Credit Author statement

Sample containing the analytes → Stirring → GO film immersion → Analytes adsorbed on the GO film → LIBS analysis
Evaluation of Thin Film Microextraction for trace elemental analysis of liquid samples using LIBS detection

L. Ripoll\textsuperscript{1*}, J. Navarro-González\textsuperscript{2}, S. Legnaioli\textsuperscript{3}, V. Palleschi\textsuperscript{3}, M. Hidalgo\textsuperscript{1*}

\textsuperscript{1}Department of Analytical Chemistry and Food Sciences and University Materials Institute, University of Alicante, E-03690, Alicante, Spain

\textsuperscript{2}Image Processing Laboratory, University of Valencia, E-46980 Paterna, Valencia, Spain

\textsuperscript{3}Institute of Chemistry of Organometallic Compounds, National Research Council (CNR), Research Area of Pisa, Applied Laser Spectroscopy Laboratory, via G. Moruzzi, 1, 56124 Pisa, Italy

E-mail addresses: montserrat.hidalgo@ua.es (M. Hidalgo), laura.ripoll@ua.es (L. Ripoll)

Abstract

An analytical methodology based in the combination of Thin Film Microextraction with Laser-induced Breakdown Spectroscopy (TFME-LIBS) was investigated, for the first time, for detection of Cu, Cr, Ni and Pb in aqueous solutions. In this methodology, the analytes were extracted in a thin film of adsorbent material deposited on a solid support, which was introduced in the sample to analyse. After extraction, the analytes retained in the adsorbent were analysed by LIBS. In order to obtain adsorbent films useful for the microextraction step, two different experimental procedures for film generation, denoted as Drop Casting Deposition and Mould Deposition, were evaluated. In both cases, graphene oxide was used as adsorbent material. The mould deposition procedure was found to produce more homogeneous graphene oxide layers, leading to more uniform distribution of the adsorbed analytes on the graphene oxide surface. Experimental parameters affecting the TFME procedure, such as the adsorbent amount and extraction time, were studied. Under optimum microextraction conditions, the analytical figures of merit of the proposed TFME-LIBS method were evaluated, leading to limits of detection ranging from 41\(\mu\text{g kg}^{-1}\) and 52\(\mu\text{g kg}^{-1}\). Method trueness, evaluated from the analysis of a real sample of bottle water, led to recovery values about 70\%, indicating the existence of strong matrix effects probably due to the presence of major cations in the bottle water. After 50\% dilution of the sample with deionized water, recoveries values improved to 99\%-108\%.

Keywords: Thin film microextraction, graphene oxide, LIBS, trace analysis, liquid samples

Electronic supplementary material available.
1. Introduction

A number of valuable characteristics of Laser Induced Breakdown Spectroscopy (LIBS), such as portability of LIBS equipment, capability for remote, in-situ and on-line analysis; or “green” analytical capability [1], have contributed to the fast spreading of the technique to many different application areas. Nowadays, analysis of solids by LIBS is widely used for many different purposes, including on line quality control [2, 3], cultural heritage or archaeological research [4, 5], depth profile and chemical mapping [6, 7], or even Mars exploration [8], among many others.

Applications of LIBS analysis of liquid samples, in comparison to that developed for solids, are very limited. Limits of detection of LIBS in these kind of samples cannot compete with those of the most conventional techniques, such as ICP OES or ICP MS [9-12]. Therefore, at present, the most promising applications of LIBS in liquids are those requiring remote analysis in which, in addition, elemental detection at low concentration levels is not an essential need. An example can be its potential use for oceanic exploration [13-15]. Even so, and due to the capability of LIBS for fast and on-line analysis, a potential use of this technique could be as early warning system, for quality control applications or environmental monitoring, to give some examples. However, the use of LIBS in these kind of applications requires elemental detection capabilities at low concentration levels, which can be hardly reached by the technique without the application of, at least, a minimum sample preparation process. Analytical methodologies including very simple sample preparation steps, such as the presentation of the liquid sample to the LIBS measurement instrument in the form of a liquid jet or an aerosol, have been able to reduce detection limits for some alkaline or alkaline earth elements. However, for most analytes of high health and environmental impact, LODs still remain of the order of hundreds of µg kg$^{-1}$ [16-19].

Extraction procedures are traditional sample preparation strategies commonly used in analytical chemistry to separate the target analytes from interferent species in the samples and, at the same time, to concentrate these analytes to levels high enough to be analysed by the detection technique, thus improving the limits of detection of analytical methods. In particular, the recently developed microextraction procedures can offer interesting advantages over the traditional ones, since they are usually faster, cheaper and more environmentally friendly, providing, at the same time, very high preconcentration capacities [20-23]. In fact, microextraction procedures have already been combined with LIBS in previous research works, by using both liquid-phase [24-28] and solid-phase [29,30]
microextraction modalities. In all cases, limits of detection of LIBS were highly improved, reaching LOD values of few tens of µg kg^{-1} or even lower. Unfortunately, up to date, all microextraction modalities tested with LIBS technique are relatively complex and, more important, they are difficult to combine with LIBS detection in an automatic way, therefore limiting the use of the developed method for on-line applications.

There are microextraction modalities, mainly Solid Phase Microextraction (SPME) modalities, which are experimentally simple. Among them, it can be cited Rotating Disk Sorptive Extraction (RDSE) or Thin Film Microextraction (TFME) [31-34]. In particular, TFME is also easy to automate and combine with LIBS detection. In this microextraction procedure, a solid film is coated with a thin layer of adsorbent material. The coated film, attached to the end of a rigid rod, is introduced into the sample solution, which is constantly stirred during a given time (i.e., extraction time). After extraction, the analytes-enriched film is removed from the sample and, usually, a final step of analytes desorption into the detection instrument is needed. The last desorption step, however, can be avoided if LIBS is used as detection technique, due to its ability to directly analyse solids samples.

