliminate the influence of the matrix effect and improve the accuracy of quantitative analysis to a certain extent, compared with the external standard method [20]. In this work, the dominant element C (C I 247.856 nm) is chosen as the reference element because it can be assumed constant in all eight Pu'er tea leaf samples.

Multiple regression analysis. Both the external and internal standard methods are univariate calibration models. The univariate calibration takes into consideration the objective element only. Still, the other matrix elements can disturb the calibration of the objective element. The multiple linear regression method can take advantage of the information of more correlative spectral lines and partly avoid the matrix effect. Therefore, the multiple linear regression method can effectively improve the performance of quantitative analysis by LIBS in complex natural multi-matrix plant samples.

For a complex multi-component material, the measured signal is often related to multiple factors. Assuming the response is y_i , and the factors are x_1, x_2, \dots, x_n , respectively, the set of observed values of the measurement data y_i can be expressed as [21]

$$y_i = b_0 + b_1x_{i1} + \dots + b_mx_{im} + e_i \quad i = 1, 2, \dots, n,$$

where e_i is the residual error or residue, and i and m are the number of times of the measurement and the number of variants, respectively. The parameter \hat{y}_i is the estimated value of y_i , namely:

$$\hat{y} = b_0 + b_1x_{i1} + \dots + b_mx_{im} + e_i \quad e_i = y_i - \hat{y}_i, \quad i = 1, 2, \dots, n,$$

$$Q = \sum_{i=1}^m e_i^2 = \sum_{i=1}^m (y - \hat{y})^2.$$

In the equation above b_0, b_1, \dots, b_m can be calculated when the Q value achieves its minimum. This shows that the influencing factors of the response should be ascertained.

During the LIBS analysis, the results of determination can be influenced by the concentrations of objective elements. Other correlative elements contained in the samples also play important roles in the quantitative analysis. The concentration of the objective element (C_i) can be considered to be related to the spectral line intensities of the objective element and other correlative elements (I_i), as given by the following [22]:

$$C_i = f(I_1, I_2, \dots, I_i),$$

where C_i refers to the predicted concentration of the objective element, and I_i is intensity of the spectral line of the objective element and correlative elements in the sample [18].

For this work, the Pb element (Pb I 405.78 nm) in Pu'er tea leaves is chosen as the objective element to prove the feasibility of LIBS in quantitative analyzing heavy metals in plant products. In the wavelength range of 400–410 nm, the characteristic lines of Mn are abundant. The concentrations of Mn and C can be assumed to be the same across the eight Pu'er tea leaf samples; therefore, Mn and C are chosen as the correlative elements in this work. After screening, the intensities of C I 247.856 nm, Pb I 405.78 nm, and the sum of the three intensities of Mn I 403.076 nm, Mn I 403.307 nm, and Mn I 403.449 nm are chosen as the independent variables for the quantitative analysis. Then

$$C_{Pb} = f(I_{Pb}, I_C, I_{\Sigma Mn}),$$

where I_C, I_{Pb} , and $I_{\Sigma Mn}$ are the intensities of the C I 247.856 nm, Pb I 405.78 nm, and the sum of the three spectral intensities of Mn (Mn I 403.076, Mn I 403.307, and Mn I 403.449 nm), respectively. Therefore, the multiple linear regression method is a ternary linear regression analysis.

Results and Discussion. In this work, eight Pu'er tea leaf samples are calibrated using three methods: the external standard method, the internal standard method, and the multiple linear regression method. In order to comprehensively

