QUANTIFICATION OF ELEMENTS IN COW FUR BY LASER INDUCED BREAKDOWN SPECTROSCOPY **

I. Cherni [1,2], S. Hamzaoui [2*], N. Jaïdane [2]

[1] Higher Institute of Medical Technologies of Tunis, University of Tunis El Manar, Tunis, Tunisia
[2] Faculty of Sciences of Tunis, University of Tunis El Manar, Tunis, Tunisia; e-mail: sami.hamzaoui@istmt.utm.tn

The purpose of this work is to report an analytical procedure to prove the validity of the hypothesis of the representativeness of the mass vaporized in the plasma plume of the studied sample. To achieve that, we used the laser induced breakdown spectroscopy (LIBS) technique to analyze some minerals and trace elements in cow tail hair. First, hair samples were dissolved in nitric acid; then the solutions were analyzed by atomic absorption spectrometer. Ca, Mg, and Na mass concentrations were determined for the 25 hair samples. Finally, a small amount of hair from every strand was cut in very small pieces and mixed with potassium bromide to make 12 mm diameter pellets. The laser was focused on the pellet surfaces, and the intensities of the emission lines of the studied elements were related to their absolute mass concentrations already measured. Experimental conditions were chosen to guarantee the reproducibility of ablations and to minimize the fluctuations of the ablated mass. In addition, local thermodynamic equilibrium was verified to prove the possibility of use of the theoretical model to obtain the variation of the emission line intensity as a function of the species concentration in the plasma plume.

Keywords: *laser induced breakdown spectroscopy, wool, representativeness, calcium, sodium, magnesium.*

ОПРЕДЕЛЕНИЕ КОНЦЕНТРАЦИИ ЭЛЕМЕНТОВ В ОБРАЗЦАХ КОРОВЬЕЙ ШЕРСТИ МЕТОДОМ ЛАЗЕРНОЙ АТОМНО-ЭМИССИОННОЙ СПЕКТРОСКОПИИ

I. Cherni [1,2], S. Hamzaoui [2*], N. Jaïdane [2]

УДК 621.375.826;543.42;533.9

[1] Институт медицинских технологий Туниса, Тунис
[2] Университет Туниса Эль-Манар, Тунис; e-mail: sami.hamzaoui@istmt.utm.tn

(Поступила 25 марта 2019)

Разработана аналитическая процедура доказательства обоснованности гипотезы о репрезентативности испарившейся массы в плазменном факеле исследуемого образца. Использован метод лазерной атомно-эмиссионной спектроскопии и выполнен независимый анализ некоторых минералов и микроэлементов в шерсти коров. Образцы растворялись в азотной кислоте, растворы проанализированы с помощью атомно-абсорбционного спектрометра. Массовые концентрации Ca, Mg и Na определены для 25 образцов. Небольшое количество шерсти разрезано на кусочки и смешано с бромистым калием для получения гранулы диаметром 12 мм. Лазерный пучок фокусировался на поверхности гранул, интенсивность линий излучения исследуемых элементов сопоставлена с результатами независимых измерений абсолютных массовых концентраций. Экспериментальные условия выбирались такими, чтобы гарантировать воспроизводимость испаряемой массы и минимизировать ее флуктуации. Проверено наличие локального термодинамического равновесия в плазменном факеле

** Full text is published in JAS V. 87, No. 4 (http://springer.com/journal/10812) and in electronic version of ZhPS V. 87, No. 4 (http://www.elibrary.ru/title_about.asp?id=7318; sales@elibrary.ru).

для доказательства возможности использовать теоретическую модель, дающую изменение интенсивности линии излучения в зависимости от концентрации частиц.

Ключевые слова: *лазерная атомно-эмиссионная спектроскопия, шерсть, репрезентативность, кальций, натрий, магний.*

Introduction. Many authors believe that hair analysis can be a complement to the conventional analysis of blood and urine [1, 2]. In fact, hair has several advantageous properties that make it a potential material of choice for a mineral analysis such as simple and non-invasive sampling and easy and inexpensive transport and storage [3, 4] compared to traditional biological matrices (urine or blood).

Hair provides historical data on minerals and trace element levels in the body as well as nutritional status over a long period [5, 6]. It is considered to be a reliable indicator of the effects of toxic metals on human health [7, 8]. In fact, hair concentrations of minerals and trace elements are stable because of their inert and homogeneous appearance [9].