In this work, the analytical capability of a methodology based on the combination of TFME with LIBS detection (TFME-LIBS) was evaluated for trace elemental analysis in aqueous samples. To this end, two different experimental procedures for producing the thin films to be used in the microextraction step were tested: (i) drop casting deposition and (ii) a modification of the previous one denoted as mould deposition. In both cases, graphene oxide (GO) was selected as adsorbent material. Selection of this adsorbent was based on its large surface area and on the elevated presence of oxygen-containing functionalities on its surface, which allows successful removal of metal ions from aqueous solutions without the need of any previous metal complexation step [35-38]. After selecting the best-suited method for producing the films, some experimental factors affecting the TFME procedure were optimized. Finally, different analytical figures of merit of the TFME-LIBS procedure were evaluated. Method trueness was estimated by addition and recovery experiments on a real sample of bottled water.

2. Experimental

2.1. Instruments and apparatus

2.1.1 LIBS experimental setups

Two different LIBS instruments were used in this work (Figure S1): (i) a double pulse µ-LIBS system (Figure S1a) [39] and (ii) a conventional single pulse LIBS system (Figure
S1b). The µ-LIBS system was used during the first part of the experimental work, for evaluation of the adsorbent films for microextraction and for optimization of the microextraction experimental parameters, while the conventional LIBS system was employed for subsequent quantitative studies carried out during the second part of the experimental work.

The µ-LIBS is a MODI (Mobile Dual-Pulse Instrument, Marwan Technology, Italy) commercial LIBS equipment. It comprises a double-pulse Nd:YAG laser, emitting at the fundamental wavelength with a nominal pulse width of 15 ns FWHM, and a compact time-integrated spectrometer (Dual channel spectrometer, model AvaSpec-2048-2, Avantes, Eerbeek, The Netherlands) with spectral coverage from 180 to 900 nm. In this equipment, the laser pulses are sent through the MODI articulated arm into an optical microscope with a 10x magnification objective lens and 10 mm focal length (Zeiss Axio Lab. A1 Carl Zeiss Microscopy GmH, Deutschland) and then focused onto the sample. The sample is placed on an x-y motorized stage that can be programmed to perform a 2D movement to integrate the entire surface with a maximum lateral resolution of 30 µm. The LIBS signal is collected and sent to the double channel spectrometer by a bifurcated optical fibre, which is placed at 45 degrees with respect to the laser beam axis. The tip of the optical fibre is placed about 1 cm from the surface to collect the signal from the whole laser-induced plasma.

In this work, the laser was always operated in co-linear double pulse mode, with pulses of energy 13.8 mJ (i.e., 5.4 + 8.4 mJ in double pulse operation). LIBS spectra were acquired by applying a delay of 1 µs between the two pulses, with 1.26 µs delay time after the second laser pulse. The integration time was 2 ms.

In order to perform surface mappings, the motorized stage movement for sample positioning was controlled by a dedicated software programmed in LabVIEW (LabVIEW v.8.6, National Instruments, Mopac Expwy, Austin, USA). This software allows programming both, the number of y and x laser firing positions on the mapped surface and the separation between adjacent y and j positions. As a result, a matrix M(yx) is programmed, representing a total of y×x positions for laser firing on the sample surface. Data acquisition of the programmed laser shots was controlled by the spectrometer software (AvaSoft©, v 7.5.3, Avantes, Eerbeek, Netherlands). The delay system of the commercial equipment allowed synchronization of sample positioning, laser firing and data acquisition.

The conventional single pulse LIBS instrument uses a Nd:YAG laser (Handy-YAG model HYL 101, Q-switched, Quanta System S.P.A., Varese, Italy), emitting at the fundamental wavelength with a nominal pulse width of 6 ns FWHM, and a compact time-integrated spectrometer (five channel spectrometer, model AvaSpec-2048-SPU, Avantes,
Eerbeek, The Netherlands). In this system, the laser is focused on the sample by a 60 mm focal length biconvex lens. The sample is placed on an x-y translation stage, which is manually moved to interrogate different positions of the solid surface. Plasma emission is collected and sent to the entrance slit of the five-channel spectrometer by a five-furcated optical fibre (5 × 400 μm fibre optic cable, model FC5-UV400-2, Avantes, Eerbeek, Netherlands), which is positioned at 60° with respect to the laser beam axis. In this system, the tip of the optical fibre is placed about 2 cm from the sample surface to collect the signal from the whole laser-induced plasma. LIBS measurements are externally controlled by manually triggering the laser firing (i.e., external triggers to laser flashlamp and Q-switch) with two pulse generators (Digital delay/pulse generator, model DG 535, Stanford Research Systems, Inc.; and 1 MHz–50 MHz pulse/function generator, model 8116A, Hewlett Packard/Agilent Technologies, Santa Clara, USA). Synchronization of laser firing and data acquisition is performed with the same two-pulse generators system and with the aid of the spectrometer software (AvaSoft®, v 8.5.0.0, Avantes, Eerbeek, Netherlands).

In this study, laser pulses of 180 mJ energy were used. All LIBS spectra were collected 1.3 μs after the plasma generation, with 1 ms acquisition time. A total of 25 independent single shots LIBS spectra were acquired per sample, by using the AvaSoft® spectrometer software.

