TRACE ELEMENT DISTRIBUTION MAPPING IN PHARMACEUTICAL AND BIOLOGICAL SAMPLES USING LASER-INDUCED BREAKDOWN SPECTROSCOPY

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Introduction

Laser-induced breakdown spectroscopy (LIBS) has numerous advantages like determination of ppb-ppm level concentrations, microdestructivity, the ability of making high resolution laterally (μm) or depth-resolved (100 nm) analysis, sensitive measurement of heavy and light elements and direct analysis of solid samples with minimal sample preparation. The aim of the present work was to demonstrate that LIBS is a suitable analytical technique to assess the trace element distribution of pharmaceutical and biological samples. As test samples, we used specimens of chickpea milkvetch **(***Astragalus cicer*), a perennial plant with excellent nutritional value and a commercially available dietary supplement pill (Supradyn).

Experimental

The LIBS spectra were taken by using the J200 LA-LIBS tandem spectrometer (Applied Spectra Inc., USA) with the following settings: laser pulse energy 14 mJ, spot size 60 μm, integration time 1.05 ms, gate delay 0.5 μs, repetition rate 10 Hz. The laser parameters were set to collect data from the whole sample surface with no overlapping measurement points, and from each location, only one spectrum were recorded.

The only sample preparation in the case of pill was cutting it in half with a sharp scalpel and embedding it in bluetech glue in order to fix them in the sample holder. Seeds of *Astragalus cicer* were surface sterilized with 20% (v/v) sodium hypochlorite for 20 min, and washed with sterile distilled water four times in 20 min. Seeds were dried on a sterile metal filter and we polished them one by one using P-400 sanding paper in order to scratch the external seed coat. Seeds were placed on agar medium (the scratched surface of the seeds contacted the medium). Plastic, square Petri dishes contained half-strength Murashige and Skoog medium (0.8% (v/v) agar, 1% sucrose). Samples were grown under controlled conditions (12 h/12 h light/dark cycle, relative humidity 55–60% and temperature 25 ± 2°C) for 14 days. When the plants grew big enough, the plantlets were carefully removed with tweezers and their roots were rinsed with deionized water to wash away the remaining of the medium in which they were cultivated. Plantlets were then pressed, dried and mounted on a glass microscope slide with double-sided foam tape.

Results and discussion

In Figure 1., the elemental distribution maps of five trace elements (Ca, Mg, Cu, Mo and Zn), are presented in the pill. On the elemental distribution maps, the colour scale ranges from blue to red, where blue indicates low intensity and red indicates high intensity. As it ought to be, the

images testify that all of the elemenst are distributed in a more or less homogenous way troughout the pill. Based on the certificate of the manufacturer, Ca and Mg are present in the pill at the highest concentration (51.3 and 21.2 mg per pill), whereas Cu, Mo and Zn are present at least an order of magnitude lower concentration. This also reflects in the intensities of the elements in the maps, however please note that there are significant differences between the sensitivity of the spectral lines used for plotting as well. Since the pills are made by mixing and pressing together powdered chemicals containing these elements (and others), whether we can detect an element or not also depends on the size of the grains. Interestingly, Cu and Zn appears to be present in a significant amount in the coating of the pill as well, as opposed to the other elements which occur only in the main body of the pill. Ca, Mg and Mo seems to be present in larger grains than the other two elements. These information can help the formulation and quality control of the manufacturing of such pills.

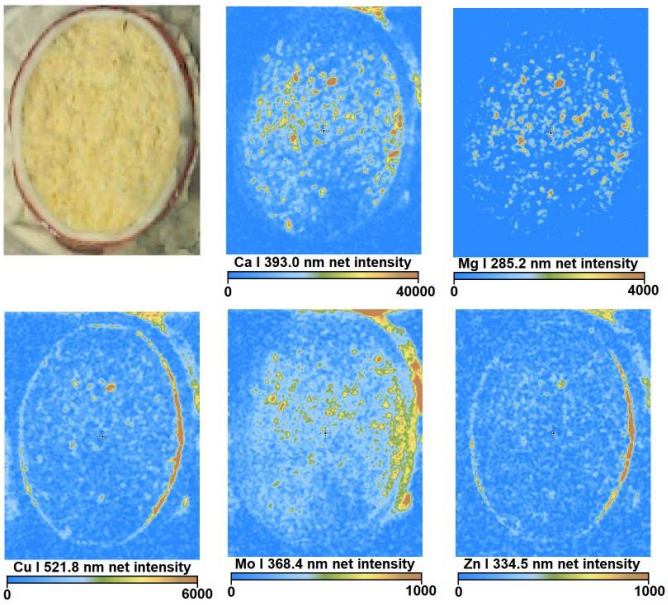

Figure 1. Optical microscopy image (top left corner) and LIBS elemental distribution maps of some of the trace elements in a multivitamin dietary supplement pill.