For these reasons, hair has been the subject of frequent researches for more than a quarter of a century [10, 11], and several techniques have been used to determine the concentrations of minerals and trace elements, such as X-ray emission spectrometry [12], flame atomic absorption spectrometry (FAAS) [13], cold vapor atomic absorption spectrometry (CV-AAS) [14, 15], and the atomic fluorescence spectrometry (AFS) [16].

However, these methods present some disadvantages; the pretreatment of the samples can be expensive. They can also take a lot of time and require the use of damaging chemicals [17, 18]. For these reasons, many authors opted for the use of Laser Induced Breakdown Spectroscopy (LIBS), which is non-invasive, multielemental, and fast and applicable to all type of samples such as solid, liquid, and gaseous states [19–22].

In this context, one must note that LIBS is a qualitative technique, but to obtain reproducible quantitative results, new procedures must be developed. Researchers have proposed several approaches to obtain quantitative information from LIBS measurements: LIBS analysis using calibration curves generated via concentration measurements with standard analysis techniques such as micro X-ray Fluorescence Spectroscopy [23], ICP-AES [24] and ICP-MS [25].

In another approach, standard samples were used to calibrate LIBS measurements [26]. Some authors have prepared the standard samples themselves by synthesizing components with well-defined composition ratios [27]. We must specify that the use of standards is effective when the sample matrix is known [28, 29].

The most widely used quantitative approach is Calibration-Free Laser-Induced Breakdown Spectroscopy (CF-LIBS). This technique, applied to model samples [30, 31], is based on the measurement of line intensities and plasma properties (plasma electron density and temperature) on the assumption of a Boltzmann population of excited levels**.** For hair analysis, CF-LIBS has not been widely used [32].

All these quantitative procedures using the LIBS technique suppose the representativeness of the vaporized mass in the plasma plume of the studied sample. We propose in this work an experimental study to prove the validity of this hypothesis in the case of laboratory plasmas.

Experimental setup. The experimental set-up used in these experiments is shown in Fig. 1. A standard Nd:YAG laser (Brilliant, Quantel), was used to generate the plasma. It ran in its second harmonic at 532 nm with at a repetition rate of 2 Hz. The individual laser pulses had pulse duration of approximately 4 ns. The pulse energy was 30 mJ. One mirror was used to direct the laser beam onto the target surface with an angle of incidence of 45°. The laser light was focused by a f_l = 10 cm lens to a spot of approximately 1 mm^2. The distance between the plasma plume and the entrance slit of the monochromator was 40 cm. The light from the plasma was collected by a lens of focal length f_l = 10 cm placed at the same distance from the plasma plume and the entrance slit of the monochromator, and the image magnification was 1:1. The system used for spectral analysis consisted of a Jobin Yvon THR 1500 monochromator equipped with a 1200 grooves/mm grating.

A fast intensified charge coupled device (ICCD, Andor Technology, model DH520-25F-03) was used for photon detection. Under our experimental conditions, the overall spectral resolution of the spectroscopic system used was estimated to be 0.05 nm. Synchronization between the laser and the ICCD detector was ensured by a microcomputer via a pulse delay generator (Model DG 535, Stanford Research Systems, Inc). A microcomputer was equipped with software for data acquisition and analysis of spectra.

Data acquisition was performed by averaging the signal over 10 successive laser shots, and it was verified that the plasma was reproducible by recording the same spectrum several times. The delay time was fixed to 2 μs and the gate width to 0.5 μs. This delay was fixed a short amount of time after the onset of the laser breakdown because the fast dynamics of the plasma do not allow the system to reach thermodynamic

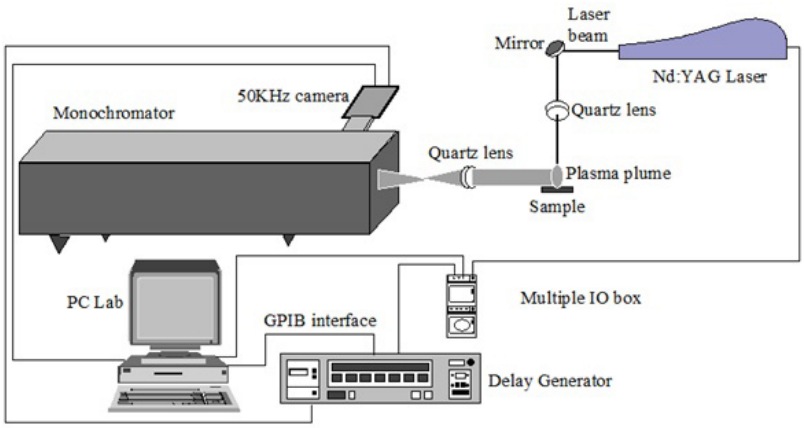

Fig. 1. Schematic diagram of the experimental arrangement.

equilibrium. It also allows the minimization of the auto-absorption effects. The simple criterion of the McWhirter formula was applied to judge the existence of LTE in the present work.

