Laser-induced breakdown spectroscopy of $\gamma$-Fe$_2$O$_3$ nanoparticles in a biocompatible alginate matrix

E. Brown, S.J. Rehse *

Department of Physics and Astronomy, Wayne State University, 666 W. Hancock, Detroit, MI 48201, United States

Received 21 November 2006; accepted 12 October 2007
Available online 23 October 2007

Abstract

An intensive multi-disciplinary research effort is underway at Wayne State University to synthesize and characterize magnetic nanoparticles in a biocompatible matrix for biomedical applications. The particular system being studied consists of 3–10 nm $\gamma$-Fe$_2$O$_3$ nanoparticles in an alginate matrix, which is being studied for applications in targeted drug delivery, as a magnetic-resonance imaging (MRI) contrast agent, and for hyperthermic treatments of malignant tumors. In the present work we report on our efforts to determine if laser-induced breakdown spectroscopy (LIBS) can offer a more accurate and substantially faster determination of iron content in such nanoparticle-containing materials than competing technologies such as inductively-coupled plasma (ICP). Standardized samples of $\alpha$-Fe$_2$O$_3$ nanoparticles (5–25 nm diameter) and silver micropowder (2–3.5 $\mu$m diameter) were created with thirteen precisely known concentrations and pressed hydraulically to create solid “pellets” for LIBS analysis. The ratio of the intensity of an Fe(I) emission line at 371.994 nm to that of an Ag(I) line at 328.069 nm was used to create a calibration curve exhibiting an exponential dependence on Fe mass fraction. Using this curve, an “unknown” $\gamma$-Fe$_2$O$_3$/alginate/silver pellet was tested, leading to a measurement of the mass fraction of Fe in the nanoparticle/alginate matrix of 51±3 wt.%, which is in very good agreement with expectations and previous determinations of its iron concentration.

© 2007 Elsevier B.V. All rights reserved.

Keywords: Laser-induced breakdown spectroscopy; LIBS; Iron-oxide nanoparticles; Alginate matrix; Inductively-coupled plasma spectroscopy

1. Introduction

There is a growing interest in the use of magnetic nanosized particles in biomedical applications. In particular iron-oxide nanoparticles have been used as magnetic-resonance imaging contrast agents [1,2], for targeted drug delivery [3], to deliver and concentrate drug molecules, or to tag, capture, isolate or immobilize bio-materials such as antibodies and DNA [4,5]. Thorough reviews of the biomedical uses of such magnetic nanoparticles may be found elsewhere [6,7]. Quite recently, the fabrication and magnetic characterization of iron-oxide ($\gamma$-Fe$_2$O$_3$) nanoparticles precipitated inside alginate hydrogels has been reported [8]. These magnetic alginate hydrogels are polysaccharides (a naturally occurring carbohydrate-based polymer) and are thus biocompatible and hydrophilic – important materials properties in medicine and biomedical engineering – and their magnetic properties may be optimized or tailored depending on their Fe concentration.

Typically such biocompatible magnetic hydrogels are prepared through a wet-chemical technique with the iron-oxide nanoparticles formed in situ at cross-linking sites of the polysaccharide alginate matrix. This method of preparation allows for a wide range of Fe concentrations in the resulting hydrogel (10–50 wt.% of Fe) after it has been dehydrated for magnetic testing. Because of this high-degree of variability, an accurate assessment of the actual Fe content must be performed on each newly created batch of hydrogel to make the magnetic characterization measurements meaningful. The elemental composition has typically been determined by inductively-
coupled plasma (ICP) spectroscopy performed in a commercial analysis laboratory. Unfortunately, this testing could not be done on-site, was expensive, and had in the past yielded inconsistent results on identical samples obtained from the same batch (G. Lawes, private communication).

The ability to quickly and accurately determine the elemental concentration of the magnetic component of this new class of biocompatible hydrogels would be a useful tool for those scientists and engineers interested in this emerging nanoparticle field. These nanoparticles are relatively straightforward to synthesize, but usually very difficult to characterize and quantify. In addition, the flexibility of the LIBS analysis technique would be useful as additional elements (such as cobalt) are added to these hydrogels to tailor their magnetic properties.