Spectra stored with either the µLIBS or the conventional LIBS system were further processed by a dedicated software for single shot LIBS analysis (SS_LIBS) written in MATLAB (MathWorks, Inc. Natick, Massachusetts, USA). This software allows performing, in a given set of single shots LIBS spectra, the individual processing of different spectral regions of interest for each single shot spectrum. By selecting the desired wavelength intervals for the different emission lines under study, SS_LIBS generates a previous 3D plot (wavelength-intensity-number of single shot spectrum) for each of the selected analyte emission lines. Inspection of these previous 3D plots allows both checking whether the correct spectral region has been entered and discarding possible “null” LIBS spectra in the analysed spectra set (i.e., LIBS spectra showing no signal for all analyte lines tested). After proper corrections, the SS_LIBS software extracts several features of the spectral signals, such as centre of the spectral peaks, integrated intensity for the different analyte lines in the different single shot spectra, and the mean integrated intensity over the set of spectra for the different analyte lines, among others.

In this work, Cr I (359.350 nm), Ni I (352.454 nm), Cu I (327.396 nm) and Pb I (368.346 nm) were the emission lines evaluated.
2.1.2. Preparation and study of the graphene oxide films

An ultrasound bath (model Transsonic TP690, Elma Company, Germany) was used to form stable suspensions of GO in water and water/MeOH solvents. A hot plate (model 501, Darlab Egara S.L.) was used to dry the graphene oxide films after deposition of the adsorbent on the solid substrates (i.e. ceramic or glass slides).

The morphology and homogeneity of the obtained GO films were examined with the use of a Scanning Electron Microscope (SEM) (model S3000N, Hitachi High-Technologies Corporation, Tokyo, Japan) and a micro X Ray Fluorescence (μ-XRF) analyser (model Orbis, EDAX, Mahwah, Japan) with a LiSi detector (Rhodium Tube (Rh), 35 kV and 960 µA).

2.1.3. Microextraction procedure

Adjustment and measurement of the solutions pH was carried out using a pH meter (Basic 20+, Crison Instrument, Spain). The same hot plate stirrer already mentioned in the previous section (model 501, Darlab Egara S.L.) was used to accelerate the mass transfer during the microextraction processes, and also to dry, during a few seconds, the analyte-enriched adsorbents after the extraction process and prior to LIBS analysis.

2.2 Reagents, solutions and materials

All solutions were prepared with analytical grade chemicals and in distilled deionized water (DDW, 18.3 MΩ cm⁻¹). Standard aqueous solutions containing Cu, Cr, Ni and Pb were prepared by appropriate dilution of 1000 mg L⁻¹ mono-element stock solutions (High-Purity mono-element standard solutions, Charleston, UK). Acetic/acetate buffer solution at pH 5 was prepared from glacial acetic acid 99.8 % (Sharlau Chemie S.A. Sentmenat, Spain) and ammonium acetate 98% (Sigma-Aldrich Sant Lois, Missouri, USA). A real sample of mineral water (Recoaro©, Italy. Total solid content ~150 mg L⁻¹ and major cation content Ca²⁺ ~ 35 mg L⁻¹, Mg²⁺ ~ 14 mg L⁻¹, K⁺ ~ 0.3 mg L⁻¹) was used for evaluation of the analytical method trueness.

Commercial GO powder (Graphene oxide flakes, Sigma Aldrich, St. Louis, USA) was used for preparation of the microextraction films. Depending on the experiment, different GO dispersions were prepared: (i) a 5 mg mL⁻¹ GO dispersion in ultrapure water or (ii) a 2.5 mg mL⁻¹ GO dispersion in a 50% (v/v) water-EtOH solution (EtOH 96% (v/v) for analysis Panreac Química S.L.U. Barcelona, Spain). A 2% (w/w) carboxymethyl cellulose/water solution, prepared from carboxymethyl cellulose sodium salt (Sigma-Aldrich Sant Lois, Missouri, USA) was used as binder to ensure adhesion of the adsorbent material to the underlying solid.
substrate. Ceramic sheets (10 × 34 mm) obtained from commercial ceramic screen-printed electrodes (Metrohm DropSens, Llanera (Asturias), Spain) and glass slides (13 × 26 mm) obtained from commercial microscope slides (Linealab, S.L, Badalona (Barcelona), Spain) were used as solid substrates. Square 10 × 10 mm silicone moulds, fabricated from commercial silicone sheets (Violett Lifestyle, Murga (Alava), Spain), were used in the experiments performed with glass substrates, in order to maintain the GO coating contour by avoiding its spreading over the surface of the slide.

2.3 Experimental procedure

In order to evaluate the combination of Thin Film Microextraction methodology with LIBS detection for elemental analysis of liquid samples, two different experimental procedures for production of microextraction films, denoted as “drop casting deposition” (DCD) and “mould deposition” (MD), were studied and compared (Section 3.1). By using the most adequate thin film generation procedure among those studied, different experimental parameters influencing the TFME process were evaluated, in order to select the most suited working conditions (Section 3.2.). Finally, several analytical figures of merit of the proposed TFME-LIBS method were estimated, in order to assess its analytical capability for trace metals detection in water samples (Section 3.3.).

2.3.1. Generation of microextraction films

Figure 1 shows the experimental procedures for generation of GO films using DCD (Figure 1a) and MD (Figure 1b).

In the DCD procedure, ultrapure water GO dispersion and ceramic sheet were used as adsorbent film precursor and solid substrate, respectively. A 5 mg mL⁻¹ GO aqueous dispersion was prepared by mixing 50 mg of GO powder with 10 mL of deionized water. The so prepared dispersion was sonicated during 30 min in an ultrasound bath immediately prior to use, in order to separate the graphene oxide layers and to obtain a well-distributed dispersion. Before depositing the GO adsorbent on the ceramic sheet, the surface of this solid substrate was covered with a thin layer of aqueous carboxymethyl cellulose binder solution. The so prepared ceramic sheet was placed on a hot plate kept at a constant temperature of 100 °C. Afterwards, the desired quantity of GO was deposited on the ceramic surface by adding and drying, one over the other, several small aliquots of the GO dispersion (50 - 60 µL each) on the heated solid, until a final volume of 160 µL of GO dispersion was
added. By this process, DCD films containing 800 µg of adsorbent were obtained, as shown in Figure 1a.