Concerning the samples, our choice was hair from the cow's tail. The chosen cows are from the same herd. We made this choice to ensure that the differences between elemental concentrations for cows are not related to differences in diet or to other therapeutic treatments.

Twenty-five samples were cleaned with acetone and distilled water to remove waste. We started analyzing hair by the atomic absorption technique. The obtained absolute concentrations were used as references for hair analysis by the LIBS technique. For this analysis, 0.1 g of every strand of hair was dissolved in 5 mL heated nitric acid. The obtained solutions were diluted by adding 20 mL of demineralized water, after which they were analyzed using a flame atomic absorption spectrophotometer.

To analyze the same hair samples using the LIBS technique, 0.01 g from every strand of hair was cut in very small pieces and mixed with potassium bromide. The mixture was crushed and homogenized, and 12 mm pellets were obtained with a manual Retsch pellet press under 20 tons pressure. The nanosecond pulsed laser was focused on the pellet surfaces. To modify the impact point of the laser pulses on the pellet surfaces, the pellets were mounted on an X and Y table. Each spectrum was the result of the signal accumulation of 25 successive pulses. The studied lines are Ca II (393.36 nm), Na I (588.99 nm), and Mg I (285.21 nm).

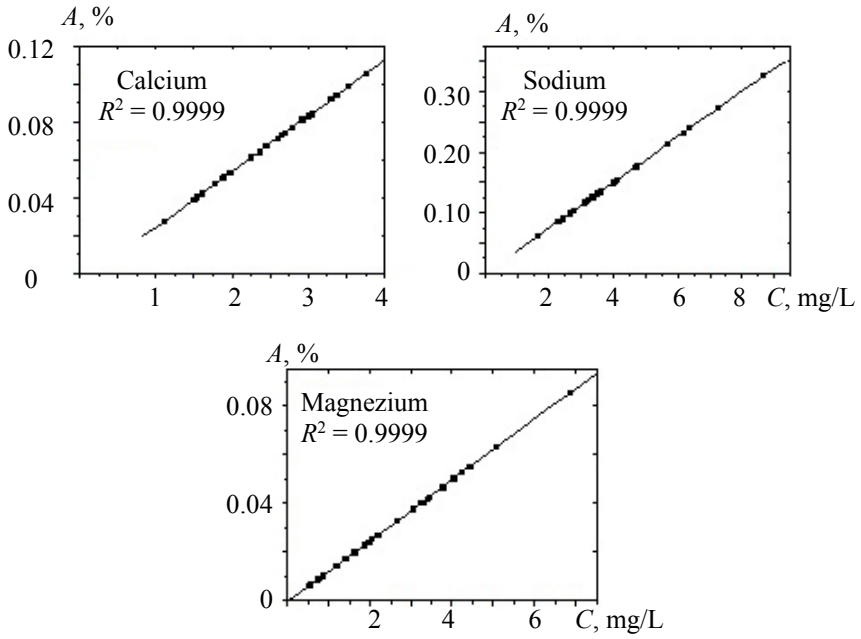

Fig. 2. Variation of absorbance in function of mass concentrations.

Results and discussion. *Analysis by the atomic absorption technique.* The differences between concentration values for each studied element prove that there is a linear increase in absorption as a function of concentration. Values are presented as a straight line, where the ordinate axis designates the absorption and the abscissa axis designates the concentrations found by atomic absorption spectrometer (Fig. 2).

Analysis by the LIBS technique. As already mentioned, the hair samples were made and analyzed in pellet form. For all samples and the studied species, we measured the emission line intensities obtained by the LIBS technique, after which we related these emission line intensities to the corresponding absolute mass concentrations previously found by the atomic absorption technique (Fig. 3). The obtained results show linear dependences of the emission line intensities of the studied species as a function of their mass concentrations in the hair samples. Some fluctuations on both sides of the linear fit are noted, caused essentially by the inhomogeneity of the pellet surfaces and by micro-changes of their flatness. These fluctuations stay in the acceptable relative error ranges $0.9594 \le R^2 \le 0.9813$.