The objective of this study was to determine if laser-induced breakdown spectroscopy (LIBS) could be utilized to replace ICP for the rapid, accurate “in-house” determination of absolute iron concentration in such dehydrated magnetic hydrogels. To this end, calibrated “standard” samples of various known Fe concentrations were created for the construction of a calibration curve that related the intensity of Fe emission in a LIBS plasma to the absolute wt.% of Fe in the sample. The Fe emission from a nanoparticle-containing alginate hydrogel was then measured utilizing the same LIBS analysis and the absolute Fe concentration of the magnetic hydrogel was determined. Lastly, the results of our measurement were compared to an independent measurement made with ICP spectroscopy at an outside laboratory.

2. Experimental

2.1. Preparation of alginate hydrogels and standard samples

The precipitation of the iron-oxide nanoparticles in the biocompatible hydrogels was accomplished by following the method outlined by Kroll et al. [9] and is discussed in detail elsewhere [8]. This precipitation was accomplished by cross-linking sodium alginate with Fe$_{2+}$ and Fe$_{3+}$ in a methanol–water solution. The cross-linked alginites were then oxidized at room temperatures under alkaline conditions during which the ions of Fe$_{2+}$ or Fe$_{3+}$ formed γ-Fe$_2$O$_3$. Multiple exposures (“loadings”) of the hydrogel to the Fe$_{2+}$ solution increased the size and concentration of the iron-oxide nanoparticles, resulting in Fe$_2$O$_3$/Fe$_3$O$_4$ nanoparticles with spherical diameters ranging from 3–10 nm. A histogram of measured particle sizes showed a symmetric distribution, with a mean of 5.5 nm.

The most serious impediment to the construction of an Fe concentration curve for such samples was the inability to obtain “standard” hydrogels with known Fe concentration. As described above, the chemical fabrication procedure yielded highly inconsistent iron concentrations and only a small volume of externally-calibrated material was made available to us for LIBS testing. Additionally, the biocompatible alginate hydrogel was never fabricated without Fe, so it was not possible to construct an appropriate set of standards by custom-doping the alginate matrix with known quantities of Fe$_2$O$_3$. We were therefore presented with the challenge of confirming a single external measurement on a small volume of material without significant access to the original source materials. Nonetheless, it was decided to fabricate a set of standards utilizing nanoparticle Fe$_2$O$_3$ as a proof-of-concept experiment to determine if additional work on the construction of more realistic standard samples was warranted. Within these limitations, it was decided to prepare the standard samples utilizing commercially available α-Fe$_2$O$_3$ powder rather than γ-Fe$_2$O$_3$, α-Fe$_3$O$_4$ (hematite) and γ-Fe$_3$O$_4$ (magnetite) are different crystal structural forms of the same compound, Fe$_2$O$_3$ (iron-oxide) [10]. The α-Fe$_2$O$_3$ powder was less expensive and was easier to obtain than the γ-Fe$_2$O$_3$ and the ratio of Fe to O was the same for both structural forms. As mentioned above, it was not always completely known whether the structural form of Fe in the cross-linked hydrogels was Fe$_2$O$_3$, Fe$_3$O$_4$, or a mixture of the two. Powder X-ray diffraction patterns showed broad peaks corresponding to either Fe$_2$O$_3$, Fe$_3$O$_4$, or both, but the orange–red color of the hydrogels suggested they were predominantly γ-Fe$_2$O$_3$. It was therefore decided that the particular structural form of Fe$_2$O$_3$ used in the construction of the standards (gamma or alpha) was not nearly as important as the overall mass fraction, which was identical for the two.

The standard samples to be tested via LIBS needed to be similar to the hydrogel samples that were produced for the determination of their magnetic properties. Specifically, the magnetic properties were characterized in a superconducting quantum interference device (SQUID) at temperatures from room temperature to 5 K [11]. To fit in the cryogenic SQUID, the hydrogel samples were typically dehydrated, powdered, and mixed with Ag powder prior to pelletization. The Ag provided a robust non-magnetic matrix to support the nanoparticle-containing alginate hydrogel. It was therefore decided to also perform LIBS on pelletized mixtures of Ag powder and Fe$_2$O$_3$ powder for mechanical convenience and to most closely emulate the hydrogel samples used in those previous experiments.

