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Critical review and advices on spectral-based normalization methods for LIBS quantitative analysis

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Abstract

As it is the case for any spectroscopic technique, laser-induced breakdown spectroscopy (LIBS) is strongly influenced by the signal fluctuations, and the LIBS spectra need to be normalized to obtain enhanced analytical performance. Nowadays, normalization in LIBS remains an open question and, in the present review, the normalization methods commonly applied to LIBS are presented and discussed, in particular those based on background, total area, internal standard, and Standard Normal Variate. We emphasize that the figures of merit, namely the coefficient of determination, the root-mean square error of prediction and the limit of quantification used to assess the advantages of processing normalized instead of non-normalized LIBS spectra, in a context of quantification, must be calculated in a rigorous way to be able to draw conclusions. We thus propose advices and good practices to achieve a rigorous comparison between quantitative models involving various normalization approaches, the final choice of the best normalization being ultimately driven by the analytical context. In order to take the best advantage from normalization in LIBS and thus increase the analytical performance of this technique, we encourage the analyst to thoroughly compare different normalization methods.

Keywords: LIBS, normalization, assessment, good practices, figures of merit

Introduction

As it is the case for any spectroscopic technique, laser-induced breakdown spectroscopy (LIBS) is strongly influenced by signal fluctuations [1]. Indeed, the LIBS spectra are correlated to the plasma properties, themselves correlated to: i) the measurement conditions such as laser energy, lens-to-sample distance, and spectrometer calibration, and ii) the physical properties of the sample such as roughness, hardness, and porosity.

Reducing the signal fluctuations allows increasing the quality in LIBS, which is of major importance for the development of this technique. The first strategy in order to reduce fluctuations is to build LIBS experiments with a high degree of control and stabilization. In this case, stabilizing the laser energy, the lens-to-sample distance as well as the spectrometer calibration allows for significantly reducing the signal fluctuations [2]. However, all the laboratory setups don't share the same level of control and thus don't allow for reaching the same reduction of signal fluctuations. In addition, in the case of on-site analyses, involving

compact LIBS systems [3], for either stand-off or handheld operation, the degree of control and stabilization is expected to be much lower, and the related level of fluctuations much higher compared to laboratory instruments. In this context, normalization approaches have been proposed in order to reduce the fluctuations observed in the raw LIBS data. A first type of normalization consists in monitoring other data in addition to the LIBS spectrum, either simultaneously, like the acoustic signal induced by the shock-wave [4, 5, 6], or after, like the ablated mass [7]. Then, the LIBS signal is corrected thanks to this additional information, namely divided by a factor related to this external parameter. Then, if the variation of the monitored parameter is correlated with those of the LIBS signal, this normalization process is expected to efficiently reduce the signal fluctuations. However, most of the LIBS instruments don't allow to monitor additional parameters during or after the measurement and the analyst has to treat the raw LIBS signals, without any extra information. In this last case, normalization simply consists in a mathematical transformation of the raw data.

Data normalization is expected to have a positive impact on both quantification and sorting models. Nevertheless, we decided to focus the present discussion only on quantification since this is, by principle, the top objective of any analytical method. In addition, quantification in LIBS can be addressed by building either a calibration model from a series of samples with known concentration values, or a physical model from the plasma properties and spectroscopic data, without any calibration, and well-known as calibration-free (CF-LIBS) [8]. It is interesting to note that signal fluctuations due to a change in plasma properties are naturally taken into account by CF-LIBS. Thus, any change in electron density and temperature induces complex modifications of the calculated LIBS spectra, which cannot be described by a simple multiplicative coefficient. More precisely, the components of the LIBS spectra, namely the continuum as well as the ionic, neutral, and molecular emission lines are changing in non-proportional ways according to the changes in the plasma properties. The CF-LIBS approach thus includes a kind of self-normalization process that enables to correctly interpret each single LIBS spectrum individually. Consequently, in the present review, we will focus our discussion only on the case of calibration models. In this case, the transformations to be applied to the raw LIBS spectra for normalization purposes are quite basic, but to date there is no normalization approach considered as being more efficient than the others to treat the LIBS data. Even more, the advantage of normalization in LIBS is sometimes questionable, making this topic still an open question. In the critical review proposed here, a state-of-the-art about normalization in LIBS is presented in the first section, with a particular emphasis on the four methods of normalization the most often adopted in LIBS, specifically normalization by: i) background, ii) total area, iii) internal standard, and iv) standard normal variate (SNV). Other factors were also tested to normalize the LIBS spectra such as the Euclidian norm, the maximum and the minimum intensity values of each individual spectrum [9, 10, 11]. However, the related normalization approaches can be considered as quite similar to the four methods previously mentioned, and they are finally rarely used and not detailed in this review. Then, in the second section of this review, in order to properly compare the performance of the different normalization approaches between them, good practices and significance of selected figures of merit, namely the coefficient of determination (R^2); the root mean square error (RMSE) declined as the root mean square error of calibration (RMSEC) when strictly applied to the calibration set, the root mean square error of prediction (RMSEP) when strictly applied to the validation set not involved in the construction of the model, and the root mean square error of cross-validation (RMSECV)

after a cross-validation process; the limit of detection (LOD), and the limit of quantification (LOQ) are presented as a key-step to make accurate decisions. Finally, we have decided to illustrate the present discussion through the quantitative analysis of a selected dataset after different normalization approaches, and we have accurately compared the related figures of merit to conclude if a given normalization method can be finally recommended in a general context.

1. State of the art

The need for normalization concerns not only LIBS but all spectroscopic techniques. And the most efficient strategies of spectra normalization in general are well documented in the scientific literature and routinely applied, especially for Near-Infrared Spectroscopy (NIR), and inductively-coupled plasma atomic emission spectroscopy (ICP-AES), selected here as two emblematic examples of the spectroscopic techniques, and detailed below.