We also remark that deviations from linear fit were observed mainly for higher concentrations. This can be explained by the fact that the self-absorption effect increases with the abundance of the specie(s) in the sample and in the plasma. Its effect is to decrease the line intensity by the absorption of emitted photons in the cold peripheral zones of the plasma. On the other hand, these results show the capability of the LIBS technique to discriminate concentration variations that do not exceed 1%.

Finally these results show that when the experimental conditions are well controlled to guarantee the reproducibility of the laser pulses, we can consider that the ablated mass is the same between the different laser pulses, and the increase in the emission intensities of different species is only a function of their concentrations in the different hair samples. Thus, one can confirm that there is a representativeness of the vaporized mass of hair in the plasma plume of the studied sample; in fact, the ablated mass is the same between the different laser pulses, but the species concentrations vary between hair samples. According to the latter results, we can consider that the concentrations of species in plasma plumes are equivalent to their concentrations in hair samples.

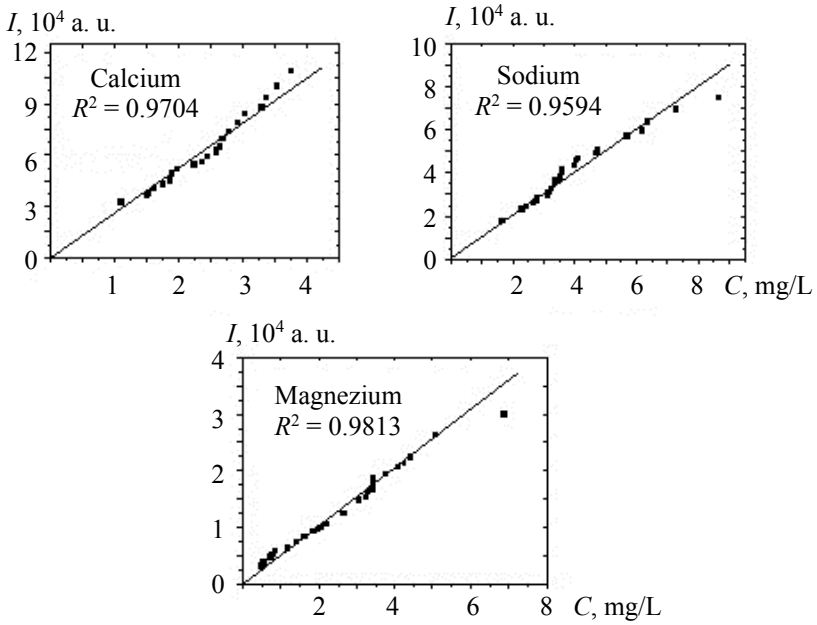

Fig. 3. Variation of the emission lines intensities in function of mass concentrations.

Mathematical illustration of results. The obtained results are in a good agreement with the theoretical expression of the emission line intensity as a function of the species concentration in the plasma plume as given by [33]:

$$I_e = KC_eM_v\exp\left(-E/kT\right), \tag{1}$$

where K is a constant factor whose value depends on the observed transition and the collection efficiency; C_e is the concentration of the studied element in the plasma; M_v is the total mass of hair vaporized in the plasma plume; E and T are respectively the transition energy and the excitation temperature; k is Boltz-

mann's constant. As mentioned previously, under our experimental conditions the total mass of hair vaporized in the plasma plume (M_v) can be considered constant and the emission line intensity varies only with the species' concentration. Finally, to validate the use of the Eq. (1) for our plasma we must prove that it works under LTE condition.

Local thermodynamic equilibrium (LTE). To verify the validity of McWhirter's criterion, the formula requires estimation of the electronic density and electronic temperature. For this, the electron density is estimated as

$$\Delta\lambda_{1/2} = 2\omega\frac{N_e}{10^{16}}, \tag{2}$$

were $\Delta\lambda_{1/2}$ is the full width at half maximum (FWHM) of the Stark broadening profile of a particular transition, ω (cm) is the electron impact parameter, and N_e (cm^{-3}) is the electronic density. Note that we neglected broadening due to ions.