In the MD experimental procedure, the adsorbent film precursor and solid substrate were ultrapure water/EtOH GO dispersion and glass slide, respectively. In this case, a given volume of the GO aqueous dispersion (previously sonicated) was 50% diluted by adding the same volume of EtOH. The resulting GO water/EtOH dispersion, containing 2.5 mg mL$^{-1}$ GO, was again sonicated during 10 min immediately prior to use. After preparing the glass slide with the carboxymethyl cellulose binder, a silicone mould was placed on top of the glass substrate and the mould cavity (a 10 x 10 mm square) was filled with the desired quantity of GO. To this end, GO water/EtOH dispersion was deposited and dried on the glass substrate following the same procedure than in the previously described DCD approach. In this case, however, aliquots of 40 µL each were successively loaded into the mould and dried on the glass surface until a final volume of 320 µL of the GO water/EtOH dispersion was added, therefore leading to the generation of MD films containing the same quantity of GO adsorbent than the DCD films (i.e., 800 µg. See Figure 1b). In the MD method, the hot plate temperature was set at 70ºC.

2.3.2. Microextraction procedure

The general experimental procedure for extraction of the analytes from aqueous samples by TFME is illustrated in Figure 2. In this procedure, 20 g of sample was placed in a 50 mL vial. The sample pH was set at 5 by addition of 2 mL acetate/acetic buffer solution, and then the solution weight was brought to 25 g with deionized water. The film, coated with the GO adsorbent by any of the aforementioned methods, was suspended from the end of a vertical rod. With the aid of the rod, the film was introduced into the sample, which was constantly stirred during the extraction process in order to accelerate the mass transfer. After extraction, the analyte-enriched adsorbent was removed from the solution and was dried for approximately 20-30 seconds in a hot plate. The dried films were finally analysed by one of the LIBS systems described in Section 2.1.1 above. The particular experimental conditions used for LIBS analysis of the films are specified in the corresponding sections below.

3. Results and discussion

3.1 Generation of the GO films

In order to select the most adequate adsorbent film to use with the proposed TFME-LIBS method, GO layers obtained by the previously described DCD and MD approaches
were studied by using scanning electron microscopy, micro X-ray fluorescence and LIBS. The LIBS equipment used in this initial part of the experimental work was the µ-LIBS system described in Section 2.1.1 above. SEM analysis was carried out with the aim of studying the homogeneity of the adsorbent material spread on the surface of the solid substrate. Therefore, this study was carried out on the generated films prior to their use in microextraction procedures. µ-XRF and LIBS analysis were both carried out after using the films for extraction of different analytes from model aqueous solutions, in order evaluate the homogeneity in the distribution of the extracted analytes on the adsorbent material.

### 3.1.1. Drop casting deposition

Drop casting deposition method was selected due to its great experimental simplicity [40]. For generation of the DCD films, 160 µL of the aqueous GO dispersion was deposited and dried on the ceramic substrates as described in the previous Section 2.3.1., resulting in films having 800 µg of adsorbent. With a temperature setting of 100 ºC in the hotplate, the drying of the aqueous graphene oxide dispersion on the ceramic substrate was complete in nearly 10 minutes. With the DCD method, the deposited droplets of GO dispersion spread freely on the ceramic support, forming a rounded solid film perfectly adhered on the surface after its drying (see Figures 2 and 3).

Figure S2 shows SEM images of a DCD film of GO acquired with magnification 12x (Figure S2a), 33x (Figure S2b) and 60x (Figure S2c). As can be appreciated, the surface of the adsorbent material is characterized by a large number of pores and irregular features, which could be even visible with the naked eye.

Inhomogeneity in the GO layer can have a considerable influence on the distribution of analytes on the surface of the adsorbent when the generated films are used in microextraction processes, which furthermore can led to irreproducible results in the subsequent detection step. This fact can be especially problematic when LIBS is used as detection technique, since its punctual nature implies the local analysis of microregions of the adsorbent surface.

In order to study the distribution of the adsorbed analytes on the surface of the GO layer, different metals (i.e., Ni, Cr, Cu and Pb) were extracted from a model aqueous solution following the microextraction procedure already described in the previous Section 2.3.1. Afterwards, the analyte-enriched DCD films were analysed by LIBS and by μXRF. Concentration of analytes in the model aqueous solution used in this study was 0.5 mg kg\(^{-1}\), and the extraction time was 20 min.
LIBS analysis of the analyte-enriched films was carried out with the µLIBS system described in Section 2.1.1. above. Figure S3 shows a comparison of LIBS spectra obtained from the analysis of an analyte-enriched DCD film (Figure S3a) and a ceramic substrate (Figure S3b). The presented LIBS spectra are the mean of 30 laser pulses fired in 30 different positions on the samples surface, which were located approximately in the central area of the GO circular residue (Figures S3a), or in a nearly equivalent position in the ceramic substrate (Figure S3b). The separation between adjacent laser spots was 200 µm. As observed, multiple emission lines were present in the spectrum of the ceramic substrate, in particular, in the spectral regions comprising the most intense emission lines of the selected analytes. The same emission lines from the ceramic substrate were also observed in the spectrum of the DCD film after extraction, indicating that a single laser shot on the surface of the analyte-enriched GO was able to completely penetrate the GO layer reaching the ceramic substrate (see for instance, titanium or aluminium emission lines in both spectra). The insets at the bottom of the figure are enlarged spectra showing the particular spectral regions of the analytical lines, in which spectra of Figures S3a and S3b appear overlapped. As can be appreciated, all analyte lines, with exception of Ni I (352.454 nm), suffered from spectral interferences from the substrate. In the case of lead, the Pb I (368.346 nm) line was not even visible due to the interference of titanium from the ceramic substrate. In addition, and according to the previously presented SEM study, the highly inhomogeneous distribution of the GO layer obtained in the DCD films could also led to variations in the spectral contribution from the ceramic substrate, because of the different GO layer thickness in different positions irradiated by the laser.