The presence of the Ag binder also provided convenient reference lines in the LIBS spectrum which were used to normalize the intensity of the Fe emission lines. The presence of silver and the absence of the alginate hydrogel in the sample introduced the problem of the “matrix effect” which has been addressed in many publications, particularly in regards to powdered (i.e. soil, sand, or concrete) samples, heterogeneity effects, and efforts toward effective normalization [12–16]. As observed by Gornushkin et al. [17], the accuracy of LIBS measurements can, in general, be expected to degrade when calibrations are not matrix-specific. We have therefore limited our calibration to standard samples which resemble the alginate hydrogel samples as closely as possible within the constraints outlined above.

Thirteen calibrated “standard” samples were prepared by combining precisely measured masses of research-grade α-Fe$_2$O$_3$ nanopowder (544884, Aldrich) and Ag powder (327085, Aldrich). The composition of the thirteen samples is shown in Table 1. Standard samples possessing a wide range of Fe mass fractions were prepared to completely straddle the anticipated Fe mass fraction of the γ-Fe$_2$O$_3$/alginate mixture which was expected to vary anywhere from 5% to 50%. The α-Fe$_2$O$_3$ nanopowder had an average particle size of 5–25 nm and the Ag powder had an average particle size of 2–3.5 μm. The powders
were combined and mechanically agitated by hand for up to one-half hour. The mechanical action was not intended to alter the size of the particulates (as crushing with a mortar and pestle would do) but merely to minimize the heterogeneity of the sample. After mechanical agitation, the samples were placed in sealed glass vials and agitated in an ultrasonic water-bath to further uniformly distribute the powders.

The powder mixtures were compressed in a hardened machine-steel dye by a 20 ton hydraulic press. The resulting pellets were circular with a 5 mm diameter, 200 μm thickness, and a total mass of ~ 60 mg. The samples with high Ag mass fraction (samples 1, 2, etc.) were very robust and exhibited excellent cohesion due to the malleability of the Ag. The samples could be handled repeatedly with no effect. The samples with the highest Fe mass fraction, however, (samples 13, 12, etc.) tended to resist pelletization and remained very brittle and prone to fracturing or crumbling upon removal from the dye or transfer to the ablation sample holder. The presence of the Ag binder (or some form of binder) was therefore crucial to the creation of the ablation sample holder. The presence of the Ag binder (or some form of binder) was therefore crucial to the creation of the ablation sample holder. The presence of the Ag binder was therefore crucial to the creation of the ablation sample holder. The presence of the Ag binder was therefore crucial to the creation of the ablation sample holder. The presence of the Ag binder was therefore crucial to the creation of the ablation sample holder.

The pelletized standard samples were placed on a magnetic sample holder composed of a flat 2 cm² sheet of tantalum metal adhered to a permanent magnet with double-sided tape. All the standard samples were sufficiently magnetic to experience a substantial attraction to the permanent magnet, effectively immobilizing them on the surface of the tantalum. The absence of clips holding the sample in place allowed the entire surface area of the sample to be accessed with the ablation laser and ensured a high-degree of sample flatness in the focal region of the laser-focusing microscope objective. No motion of the sample pellet on the surface of the tantalum sheet was observed as a result of laser ablation or sample translation. The tantalum sheet was utilized to prevent any possible contamination of the sample’s spectrum from the iron-oxide magnet. A sample after laser ablation is shown on this holder in Fig. 1.

The experimental apparatus used for LIBS in all the experiments is shown schematically in Fig. 2. Fig. 2(a) shows an overhead view of the apparatus. A Q-switched Nd:YAG laser (Spectra-Physics LAB-150) operating at its fundamental wavelength of 1064 nm generated laser pulses of 10 ns duration. This laser was capable of running at a repetition frequency of 10 Hz, but typically only 5 consecutive shots at 10 Hz were utilized. Pulse energies were varied by attenuating the beam with a half-waveplate and a Glan-laser polarizer.

To improve the focusing ability of the system, a spatial mode cleaner consisting of a 3× telescopic beam expander (AR coated lenses) was used to expand the beam from its nominal beam diameter of 9mm to a final beam diameter of 27 mm. An iris with a 9 mm opening immediately following the beam-expander sampled the inner one-third of the beam diameter. This LAB-150 specifies a >70% near-field (1 m) Gaussian fit to the actual spatial mode energy distribution, with this specification increasing to >95% in the far field (>6 m). The mode cleaner was located 1.3 m after the laser and transmitted approximately 15% of the energy incident upon it.