We selected the Mg I 285.21 nm spectral line; this line is well isolated from other spectral lines. Its shape was corrected by simply subtracting the contribution of the instrumental line broadening through the relation $\Delta\lambda_{1/2} = \Delta\lambda_{obs} - \lambda_{inst}$. The instrumental line broadening was found to be 0.08 nm as determined by measuring the FWHM of the narrow He-Ne laser line at 632.6 nm. The FWHM of the corrected line was calculated by Lorentzian fitting; its value is $\Delta\lambda_{1/2} = 0.053$ nm, and the electron density is estimated as $N_e = 3.69\times10^{16}$ cm^{-3}.

For the estimation of the electronic temperature, we used the Boltzmann pair line method for species that have the same stage of ionization [34]:

$$\frac{I_1}{I_2} = \frac{g_1A_1\lambda_2}{g_2A_2\lambda_1}\exp\left(-\frac{|E_1 - E_2|}{kT_e}\right). \tag{3}$$

The two Mg II lines at 279.07 nm ($E = 8.8637$ eV) and 280.27 nm ($E = 4.4224$ eV) for relevant spectral segments were used (Fig. 4). The electron temperature for the fixed gate delay was estimated as $T_e = 9852$ K. To examine the local thermodynamic equilibrium condition, we used the McWhirter criterion given as [35]:

$$N_e \geq N_c = 1.6\times10^{12}T^{1/2}(\Delta E)^3, \tag{4}$$

where ΔE is the energy of the investigated transition and T is the estimated electronic temperature. Using T and ΔE in Eq. (3), one can easily calculate the critical electron number density $N_c = 1.31\times10^{16}$ cm^{-3}, which is much lower than the estimated electron density, and consequently our plasma satisfies the LTE condition.

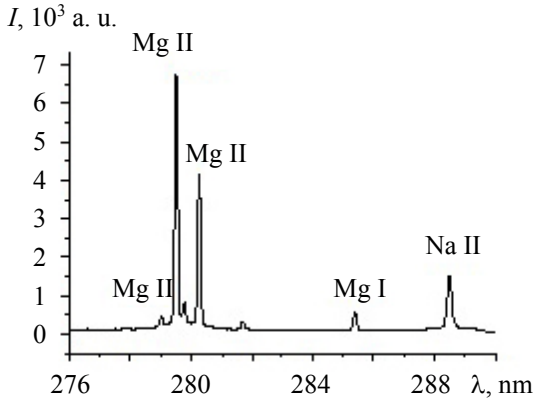

Fig. 4. Used lines for the electronic density and temperature calculation.

Conclusions. The purpose of this work was to prove the usefulness of the LIBS technique in quantitative analysis and the validity of the hypothesis of the representativeness of the mass vaporized in the plasma plume of the studied sample. In fact, one cannot deduce the absolute concentration of one species by using the intensity of its emission line without making sure of the validity of this hypothesis.

For that we analyzed hair from the cow's tail by using of LIBS technique. We started by analyzing these hair samples by the atomic absorption technique to find the mass concentrations of Ca, Na, and Mg. These hair samples were also analyzed by the LIBS technique, and emission line intensities were related to the obtained mass concentrations. Results showed that these emission line intensities increase linearly with their mass concentrations obtained by atomic absorption.

The results are in a good agreement with the theoretical model giving the evolution of the emission line intensity as a function of the species concentration. Here we note that, under our experimental conditions, the ablated mass was consider constant by minimizing the energy ablation fluctuation.

Finally, we proved the possibility of use of the theoretical model by demonstrating the validity of the local thermodynamic condition for our plasma.