Figure 3 shows an example of the inhomogeneous distribution of the analytes emission intensity over the GO layers obtained with the DCD methodology. This analysis was carried out by programming a matrix of 5×50 positions for laser firing on the GO surface with the µLIBS system (i.e., a total of 250 different positions on the GO layer). The separation between adjacent laser spots was 200 µm. The selected positions for LIBS analysis corresponded to the central part of the GO circular residue (see Figure 3a). Results presented in this figure correspond to Ni I (352.45 nm), since this was the emission line less interfered by the ceramic substrate (see insets at the bottom of the previously mentioned Figure S3). Results obtained from Cu and Cr were inconclusive due to the strong spectral interference from the substrate, and Pb was undetectable. As observed from this figure, emission signal at the wavelength corresponding to Ni I (352.45 nm) was highly irreproducible (Figure 3b), with % RSD over 50 laser shots ranging from 23% to 29% for the gross Ni I signal, and from 24% to 34% for the net (i.e., baseline-subtracted) signal (Figure
A noticeable signal depression can be also observed in Figure 3b for LIBS analysis performed in \( y \) positions from 2 to 4 and \( x \) positions from 13 to 16 (i.e., positions marked with dotted rectangles). According to the 3D plots in Figure 3c, in which these particular zones have been enlarged, no Ni emission was observed in these \( xy \) positions of the GO layer. Since, according to the results shown in Figure S3, Ni emission was not visible in the ceramic substrate, this signal depression could correspond to a zone of reduced GO thickness (i.e., a hole), with less amount of adsorbed analyte ablated by the laser.

Results obtained by µ-XRF analysis also confirm the inadequacy of the DCD methodology for generating homogeneous films to be used in TFME. Figure 4 shows elemental µ-XRF mappings of the GO layer for the extracted Ni, Cr and Cu. An image of the GO layer is also included for comparison purposes (Figure 4a), as well as a mapping of Al as constituent of the ceramic substrate (Figure 4e). In this analysis, Pb could not be detected due to its low x-ray fluorescence intensity. However, as can be observed for the other three elements, the distribution of emission intensity of the different analytes over the adsorbent surface was highly inhomogeneous. The darker zones in Figures 4b to 4d, which correspond to positions on the XRF surface mappings producing lower fluorescence intensities (see intensity scale on the right), are roughly coincident with the positions of the different pores on the material observed in the sample image of Figure 4a. In contrast, aluminium emission, arising mainly from the ceramic substrate, appears as a negative image of those of the adsorbed analytes.

These results were in good agreement with that obtained from LIBS analysis regarding the inhomogeneous distribution of analytes in the DCD films. This problematic, in addition to the drawback arising from the spectral interferences from the ceramic substrate, led to discard the DCD approach for generation of the adsorbent films.

### 3.1.2. Mould deposition

The mould deposition experimental procedure (MD) was a modification of the previous approach, in which several improvements were introduced in order to ensure the viability of the obtained films for the proposed TFME-LIBS analytical methodology. The main differences with the DCD approach were: (i) the substitution of the ceramic substrates by glass substrates for deposition of the GO adsorbent, (ii) the use of water/EtOH mixture as solvent for the GO dispersion, (iii) the use of a silicone mould for the generation of the GO layer on the glass substrate and, (iv) the use of a lower temperature for drying the GO suspension on the substrate.
Figure S4 shows a comparison of LIBS spectra obtained from a ceramic substrate and a glass substrate with the µLIBS system. These spectra are the mean of 30 laser shots performed on the solids (i.e., a programmed 3×10 matrix for the sample movement, with 200 μm shot-to-shot spatial separation). As observed, the glass substrate resulted in a less congested spectrum compared to ceramic, in particular in the spectral regions of the selected Cu, Ni, Pb and Cr emission lines, therefore eliminating, or greatly reducing, the spectral interferences from the substrate in LIBS analysis of the analyte-enriched films.

The use of a water/EtOH mixture as solvent for the GO dispersion was implemented with the aim of reducing its surface tension. As observed from Figure S5, the surface tension of the GO dispersion was highly reduced when water was substituted by a 50% v/v water/EtOH solution. This fact contributed to a better spreading of the GO on the surface of the glass substrate and, therefore, to the generation of more homogeneous GO layers. In this case, however, it was necessary the use of a silicone mould in order to avoid an uncontrolled spreading of the suspension over the glass surface, because of its low surface tension, and to obtain reproducible GO layers. In addition, the use of a lower temperature of 70ºC in the drying step led to a more gradual drying of the solid without a significant increment in the drying time, due to the lower boiling point of the water/EtOH solution.

As with the DCD films, the generated MD films were first investigated by SEM and, after their use in microextraction processed, by LIBS and µXRF. In this latter studies, the experimental conditions used with the previous DCD films, for both the microextraction and the detection steps (LIBS and µXRF), were kept for the MD analyses.

Figure S6 shows SEM images, at different magnifications, obtained from a GO layer prepared with the MD approach. As observed, even if some imperfections on the GO surface can still be appreciated from these images, the homogeneity of the adsorbent layer was clearly improved in comparison to that obtained with the previously tested DCD methodology.

