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# Qualitative evaluation of maternal milk and commercial infant formulas via LIBS

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ABSTRACT

This study focuses on the use of laser-induced breakdown spectroscopy (LIBS) for the evaluation of the nutrients in maternal milk and some commercially available infant formulas. The results of such evaluation are vital for adequate and healthy feeding for babies during lactation period. Laser-induced breakdown spectroscopy offers special advantages in comparison to the other conventional analytical techniques. Specifically, LIBS is a straightforward technique that can be used *in situ* to provide qualitative analytical information in few minutes for the samples under investigation without preparation processes. The samples studied in the current work were maternal milk samples collected during the first 3 months of lactation (not colostrum milk) and samples from six different types of commercially available infant formulas. The samples' elemental composition has been compared with respect to the relative abundance of the elements of nutrition importance, namely Mg, Ca, Na, and Fe using their spectral emission lines in the relevant LIBS spectra. In addition, CN and $C_2$ molecular emission bands in the same spectra have been studied as indicators of proteins content in the samples. The obtained analytical results demonstrate the higher elemental contents of the maternal milk compared with the commercial formulas samples. Similar results have been obtained as for the proteins content. It has been also shown that calcium and proteins have similar relative concentration trends in the studied samples. This work demonstrates the feasibility of adopting LIBS as a fast, safe, less costly technique evaluating qualitatively the nutrients content of both maternal and commercial milk samples.



## 1. Introduction

Maternal milk is universally accepted as the unique food for infants that provides all their nutritional and immunological requirements. During the first months of life, milk is the only source of nutrients and, therefore, it plays an essential role in terms of body growth and development of newborns [1]. Human milk provides an adequate intake of macronutrients (proteins, lipids, and carbohydrates) and micronutrients (minerals, vitamins, and enzymes), its composition changes along lactating period from colostrum to mature milk. Maternal milk is normally used as a nutritional reference to develop infant formulas with a similar composition [2–4]. Manufacturers are doing their best in optimizing percentages of elements and proteins in infant formulas to mimic maternal milk contents more closely.

Nutrient levels in infant formulas are generally modeled on the composition of human milk and one goal of the improvement of such commercial formulas is to make them the closest alternative to human milk [5]. However, this goal is still far from being attained due to the insufficient data available about “mother's milk” which can be used as reference. Constituents of maternal milk have been measured in many published studies [6,7], but data that could be used for comparison of samples is limited, since researchers often use pooled milk samples and do not have a well-defined study.

To have a better insight about the dietary course of infants during lactation period, it is essential to learn more about trace elements and proteins contents of maternal milk. This information can be also very helpful in developing commercial infants formulas which simulate as close as possible the mother's milk. Bocca et al. [7] reported that whilst the total content of the elements in human milk has been assayed repeatedly in recent years, literature on element speciation in this biological fluid is still scarce [8–10]. However, the high probability of samples contamination should be taken into consideration as well as the possible modification of their composition during speciation analysis. Therefore, there is a rising need for a sensitive and reliable analytical technique for identification and quantification of the elements and proteins in maternal milk and infants milk formulas.

The LIBS technique is a powerful spectrochemical analytical technique to assess the elements in biological samples [11–13]. The technique has been developed for fast and sensitive elemental

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analysis of solid, liquid, and gaseous samples over the past 3 decades mainly because of its simplicity and lack of time-consuming sample preparation procedures [14].

Most studies focusing on trace elements in milk adopt conventional techniques such as Proton-Induced X-ray emission (PIXE) [15], ICP-MS and SEC (size exclusion chromatography)–ICP-MS [4], ICP-AES [6], UN (ultrasonic nebulization)–ICP-AES [7], and CF-LIBS with ICP-AES [16]. LIBS techniques can be used successfully to assess the elements in milk; in addition, molecular bands of CN and $C_2$ in LIBS spectra can be exploited for the relative evaluation of proteins and organic materials in milk.