In the case of NIR, the spectra can significantly be influenced by light scattering through a baseline change, known as the multiplicative effect, and non-linear variations of intensity. Therefore, the most used normalization techniques in NIR are divided into two groups [12]: i) the scatter-correction methods, including *Standard Normal Variate* (SNV) and *Multiplicative Scatter Correction*, and ii) the signal derivative based on algorithms such as *Savitzky-Golay* or *Norris-Williams*. The review made by Rinnan *et al.* [12] about the pre-processing methods in NIR, theoretically describes each of these normalization techniques as well as their advantages and limitations. Nowadays, all the NIR spectra are systematically normalized and the normalization process is well-established [13, 14].

In the case of ICP-AES, the plasma fluctuations are eliminated by the use of the internal standard method [15]. It means that a chemical element is chosen for its compatibility with the matrix of interest, and by the presence of an atomic emission line exhibiting good signal-to-noise ratio and without any risk of spectral interference with emission lines related to other elements. The selected element is then used as a dopant and introduced under the same concentration into each solution prepared for quantification. In this context, the elements Yttrium (Y) and Scandium (Sc) are often selected as internal standards for these reasons and also because they are absent from almost all the *real-life* samples. Then, the emission line selected for the normalization process is exploited as follows: its peak intensity is measured for each sample, including the blank; and then, the ratio to the intensity of the blank is calculated for this emission line for each sample. Finally, the peak intensities related to all the other elements are multiplied by this ratio. This method is well-established and has been routinely applied to ICP-AES for many years [16].

Regarding LIBS, it is worth noticing that, despite many attempts of normalization during these last years, there is no clear preference for one type of normalization, the analysts adopting the method considered as the most efficient for their specific application. In 2010, Zorov *et al.* [5] described the strategies of normalization to be applied to LIBS and other spectroscopic techniques based on laser sampling, such as ICP-AES, atomic absorption spectroscopy and laser-enhanced ionization spectroscopy. They presented examples of LIBS data successfully normalized [17, 18] as well as cases for which the normalization was not efficient [19, 20], confirming that determining the right normalization method for LIBS is not

an easy task. In 2012, Hahn and Omenetto [4] emphasized that it was almost impossible to apply the internal standard method to normalize the LIBS data since many important criteria couldn't be satisfied. Furthermore, Tognoni and Cristoforetti [6] proposed three types of normalization approaches, namely the internal standard method, the normalization by the background and finally a normalization based on the calculation of the plasma properties, similar to the CF-LIBS method. The authors finally recommended to select the most relevant normalization strategy among the three, based on each single experimental context.

To go further into details, an extended selection of articles dealing with normalization in LIBS is presented in Table 1. The aim of this list is to: i) show which normalization methods have been investigated and finally selected, ii) point out that some authors have demonstrated the advantage of treating normalized compared to uncorrected data, while some others haven't, and iii) present the figures of merit that have been used to determine the prediction ability of each model, and thus the advantage of applying normalization.

Table 1: Selection of articles dealing with normalization in LIBS. The investigated and selected normalization methods are presented as well as the figures of merit used to assess the prediction ability of each model. IS: internal standard; SNV: standard normal variate; I_{max}: maximum intensity; I_{min}: minimum intensity.

| Investigated normalization method | Selected normalization method | Comparison normalized/ uncorrected data | Figures of merit | References |
|--|-------------------------------|---|--------------------------------|------------------------------|
| IS, Background, Total area | IS | yes | RMSECV, LOD | 2018, Thomas et al. [27] |
| IS, Total area, I _{max} , Norm | IS | yes | R ² , RMSEC | 2019, Andrade et al. [28] |
| Background | Background | no | R ² , LOD | 2011, Dell'Aglio et al. [30] |
| Background | Background | no | R ² , RMSEC | 2009, Senesi et al. [31] |
| Total area | Total area | no | R ² , RSD | 2014, Fabre et al. [22] |
| Total area | Total area | no | R ² , RMSE | 2017, Payré et al. [61] |
| Total area | Total area | yes | R ² , RMSECV | 2018, Takahashi et al. [26] |
| IS | IS | no | RSD | 2008, Juvé et al. [21] |
| IS | IS | yes | R ² | 2011, Gupta et al. [48] |
| IS applied to Fe, Mg, Al, Si | IS (Fe line) | yes | R ² | 2009, Kwak et al. [35] |
| IS applied to Ba, Si, B | IS (Ba line) | no | R ² , RMSECV | 2014, Sarkar et al. [36] |
| SNV | SNV | no | RSD | 2011, Ismael et al. [23] |
| SNV, IS | SNV | yes | R ² , LOD | 2015, Syvilay et al. [42] |
| Total area, IS, I _{max} , norm | Element dependent | yes | RMSEC, RMSECV, RMSEP, LOD | 2016, Castro et al. [45] |
| Total area, Background | Total area | yes | RSD, RMSEP | 2001, Body and Chadwick [17] |
| Total area, IS, Calibration-free | Element dependent | yes | R ² | 2006, Sallé et al. [47] |
| Total area, IS, I _{max} , norm | Element dependent | yes | R ² , RMSEC, RMSEP | 2017, Augusto et al. [43] |
| IS, I _{max} , norm | Element dependent | yes | RMSEC, RMSECV, RMSEP, LOD, LOQ | 2016, Andrade et al. [46] |
| Total area, Background, IS | IS, total area | no | R ² , RMSEP, RSD | 2016, Karki et al. [24] |
| I _{min} , I _{max} , IS, norm | Norm | no | REP, RMSEP | 2015, Sarkar et al. [44] |

Seven normalization methods are reported in Table 1, namely normalization to the maximum, minimum, background, total area, Euclidian norm, internal standard and SNV. Details about the calculations related to these methods can be found in the corresponding

references. Table 1 also reveals that the comparison between normalized and uncorrected data has not been systematically presented, some of the authors assuming that normalization was expected to provide *a priori* better analytical results than uncorrected data, but without verifying if this statement is always true. The figures of merit that have been used to assess the normalization methods are also given in this table. First, the reduction of the variations of the LIBS signals was presented by some authors [21, 22, 23, 17, 24]. The coefficient of determination R^2 has been very often used, either alone, or in complement to other figures of merit such as the root-mean square error (RMSE) declined as RMSEC (calibration set), RMSEP (validation set), and RMSECV (cross-validation), as well as the limits of detection (LOD) and quantification (LOQ). It is worth noticing that these figures of merit should be calculated with an extreme thoroughness since they allow not only for demonstrating if the normalization enables to reach better analytical performances than by uncorrected data, but also to determine if a normalization method provides a better correction than the others. In addition, it is worth pointing out that very recent publications [26, 27, 28] were partly dedicated to the selection of the best normalization approach, revealing it is still an open question. From Table 1, it is also interesting to notice that the *best* normalization method seems to depend on the element and the sample. However, in order to push further the discussion, the principles and advantages of the four most common normalization methods applied to LIBS are presented hereafter.