Figure S7 and Figure 5 show the results obtained in the LIBS study. As observed from Figures S7a and S7b, emission from Si, which could be unequivocally attributed to the glass substrate, can be appreciated in both spectra, indicating that also in this case a single laser shot was able to completely ablate the GO layer reaching the substrate (see SEM image in Figure 5a). Even so, as can be observed from the insets at the bottom of Figure S7, Pb I (368.346 nm) emission line was clearly visible when glass, instead of ceramic, was used as substrate in the films. Moreover, with exception of this emission line, which still appears to be slightly interfered by a titanium line from glass, the other analyte emission lines were free from interferences.
The homogeneity in the distribution of the adsorbed analytes over the GO layer was also improved using the MD films, as can be appreciated by comparing the results presented in Figure 5 with that of Figure 3 for Ni I (354.454 nm) emission line. A significant decrease in % RSD of both gross and net emission signals was obtained. In addition, contrary to the observed by comparing % RSD of gross and net signals in LIBS analysis of the DCD films, % RSD in LIBS analysis of MD films did not changes significantly after baseline subtraction, probably due to the absence of nearby emission lines interfering the analyte emission.

Results obtained by LIBS were also corroborated by µXRF analysis (Figure 6). As observed, even if a completely homogeneous distribution of analytes over the GO layer was not obtained with the MD approach, comparison of this Figure with µXRF results in Figure 4 demonstrated a considerable improvement over the previously tested DCD methodology. Consequently, MD was the experimental procedure used for generation of adsorbent films in the following experiments.

3.2 Study of experimental parameters in TFME

Two different experimental parameters affecting the microextraction process were optimised: (i) the amount of GO adsorbent used in the generation of the MD films and (ii) the extraction time. Sample pH, which is a critical parameter influencing metals extraction by graphene oxide, was optimised in a recent study carried out by our research group on the same GO adsorbent and, therefore, the optimized pH value (i.e., pH 5) was kept the same in the present work [30]. For optimization studies, the test samples consisted in model aqueous solutions containing 0.5 mg kg⁻¹ of the different target analytes. After extraction, the analyte-enriched films were analysed by the µLIBS system. In this analysis, a 10×10 matrix was programmed for sample movement, resulting in 100 laser shots on the sample surface separated a distance of 500 μm. The obtained 100 LIBS spectra were finally averaged. C I (247.856 nm) emission line was used as internal standard in the data processing process and, thus, the presented signals in Figures S8a and S8b correspond to the ratio of the integrated intensity of the analyte line to the integrated intensity of the internal standard. Carbon line normalization of the emission signals was used in order to compensate signal fluctuations, from sample to sample, due to small variations in the focusing conditions when different replicate samples were positioned in µLIBS system. In all cases, three independent replicate samples were analysed, with each replicate sample encompassing the whole process from film generation to LIBS measurement of the enriched film. Error bars in the presented Figures S8a and S8b correspond to ± one standard deviation.
For optimization of the adsorbent amount, MD films containing GO amounts ranging from 0.5 mg to 1 mg were prepared. Preparation of the films was carried out following the procedure already described in Section 2.3.1. above, by appropriately increasing the volume of the 2.5 mg mL\(^{-1}\) graphene oxide dispersion deposited on the glass substrate. The prepared films were used for extraction of the analytes from the test samples following the procedure described in the previous Section 2.3.2. The extraction time was fixed in 20 min. Figure S8a shows the results obtained in this study. As observed, signal intensity increased with adsorbent amount up to a value of 0.8 mg. A further increase in adsorbent amount did not produce a significant increment in adsorbed analytes leading to higher LIBS signal and, therefore, 0.8 mg of graphene oxide in the MD films was selected as the optimum.

Optimization of the extraction time was evaluated within the time interval from 3 to 40 minutes, with MD films containing 0.8 mg of GO. Result of this analysis is shown in Figure S8b. Adsorption of analytes was observed to continuously increase with extraction time. However, a negligible LIBS signal increment was observed when increasing the extraction time from 30 to 40 minutes. In the case of Cu and Pb, LIBS signal variation was even within the uncertainty interval due to measurement repeatability. For this reason, extending this optimization study to extraction times longer than 40 minutes was not considered, in order to minimize as much as possible the total analysis time of the TFME-LIBS procedure. Therefore, 40 minutes was selected as optimum extraction time.

In summary, the extraction conditions finally chosen after all the previously mentioned studies were: 0.8 mg of GO in extraction films prepared by the MD approach, extraction of analytes from 20 g of sample, sample pH adjusted to 5 and 40 minutes of extraction time.

### 3.3 Analytical figures of merit

With the selected film generation method and optimized TFME conditions, the quantitative capability of the proposed TFME-LIBS method was tested by using, in the detection step, the conventional single-pulse LIBS instrument described in Section 2.1.1. To this end, analytical figures of merit, such as sensitivity, limit of detection (LOD), limit of quantification (LOQ) and method trueness, were evaluated.

In these studies, LIBS analysis was carried out by averaging the spectra obtained from 25 laser shots on the surface of the analyte-enriched GO adsorbent. Due to the larger laser spots generated with the conventional LIBS system, in comparison to the previously used µLIBS (i.e., ~250 µm instead of ~ 100 µm), the distance between adjacent laser firing positions was incremented to 2 mm. This increased distance avoided possible interferences due to deposition of ablated material from nearby irradiated positions. With this setting, 25
Laser shots were enough to cover the approximately 100 mm$^2$ adsorbent area avoiding laser shots on the edges of the adsorbent. With the conventional LIBS system, tiny adjustment of the focusing conditions from sample to sample was not critical, and the use of C I (247.856 nm) emission line as internal standard was not observed to provide any substantial advantage over the processing of uncorrected emission signals. For this reason, integrated emission intensity of the different analyte lines were considered in the quantitative studies.

