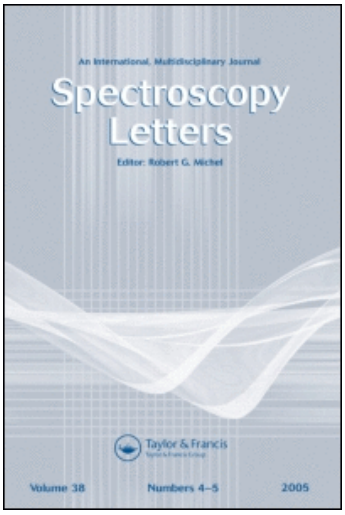

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Atomic Absorption Assessment of Mineral Iron Quantity in Ferritin

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ABSTRACT The goal of this work is to study the possibilities for quantitative determination of the number of iron atoms in the mineral core of ferritin by atomic absorption spectroscopy (AAS). Distinctive AAS measurements show iron content between 1000 and 4500 atoms per molecule ferritin.[1–6] This motivated us to investigate the amount of iron in the horse spleen ferritin with atomic absorption spectroscopy under application of the Bulgarian standard *BDS EN 14082/2003 Foodstuffs—Determination of Trace Elements—Determination of Lead, Cadmium, Zinc, Copper, Iron and Chromium by Atomic Absorption Spectrometry (AAS) after Dry Ashing*.[7]

We have obtained as a result 1792 atoms in one ferritin molecule. This result is in accordance with previous results, published by leading researchers.[4,5] The investigation of the iron content with AAS under the use of the Bulgarian standard can be a good opportunity to study many other objects of biological interest.

KEYWORDS atomic absorption spectroscopy, ferritin, iron

INTRODUCTION

Ferritins are members of a family of iron storage proteins in living organisms from bacteria to human. The ferritins are most commonly found in the liver and in the spleen. The main functions of ferritins are the reversible formation and dissolution of a solid nanomineral-hydrated iron oxide, which additionally detoxifies the excess iron and reactive oxygen species.[8–10] The Fe is deposited in micellar form in the center of the protein spherical shell.[6] This spherical space can be filled up with as many as 4500 iron atoms, which is equivalent to an iron concentration of ~0.25 M. Iron is stored within the ferritin as an Fe(III) oxohydroxide structure that contains some phosphate and is similar to the rust. The overall stochiometry of the iron core is $[FeO(OH)]_8[FeO(H_2PO_4)]$,[11] which is structurally similar to the mineral ferrihydrite.

Channels, i.e., pores through which certain ions or molecules can pass through the shell in both directions, are formed at the intersections of the peptide subunits. These pores are of crucial importance for ferritin's ability to release or deposit iron in a controlled way. There are two types of pores: fourfold, occurring in the intersection of four peptide subunits and threefold, occurring at the intersection of three peptide subunits. The two

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kinds of channels reveal different chemical properties; hence, they perform different functions. Their size was evaluated to be about 3–5 Å,[12,13] but it can vary due to the intramolecular dynamics of the subunits with corresponding rearrangement of the quaternary structure. Moreover, some experimental results, which show differential accessibility for the dissoluble molecules, support the idea of a "gate" in the pores regulating their opening and closing.[14]

The iron in the ferritin core is stored as insoluble Fe(III) in a crystalline solid form. Thus, in order to be released, the mineral lattice must be dissolved. This is accomplished by reducing iron from Fe(III) to Fe(II). In the Fe(II) state, iron becomes soluble as a hydrated Fe^{2+} ion, $Fe(H_2O)_6^{2+}$, and can leave the protein via the threefold channels, which are covered inside with hydrophilic side chain groups.

It is conceivable that understanding of the iron transformation processes managed by ferritins is of general significance for many areas in the life sciences. Along with other unresolved issues regarding supramolecular assembly and gene regulation, this could be one of the main goals of most ferritin research programs.

Atomic absorption spectroscopy (AAS) is a method used in many studies for determination of the iron quantity in the ferritins. Different authors have found this amount to be between 1000 and 4500 atom per molecule.[1–6] This encouraged us to investigate the iron amount in the horse spleen ferritin using atomic absorption spectroscopy.

MATERIALS AND METHODS

Materials

Horse spleen ferritin (HoSF Type I, Merck, Germany) was analyzed for iron content. Hydrochloric acid (HCl, Merck) at concentration of 3 M was used to dissolve dry ash ferritin material.

Instrument

In the present analysis the atomic absorption spectrophotometer Perkin Elmer 3030 (Germany) was used. A hollow cathode lamp was used as a primary source of light with the wavelength $\lambda = 248.3$ nm. The evaporating flame was an oxidizing acetylene/air flame. Only class A glassware was used in this study.

Method

The preparation of the probe and the analysis were carried out in compliance with Bulgarian standard *BDS EN 14082/2003 Foodstuffs—Determination of Trace Elements—Determination of Lead, Cadmium, Zinc, Copper, Iron and Chromium by Atomic Absorption Spectrometry (AAS) after Dry Ashing*.[7]

RESULTS AND DISCUSSION

Two parallel probes were checked out. In both cases a precisely measured quantity of 0.1 mL ferritin solution was put in a quartz crucible. Its corresponding weight was measured in grams. The weights of the first and second probe were 0.09 and 0.10 g, respectively. The solution was transformed to a visible dry state by carefully heating up to 60°C in order to avoid boiling. The drying procedure continued at 105°C for 1 hr. In the next step the quartz crucible was carefully placed on an electrical heater and the substance was brought to a proper degree of ash within 4 hr. Then it was placed in a muffle oven where it withstood at 440°C for ca. 12 hr. The ash remnant was transferred into a measuring flask of class A with a volume of 25 mL and dissolved in 3 M HCl. The obtained solution was yellowish and clear, without opalescence. A quantity of 1 mL of this solution was diluted with bidistilled water in a flask up to 10 mL. Then 2 mL of the solution were diluted again up to 10 mL. Thus, the overall dilution degree of the initial ferritin solution was 12,500. This was the final solution for assessment of iron content using an atomic absorption spectrophotometer.