To visualize the laser beam location after focusing, a continuous wave (cw) alignment helium–neon laser at 632.8 nm was overlaid with the infra-red laser beam with a 50:50 633 nm beamsplitter. The coating-specific reflectivity did not reflect a significant fraction the incident infra-red radiation. The collinear visible and infra-red laser beams were then reflected vertically via a periscope assembly, Fig. 1(b).

After reflection from the periscope’s vertical turning mirror, the two laser beam passed through a broadband visible wavelength beamsplitter. This beamsplitter was used to allow a CCD camera (Everfocus EX100) to image the magnified target. The magnified view was displayed on a TV monitor for visual placement of the sample target in the focal region. The transmission of all optical elements prior to focusing was approximately 8%.
Both laser beams were focused by a high-damage threshold 5× infinite-conjugate microscope objective (LMH-5X-1064, OFR). This objective had an aperture of 10 mm, an effective focal length of 40 mm, a numerical aperture of 0.13 and a working distance of 35 mm. This unusually large working distance allowed easy sample manipulation and plasma light collection. The minimum theoretical spot size from this objective assuming a perfectly Gaussian mode and a completely filled aperture was 12 μm.

Visual examination of single-shot ablation craters on a variety of substrates yielded an average diameter of 100 μm in the objective’s focal plane. The AR coatings of the objective had the same damage threshold as the coating on the Glan-Laser polarizer, 500 MW/cm², which corresponds to an energy density of 50 MJ/cm²/pulse. Typical laser pulse energies used in this experiment (9 mJ/pulse at the sample target) were far below this threshold.

The samples were placed under this microscope objective on a manual “z-axis” vertical translation stage for precise focusing of the laser beam onto the surface of the target. The vertical translation stage was mounted on two crossed-axis translation stages driven by piezoelectric actuators (Picomotor, New Focus) capable of sub-micron translation of the target in the x–y plane. In practice, the sample was translated by approximately 150 μm between measurements to yield well-separated ablation craters.

A one meter long 600 μm quartz optical fiber located approximately 30 mm away from the ablation microplasma collected and transmitted the plasma emission to an Echelle spectrometer with fiber-coupled input slits (LLA, ESA3000). The fiber was angled at 30° relative to the sample normal and was aligned by illuminating the far exit end with a second helium–neon laser and overlapping the resulting cone of light from the fiber entrance with the alignment laser spot on the sample.

The Echelle spectrometer utilized a 1024 × 1024 CCD-array (24 micron x 24 micron pixel area) with an image-intensifier (ICCD) and was controlled by a PC running manufacturer-provided software. The PC controlled not only the gating (shuttering) of the ICCD, but also controlled operation of the pulsed-laser via an on-board fast pulse generator to eliminate jitter in the time between laser pulse and plasma observation. The useful spectral range of the spectrometer was 200–834 nm with a 0.005 nm resolution in the UV.

To perform LIBS, the spectra from 5 individual laser pulses were accumulated on the CCD camera chip prior to read-out. These on-chip accumulations (OCA) allowed a rapid averaging of the emission spectra, provided the resulting intensity did not exceed the dynamic response of the CCD chip. The sample was then translated 150 μm, another 5 ablation shots fired, and the resulting spectrum was averaged in software with the previous 5-shot accumulation. In this way 10 accumulations of 5 laser shots (50 laser shots total) were made on each sample. The 10 measurements were not averaged together to create one spectrum. Rather the scatter in the results from the 10 measurements allowed us to calculate a one-sigma standard deviation uncertainty for our results. The spectra were obtained at a gate delay time (the time between firing of the ablation pulse and electronic activation of the CCD camera’s image-intensifier) of 1 μs with a gate width (integration time) of 20 μs. These values were not varied during the experiment.
3. Results and discussion

Ten measurements of the ratio of the intensity of the Fe(I) emission line at 371.994 nm to that of the Ag(I) line at 328.069 nm for each of the 13 standard samples were used to create a calibration curve. The intensity of the emission line was obtained by fitting a Lorentzian lineshape to the region of the spectrum containing the emission profile utilizing a non-linear least squares fitting routine. The area of the fit Lorentzian line shape was used as the line intensity. Prior to the construction of the calibration curve, an investigation into the use of other lines was conducted. While the UV and visible wavelength LIBS spectra from the samples were very dense with emission lines from Fe and to a lesser extent Ag, the number of lines appropriate for construction of a calibration curve was not large. We required the strongest possible emission line that exhibited good signal-to-noise in samples with low concentration yet did not exhibit saturation or self-absorption in samples of intermediate or high concentration. Also, both lines were required to be well-separated from any nearest-neighbor lines, to allow an accurate determination of the area under the curve. This was particularly problematic in such a dense spectrum.