This work aims to make use of LIBS as a fast, sensitive, and less costly elemental analysis technique for evaluating the nutrient content of maternal milk qualitatively (of young and old mothers) in comparison with six different commercially available infants formulas. Spectral emission lines of Mg, Ca, Na and Fe as well as the molecular bands of CN and $C_2$ in the LIBS spectra of milk samples have been exploited to perform such qualitative evaluation.

## 2. Materials and methods

### 2.1. Milk samples

Sixty milk samples were randomly collected from 60 lactating mothers (within the first 3 months) with 30 of them being younger than 30 years and the other 30 above 30 years of age. The commercial infants formulas samples were gathered from the top six most sold brands that have the highest sell volume on the markets for newborns (first 3 months of age) meeting the nutrient standards set by the FDA. All human milk samples were collected from healthy mothers visiting the National Family Centers in Cairo, Egypt, during the summer season. Considerable precautions have been made to optimize the experimental procedures conditions by following proper criteria. The samples were collected in clean tubes then frozen until the total number of samples were gathered to start their analysis at the same time. Mothers were asked to fill out fully informed consent forms including all the required data; (date, time of collecting milk, age, smoking habits, vitamin intake, etc.).

Frozen milk was thawed using tap water, and droplets of samples (0.5 mL/droplet) were taken onto an ashless filter paper, the samples were left for about 15 min in clean air to ensure that the milk has been homogeneously expanded and absorbed in the filter paper before further LIBS analysis. Formulas were prepared according to the instructions written on the can of each type then droplets from each prepared formula were taken onto the same type of filter paper and undergoing the same procedure for maternal milk.

### 2.2. LIBS arrangement

A typical LIBS experimental setup has been used throughout the present measurements. Detailed description of the instrumentation is given elsewhere [13]. The laser used was a Q-switched Nd:YAG laser (BRIO, Quantel, France) operating at its fundamental wavelength ($\lambda = 1064$ nm), producing pulses each of 5 ns duration and 100 mJ energy at a repetition rate of 1 Hz. The laser beam was focused onto the sample surface by means of a planoconvex quartz lens ($f = 10$ cm). The laser irradiance was thus estimated to 340 GW/cm². The sample under investigation was mounted on an X–Y micrometric translation stage. To avoid electronic interference and jitters, the CCD intensifier high voltage was triggered optically at a typical optimized delay time of 2000 ns with integration time 2000 ns. To optimize the signal-to-noise ratio and guarantee results reproducibility LIBS spectra were collected from three fresh spots of the same sample, spectra of five consecutive shots were recorded for each spot. The spectrometer used was a Mechelle spectrometer (Mechelle 7500, Multichannel, Sweden) coupled to a gateable ICCD camera, DiCAM-Pro (PCO, computer optics-Germany) with the GRAM/32 software. The used ICCD is UV-enhanced and the spectroscopic system covers the spectral range from 200 nm up to 700 nm. The obtained spectra were displayed on a PC for further processing and spectroscopic analysis adopting the proper computer programs and relevant data base.

## 3. Results and discussion

All spectra have been averaged over the total number of measured samples in each case and were normalized against the intensity of the carbon spectral line at 247.8 nm to compensate for any experimental fluctuations and to reduce the matrix effect. LIBS spectra depicting the spectral lines of Mg, Ca, Fe and Na in the investigated samples are shown in Fig. 1. It is clear that the intensities of such spectral lines are the highest for maternal milk samples compared to the infant milk formulas samples. However, the intensity of the magnesium line at 279.5 nm is also high in infant milk sample (S-26) and approaches that of the human milk. It is well known that calcium is an essential element for the growth and development of infants, but maternal milk and infant formulas differ generally in their Ca content as has been mentioned by Fomon and Nelson [17].

Iron is one of the essential trace elements required for healthy growth of infants. It exists in all living cells where it plays several vital roles. Fe deficiency may cause developmental delays and behavioral disturbances especially when accompanied by iron-deficiency anemia [18,19]. On the other hand; excess iron intake may interfere with copper and zinc absorption favoring oxidative stress and infections [8]. In general the Fe content in human milk is much higher than that in commercial infant formulas as shown in Fig. 1(b). Using LIBS, it is also possible to evaluate maternal milk of mothers of different ages and probably different health statuses. Fig. 2 shows a comparison of trace elements content in human milk between two groups of mothers of different ages (above 30 years and under 30 years). The results obtained show that milk of young mothers is of much higher quality than that of older mothers.