In order to evaluate the different analytical figures of merit, calibration plots were generated from triplicate analysis of seven standard solutions having analyte concentrations ranging from 0.03 to 0.5 mg kg$^{-1}$. Sensitivity was evaluated from the slope of the obtained calibration graphs, which are presented in Figure S9. LOD and LOQ were calculated following the 3$\sigma$ and 10$\sigma$ IUPAC recommendation, respectively, with $\sigma$ the standard deviation of six replicate analysis of the less concentrated standard (i.e., 0.03 mg kg$^{-1}$). Method trueness was evaluated from addition and recovery experiments performed on a real sample of mineral water (Recoaro, Mineral water, Italy). As observed from the calibration plots in Figure S9, Emission signal of all analytes showed a linear behaviour in the concentration interval evaluated, with $R^2$ in the range 0.9803 - 0.9909. Signal repeatability in the analysis of the different standards ranged from 1% up to 25% RSD. It was found to be worst for Ni and Pb, with mean % RSD values of 12 % and 14 %, respectively, and considerably better for those analytes presenting higher analytical sensitivity (i.e., 5 % and 9 % mean % RSD for Cu and Cr, respectively). As can be observed from the results presented in Table 1, the obtained LODs and LOQs were at the µg kg$^{-1}$ level for all the tested elements. LODs ranged from 41 µg kg$^{-1}$ for Cu to 52 µg kg$^{-1}$ for Ni, whereas LOQs ranged from 136 µg kg$^{-1}$ to 173 µg kg$^{-1}$ for the same two elements.

For evaluation of the TFME-LIBS method trueness, the Recoaro$^\circledR$ mineral water sample was firstly analysed by ICP OES in order to estimate the concentration level of the target analytes in the sample. This concentration level was found to be below the limit of detection of the ICP OES method for all target elements. After this preliminary study, the sample was spiked with 0.2 mg kg$^{-1}$ of the different analytes. Then, un-spiked and spiked samples were analysed in triplicate by TFME-LIBS in order to estimate the recovery for the different metals. Results of a first trueness evaluation led to unsatisfactory recoveries, with values ranging between 66% and 75% (see Table 1). The reason for these low recoveries was hypothesized to be due to the existence of competitive adsorption in the microextraction step. Competitive processes are typical in Solid Phase Microextraction (SPME) procedures, in which a solid adsorbent with only a limited surface area is available for adsorption [41-44]. The presence of other cations, such as Ca$^{2+}$, Mg$^{2+}$, and K$^+$ among others, at mg kg$^{-1}$
Conclusions

In this work, TFME procedure with the use of graphene oxide as adsorbent material has been evaluated as sample preparation method for LIBS analysis of aqueous samples. According to the presented results, the experimental procedure for preparation of the adsorbent films to be used in the extraction process has a strong influence on the subsequent LIBS analysis of the adsorbed analytes. On the one hand, the procedure used for deposition of the adsorbent material on a solid substrate can affect the homogeneity of the generated adsorbent layer and, in consequence, the distribution of the extracted analytes over the adsorbent material and the precision of the obtained LIBS signal. On the other hand, the solid substrate selected for deposition of the adsorbent material can influence LIBS analysis of the analyte-enriched adsorbent, due to possible spectral interferences from the substrate material. The Drop Casting Deposition (DCD) method used in this work for generating adsorbent films can be probably considered one of the experimentally simplest approaches, with a capability for generating homogeneous adsorbent layers that is highly dependent on the experimental procedure used. As demonstrated in this work, the Mould Deposition (MD) experimental procedure, consisting in a slight experimental modification of the DCD approach, can led to a considerable improvement in the quality of the generated adsorbent layers.

With the use of adsorbent films produced by the MD experimental procedure, the proposed TFME-LIBS method was demonstrated to be suitable for the detection of several analytes in water samples at the low μg kg\(^{-1}\) concentration level. The developed method, however, was observed to suffer from matrix effects when applied to the analysis of a real water sample, probably due to saturation of the adsorbent material by concomitant cations. This result pointed out the necessity of further studies aimed to improve the method...
trueness, in order to ensure its applicability to real samples analysis without the need of a previous sample dilution. Among others, a more adequate adjustment of the adsorbent amount used in the preparation of the films, or the use of a more selective adsorbent material (e.g., functionalized GO), can be possible solutions to the problem.

Despite the difficulties encountered in this preliminary evaluation of the TFME-LIBS method, it can be said, as a general conclusion, that the proposed method could provide a substantial advance toward the development of an automatic analytical system useful for online analysis of liquid samples. On the one hand, TFME procedure is easy to automate, as well as the combination of the two independent processes of TFME and LIBS detection. On the other hand, the presented results could be further improved by using new ways of thin films preparation, in order to improve the homogeneity of the adsorbent layer and/or increase the interfacial area for adsorption, or by selecting new adsorbents from the wide diversity of existing materials.

Conflicts of interest

There are no conflicts of interest to declare.

Acknowledgements

This work was supported by the Spanish Ministry of Economy and Competitiveness [CTQ2016-79991-R], the Regional Government of Valencia (Spain) [PROMETEO/2013/038 and PROMETEO/2018/087]; and the University of Alicante [UAUSTI17-04 and UAUSTI18-04]. L. Ripoll is grateful to the Ministry of Economy and Competitiveness for her PhD fellowship (FPI-MICINN (BES-2012-058759)).
References