Normalization to the background

The background emission is theoretically considered to be a relevant indicator to monitor the plasma properties. In 2008, De Giacomo *et al.* [32] demonstrated the existence of a correlation between the intensity of the continuum radiation, i.e the background intensity, and the plasma density, itself correlated to the amount of emitters in the plasma. In the case of normalization to the background, the peak intensity value of the emission line of interest is divided by the value of the background emission. This one is recorded at the vicinity of the emission line of interest, either from the intensity value at a single wavelength, or from the average value over a selected spectral window [6]. It is worth pointing out that the background emission must be carefully recorded. In practice, it means that the detector dark current must be separately recorded and then subtracted prior to such signal processing. This point has been deeply discussed in ref. [6, 66] and is crucial to take into account the actual background signal. Zorov *et al.* [5] attributed the first results about the use of normalization to the background to Xu *et al.* [29], but the corresponding work was quickly disapproved by other authors [19], which clearly revealed a lack of generalization ability. However, this type of normalization has been successfully applied to several cases [5, 30, 31]. In the frame of analysis of soils polluted with heavy metals, Dell'Aglio *et al.* [30] adopted the normalization to the background for emission lines related to several elements (Cr, Cu, Pb, V, Zn) in order to reduce matrix effects. They concluded that the correlation between the data derived from ICP-AES and the normalized LIBS signals was quite satisfactory. Senesi *et al.* [31] also adopted the normalization to the background to quantify the concentrations of heavy metals in soils, with a particular focus on chromium. However, despite significant reduction of the fluctuations and high correlation between the normalized LIBS and ICP-AES data, it is very important to notice that only 3 to 4 points were taken into account to build the quantitative models in this study. The conclusions are thus likely to be non-robust.

Normalization to the total area

In the case of normalization to the total area, for each LIBS spectrum, the peak intensity value of the emission line related to the analyte is divided by the value of area of the spectrum over the whole spectral range. Here again, the detector dark current must be separately measured and subtracted prior to such signal processing, to deal with real LIBS spectra. The total area is calculated as the sum of all the intensity levels describing the LIBS spectrum, once the detector dark current has been removed, and is considered to be a good indicator of the plasma properties. Thus, Body and Chadwick [17] demonstrated the existence of a strong correlation between this area and the laser energy. Then, Fabre *et al.* [22] applied this normalization method to the analysis of Martian rocks and soils by ChemCam onboard the MSL Curiosity rover. In this case, three separate spectrometers were operated and the normalization to the total area was implemented by considering only the spectrometer for which the emission line of the analyte was detected. Therefore, all the results of quantification obtained from the ChemCam data, and based on Partial Least Squares regression (PLS) for the major elements and univariate analysis for the minor and traces, were entirely based on normalized data. In 2010, Zorov *et al.* [5] reported that J.A. Bolger [33] proposed the normalization method to the total area for the first time in LIBS, which allowed to improve the linearity of the calibration curves of Cu, Fe, Ni, Mn and Cr in mineral rocks. However, it is interesting to notice again that the calibration curves were built from a very small number of points, making the conclusion of this study potentially non-robust. Finally, Yu *et al.* [25], in the context of sorting certified soils by chemometric tools, examined the compensation of matrix effects and changes in experimental conditions, mainly temperature, through the normalization to the total area. They presented this normalization as a standard step of pre-processing but without comparing the corresponding analytical results to those obtained from uncorrected data. More generally, most of the articles about the normalization to the total area have concluded that this pre-processing step provides a benefit.

Normalization to an internal standard

The internal standard (IS) method is frequently adopted by the LIBS analysts. It consists in dividing the peak intensity (or area) of the emission line related to the analyte by the peak intensity (or area) of a selected emission line related to the internal standard. The concentration of the internal standard is supposed to remain constant for the whole set of samples, or at least to be known by the analyst. The underlying hypothesis is that the variations of the peak intensity related to the internal standard are only due to changes in the plasma properties. This is expected to be true when all the samples considered to build a calibration model are characterized by a single matrix in order to avoid any risk of bias linked to matrix effects. But it is interesting to notice also that this normalization method can be indifferently applied to any kind of matrix [21, 36, 48, 63, 64, 65]. Moreover, it requires the use of another analytical technique to measure the concentration values of the internal standard inside each sample.