Additional calibration curves were constructed from neutral Fe emission lines that did and did not terminate in the ground state of the atom. Specifically, calibration curves were constructed using the Fe(I) 382.444 nm and 385.991 nm resonance lines which terminate in the ground state and the Fe (I) 367.992 nm, 373.714 nm, and 385.638 nm lines which do not terminate in the ground state. These curves exhibited a near-identical dependence on Fe mass fraction to the final curve constructed from the Fe 371 nm line and the Ag 328 nm line, but in general possessed a greater amount of statistical scatter around each calibration point. A few calibration curves were constructed using smaller Ag lines. These curves possessed qualitatively similar behavior but did not cover the same range of Ag mass fraction, due to the prohibitively small size of the alternative Ag peaks at low Ag mass fraction.

In general, this investigation proved that the ratio of almost any well-separated Fe(I) line to any Ag(I) line behaved in a predictable and consistent manner and could reliably be used as a calibration curve. Typically, any combination of atomic and ionic lines yielded fairly inconsistent results. The ratio of any two ionic emission lines did behave in a suitably reproducible fashion, but the lines became unacceptably small in samples of low concentration. Therefore the use of two neutral lines in the construction of the calibration curve was required, and the choice of the neutral resonance lines was predicated on statistical reproducibility.

3.1. Fe emission saturation

Prior to the creation of the calibration curve, 10 samples of varying Fe mass fraction were created and LIBS was performed to study the dependence of the absolute emission intensity of the two calibration lines on the Fe (Ag) mass fraction. The results of this study are shown in Fig. 3. Fig. 3(a) shows the integrated area under the line profile (emission intensity) in arbitrary units for both the Fe (open circle) and the Ag (filled circle) as a function of that species’ mass fraction in the sample. The sample number (1 through 10) is indicated in the graph. For example, sample number 1 had low Fe mass fraction (0.12) and high Ag mass fraction (0.82) while sample 10 had high Fe mass fraction (0.57) and low Ag mass fraction (0.19). A linear fit to the Ag data is shown as a dotted line and indicates a fairly linear behavior of the Ag emission intensity as a function of its concentration in the sample. Two linear fits are shown for the Fe data. The dashed line is a fit to all 10 data points. Not only are these data points not described well by this linear fit (poor $\chi^2$), but they also show a clear deviation from any linear behavior for Fe mass fractions greater than 0.45. The solid line is a fit to the data from samples one through seven only and shows a much more consistently linear behavior for these points. Fig. 3(b)
shows the ratio of the Fe 371.994 nm emission intensity to that of the Ag(I) emission intensity at 328.069 nm for the 10 samples. The lines are an exponential function fit to the data. The dashed line is a fit utilizing all 10 samples, while the solid line is a fit to only the first 7 samples. Again the consistent behavior of samples with an Fe mass fraction less than 0.45 is evident. In particular, for samples with an Fe mass fraction greater than 0.45, the apparent Fe/Ag ratio actually decreases significantly, which is a non-physical result not suitable for use in construction of a calibration curve. It was decided to only use standard samples with an Fe mass fraction of less than 0.45 (45 wt.%). Accordingly, an additional six standard samples were created according to the procedure described above, bringing the total number of standards to 13. Eventually 15 measurements were performed on these 13 standard samples.

The behavior of the emission lines utilized in the construction of the calibration curve was studied as a function of all Fe mass fractions. For no mass fraction of Fe or Ag was any self-reversal of either line noted. As a further test, the ratios of the intensity of these lines to the intensity of other lines originating in different levels of the same upper state multiplet and terminating in the same lower state (the ground state in both cases) were also calculated for all mass fractions. Specifically, the intensity of the Fe(I) resonance line at 338.289 nm (5p2P1/2 → 5s2S1/2) was compared to the intensity of the other resonance line at 338.289 nm (5p2P1/2 → 5s2S1/2). No dependence or change in this ratio as a function of Ag mass fraction (from 0.4 to 0.9) was observed. The intensity of the Fe(I) 371.994 nm line (4s4p(3P)5D4 → 4s2 5D4) was compared to another line from the 4s4p(3P) multiplet, namely the 367.991 nm line (3P 5F4 → 5s2S1/2). The ratios of these lines were constant within their uncertainty as a function of all Fe mass fractions (0.09–0.42). This calculation was not performed for the samples with Fe mass fraction greater than 0.45 which were not included in the calibration curve.