On the elemental distribution maps, presented in Figure 2., the distribution of four selected essential micro Fe, Mn and macro K, Mg nutritious elements can be seen in *Astragalus cicer* seedlings. The macroelements are presented in the plants in the range of mg/g concentration whereas the

microelements are in the range of µg/g. It appears that the distribution of these elements is nearly homogenous throughout the whole plant except in the case of Mn. Manganese is mainly enriched in the roots and to some extent also in the lower part of the stem. The cotyledons do not show significant manganese concentration. Mn is presented in a lowest concentration in the plants thus it has the lowest intensities from these four elements. K is an important macroelement involved in osmoregulation that therefore has the highest concentration therefore it is represented with the highest intensity. Similarly, the macroelement Mg being a structural element in chlorophyll pigments and involved in biochemical processes show consistent distribution in the seedlings. Regarding Fe, a similarly equable distribution could be observed reflecting the general necessity of this microelement in plant organs. The distribution of all of the four selected elements shows a common feature: higher concentrations were recorded for the center (inner part) of the root and the cotyledons and they were found to decrease towards the edges. It is clearly visible that these elements are more concentrated in the root than in the cotyledon. Each map has a well-illustrated shape of the root of the plant. With this non invasive and in situ technique we can get extra information from the distribution of the elemental content

of the plants with minimal sample preparation compared to classical ICP-MS measurements where complicated sample preparation when digestion of the sample is a must.

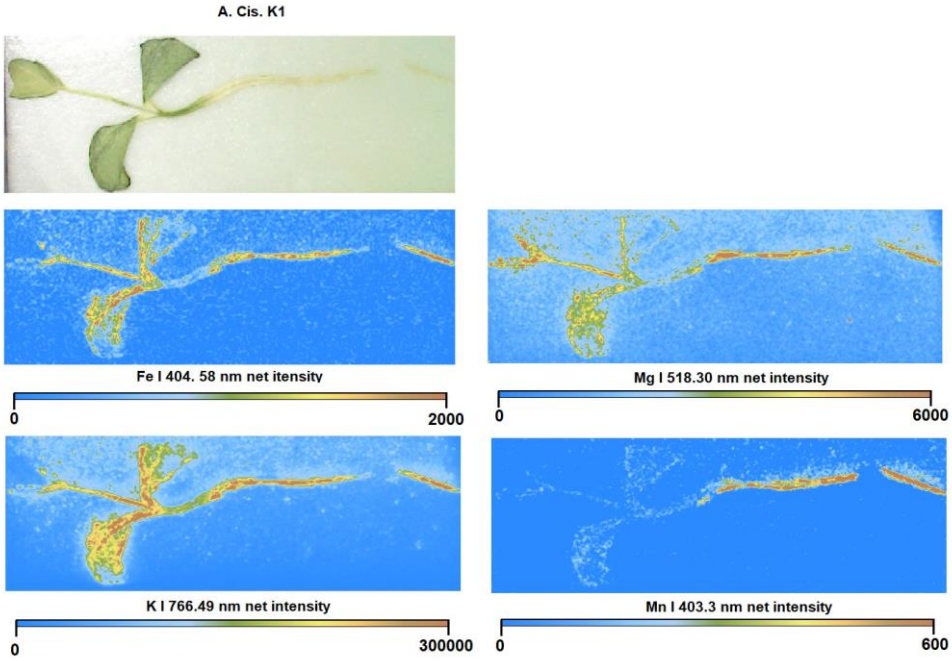

Figure 2. Optical microscopy image (top) and LIBS elemental distribution maps of the plant sample

Acknowledgements

The financial support from the National Research, Development and Innovation Office of Hungary via projects No. EFOP-3.6.2-16-2017-00005 and K135303, K129063 are kindly acknowledged.