The list of criteria defined by Barnett *et al.* [34] to be verified in order to identify a reliable internal standard, have been applied to LIBS [4]. Three of these criteria are related to the choice of the element playing the role of internal standard, since the analyte and the internal standard are expected to share similar: i) volatilization rates; ii) ionization energies; and iii) atomic weights. Then, three other criteria are focused on the selection of the spectral line, which is expected to: i) have the same excitation energy than the one of the analyte; ii) be not affected by self-absorption; iii) have a similar intensity as the one of the analyte. In practice, this list of criteria seems to be almost impossible to fulfill, especially in the case of natural and complex matrices, driving to conclude that the internal standard method is generally not ideal to LIBS analysis. Despite this statement, several studies have concluded on the advantages of applying the internal standard method to LIBS, even if the choice of the internal standard according to the criteria previously mentioned was not always clearly presented. Thus, Juvé *et al.* [21], selected the spectral line of carbon at 247.86 nm considered as an internal standard, to process the space-resolved analysis of trace elements (Mg, Al, Ca, Ti, Mn, Fe) in fresh vegetables. The choice of carbon was based on the fact that it was found under constant concentration in the studied samples but no reference to the selection criteria neither for the elements nor for the spectral lines can be found in their article. Thus, the advantage of adopting internal standard normalization reported in this article might be due to chance. Similarly, Kwak *et al.* [35] did not consider the criteria related to the selection of internal standard. In the case of quantification of arsenic in soils, they normalized the intensity of the As I line at 228.812 nm to the one of the Fe I line at 248.8 nm and also to the Mg I line at 285.2 nm, then the Al I line at 309.3 nm, and also the Si I line at 288.2 nm. The only criterion to select these lines was the increase of the coefficient of determination. Indeed, starting from 0.22 without normalization, $R^2=0.96$ after a normalization to Fe, 0.91 with Mg, 0.75 with Al, and 0.58 with Si, demonstrating the advantage of normalizing the LIBS signal to the Fe I line at 248.8 to reach the best performance for the calibration model. Moreover, Sarkar *et al.* [36], in the context of quantification of uranium in a barium borosilicate glass matrix, selected barium as internal standard, after comparison with other elements, such as silicon and boron. Then, they selected the emission line of Ba I at 649.786 nm, which was satisfying to all the given criteria. Finally, by comparing the figures of merit (R^2 , RMSECV and the slope of the calibration curve), obtained after this normalization to the selected Ba I line, to the ones derived from other elements and spectral lines, they concluded that the best normalization according to theoretical criteria was also the best one from the experimental results.

To conclude, normalization to an internal standard has been demonstrated to reduce the LIBS signal fluctuations and thus increase the analytical performance of the related quantitative models. However, in addition to the cases exhibiting a successful effect of this type of normalization on the analytical performance, it is worth noticing that LIBS is also often applied to analysis of samples for which it is impossible to find a proper internal standard, according to the list of criteria previously discussed. This normalization approach can't thus be considered as a general strategy for LIBS analysis, and should only be applied to specific cases.

Standard Normal Variate - SNV

Standard Normal Variate (SNV) is one of the most used normalization techniques in near-infrared spectroscopy (NIR) [37, 38, 39] and Raman spectroscopy [40, 41]. SNV calculation is processed on each spectrum individually by centering the spectrum on the mean value and then dividing the corrected spectrum by the standard deviation of the original spectrum (Equation 1):

$$I_k^{\text{snv}} = \frac{I_k - I_{\text{mean}}}{s} \quad \text{Equation 1}$$

where I_k represents the intensity value at the wavelength k of the original spectrum; I_k^{snv} , the corresponding SNV normalized intensity value; I_{mean} , the mean of the intensity values of the original spectrum; and s , the standard deviation of the original spectrum.

In the context of in-situ quantitative LIBS analyses of polluted soils involving Pb, Cu and Fe, Ismaël *et al.* [23] obtained good correlations between the SNV-corrected LIBS data and the reference concentration values derived from ICP-AES, for Pb (405.78nm), Cu (327.39nm) and Fe (344.08nm) and observed that SNV allowed to reduce the standard deviation after a series of repetitions. Then, Syvilay *et al.* [42] demonstrated in the context of quantitative analysis of Ag, Bi, Cu and Sn in lead samples, that the univariate models built from SNV-corrected data provided better figures of merit (R^2 and LOD) than those built from either raw data or data normalized to a selected emission line of lead, considered as internal standard. To conclude, the examples of LIBS studies reported in Table 1 indicate that SNV might be an efficient normalization technique for processing the LIBS data and we point out that the advantages of the SNV normalization is worth being accurately assessed in future works.

As a general conclusion, the four normalization methods presented in the present review have been successfully applied to LIBS data, meaning they have permitted to reduce the signal fluctuations and by consequence, improve the calibration performances.

2. Recommendations

To support the advantages of normalizing, we recommend to systematically compare the figures of merit related to the models built from either normalized or uncorrected data. Indeed, from Table 1, one can notice that this simple comparison was not made in all the cases. In addition, it is worth pointing out that, in some cases, normalization might negatively impact the analytical performance of the models [19, 36, 43]. Based on the works reported in Table 1, it is not possible to conclude if one of the normalization methods might be advantageously adopted whatever the element and application. Thus, we recommend the LIBS analyst to test different normalization methods and then adopt the one offering the best analytical performance. This strategy of comparing different normalization methods has been adopted by some authors [44, 45, 24, 43, 46], who concluded that, in some cases, uncorrected data provide better analytical performances than normalized ones, and also that the normalization method should be selected on a case-to-case basis. Regarding the figures of merit calculated to assess the performance of each model, the coefficient of determination R^2 has been widely used. However, in some cases, R^2 was the only indicator considered for the assessment [35, 47, 48], which is clearly insufficient to establish robust conclusions, as it will be further discussed in this section.

Minimum number of points to build a reliable calibration model

In order to determine what is the best normalization approach for LIBS, we recommend to strictly apply the basic rules allowing to calculate correct values for the figures of merit dedicated to assess the analytical performances of the models. Our first advice concerns the minimum number of points (concentration values) to build a robust linear calibration model. This number is not defined in a unique way by the organizations in charge of standardization and traceability of chemical measurements. The ISO standard 11095:1996 [49], namely the standard dedicated to linear calibration using reference materials published by the International Organization for Standardization (ISO), advises to include more than three points to build a reliable model. Moreover, through a guide named “The Fitness for Purpose of Analytical Methods” [50], the European network of organizations EURACHEM, promoting good quality practices about chemical measurements, recommends to include a minimum of 6 concentration levels plus blank. In the same way, a commission decision (2002/657/EC), edited by the commission of the European communities [51], about the performance of the analytical methods and the interpretation of results advises to include at least 5 points, i.e. concentration levels, including zero. Based on these references, the minimum number of points to build a reliable calibration model should be around 5 or 6. Furthermore, these points should be, ideally, equally distributed over the concentration range related to the calibration to obtain the most representative calibration model related to the analytical context. But, despite of its major importance for quality purpose, this last criterion is generally under-estimated or simply not considered by the LIBS analysts. We have thus decided to illustrate how the use of an unappropriated dataset to build the calibration models might drive to non-robust conclusions.