In addition, a Boltzmann plot was constructed by plotting the log-rescaled intensities of 23 Fe(I) emission lines vs. the energy of the upper state of the transition in eV (Fig. 4). This plot was made from data obtained from sample #2 (Fe mass fraction 0.1263) but is very representative of plots created from samples of all Fe mass fraction. The lines used in the construction of this plot are given in Table 2. The three resonance line that were examined for use in calibration curves are shown as solid circles. While the Fe(I) 371.994 nm line used in the final calibration curve seems to exhibit the greatest deviation from the Boltzmann exponential fit to the data (solid line) it’s behavior was consistent for all Fe mass fraction samples and was not found to deviate from the other relative intensities at even the highest mass fractions.

Typically Boltzmann plots of the type shown in Fig. 4 are utilized to calculate plasma temperatures assuming conditions of local thermodynamic equilibrium. Due to the long integration window used in this experiment (20 µs) the plasma temperature would have changed significantly during the time of spectral integration (by thousands of degrees Kelvin) and thus no such temperature can be implied by the graph shown in Fig. 4. Nonetheless, the construction of the graph does imply a Boltzmann distribution of energy states throughout the integration time and provides confidence in the choice of spectral lines used in the calibration curve.

### 3.2. Calibration curve

A calibration curve of the ratio of the emission intensity of the Fe(I) line at 371.994 nm to the Ag(I) line at 328.069 nm was constructed from 15 measurements taken over the course of one month on 13 standard samples. The data span an Fe mass

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>Energy of upper state (eV)</th>
<th>Rescaled line intensity (a.u.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>385.991 a</td>
<td>3.211</td>
<td>−8.07</td>
</tr>
<tr>
<td>382.444 a</td>
<td>3.241</td>
<td>−7.72</td>
</tr>
<tr>
<td>385.638</td>
<td>3.266</td>
<td>−7.87</td>
</tr>
<tr>
<td>389.566</td>
<td>3.292</td>
<td>−7.76</td>
</tr>
<tr>
<td>371.994 a</td>
<td>3.332</td>
<td>−9.01</td>
</tr>
<tr>
<td>367.992</td>
<td>3.368</td>
<td>−7.85</td>
</tr>
<tr>
<td>373.713</td>
<td>3.368</td>
<td>−8.64</td>
</tr>
<tr>
<td>349.057</td>
<td>3.603</td>
<td>−8.37</td>
</tr>
<tr>
<td>344.388</td>
<td>3.687</td>
<td>−8.58</td>
</tr>
<tr>
<td>346.586</td>
<td>3.687</td>
<td>−8.51</td>
</tr>
<tr>
<td>293.69</td>
<td>4.221</td>
<td>−9.95</td>
</tr>
<tr>
<td>364.784</td>
<td>4.312</td>
<td>−9.95</td>
</tr>
<tr>
<td>440.475</td>
<td>4.372</td>
<td>−9.84</td>
</tr>
<tr>
<td>427.176</td>
<td>4.387</td>
<td>−9.56</td>
</tr>
<tr>
<td>356.537</td>
<td>4.435</td>
<td>−10.06</td>
</tr>
<tr>
<td>420.203</td>
<td>4.435</td>
<td>−9.78</td>
</tr>
<tr>
<td>404.581</td>
<td>4.549</td>
<td>−10.22</td>
</tr>
<tr>
<td>400.524</td>
<td>4.652</td>
<td>−9.97</td>
</tr>
<tr>
<td>407.173</td>
<td>4.652</td>
<td>−10.20</td>
</tr>
<tr>
<td>495.760</td>
<td>5.309</td>
<td>−10.63</td>
</tr>
<tr>
<td>282.328</td>
<td>5.349</td>
<td>−11.34</td>
</tr>
<tr>
<td>284.397</td>
<td>5.349</td>
<td>−11.24</td>
</tr>
<tr>
<td>322.578</td>
<td>6.242</td>
<td>−12.55</td>
</tr>
</tbody>
</table>

aIndicates resonance lines.
fraction from 0.09 to 0.42 and possess a clear exponential (not linear) dependence on Fe mass fraction over this broad range of concentrations. This functional dependence is not surprising considering that a linear intensity dependence on concentration is usually only observed for very low concentrations (e.g. at the sub-percent or part-per-million concentration level in liquid systems with trace elements), which also assumes an unchanging matrix concentration. The samples studied in this work possessed a very broad range of Fe and matrix concentrations and thus a linear dependence over the entire range was not expected.