Milk proteins, particularly caseins, have an appropriate amino acid composition for growth and development of the newborn. The caseins comprise the major protein component of milk of most mammals and represent the family of phosphoproteins synthesized in the mammary glands in response to lactogenic hormones and other stimuli and secreted as large colloidal aggregates termed micelles, which are responsible for many of milk unique physical properties [20].

As mentioned above, LIBS is essentially an elemental analysis technique. However, it is possible to make use of LIBS spectra of organic materials to follow up some molecules through the presence of their molecular emission bands such as CN, $C_2$ and OH [12,21,22]. The appearance of CN and $C_2$ bands in LIBS spectra is mainly due to two mechanisms. The first is recombination of $C_2$ with nitrogen derived from the target or from air, and recombination of atomic and ionic carbon with atomic or molecular nitrogen to form CN while fragmentation dominates $C_2$ production in molecules containing carbon–carbon double bonds [23,24]. The second mechanism is the direct ablation of native CN and $C_2$ molecules from the milk samples [12]. It is, therefore possible, to evaluate proteins in milk samples through CN and $C_2$ bands in both maternal and commercial formulas samples LIBS spectra. Casein which is the major milk protein is bound to calcium [23], this relation between calcium and casein will be demonstrated later through the relation between calcium and CN spectral lines

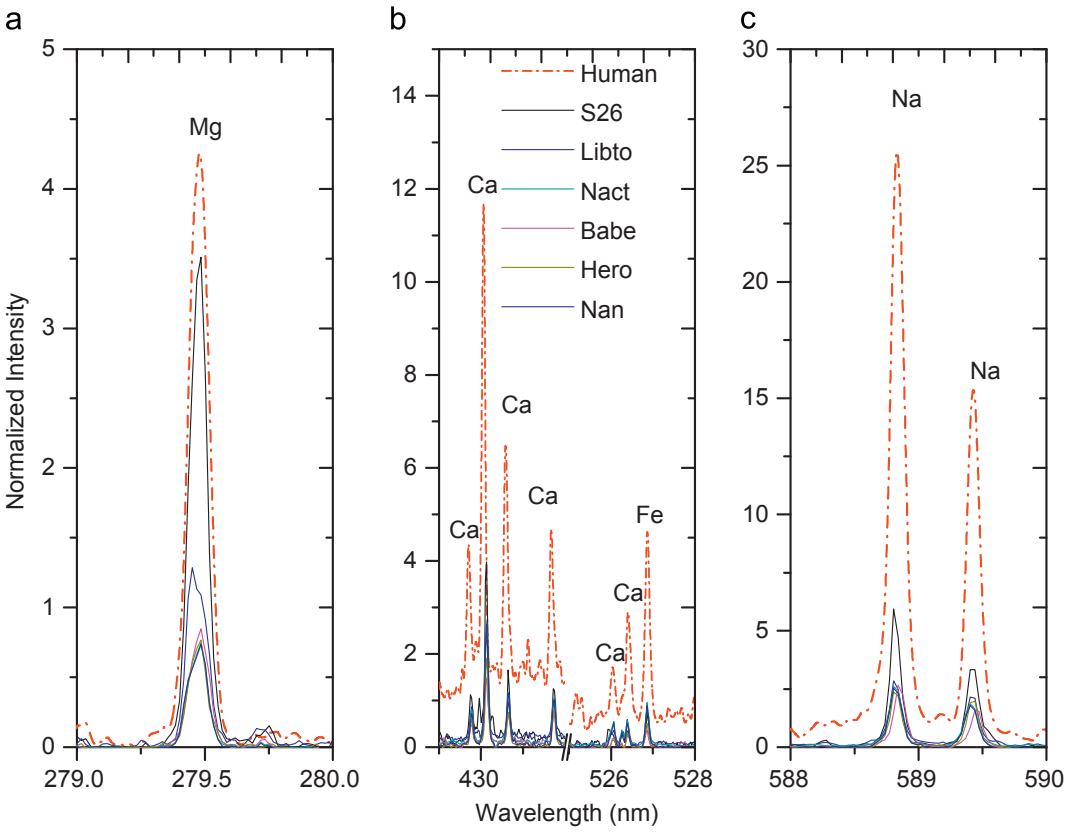


**Fig. 1.** Comparison between LIBS spectral lines normalized intensities of Mg, Ca, Fe and Na of human milk (below 30 years old mothers) and six different types of infants milk formulas.