Coefficient of determination, R^2

The coefficient of determination, R^2 , is the figure of merit the most commonly used (cf. Table 1) to assess the quality of a calibration model. However, this indicator is reliable only when the points are equally distributed over the concentration range and when there is no extreme point, very far from the other ones. At the opposite, if one of these conditions is not fulfilled, the decision based only on the parameter R^2 might be non-robust and this statement seems not always be taken into account in the LIBS literature [31, 33], still nowadays [52].

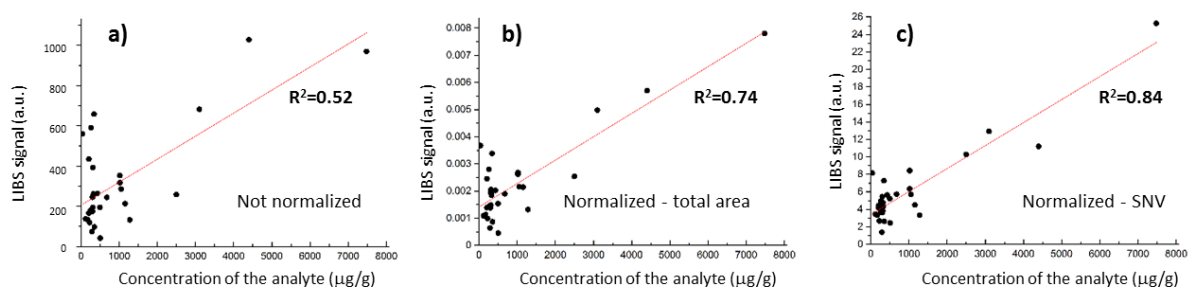


Figure 1: Linear calibration models (red lines) obtained (after baseline-subtraction) for: a) not normalized data, b) data normalized to the total area, and c) data normalized by SNV, with the corresponding R^2 values.

Figure 1 illustrates the case of univariate calibration models built from a set of 36 LIBS spectra. This dataset has been specially selected to illustrate the case of non-satisfying conditions to build a robust model. Thus, all the details about the samples, the analyte, and the experimental setup are voluntary not provided here, to highlight the general conclusions of

this discussion and not consider it as a specific case-study. For each model displayed in Fig. 1, the baseline-subtracted signal (in arbitrary units, a.u.) was plotted on the vertical axis as a function of the concentration of the analyte ($\mu\text{g/g}$) on the horizontal axis in three cases: a) without normalization; b) after normalization to the total area of the spectrum; c) after normalization by SNV. In agreement with the *a priori* expectation, the R^2 values are higher after normalization. However, it is worth noticing that, considering a range of calibration from 0 to 8000 $\mu\text{g/g}$, 89% of the points of the calibration set correspond to concentration values lower than 1500 $\mu\text{g/g}$. Moreover, for these points, whatever the normalization process, there is no correlation between the LIBS signal and the concentration values. In such conditions, the increase of the R^2 value from 0.52 (without normalization) to 0.84 (SNV) should be considered with caution, since the regression models over the full concentration range are, in this case, not robust. Indeed, none of the points from the calibration set is between 1500 and 2500 $\mu\text{g/g}$ and between 4500 and 7500 $\mu\text{g/g}$. Moreover, in such conditions, the coefficient of determination value is highly sensitive to extreme points, considered as *outliers* [53]. For instance, in this case, the last point of the calibration curve corresponds to a concentration value of 7500 $\mu\text{g/g}$, quite far from the previous one, which corresponds to 4500 $\mu\text{g/g}$. Indeed, after removing this last point from the calibration set, the new R^2 values become 0.38, 0.53 and 0.59, for the non-normalized data, data normalized to the total area, and data normalized by SNV, respectively. Finally, the example given here aims at warning the LIBS analyst about the danger of looking only at R^2 [54] to characterize the calibration performances of a model, whatever the method of normalization. It also means that outliers should induce additional investigation through either new measurements, or new steps in data processing.

Root Mean Squared Error of prediction (RMSE)

In addition to the R^2 parameter, the prediction ability has to be determined. It consists in calculating the common Root Mean Squared Error (RMSE) [55] resulting from the predicted concentration values from a series of samples. This indicator is expected to be as low as possible for a reliable model, and is mathematically written as in Eq.2.

$$RMSE = \sqrt{\frac{\sum_{p=1}^N (c_p - \hat{c}_p)^2}{N}} \quad \text{Equation 2}$$

Where c_p is the predicted concentration value, \hat{c}_p the reference concentration value of the sample p, and N the number of samples taken into account. It is worth noticing that, similarly to the case of R^2 , this figure of merit should be considered with extreme attention, as discussed below.

When all the samples belonging to the calibration (C) set are simultaneously taken into account, the resulting *RMSE* value is referred to as *RMSEC*. However, *RMSEC* might not be a reliable indicator of the calibration model in case of the presence of extreme points, namely points exhibiting very high concentration values compared to the other ones [55]. To overcome this drawback, the *RMSE* value should be calculated on the basis of a cross-validation (CV) process, and referred to as *RMSECV*. Among the different techniques of cross-validation, the Leave-One-Out (LOO) method is the most common. It consists in

removing one point from the dataset and then calculate the *RMSEC* value from the regression built from the N-1 remaining points. Then the excluded point is reintroduced, another point is excluded, and a new *RMSEC* value is calculated, and so on, until all the points of the calibration set have been excluded. Finally, *RMSECV* is the average value of the N values of *RMSEC* calculated during the cross-validation process. *RMSECV* is expected to better reveal the limitations of the calibration model than *RMSEC*. When the calibration set is big, another method of cross-validation, known as *bootstrapping*, is generally preferred to the LOO, and consists in excluding not only one single point at a time but many. Finally, to fully assess a calibration model, it is necessary to predict the concentration values of a set of *known* samples different from the calibration set. Thus, it has been suggested [56] to split the original dataset into the calibration set (2/3) and the validation set (1/3), both of them equally spread over the concentration range. Then, the RMSE value is calculated over the validation set, and referred as *RMSEP*, with (P) related to the prediction of the concentration values of the samples belonging to the validation set. Comparing *RMSEC* and *RMSEP* allows to evaluate if there is a risk of overfitting (if $RMSEC \ll RMSEP$), before comparing different strategies of normalization. Note also that *RMSECV* and *RMSEP* are commonly used in the frame of multivariate regression, not only to evaluate the model performances but also to select the best number of components, which can easily become user dependent. Finally, the validation step in multivariate analysis is not the aim of this present review, but many information can be found in the following studies [55, 56, 57].