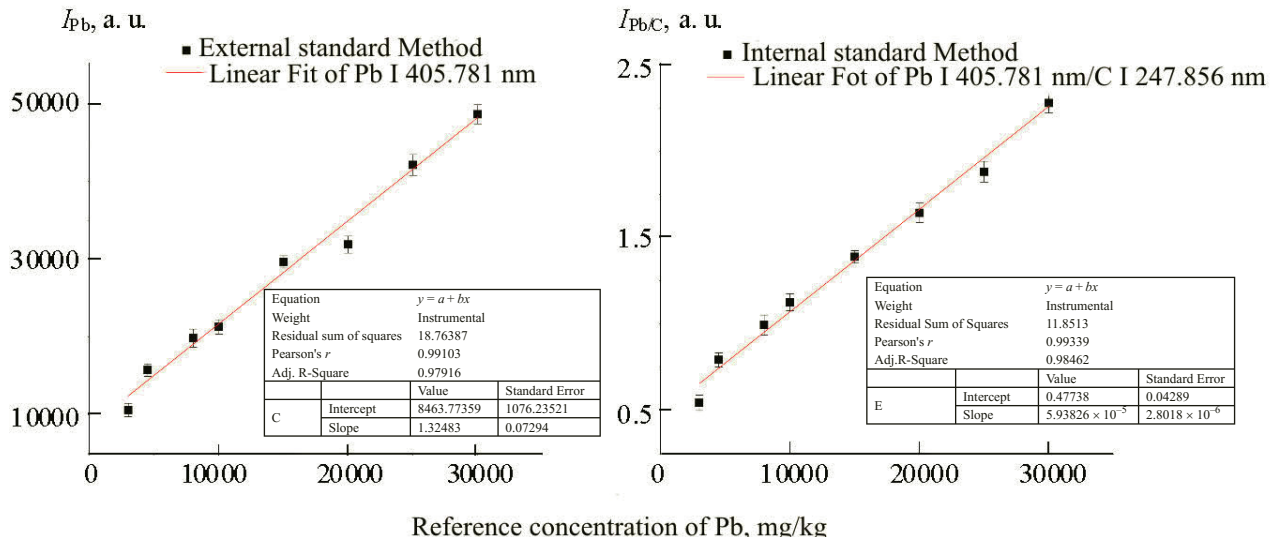


Fig. 2. Linear calibration models of the external standard method and the internal standard method.

TABLE 1. Predicted Concentrations of Pb in Samples

Sample	S1	S2	S3	S4	S5	S6	S7	S8
Standard Reference Value	3000	4500	8000	10000	15000	20000	25000	30000
Predicted Value								
External Standard Method	1628.4	5498.6	8601.7	9686.3	15999.9	17681.5	25402.0	30319.0
Internal Standard Method	1126.1	5307.6	8694.7	10923.3	15346.9	19629.4	23640.7	30331.4
Multiple Linear Regression Method	3078.5	4631.2	8119.3	10286.8	15127.3	19012.9	24315.3	30928.5

compare the performances of the three methods, all eight samples are used as both the training set and the prediction set to predict the concentrations of Pb. The Pb I 405.781 nm spectral line is chosen for the analysis. The intensities of C I 247.856, Mn I 403.076, Mn I 403.307, and Mn I 403.449 nm are chosen as the other independent variables in the multiple linear analysis. Linear calibration models of the external standard method and the internal standard method are evaluated, as shown in Fig. 2.

The limit of detection (LOD) can be obtained by linear fitting of the external standard method:

$$\text{LOD} = 3\sigma/k,$$

where σ is the standard deviation of the spectral background at 405.781 nm, and k is the slope of the linear fittings of the external standard method. The LOD of Pb is 47.22 mg/kg in this work.

When obtaining the predicted concentrations of the three methods, all eight samples are used as the prediction set to get predicted concentrations of Pb. The predicted concentrations of Pb of the three methods among the eight Pu'er tea leaf samples are listed in Table 1.

To assess the quantitative performances of the three methods, three linear fittings of the predicted concentrations versus the standard reference concentrations are established, as shown in Fig. 3.

Five parameters are calculated in order to verify the accuracy and stability of the predicted concentrations of the three methods: the linear correlation coefficients (R^2) of linear fitting of the predicted concentrations versus the standard reference concentrations, the average value of relative errors (AREs), the maximum value of relative errors (MREs), the average value of relative standard deviations (ARSs), and the maximum value of relative standard deviations (MRSs) of the predicted