REFERENCES

1. T. H. Maugh, *Science*, **202**, 4374, 1271–1273 (1978).
2. G. V. Iyengar, Element Analysis of Biological Samples: Principles and Practices, Vol. I, CRC Press, Boca Raton, Florida, 242 (1989).
3. D. Xiaonan, N. Yuzuru, H. Keizo, *Anal. Sci*., **3**, 195 (1998).
4. A. Khuder, M.A. Bakir, R. Hasan, A. Mohammad, *Environ. Monit. Assess,*, **143**, N 1-3, 67–74 (2008).
5. D. Combs, R. Goodrich, J. Meiske, *J. Anim. Sci*., **54**, 391–398 (1982).
6. R. Kellaway, P. Sitorus, J. Leibholz, *Res. Vet. Sci*., **24**, 352–357 (1978).
7. A. R. Grabeklis, A. V. Skalny, S. P. Nechiporenko, E. V. Lakarova, *J. Trace Elem. Med. Biol.*, **25**, N 1, 41–44 (2011).
8. N. A. Gres, T. M. Yuraga, A. G. Romanyuk, S. Hamad, V. P. Sokol, *Lab. Diagn. East Eur*., **4**, N 4, 62–72 (2012).
9. M. S. Blandin, *Biocontact Miner. Anal. Hair*, **60**, 226 (2012).
10. L. Kopito, R. K. Byers, I. N. Shwackman, *EnRI J. Mcd*., **276**, 949 (1967).
11. A. Taylor, *Ann. Clin. Biochem*., **23**, 364 (1986).
12. K. Asano, K. Suzuki, M. Chiba, K. Sera, R. Asano, T. Sakai, *J. Vet. Med. Sci*., **68**, 769–771 (2006).
13. M. D. L. Priya, A. Geetha, *Trace Elem. Res*., **142**, 148–158 (2011).
14. K. Chojnacka, A. Zielińska, I. Michalak, H. Górecki, *Environ. Toxicol. Pharmacol*., **30**, 188–194 (2010).
15. I. Michalak, K. Chojnacka, A. Saeid, *Chin. Sci. Bull*., **57**, 3460–3465 (2012).
16. S. Ni, R. Li, A. Wang, *Sci. China Earth Sci*., **54**, 780–788 (2011).
17. A. Bobrowski, A. Królicka, J. Zarębski, *Rev. Electroanal*., **21**, 1449–1458 (2009).
18. M. S. El-Shahawi, S. S. M. Hassan, A. M. Othman, M. A. El-Sonbati, *Microchem. J.*, **89** 13–19 (2008).
19. M. A. Gondal, M. A. Shemis, A. A. I. Khalil, M. M. Nasr, B. Gondal, *J. Anal. Spectrom*., **31**, 506–514 (2016).
20. M. A. Gondal, T. Hussain, Z. H. Yamani, M. A. Baig, *Talanta*, **69**, 1072–1078 (2006).
21. M. A. Gondal, M. M. Nasr, Z. Ahmed, Z. H. Yamani, *J. Environ. Sci. Health, A: Tox. Hazard. Subst. Environ. Eng*., **44**, 528–535 (2009).
22. M. A. Gondal, Z. S. Seddigi, M. M. Nasr, B. Gondal, *J. Hazard. Mater*., **175**, 726–732 (2010).
23. B. E. Naes, S. Umpierrez, S. Ryland, C. Barnett, J. R. Almirall, *Spectrochim. Acta B*, **63**, 1145–1150 (2008).
24. D. A. Rusak, A. E. Zeleniak, J. L. Obuhosdky, S. M. Holdren, C. A. Noldy, *Talanta*, **117**, 55–59 (2013).
25. A. O. Mehder, Y. B. Habibullah, M. A. Gondal, U. Baig, *Talanta*, **155**, 124–132 (2016).
26. K. Müller, H. Stege, *Archaeometry*, **45**, 421–433 (2003).
27. I. Choi, G. C. Y. Chan, X. Mao, D. L. Perry, R. E. Russo, *Appl. Spectrosc*., **67**, 1275–1284 (2013).
28. A. M. Matiaske, I. B. Gornushkin, U. Panne, *Anal. Bioanal. Chem*., **402**, 2597–2606 (2012).
29. E. C. Jung, D. H. Lee, J. I. Yun, J. G. Kim, J. W. Yeon, K. Song, *Spectrochim. Acta B*, **66**, 761–764 (2011).
30. A. Ciucci, M. Corsi, V. Palleschi, S. Rastelli, A. Salvetti, E. Tognoni, *Appl. Spectrosc*., **53**, 960–964 (1999).
31. M. Noda, Y. Deguchi, S. Iwasaki, N. Yoshikawa, *Spectrochim. Acta B*, **57**, 701–709 (2002).
32. M. Corsi, G. Cristoforetti, M. Hidalgo, S. Legnaioli, V. Palleschi, A. Salvetti, E. Tognoni, C. Vallebona, *Appl. Opt*., **42**, N 30, 6133–6137 (2003).
33. C. Chaléard, P. Mauchien, N. Andre, J. Uebbing, J. L. Lacour, C. Geertsen, *J. Anal. Atom. Spectrom*., **12**, 183–188 (1997).
34. H. R. Griem, *Plasma Spectroscopy*, Mc Graw-Hill, New York (1964).
35. W. Lochte-Holtgreven, *Plasma Diagnostics*, North-Holland, 135 (1968).