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Analytical Note

A novel strategy for preparing calibration standards for the analysis of plant materials by laser-induced breakdown spectroscopy: A case study with pellets of sugar cane leaves

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ABSTRACT

Calibration is still a challenging task when dealing with the direct analysis of solids. This is particularly true for laser-induced breakdown spectroscopy (LIBS), and laser ablation inductively coupled plasma optical emission spectrometry/mass spectrometry, when the calibrations are matrix-dependent and/or appropriate certified reference materials are generally not available. Looking at the analysis of plant materials in the form of pressed pellets by LIBS, a new method to overcome and/or minimize this difficulty is proposed by keeping the matrix constant in order to produce matrix-matched calibration pellets. To achieve this goal and to test this novel approach, ground sugar cane leaves were chosen and submitted to acid extractions for obtaining the corresponding blank or a material containing very low concentrations of the analytes. The resulting dried solid material was used either as a blank or a low concentration standard, and also homogeneously mixed with the original plant material at appropriate ratios as well. The corresponding pellets were used as calibration standards and ablated at 30 different sites by applying 25 laser pulses per site with a Q-switched Nd:YAG at 1064 nm. The plasma emission collected by lenses was directed through an optical fiber towards a spectrometer equipped with Echelle optics and intensified charge-coupled device. Delay time and integration time gate were fixed at 2.0 and 5.0 μs, respectively. This calibration strategy was tested for the determination of Ca, Mg, K, P, Cu, Mn, and Zn by LIBS in pellets of leaves from 17 varieties of sugar cane and good correlations were obtained with inductively coupled plasma optical emission spectrometry results in the corresponding acid digests. The proposed approach was also useful to estimate the limits of detection based on measurements of blanks, as recommended by IUPAC, or with the aid of a low concentration standard.

1. Introduction

Laser-induced breakdown spectroscopy (LIBS) has been evaluated as an alternative to wet acid digestions based methods for the determination of macro- (P, K, Ca and Mg) and micronutrients (B, Fe, Cu, Mn and Zn) in plant materials. The analyses are carried out directly in the form of pressed pellets prepared from dried, ground and comminuted leaves, with further interrogation by nanosecond laser pulses [1]. In spite of the various advantages and versatility of LIBS, one must pay attention to some boundary conditions for appropriate quantitative analysis. In most cases, these conditions are associated with the test sample presentation, with the quality of the test sample and with the calibration itself [1]. Furthermore, as stated by Hahn and Omenetto [2], difficulties related to quantitative LIBS may be attributed to the complex nature of the laser–sample interaction processes, which depends upon both laser characteristics and sample properties, and to the plasma–particle interaction processes, which may affect the results due to corresponding matrix effects.

While the LIBS qualitative analysis is rather a straightforward task, quantitative results on elemental composition require more efforts [3]. A common approach to quantitative analysis relies on the use of calibration curves prepared with certified reference materials (CRMs) [4], and it has been used for plant analysis purposes [1]. However, in practice, the similarity of physical and chemical properties of CRMs, including the corresponding similarity of pellet properties, such as density and porosity, is often rare when compared to the test samples, which may impair their commutability [1]. A simple procedure for obtaining calibration curves when an appropriate set of CRMs is not available is based on mixtures at different ratios of the materials to plot additional calibration points, as carried out by Sun et al. [5] for the determination of P, Al, Ca, Cu, Mn, Zn, Mg and Fe in plant materials by LIBS. In that

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case, NIST CRMs were mixed and homogenized in a ball milling during 20 min. A similar strategy was also used for the determination of macro- and micronutrients by LIBS in pharmaceutical tablets [6]. As the concentrations of the elements were close to each other in several samples, mixtures at different ratios of the materials were prepared to obtain additional calibration samples. Other calibration points were also obtained by diluting ground samples with pure cellulose, and the homogenization was performed by using a cryogenic mill.

The use of calibration samples previously analyzed by a reference method is an alternative when appropriate CRMs are not available, as demonstrated elsewhere [7] for the determination of macro- and micronutrients in pellets of sugar cane leaves by LIBS. Laboratory samples were cryogenically ground, in order to obtain similar particle size distributions prior to pressing, and then analyzed. By choosing calibration and validation samples from 06 varieties with similar chemical and physical matrix composition, good correlation between LIBS and inductively coupled plasma optical emission spectrometry (ICP OES) results was obtained [7].

The aim of this work was to propose a calibration strategy for LIBS analysis of pellets of plant materials. To demonstrate the feasibility of the proposed approach, a blank or a material with a very low analyte mass fraction, prepared from comminuted sugar cane leaves after acid extraction of the analytes, was used. The hypothesis was based on the fact that acid extraction of essential elements from powdered plant materials was successfully employed for many analytes (e.g. Mg, Mn, Zn, etc.) [8]. In the present work, the resulting material was mixed with the original laboratory sample at appropriate ratios, homogenized and pressed into pellets to prepare the calibration standards. The method was demonstrated for quantitative determination of Ca, Mg, K, P, Cu, Mn and Zn in pellets of sugar cane leaves by LIBS and for estimating the detection limits based on the analysis of the corresponding blank.