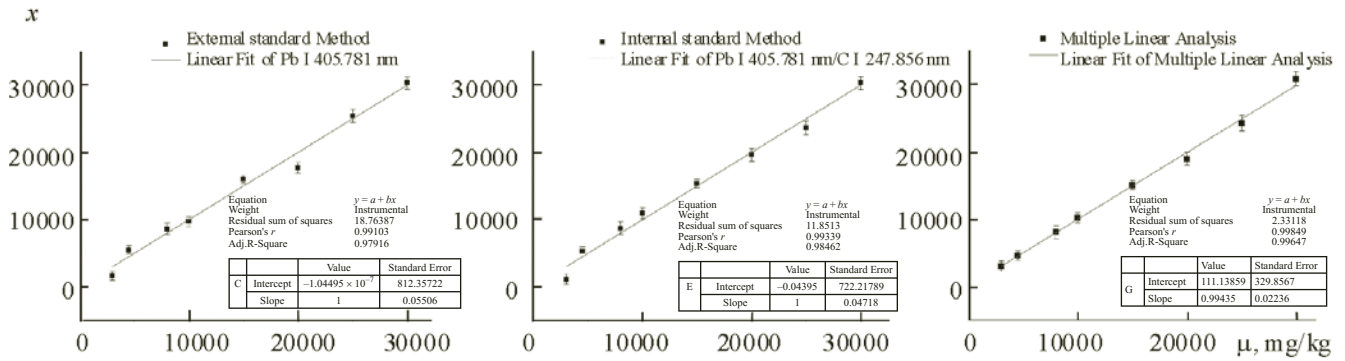


Fig. 3. Linear fittings of the predicted concentrations (x) versus the standard reference concentrations (μ).

TABLE 2. Comparison of Quantitative Performances of Three Methods for Pb

Comparison Parameters	R^2	ARE, %	MRE, %	ARSD, %	MRSD, %
External Standard Method	0.97916	12.44	45.72	10.50	40.10
Internal Standard Method	0.98462	13.63	62.46	14.28	66.30
Multiple Linear Regression Method	0.99647	2.69	4.94	9.69	24.44

concentrations of the eight samples. Here R^2 represents the accuracy, while ARE, MRE, ARSD, and MRSD represent the stability of the quantitative measurement. The relative error (RE) and relative standard deviation (RSD) can be calculated by the following formulas:

$$RE = |(\bar{x} - \mu) / \mu| \times 100\%, \quad RSD = SD / \bar{x} = (1 / \bar{x}) \sqrt{\sum_{i=1}^n (x_i - \bar{x})^2 / (n-1)} \times 100\%$$

where \bar{x} is the average value of the predicted concentration, μ is the standard reference concentration, x_i is a value of the predicted concentration, and n is the predicted times.

The five evaluation parameters (R^2 , ARE, MRE, ARSD, and MRSD) of the three methods are listed in Table 2 and Fig. 4. For the prediction accuracy, it can be observed from Table 2 and Fig. 4 that the R^2 values of the external standard method, internal standard method, and multiple linear regression method are 0.97916, 0.98462, and 0.99647, respectively. It can be concluded that the multiple linear regression method has the most accurate quantitative performance, while the external standard method provides the lowest accuracy. For the prediction stability, it can be observed from Table 2 and Fig. 4 that the ARE values of the external standard method, internal standard method, and multiple linear regression method are 12.44, 13.63, and 2.69%, respectively. The MRE values of the three methods are 45.72, 62.46, and 4.94%, respectively. The values for ARSD and MRSD are 10.50, 14.28, 9.69% and 40.10, 66.30, 24.44%, respectively. So, from this work we can conclude that the multiple linear regression method gives the most stable quantitative performance of the three methods.

Overall, the multiple linear regression method has better quantitative performance than the other two methods. Besides the intensity of the objective spectral line, it uses more information that is important to predict concentrations of the objective element, while the other two methods are unary. The internal standard method has better prediction accuracy for eliminating the influence of the matrix effect to some extent, but the internal standard method has worse stability compared with the external standard method, because the introduction of the reference element can bring instability and cause larger ARE, MRE, ARSD, and MRSD. This work has shown that the multiple linear regression method has not only the best prediction accuracy, with $R^2 = 0.99647$, but also the best prediction stability, with $RE < 5\%$ and $RSD < 25\%$, of the three methods.

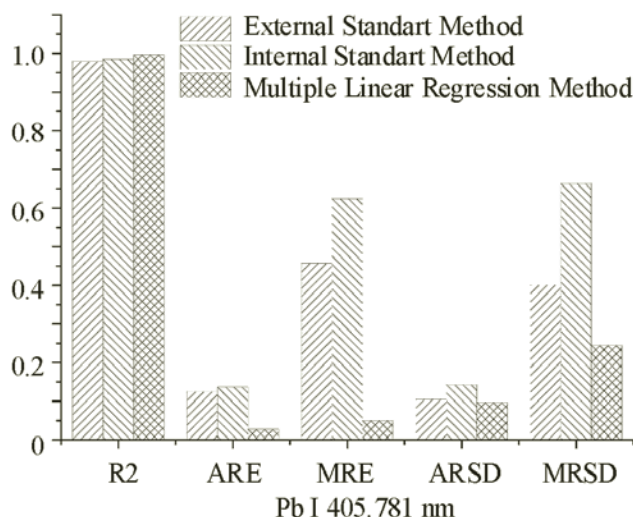


Fig. 4. Comparison of quantitative performances of three methods for Pb measurements.