The results for the first and the second probe, along with their mean values (M), standard deviations (SD), and standard errors (SE), are shown in Table 1 and Figs. 1 and 2.

TABLE 1 Measurements of 15 Separate Samples for First and Second Probe

Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	M	SD	SE
Probe 1 (mg/L)	1.61	1.68	1.61	1.63	1.63	1.65	1.61	1.63	1.62	1.66	1.69	1.69	1.71	1.72	1.70	1.66	0.04	0.01
Probe 2 (mg/L)	1.70	1.71	1.74	1.75	1.71	1.74	1.75	1.75	1.78	1.69	1.67	1.71	1.76	1.75	1.78	1.73	0.03	0.09

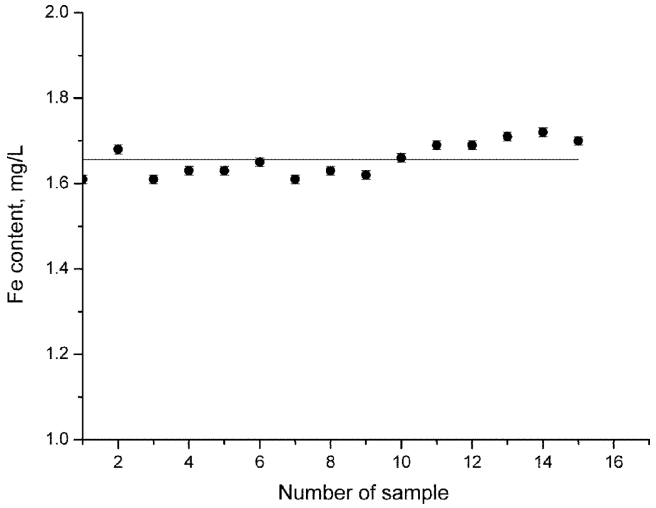

FIGURE 1 **The Fe contents of the first probe.**

Thus, taking into account the overall dilution of the original ferritin solution and the weight of the probes, we obtained the following results for the iron content:

$$\text{Probe 1}: (1.66 \pm 0.01)\ \text{mg/L} \times (0.1\,\text{mL}/0.09\,\text{g}) \times 12,500 = (2.24 \pm 0.01)\%(\text{wt/wt})$$

$$\text{Probe 2}: (1.73 \pm 0.09)\ \text{mg/L} \times (0.1\,\text{mL}/0.10\,\text{g}) \times 12,500 = (2.14 \pm 0.01)\%(\text{wt/wt})$$

The iron quantity in the initial solution of ferritin can be determined from the average value of the mean values of the two parallel assessments: $(1.69 \pm 0.01)\ \text{mg/L} \times 12{,}500 = (21.17 \pm 0.11)\ \text{g/L}$ or $(2.19 \pm 0.01)\%$ (wt/wt). The error, imposed by the procedure of manipulation of the probes, was insignificant compared to the apparatus error (0.125% according to the manual). We found the iron content to be $(2.19 \pm 0.13)\%$ (wt/wt) in the original solution offered by Merck.

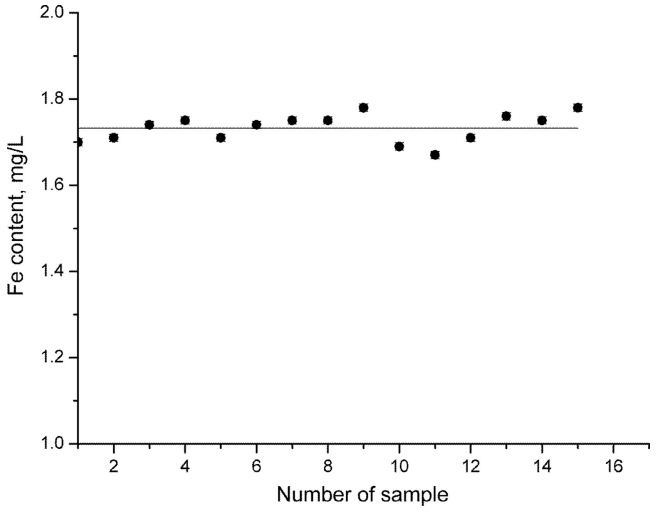

FIGURE 2 **The Fe contents of the second probe.**

Based on the above results, we can calculate the molar concentration of the iron in this solution:

$$(1.69 \pm 0.01)\ \text{mg/L} \times 12,500/56\,\text{g} = (0.378 \pm 0.002)\ \text{M Fe}.$$

Because according to the manufacturer the same solution contains 100 mg/mL of the protein ferritin, its molar concentration should be

$$100\,\text{g L}^{-1}/474,000\,\text{g mol}^{-1} = 0.211\,\text{mM ferritin}$$

Thus, we can easily estimate the number of iron atoms per molecule of protein:

$$(0.378 \pm 0.002)\ \text{M Fe}/0.211\,\text{mM ferritin} = 1792 \pm 10\ \text{atoms per molecule}$$

The obtained result shows that in our case one molecule of ferritin is loaded with approximately 1800 iron atoms. It should be mentioned that this is in good agreement with the works of other researchers, leading specialists in this area; for example, Treffry and Harrison (1730–2000 atoms/molecule)[5] and Chasteen and Theil (800–2000 atoms/molecule).[4] Therefore, we maintain that the application of the Bulgarian standard in the AAS study of iron in ferritin or other significant proteins is a reliable tool for quantitative evaluations.

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