Fig. 5 shows the LIBS calibration curve for the 13 $\alpha$-$\text{Fe}_2\text{O}_3$/Ag standard samples. In Fig. 5(a), the individual measurements (50 laser shots) are shown as an “x” and the average of the 10 measurements is shown as a closed circle. The error bars are the standard deviations of the measurements. The solid line is a weighted exponential fit to the data. (b) The same calibration curve (without the individual measurements) used to calculate the Fe mass fraction of a $\gamma$-$\text{Fe}_2\text{O}_3$/alginate/Ag pellet.

The results of the fit are given in Table 3. The $R^2$ coefficient of determination has a value of 0.974, which demonstrates the quality of the exponential fit to these data. It should be pointed out that the exponential fit was not constrained to pass through the origin, although physically it is indeed necessary to detect zero Fe emission intensity in samples with an Fe mass fraction of zero. However, it was felt that our standard sample set did not include enough samples with very low concentrations to merit an assumption of behavior in the low concentration regime approaching zero mass fraction. Also, the reduced $\chi^2$ of such a fit was significantly worse than that of the unconstrained exponential and did not even qualitatively describe the behavior of the data. Therefore we utilized the function shown in Table 3, as it was our intent to describe the behavior only of the samples we measured and this behavior was described most accurately by that function. The measurement of the Fe mass fraction in the alginate sample, described in detail below, is shown in Fig. 5(b).

3.3. $\gamma$-$\text{Fe}_2\text{O}_3$/alginate sample

A pellet containing a “6th-loaded” $\gamma$-$\text{Fe}_2\text{O}_3$/alginate hydrogel was created by combining a dried, then powdered quantity of the hydrogel with the Ag powder used in the previous studies. This pellet was composed of 31.3 mg of alginate and 31.5 mg of the powdered Ag. The mass fraction of alginate in the pellet was 0.498 (49.8 wt.%). The pellet was created in the same way as the standard pellets described in Section 2.1.

This pellet was subjected to the identical LIBS analysis utilized in the construction of the calibration curve with the $\alpha$-$\text{Fe}_2\text{O}_3$/Ag standards. A measurement of the ratio of the emission intensity of the Fe(I) line at 371.994 nm to the Ag(I) line at 328.069 nm yielded a value of 0.345±0.025. Utilizing the calibration curve constructed in Section 3.3 and shown in Fig. 4(b), this ratio corresponds to an Fe mass fraction of 0.255±0.015. The Fe mass fraction of the sample can be used to calculate the Fe wt.% of the alginate by using the simple relation (alginate Fe mass fraction)×(mass fraction of alginate in pellet)= (Fe mass fraction of pellet). Since the mass fraction of alginate in the pellet was already known (see above), the alginate Fe mass fraction was trivially computed. Propagation of uncertainties in the usual way yielded a final Fe mass fraction determination of 0.51±0.03.

| Model: exponential growth |
| Equation: $y = y_0 + A(\exp(x/C))$ |
| Weighting: $w = 1/\sigma^2$ |

