Study of Spleen Elemental Composition Using CF-LIBS

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Abstract. The aim of this work was to determine iron concentration as the trace element in spleen samples and relation of increased iron concentration to certain splenic diseases. We examined nine spleen samples—three reference samples were obtained from patients who underwent the splenectomy because of rupture or other mechanical damage to spleen with a risk of bleeding to death, another three samples were from patients with hereditary spherocytosis (splenectomy is inevitable) and the last three samples were obtained from patients with autoimmune thrombocytopenia (splenectomy is inevitable). The CF-LIBS method was used for elemental quantitative evaluation. We managed to prove increased iron concentration in the samples from patients with hereditary spherocytosis in comparison to reference samples. However, it does not apply to the samples from patients with autoimmune thrombocytopenia. The method could be used in diagnostics for detection of trace amounts of some elements in tissues after specifying certain conditions for measurements and for sample structure.

Introduction

Laser-induced breakdown spectroscopy (LIBS) is an analytical method used for qualitative as well as quantitative analysis of samples of all states of matter (solids, liquids, gases). It uses a source of highly powerful laser pulse. With a system of optical components, this pulse is transmitted and focused on the surface of a sample. The sample atomizes due to the effect of the pulse and forms neutral atoms and ions. Plasma with an electron temperature of 10 000–20 000 K is generated at the focusing point of the laser beam on the surface of the sample. It causes sample material evaporation and sublimation up to complete dissociation to atoms, following by their ionization and excitation. They emit photons of characteristic wavelength and energy. Every element has a characteristic emission spectrum. Emission spectra are collected and transmitted to spectrometer by a lens and an optical fibre. Spectral lines are identified by NIST database [Ralchenko et al., 2007]. The method has many advantages over other methods of atomic emission spectroscopy, such as simplicity and rapidity of analysis or no sample preparation. Concerning biological samples, it has been used to examine hard tissues [Samek et al., 2000; Samek et al., 2001; Abdel-Salam et al., 2007] as well as soft tissues [Yueh et al., 2009].

In this work, we focused on application of this method as an analytical tool in medicine. We examined the elemental composition of human spleen samples putting emphasis on iron concentration and its relation to certain splenic diseases. Calibration-free LIBS (CF-LIBS) is a standardless method developed for quantitative analysis [Ciucci et al., 1999] which uses spectral data and plasma parameters to determine concentrations of the elements in a sample. The technique was well reviewed by Aguilera and Aragón [2007].

Experimental setup

In our experiment we have used the frequency-doubled Nd:YAG laser (QUANTEL – Brilliant EaZy, maximal energy 165 mJ in the pulse, pulse duration 4 ns, wavelength 532 nm). Output beam was deviated by the optical prism (Thorlabs, BK7) and focused using the lens (Thorlabs, focal length 4 cm) on the sample surface. The size of the focal spot on the sample is approximately 0,1 mm. Emitted light of the ignited spark was collected with the lens (Thorlabs, focal length 8 cm) into the optical fiber
connected to the echelle type spectrometer (Andor, Mechelle 5000) equipped with an intensified iCCD camera (Andor, iStar DH734i-18F-03). This spectrometer allowed a wide spectral range coverage (200–950 nm) with a relatively high resolution (Dl/l=5000) and a short focal length (178 mm). The mentioned experimental setup is showed in Figure 1. The wavelength calibration was realized using of HG-1 calibration lamp (Ocean Optics) which is argon-mercury low-pressure discharge lamp. All measured spectra were corrected according to the spectral response curve of the spectrometer which was acquired by the use of NO gamma molecular system, N2 second positive molecular system, deuterium and tungsten lamp [Rakovský et al., 2011]. In order to decrease the signal noise the iCCD camera was cooled down to –15°C. The parameters of exposure window we used for our measurements were gate delay 2 μs, gate width 5 μs, gain level 100.

**Samples**

We examined human spleen samples from 9 different patients. 3 samples were reference, 3 samples came from patients with diagnosed hereditary spherocytosis (HS) and 3 samples came from patients with diagnosed autoimmune thrombocytopenia (AITP). All samples were prepared on silicon slides using standard histological sample preparation. HS is a disease manifested by increased elimination of erythrocytes in spleen, thus the iron concentration in spleen should be also increased. AITP manifests by increased elimination of thrombocytes in spleen and could be joined with increased elimination of erythrocytes. Both diseases are hemolytic anemias.

**Results**

We examined 9 spleen samples. We identified spectral lines of C, O, H, N, Si, Na, Ca, Mg, Fe in spectra. The spectral lines of Na, Ca, Mg, Fe were used for the CF-LIBS evaluation. The spectral lines of O, H, N were not considered because our measurements were carried out in the air, thus there is a contribution of these elements from the ambient air and also we did not use spectral lines of Si because those represent contribution from the silicon slides. Spectral line of carbon was not used because of the self-absorption of the line. We compared the ratio of Fe/Mg concentration to determine proportional representation of Fe in the samples.

We made three accumulations of ten laser shots for each sample except the sample no.3 (AITP) because of the small surface and thickness of the sample. Each laser shot was focused to different spot on the sample.

Figure 2 shows the comparison of the Fe/Mg ratio in the samples and it could be seen an increased concentration of iron in the HS samples. Figures 3 and 4 show examples of spectra of all 3 types of spleen samples containing spectral lines of iron and magnesium.
Figure 2. Comparison of Fe/Mg concentration ratio in the samples (AITP samples—red, HS samples—green, reference samples—blue).

Figure 3. Comparison of the samples in the part of spectrum containing spectral lines of iron.

Discussion

The aim of this work was to determine iron concentration as the minor element in spleen samples and relation of increased iron concentration to certain splenic diseases. It is required to consider all elements present in a sample to determine their exact concentrations using CF-LIBS for evaluation. As we mentioned above, the spectral lines of O, H, N were not considered because of the contribution of the ambient air and the spectral line of C because of the self-absorption. Therefore, we compared the ratio of Fe/Mg concentration among the samples and with the work of Yoshinaga et al. [1990]. They determined concentrations of various elements in human spleen samples using ICP-MS (Inductively Coupled Plasma–Mass Spectrometry) and the concentration of Fe was 272 μg/g and the concentration of Mg was 163 μg/g (the Fe/Mg ratio = 1.67). They did not determine the concentration of Ca so we used the Fe/Mg ratio. In this work, we determined the Fe/Mg ratio 3.8 ± 1.2 for AITP samples, 4.5 ± 1.4 for reference samples and 12.1 ± 1.6 for HS samples. It shows us that the Fe concentration is higher in HS samples than in any other.
Conclusion

In this work we managed to prove increased iron concentration in HS samples in comparison to reference samples. However, it does not apply to the samples from patients with AITP. We have to perform measurements in the vacuum using more samples to further extend our results. The method could be used in diagnostics for detection of minor and trace amounts of various elements in tissues after specifying certain conditions for measurements and for sample structure.

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References