Limits of detection and quantification, LOD and LOQ

The limits of detection (LOD) and quantification (LOQ) have been discussed by Mermet in review papers [58, 59] dedicated to atomic spectrometry. In LIBS, the LOD is the most frequently reported, and defined by Eq.3 [59], for a regression model written as: $y = a + b \cdot x$

$$LOD = \frac{3s}{b} \quad \text{Equation 3}$$

where s is the standard deviation of the background signal measured close to the peak of interest. Similarly, the limit of quantification is defined by Eq. 4 [58, 59]:

$$LOQ = \frac{10s}{b} = \frac{10}{3} LOD \quad \text{Equation 4}$$

The limits of detection and quantification are calculated from the regression model. And, as already stated for the calculation of R^2 and RMSE, the calibration set needs to fulfill basic requirements to obtain reliable results for the values of these limits.

Finally, the figures of merit R^2 , RMSE, LOD (or LOQ) are perfect to assess the analytical performance of the models as well as the advantage of normalization [60]. In the next section, we exploit these figures of merit to compare, for a dataset recorded in our laboratory with a handheld LIBS instrument, the analytical performance obtained by i) non-normalized spectra, ii) spectra normalized to the total area, and iii) spectra normalized by SNV.

3. Example of normalization

The general discussion about normalization in LIBS has been presented in the state-of-the-art of this review, and then important recommendations that deserve to be completed by a case-study. It aims at illustrating the question of normalization and allows us to propose a general guideline to assess and then report the analytical performance of the quantitative models based on different normalization methods. Let's mention that this guideline could efficiently be applied to any spectroscopic technique and normalization method but it appears to be of major importance for LIBS. We consider a first set of 14 samples of plants (calibration set), sampled from field crops in France, and stored in a sample bank managed by the French National Institute for Agricultural Research (INRA), in the frame of the French national observatory network Quasaprove [62]. More precisely, these samples were analyzed by a portable LIBS instrument and the analyses presented here were focused on the quantification of magnesium. Each sample was dried and then ground by using a planetary ball mill (PM 400, Retsch), allowing to obtain a grain size smaller than 250 μm . Then, each sample was prepared as a 12-mm diameter pressed pellet after 8 tons load during 3 minutes without using any additional binder. 10 locations were randomly chosen at the surface of the sample. And at each location, 3 laser shots were considered as pre-shots and then the LIBS spectra related to the following 7 shots were recorded. The 10 locations and the 7 spectra per location gives access to 70 LIBS spectra per sample. The average spectrum over the 70 spectra was considered for the analyses. At each wavelength, the RSD value from the 70 spectra has also been calculated and reveals that each sample can be considered as homogeneous, which confirms the observation one can make with the naked eyes.

Considering the quantification of magnesium as an example for the present guideline, the concentration values of the 14 samples composing the calibration set ranged from 0.7 to 8 $\mu\text{g/g}$, according to the reference ICP-AES analyses. In addition, 4 other samples of plant, from the same bank, and not used for the calibration, were selected to validate the calibration models, based on their availability and their concentration values measured by ICP-AES.

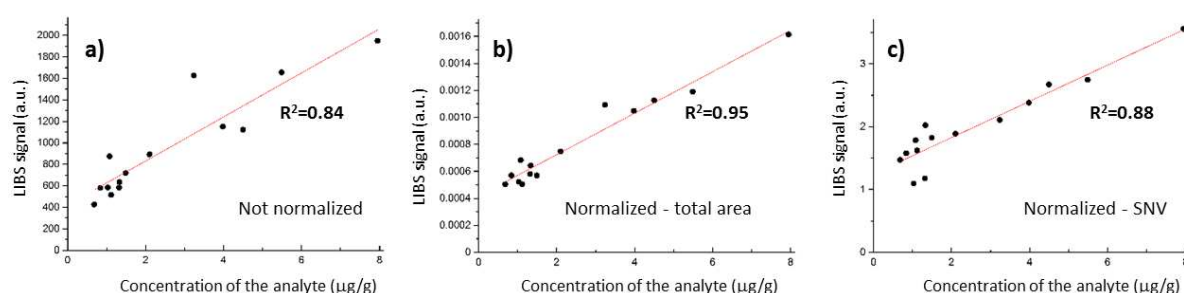


Figure 2: Linear calibration models (red lines) obtained (after baseline-subtraction) for: a) not normalized data, b) data normalized to the total area, and c) data normalized by SNV, with the corresponding R^2 values. In this case-study, the analyte was magnesium and the samples were plants.

Figure 2 displays calibration models built from; a) the baseline subtracted peak intensity value of Mg II at 280.27 nm, b) normalization by the total area of the spectrum, c) normalization by SNV. It should be emphasized that the present discussion is focused on the

methodology one should apply to be able to objectively compare the different methods of normalization between them. Thus, even if the models based on normalization to the background and internal standard could have also been presented in Figure 2, we have decided to select only two methods of normalization to illustrate our purpose. These two normalization methods were expected to reduce the fluctuations and thus to improve the analytical performance of the models, according to previous works [22, 25, 61, 26, 23, 24], and this result is again verified in the case of plant samples displayed in Figure 2. Indeed, the points related to the concentrations of magnesium larger than 3 $\mu\text{g/g}$ are much closer to the linear regression curve after normalization. At the opposite, two of the points related to concentration values close to 1 $\mu\text{g/g}$ and normalized by SNV are displayed far from the regression line and the other points.