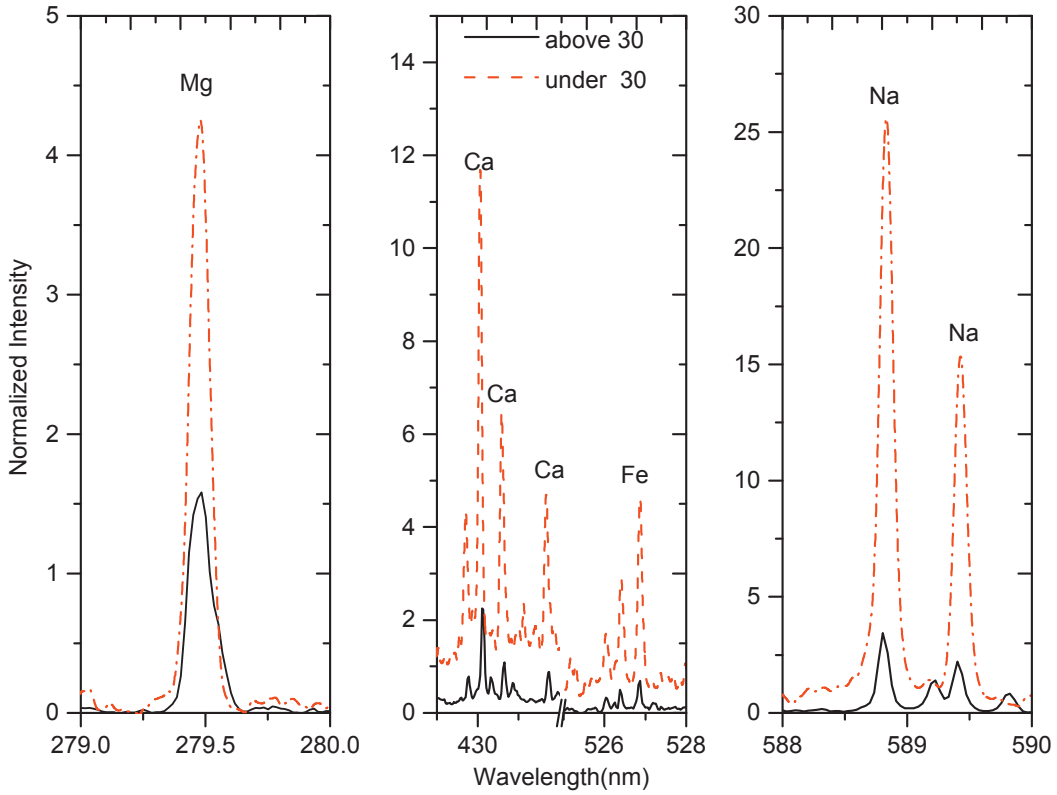


**Fig. 2.** Comparison between LIBS spectral lines normalized intensities of Mg, Ca, Fe and Na of human milk samples of young mothers (below 30 years old) and old mothers (above 30 years old).

and bands. Fig. 3(a) and (b) shows the violet CN emission bands ($B^2\Sigma^+ \rightarrow X^2\Sigma$) and the $C_2$ Swan band ($A^2\Pi \rightarrow X^2\Sigma^+$) in the visible region for maternal milk and commercial infant formulas samples respectively. As shown in Fig. 3a the high intensity of the CN band in the maternal milk indicates its high proteins, mainly caseins content compared to all types of the measured commercial milk formulas. The inset in the same figure depicts the high content of proteins in young mothers' milk than in that of older mothers. Similarly, the $C_2$ spectral band intensity shown in Fig. 3b and the inset therein confirms the same results. In fact LIBS results cannot discriminate between different types of protein but it can be useful in evaluating the total protein content in different milk samples.