2. Experimental

2.1. LIBS instrumentation

Experiments were carried out with a Q-switched Nd:YAG laser (Brilliant, Quantel, France) operating at the fundamental wavelength (1064 nm), generating 5 ns pulses up to (365 ± 3) mJ, in a 6 mm diameter beam with quality factor M^2 smaller than 2, at 10 Hz repetition rate. The laser pulse was focused on the sample pellet by a plano-convex lens with 2.54 cm diameter and 20 cm focal length (Newport, USA) positioned at 50 cm from the laser head.

Individual test samples (i.e. 15 mm pellet diameter) were placed into a plastic sample holder in a two axes manually controlled translation stage that moved in the plane orthogonal to the laser direction. Argon (5.0 L min^{-1}) was continuously fed into the ablation atmosphere by one entrance inlet positioned at the sample holder. The plasma emission was collected by an optical arrangement composed of 50 mm and 80 mm focal length fused silica lenses (LLA Instruments GmbH, Germany) and coupled to the spectrometer with an optical fiber (1.5 m, 600 μm core) matching its numerical aperture. The collecting optics position was such that the plasma emission was spatially integrated. The collection angle with respect to the laser optical axis was 25°.

A spectrometer (ESA 3000, LLA Instruments GmbH, Germany) equipped with Echelle optics and focal length of 25 cm with aperture of 1:10 was used, which provides a 24.5 × 24.5 mm^2 flat image plane. This was selected as a compromise between resolution in the wavelength range from 200 to 780 nm and resolving power ranging from 10,000 to 20,000. The linear dispersion per pixel ranges from 5 pm at 200 nm to 19 pm at 780 nm. The detector is an ICCD camera, comprised of a Kodak KAF 1001 CCD array of 1024 × 1024 pixels full frame (24 × 24 μm^2) and a microchannel plate image intensifier of 25 mm diameter coupled to an UV-enhanced photocathode. The image signals were digitalized in dynamic range of 16 bits and further processed by a computer. The dark current of the ICCD was automatically subtracted from the measured spectral data. The background signals were measured, averaged and subtracted from emission line intensity [6]. The delay time, integration time gate and the number of accumulated pulses were fixed at 2.0 μs, 5.0 μs and 25, respectively [7].

The lens-to-sample distance (LTSD) and the pulse energy were adjusted at 17.5 cm and 110 mJ, respectively, leading to 25 J cm^{-2} at the sample surface.

Thirty different sampling sites on the pellet surface (test sample) were analyzed. Each crater was obtained after 25 consecutive laser pulses in each site. A 1.5 mm distance was kept between sites in order to avoid any possible influence of re-ablation at the edges of neighbor craters.

2.2. Sample pre-treatments

Sugar cane leaves were properly collected, taking into account the agricultural recommendation for plant diagnosis [9], washed separately with running tap water, rinsed twice with distilled water and three times with ultra-pure water. The midrib of each leaf was removed [9] and drying was carried out in a forced-air oven at 60 °C.

A cryogenic mill (Spex model 6800, USA) with a self-container liquid nitrogen bath was employed for sample comminution for 40 min. A pre-cooling time of 5 min was used with further 20 cycles of 2 min grinding. After each grinding cycle, the magnetic field was turned off for 1 min to allow sample re-cooling. This procedure provides 95% of particles <75 μm [7].

2.3. Calibration standards

Ten grams of cryogenically ground samples was transferred into a 500 mL flask containing 200 mL of 0.2 mol L^{-1} HNO_3. The acid and its concentration were defined experimentally. The mixture was shaken for 5 min by using a vortex, with further settlement of the solid phase prior to filtering through a Whatman® (Maidstone, UK) filter paper (3 μm pore diameter). The fraction retained in the filter was then transferred into another flask, and a new portion of acid solution was added. The mixture was shaken and filtered. This step was repeated 5 times. The final residue was oven-dried at 60 °C and homogenized by manual mortar and pestle. This material was analyzed by ICP OES after microwave assisted acid decomposition and used as blank, or a material containing very low concentrations of the analytes. Additional calibration standards were then prepared from appropriate mixtures of the extracted material with 25, 50 and 75% m/m of the original material. These materials were homogenized by cryogenic grinding for 2 min.

2.4. Acid digestion and ICP OES

The calibration standards and a set of leaves from 17 varieties of sugar cane were microwave-assisted acid digested in closed vessels (ETHOS 1600, Milestone, Italy) in triplicate, according to the following procedure [7]: 250 mg of ground material was accurately weighed in the TFM® vessels and then 6.0 mL of 20% v v^{-1} HNO_3 and 2.0 mL of 30% v v^{-1} H_2O_2 were added. The microwave heating program consisted of 5 steps: ramping to 120 °C in 3 min (step 1) and keeping this temperature during 2 min (step 2), ramping to 160 °C in 4 min (step 3), ramping to 220 °C (step 4) and keeping at 220 °C during 15 min (step 5). Thereafter, the residual solutions were transferred to 25 mL volumetric flasks, and the volume made up with high-purity de-ionized water (resistivity > 18.2 MΩ cm). The concentrations of Ca, Mg, P, K, Cu, Mn and Zn in the final solutions were determined by a radial view ICP OES (Optima 3000 DV, Perkin Elmer, Germany) and were used as reference values for the calibration standards. The ICP OES operational parameters are described elsewhere [6].

The accuracy of ICP OES results was checked with the following CRMs: spinach leaves (National Institute of Standards and Technology—NIST