Table 2 displays the values of the figures of merit dedicated to the assessment of the quantitative models, specifically R^2 , RMSEC, RMSECV, RMSEP, LOD (reminding that LOD and LOQ are closely linked, no need to report also the values of LOQ), calculated for the three regression models displayed in Figure 2. As already discussed, even if the R^2 values are higher after normalization, it might be risky to draw a conclusion based on this single parameter. Indeed, one can observe in Table 2 that while R^2 is rising from 0.84 (without normalization) to 0.88 (SNV), RMSEP is unexpectedly increasing from 0.74 to 1.42, indicating that the regression model built after SNV normalization exhibits a lower predictive ability than the model built from non-normalized data. The SNV model might suffer from over-fitting since both the RMSEC and RMSECV values are half the RMSEP value. At the opposite, the values reported in Table 2 related to the normalization to the total area reveal for this model stronger correlation (higher R^2) and better prediction ability (lower errors of prediction). Finally, even if the values of R^2 , RMSEC, RMSECV, and RMSEP are better after normalization to the total area, the values of LOD are not significantly different from data without normalization and after normalization.

| | R^2 | RMSEC($\mu\text{g/g}$) | RMSECV($\mu\text{g/g}$) | RMSEP($\mu\text{g/g}$) | LOD($\mu\text{g/g}$) |
|----------------|-------|--------------------------|---------------------------|--------------------------|------------------------|
| Not-normalized | 0.84 | 0.90 | 0.89 | 0.74 | 0.20 |
| Total area | 0.95 | 0.45 | 0.45 | 0.57 | 0.24 |
| SNV | 0.88 | 0.76 | 0.75 | 1.42 | 0.32 |

Table 2: Figures of merit calculated to assess the performance of regression models involving different normalization strategies.

In the case of the models reported in Table 2, the normalization to the total area allows for building a calibration model with a better coefficient of determination and smaller prediction errors. This type of normalization might thus be adopted here even if it doesn't allow for lowering the limits of detection and quantification. At the opposite,—the data normalized by SNV drive to worse prediction ability, namely a higher RMSEP value, than non-normalized data.

More generally, the normalization to the total area has been considered as valuable not only in the case reported in Table 2 but also in many previous studies. It is thus *a priori* expected that this type of normalization should improve the quantification ability of any LIBS analysis. However, we recommend to demonstrate its advantage *a posteriori*, from a comparison of the values taken by the figures of merit reported in Table 2. At the opposite, the normalization by SNV appears to be more questionable. Some previous studies have

demonstrated its advantage but it was not the case in the frame of the case-study reported in Table 2.

We finally recommend, whatever the types of samples and elements to be quantified, to always compare the figures of merit related to the models calculated from not-normalized and normalized spectra, as displayed in Table 2. Let's point out that, in addition to the normalization to the total area and by SNV, the normalization to the background and to an internal standard should also be considered. Based on the methodology presented here, not only the best normalization method can be objectively selected, but the calculated values of the figures of merit can also be directly exploited to report the analytical performance of the selected model.

Conclusion

LIBS spectra are often normalized in order to take into account signal fluctuations and thus obtain enhanced analytical performance. However, normalization in LIBS remains an open question and, in the present review, we have identified and discussed the normalization methods commonly applied to LIBS. We have more specifically detailed and discussed four of them, that we consider as the most relevant, namely i) background, ii) total area, iii) internal standard, and iv) SNV. We have also highlighted that the gain resulting from normalizing LIBS spectra was often not assessed and that the rare comparisons between different normalization methods were sometimes questionable. Indeed, this review has also revealed that basic requirements to calculate robust indicators were sometimes not fulfilled. A first case-study, based on the selection of LIBS spectra and concentration values unsuitable to build a robust calibration, has been discussed to emphasize the risk of taking a decision about the advantage of normalization from only the R^2 value. Finally, advices and good practices have been proposed in order to correctly calculate the relevant figures of merit dedicated to the assessment of the performance of the quantitative models. Obviously, an efficient normalization method is expected to provide higher R^2 values, and lower RMSE and LOD or LOQ values than those obtained without normalization. However, through a second case-study, related to the quantification of magnesium in plant samples, we have demonstrated that these three figures of merit were not always simultaneously improved. Then, considering that the analytical context naturally introduces some hierarchy within the figures of merit, in the context of quantification of major or abundant elements, reducing the RMSE value becomes the top priority and reducing the LOD or LOQ value can be of secondary importance. At the opposite, for the quantification of minor or trace elements, the normalization method should be able to reduce the LOD or LOQ value while keeping a good predictive ability, i.e. a small RMSE value. Based on this methodology, it becomes possible to objectively compare different normalization methods between each other and finally select the best one according to the analytical context. Future works may adopt this methodology in order to take the best advantage from normalization in LIBS, and thus increase the analytical performance of this technique.