Fig. 4 shows a comparison between the CN spectral bands intensities at different wavelengths in the UV region at 387 nm and 383.2 nm and in the visible region at 419.6 nm and 421.53 nm for human milk and infant formulas respectively. Similarly Fig. 5 describes the behavior of $C_2$ emission bands at 512.9 nm and 516.5 nm.

It is important to pay attention to the fact that casein which is the major protein component of milk is secreted as micelles that also carry high calcium concentration [25]. To confirm this fact in the investigated samples adopting the obtained LIBS spectra, a comparison has been performed between the summation of the net intensities of six well resolved non-resonant calcium lines in the violet spectral region at 428.42, 428.56, 429.057, 430.37, 430.90, and 431.99 nm and the summation of the net intensities of six CN violet bands (function of protein content) at 387.0, 383.2, 416.65, 417.97, 419.6, and 421.53 nm of the investigated milk samples' spectra. The results shown in Fig. 6 demonstrate clearly that the higher is the calcium content, the higher is the CN and consequently the proteins content of the milk sample. This result shows that calcium and proteins have the same qualitative trend in all samples.

However, as mentioned before, this figure indicates the relation between proteins in general and calcium. It should be taken into consideration that calcium in maternal milk is essentially bound to serum proteins, whereas it is bound to casein in cow milk. This may explain the differences pronounced in calcium bioavailability in maternal and infant milk formulas since the latter is produced mainly from cow milk [23,26].

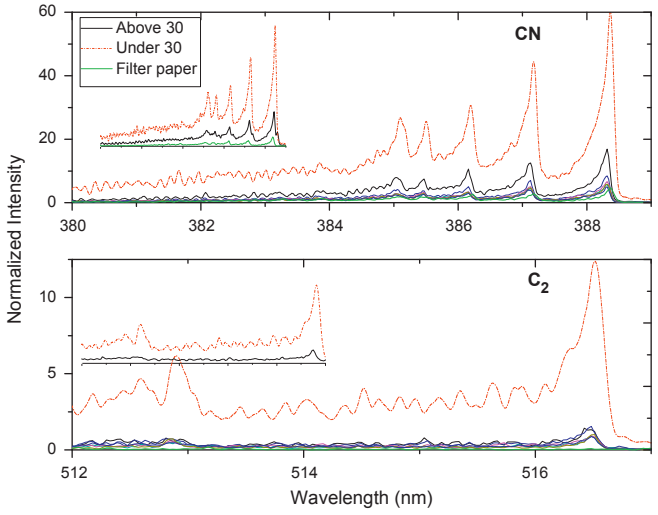


**Fig. 3.** LIBS spectra of violet CN bands and the Swan $C_2$ bands in maternal milk (mothers below 30 years old) and six different infants milk formulas. The insets depict the comparison between the same spectral molecular bands of young and old mothers' milk. The spectrum of the blank filter paper is included for comparison.

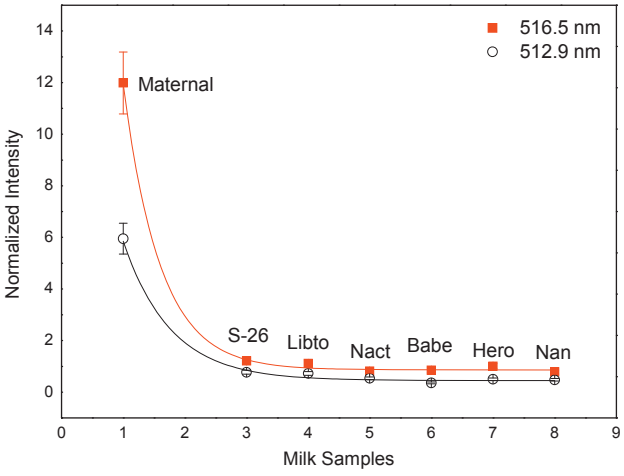


**Fig. 5.** Trends of integrated intensity values for $C_2$ Swan emission bands in the visible region for maternal milk sample and six types of commercial infant formulas.