### Fit results

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>$y_0$</td>
<td>0.065</td>
<td>0.035</td>
</tr>
<tr>
<td>$A$</td>
<td>0.061</td>
<td>0.021</td>
</tr>
<tr>
<td>$C$</td>
<td>0.167</td>
<td>0.022</td>
</tr>
</tbody>
</table>
This value of $51 \pm 3 \text{ wt.}\%$ is entirely consistent not only with previous determinations of Fe mass fractions in such dehydrated hydrogels (predicted to be in the range of $10–50 \text{ wt.}\%$ by Kroll et al. [9]) but is entirely consistent and contains a lower uncertainty than an alternative test performed on the same hydrogel batch from which our small sample was obtained. This test was performed by a private company (RTI Laboratories; Livonia, MI) utilizing inductively-coupled plasma spectroscopy which yielded an Fe mass fraction result of $53 \pm 5 \text{ wt.}\%$. The actual uncertainty on the measurement as quoted by this laboratory was significantly less than the $5\%$ quoted here, but an identical sample from the same batch sent to the laboratory yielded an Fe mass fraction that was different from the first sample by $5\%$. Both we and the fabricators of the hydrogel therefore feel that an uncertainty of $5\%$ is warranted for this determination of the Fe mass fraction in this particular batch of hydrogel. This independent measurement of the Fe mass fraction alleviated the concern that the additional presence of the alginate hydrogel material in the sample would add an unpredictable “matrix effect” and produce results inconsistent with the calibration curve which was constructed from standards that did not contain the alginate material. Unfortunately, this single sample was constructed from the only externally-calibrated batch of alginate-hydrogel made available to our group for LIBS testing, so the calibration curve cannot be confirmed with additional measurements. Nonetheless, we feel the result is a strong confirmation of the applicability of the technique and we believe that future experimentation on such nanoparticle-containing systems is warranted.

4. Conclusion

The use of LIBS as a means for the quick and accurate determination of the Fe mass fraction in chemically prepared $\gamma$-Fe$_2$O$_3$/alginate hydrogels has been demonstrated. A calibration curve was constructed from 13 “standard” samples composed of a mixture of $\alpha$-Fe$_2$O$_3$ nanopowder and Ag micropowders compressed to a homogeneous pellet. The Ag was a useful matrix with which to combine the Fe$_2$O$_3$, creating a uniform sample when pelletized, which is necessary in the low-temperature magnetic characterizations that were the motivation for the hydrogel fabrication. For the purposes of this study, the presence of the Ag matrix provided convenient “reference” emission lines against which the intensity of the Fe emission lines could be measured. Plans are currently underway to replace this Ag matrix with a similar non-magnetic material, copper, which is available in nanopowder or micropowder forms, to see what effect if any this matrix material has on the ability to determine the Fe mass fraction of the sample. However, we again reiterate that standard samples must duplicate the sample to be tested as closely as possible and must be matrix-specific. The calibration curve constructed from Fe$_2$O$_3$/Cu standards could not be used to determine the Fe concentration of alginate/Fe$_2$O$_3$/Ag test samples.

The calibration curve was used to calculate the Fe mass fraction of 31 mg of the dehydrated, powdered $\gamma$-Fe$_2$O$_3$/alginate hydrogel with a final result of $51 \pm 3 \text{ wt.}\%$. An independent ICP measurement of an identical sample from the same batch yielded a result of $53 \pm 5 \text{ wt.}\%$, confirming the validity of the calibration, the usefulness of the LIBS technique, and the accuracy of the results.

Despite the agreement between these LIBS measurements and the ICP measurements, two significant improvements would considerably assist in validating the accuracy of the measurement. First, the concentration curve would be much more appropriately constructed using standard samples produced by adding known amounts of $\gamma$-Fe$_2$O$_3$ nanopowder to alginate hydrogel samples fabricated without any iron. In this way a library of standards that almost identically matches the material to be tested could be constructed. Unfortunately, such “un-doped” hydrogel samples were not fabricated by our colleagues and were thus not available for testing.

Second, additional batches of externally-measured $\gamma$-Fe$_2$O$_3$/alginate hydrogels should be tested with the concentration curve constructed from the new standards. This would help to confirm the agreement with ICP measurements over a broader range of Fe concentrations and increase confidence of the applicability of the curve to a wider variety of samples. These additional batches are not currently available to us. This initial result, however, provides the proof-of-concept desired at the outset of the experiment and is strongly suggestive that additional work along the lines described here would be fruitful for the measurement of nanoparticle-containing biocompatible systems.

Acknowledgements

The authors would like to thank Prof. Gavin Lawes and Ron Tackett for the fabrication of the nanoparticle alginate hydrogel samples as well as the useful discussions and Prof. Boris Nadgorny for the use of the hydraulic press. S.J. Rehse was supported financially by the Wayne State University Department of Physics and Astronomy (College of Liberal Arts and Sciences) and E. Brown was supported by NSF-REU Grant #EEC-0552772 through the Wayne State University Smart Sensors and Integrated Microsystems (SSIM) program.

References