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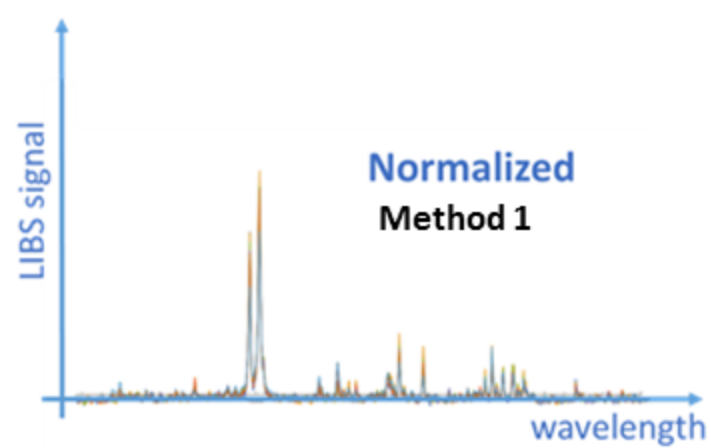
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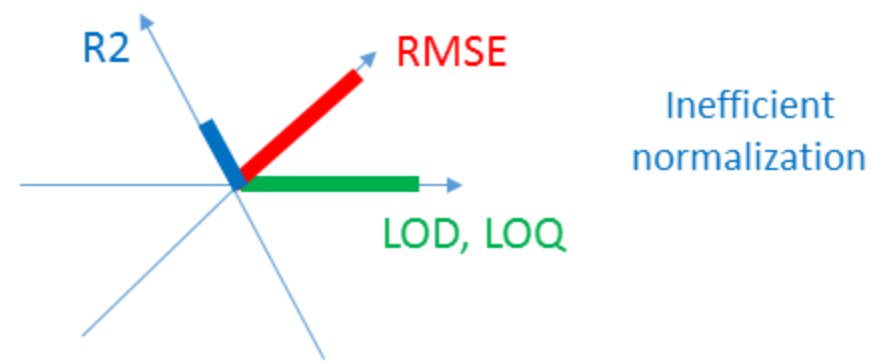
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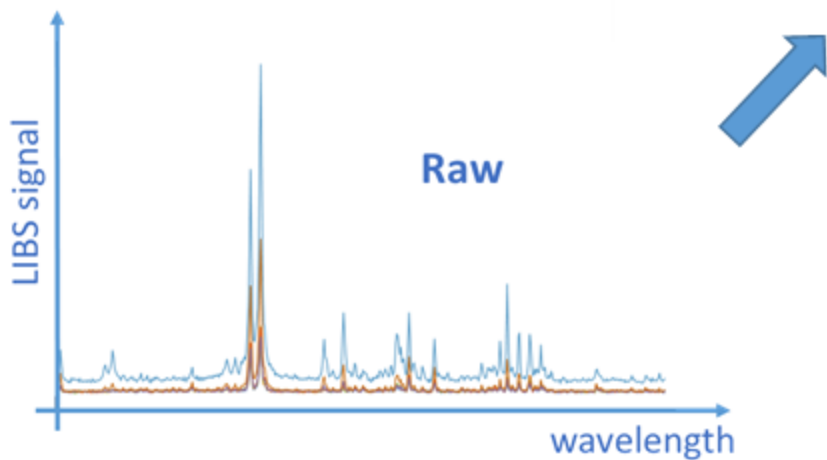
Which normalization method ?



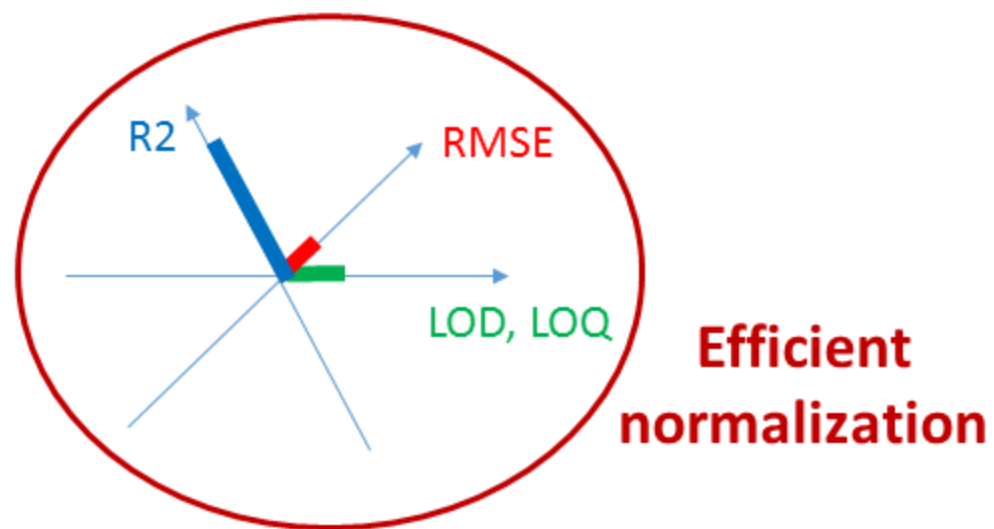
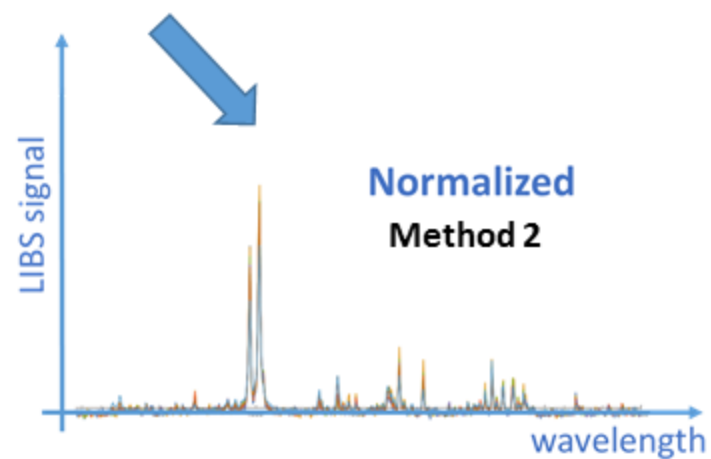
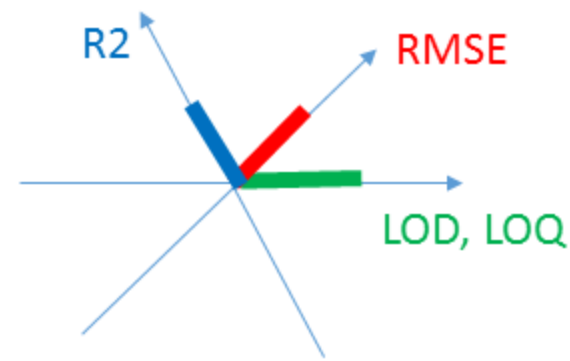
Calibration models



Inefficient normalization



Quantitative analysis



Efficient normalization