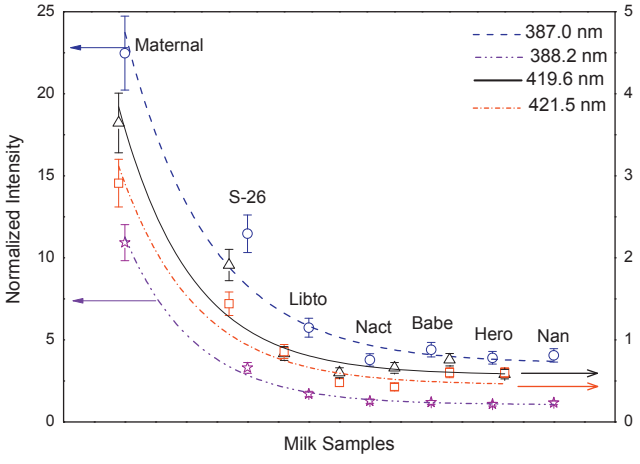


**Fig. 4.** Trends of integrated intensity values for different violet CN emission bands for maternal milk and six types of commercial infant formulas.

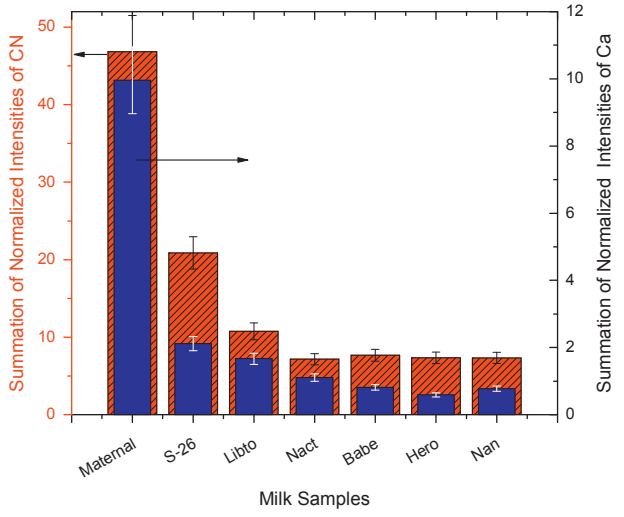


**Fig. 6.** Trends of summation of the normalized integrated line intensities for six CN and six Ca spectral lines for different samples. The error bars represent the standard deviation of the experimental data of each group.

## 4. Conclusion

It is essential to have an accurate and complete knowledge of the composition (including trace elements and proteins) of maternal milk and commercial infant formulas during lactation. This allows for better understanding of the nutritional requirements of the infant as well as for developing milk substitutes which should approximate the composition of breast milk. In the present work it has been demonstrated that LIBS can be used successfully in the qualitative evaluation of both maternal milk and infant formulas. Compared to the conventional techniques used in similar studies, LIBS is fast, safe, simple and can be used *in situ*. In the current study both trace elements and proteins have been evaluated using spectral lines of elements of nutrition importance (Mg, Ca, Fe and Na) and the molecular bands of CN and $C_2$ in the same LIBS spectra. The similarity of calcium and proteins relative concentration trends in milk samples has been also demonstrated using LIBS. This study shows that using LIBS as a spectrochemical analytical technique allows for better understanding of the nutritional requirements of the infant as well as for developing milk substitutes which should approximate the composition of breast milk. In addition, it is feasible to make use of portable LIBS systems in clinics and mothers care centers to evaluate maternal milk to adjust the infant's nutrition strategy. Using LIBS, it is also possible to evaluate milk of mothers of different ages and probably at different health statuses. Besides the importance and advantages of the present qualitative study, it is of course most beneficial to obtain quantitative information. Future work is planned for LIBS quantitative evaluation adopting authenticated samples for calibration and probably making use of other hyphenated techniques.

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