## Tables

**Table 1**: Analytical figures of merit of the TFME-LIBS method

<table>
<thead>
<tr>
<th>Emission Line (nm)</th>
<th>(^a) Sensitivity (cts·kg mg(^{-1}))</th>
<th>(^b) LOD (µg kg(^{-1}))</th>
<th>(^b) LOQ (µg kg(^{-1}))</th>
<th>Analyte concentration in bottled water (mg kg(^{-1}))</th>
<th>Recovery (%)</th>
<th>Analyte concentration in bottled water (mg kg(^{-1}))</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ni I (352.454)</td>
<td>910 ± 60</td>
<td>52</td>
<td>173</td>
<td>0.202</td>
<td>66</td>
<td>0.2009</td>
<td>100</td>
</tr>
<tr>
<td>Pb I (368.356)</td>
<td>830 ± 40</td>
<td>47</td>
<td>157</td>
<td>0.201</td>
<td>66</td>
<td>0.2012</td>
<td>102</td>
</tr>
<tr>
<td>Cr I (359.350)</td>
<td>1330 ± 80</td>
<td>48</td>
<td>161</td>
<td>0.201</td>
<td>75</td>
<td>0.2009</td>
<td>100</td>
</tr>
<tr>
<td>Cu I (327.396)</td>
<td>1270 ± 50</td>
<td>41</td>
<td>136</td>
<td>0.202</td>
<td>74</td>
<td>0.2007</td>
<td>108</td>
</tr>
</tbody>
</table>

\(^a\) Slope of the calibration plot

\(^b\) Calculated as \(\text{LOD} = 3\sigma/m\) and \(\text{LOQ} = 10\ 3\sigma/m\), with \(m\) the slope of the calibration plot and \(\sigma\) the standard deviation of 6 replicate analysis of the most diluted standard (0.03 mg kg\(^{-1}\)).

\(^c\) Concentration found using three independent replicate measurements. Reported uncertainty correspond to repeatability, expressed as one standard deviation (i.e., 68% confidence level).
Figure Captions

Figure 1. Experimental procedure for generation of GO films using (a) drop casting deposition and (b) mould deposition.

Figure 2. General experimental scheme of TFME procedure and LIBS analysis.

Figure 3. LIBS analysis of Ni I (352.452 nm) emission intensity over the surface of a graphene oxide layer obtained by the DCD experimental procedure.

Figure 4. μ-XRF mappings along the scanned sample surface on a DCD film: a) sample scanned area, b) nickel, c) chromium, d) copper and e) aluminium.

Figure 5. LIBS analysis of Ni I (352.452 nm) emission intensity over the surface of a graphene oxide layer obtained by the MD experimental procedure.

Figure 6. μ-FRX mappings along the scanned sample surface on a MD film: a) sample scanned area, b) nickel, c) chromium, d) copper and e) silicon.
(a) 10 min sonication of 50 mg graphene oxide (GO) in 10 mL of water. Ceramic/Glass substrate GO suspension (5 mg/mL) in water. Binder gel Drop casting deposition. Mould deposition 160 µL of EtOH Microdroplet deposition: addition of 50-60 µL each time until a final volume of 160 µL Heating system at 100°C

(b) 10 min sonication of 160 µL of GO water suspension (5 mg/mL) and 320 µL of GO water/EtOH suspension (~2.5 mg/mL). Heating system at 70°C Microdroplet deposition: addition of 40 µL each time until a final volume of 320 µL 800 µg of adsorbent on the glass substrates

Ceramic substrate

Aqueous solution of carboximethyl cellulose (2%) 800 µg of adsorbent on the ceramic substrates

Binder coating
Conditioning (pH = 5)
Final sample weight (25 g)

Microextraction step (40 min)

Stirrer

GO layer

on ceramic substrate

on glass substrate

Drying (20-30 s)

LIBS analysis of the analyte-enriched adsorbent
Gross Ni I (352.45 nm): 23% RSD
Net Ni I (352.45 nm): 24% RSD

Ni I (352.45 nm) line

Signal precision (% RSD) evaluated from the 50 laser shots in the rows

Gross Ni I (352.45 nm): 29% RSD
Net Ni I (352.45 nm): 34% RSD

Gross Ni I (352.45 nm): 26% RSD
Net Ni I (352.45 nm): 30% RSD

Gross Ni I (352.45 nm): 25% RSD
Net Ni I (352.45 nm): 30% RSD

Gross Ni I (352.45 nm): 26% RSD
Net Ni I (352.45 nm): 29% RSD
Emission from the substrate

Intensity

(a) Sample image

(b) Ni mapping

(c) Cr mapping

(d) Cu mapping

Emission from adsorbed analytes

Intensity

(e) Al mapping

Emission from the substrate
(a) Laser-induced crater

(b) Ni I (352.45 nm) signal in rows 1 to 50

(c) 3D view in x positions from 7 to 19

(d) Signal precision (% RSD) evaluated from the 50 laser shots in the rows

• Gross Ni I (352.45 nm): 11% RSD
• Net Ni I (352.45 nm): 12% RSD

• Gross Ni I (352.45 nm): 13% RSD
• Net Ni I (352.45 nm): 11% RSD

• Gross Ni I (352.45 nm): 12% RSD
• Net Ni I (352.45 nm): 14% RSD

• Gross Ni I (352.45 nm): 16% RSD
• Net Ni I (352.45 nm): 18% RSD

• Gross Ni I (352.45 nm): 14% RSD
• Net Ni I (352.45 nm): 56% RSD

GO layer

Spectra numbering

Laser shots

Y Position 1

Y Position 2

Y Position 3

Y Position 4

Y Position 5

X Position

Ni I (352.45 nm) line

Y Position 3
(a) Sample image  (b) Ni mapping  (c) Cr mapping  (d) Cu mapping

(e) Si mapping

Emission from adsorbed analytes

Emission from the substrate
Highlights

- TFME-LIBS, using graphene oxide as adsorbent, has been evaluated for the first time
- Thin Film microextraction is an easily automated procedure reducing the sample handling.
- Several metals in liquid samples has been detected at µg kg⁻¹ level by LIBS
- Metal chelation step has been avoided with the use of graphene oxide
Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

There are no financial interests/personal relationships which may be considered as potential competing interests