Conclusions. This work compares the performance of quantitative analysis of the toxic heavy metal element Pb in Pu'er tea leaves by the external standard method, the internal standard method, and the multiple linear regression method. Calibration models are established, and the predicted concentrations of Pb of eight samples are determined by the three methods. The limit of detection for Pb measured in this work is 47.22 mg/kg. The results obtained clearly show that the internal standard method has better prediction accuracy but worse prediction stability than the external standard method. The multiple linear regression method achieves both better prediction accuracy and stability than the other two methods in this work. It proves that the LIBS technique combined with the multiple linear regression method is a favourable method for analyzing the concentration of Pb in Pu'er tea leaves.

REFERENCES

1. S. Beldjilali, W. L. Yip, and J. Hermann, *Anal. Bioanal. Chem.*, **400**, No. 7, 2177–2183 (2011).
2. S. Sankaran, R. Ehsani, and K. T. Morganc, *Appl. Spectrosc.*, **69**, No. 8, 913–919 (2015).
3. D. K. Chauhan, D. K. Tripathi, and N. K. Rai, *Food Biophys.*, **6**, No. 3, 416–423 (2011).
4. M. Yao, L. Huang, and J. Zheng, *Opt. Laser Technol.*, **52**, 70–74 (2013).
5. H. Lin, M. Yao, and J. Lin, *J. Appl. Spectrosc.*, **80**, No. 6, 957–961 (2014).
6. Xu Yuan, Yao Mingyin, and Liu Muhua, Lin Yongzeng, *Laser Optoelectron. Prog.*, **49**, No. 9 (2012).
7. Y. Li, Y. Lu, and R. Zheng, *Spectrosc. Spectr. Anal.*, **32**, No. 3, 582–585 (2012).
8. M. Yao, J. Lin, and M. Liu, *Appl. Opt.*, **51**, No. 10, 1552–1557 (2012).
9. L. Huang, M. Yao, and Y. Xu, *Appl. Phys. B*, **111**, No. 1, 45–51 (2013).
10. D. Zhu, J. Chen, and J. Lu, *Anal. Methods*, **4**, No. 3, 819–823 (2012).
11. Y. Cai, P. C. Chu, and S. K. Ho, *Front. Phys.*, **7**, No. 6, 670–678 (2012).
12. Y. Wang, W. Liu, and Y. Song, *Chem. Phys.*, **447**, 30–35 (2015).
13. E. Jobiliong, H. Suyanto, and A. M. Marpaung, *Appl. Spectrosc.*, **69**, No. 1, 115–123 (2015).
14. Y. Zhang, G. Xiong, and S. Li, *Combust. Flame*, **160**, No. 3, 725–733 (2013).
15. Y. Yuan, S. Li, and Q. Yao, *Proc. Combust. Inst.*, **35**, No. 2, 2339–2346 (2015).
16. Y. Zhang, S. Li, and Y. Ren, *Proc. Combust. Inst.*, **35**, No. 3, 3681–3688 (2015).
17. Q. Q. Wang, K. Liu, and H. Zhao, *Front. Phys.*, **7**, No. 6, 701–707 (2012).
18. T. Chen, L. Huang, and M. Yao, *Appl. Opt.*, **54**, No. 25, 7807–7812 (2015).
19. Z. Peichao, S. Minjie, and W. Jinmei, *Plasma Sci. Technol.*, **17**, No. 8, 664–670 (2015).
20. L. Sheng, T. Zhang, and K. Wang, *Chem. Res. Chin. Univ.*, **31**, No. 1, 107–111 (2015).
21. M. Dong, J. Lu, and S. Yao, *J. Eng. Thermophys.*, **33**, No. 1, 175–179 (2012).
22. S. Palanco and J. J. Laserna, *J. Anal. At. Spectrom.*, **15**, No.10, 1321–1327 (2000).