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Atomic Absorption Assessment of Mineral Iron Quantity in Ferritin

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Abstract. Possibilities for quantitative determination of the number of iron atoms in the mineral core of ferritin by atomic absorption spectroscopy (AAS) are investigated in the work. Different measurements with AAS show an iron content from 1000 up to 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.

The obtained results give aprox. 1800 atoms per molecule Ferritin. It 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 is a good opportunity to study many other objects of biological interest.

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1 Introduction

Ferritins are family of iron storage proteins in living organisms from bacteria to human. They play a central role to the natural regulation of iron/oxygen chemistry in biology. The ferritins are most commonly found in liver and spleen. Reversible formation and dissolution of a solid nanomineral-hydrated, iron oxide is the main function of ferritins, which additionally detoxify excess iron and reactive oxygen species [7-9]. The Fe is deposited in micellar form in the centre of the protein 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 also some phosphate and is similar to rust. The overall stoichiometry of the

iron core is $[FeO(OH)]_8[FeO(H_2PO_4)]$ [10], which is similar in structure to the mineral ferrihydrite.

Channels, *i.e.* pores through which certain ions or molecules can pass the shell in both directions, are formed at the intersections of the peptide subunits. These pores are of a crucial importance to the ferritin's ability of releasing or depositing iron in a controlled fashion. There are two types of them: four-fold, occurring at the intersection of four peptide subunits and three-fold, occurring at the intersection of three peptide subunits. The two kinds of channels have different chemical properties, and hence perform different functions. Their size was evaluated to be about 3–5Å [11,12], but can vary upon the intramolecular dynamics of the subunits with corresponding rearrangement of the quaternary stricture. Moreover, some experimental results, showing differential accessibility for the solute molecules, support the idea of a "gate" in the pores regulating their opening and closing [13].

The iron in the ferritin core is stored as insoluble Fe(III) in a crystalline solid. Thus, in order for it 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 three-fold channels covered inside with hydrophilic side chain groups.

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

AAS is a method, used in many works for determination of the iron in the ferritins. The different authors found this amount to be between 1000 and 4500 atoms per molecule [1-6]. This encourages us to investigate the iron amount in the Horse Spleen Ferritin with atomic absorption spectroscopy.

2 Materials and Methods.

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

Instrument: Atomic absorption spectrophotometer "Perkin Elmer 3030" was used as the iron content determining apparatus. Its principal scheme is shown in Figure 1. In it a hollow cathode lamp is used as a primary source of monochromatic light with wavelength $\lambda = 248.3$ nm. The evaporating flame was an oxidizing acetylene/air flame. The all glassware used was class A.

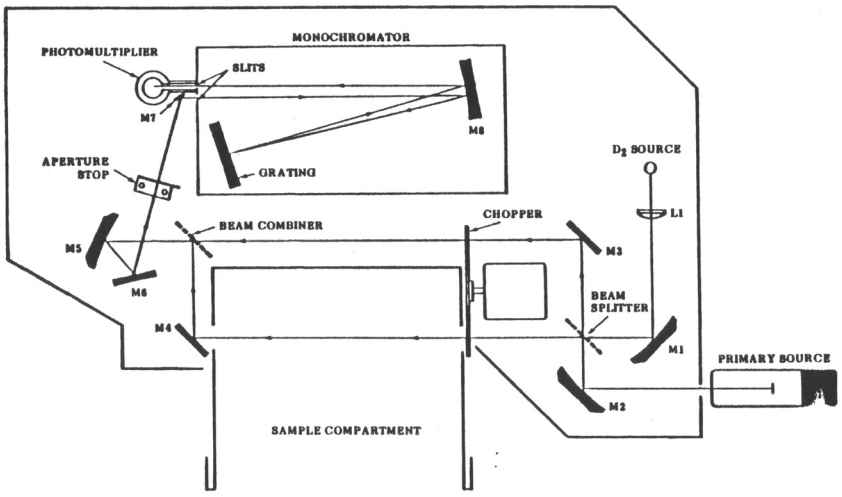

Figure 1. Scheme of Atomic Absorption Spectrometer "Perkin Elmer 3030".

Method: The preparation of the probe and the analysis were carried out according to 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 [14].

3 Results and Discussion

Two parallel probes were checked out. In both cases a precisely measured quantity of 0.1 mL ferritin solution was deposited in a quartz crucible. Its corresponding weight was measured in grams. The weight of the first and the second probe was 0.0925 g and 0.1010 g, respectively. The solution was heated at 60 ° (to visible dry state) with attention to avoid boiling. The drying procedure continued at 105° for 1 hour. In the next step the quartz crucible was carefully placed on an electrical heater and the substance was brought to proper degree of ash within 4 hours. After that it was placed in a muffle oven where it withstood at 440 ° for *ca*. 12 hours. The ash remnant was transferred in a measuring flask class A with a volume of 25 mL and was dissolved in 3M HCl. The obtained solution had a yellow-near colour and was clear, without opalescence. A quantity of 1 mL of this solution was diluted in a flask to 10 mL with bidistilled water. Then 2 mL of the solution was diluted again to 10 mL. Thus the overall dilution of the initial ferritin solution turned out to be 12500 times. This was the final solution for assessment of iron content in the atomic absorption spectrophotometer.

For each probe 10mL of the final solution was placed in the apparatus and measurements of 15 separate samples were done. The results for the first and the

Table 1. Measurements of 15 separate samples for first end second probe

	Sample	Probe 1 [mg/L]	Probe 2 [mg/L]
	1	1.61	1.70
	2	1.68	1.71
	3	1.61	1.74
	4	1.63	1.75
	5	1.63	1.71
	6	1.65	1.74
	7	1.61	1.75
	8	1.63	1.75
	9	1.62	1.78
	10	1.66	1.69
	11	1.69	1.67
	12	1.69	1.71
	13	1.71	1.76
	14	1.72	1.75
	15	1.70	1.78
Mean value M		1.6560	1.73267
Standard deviation SD		0.03924	0.0326
Standard error SE		0.01013	0.08842

second probe, along with their mean values (M), standard deviations (SD) and standard errors (SE) are shown in Table 1 and in Figures 2 and 3.

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

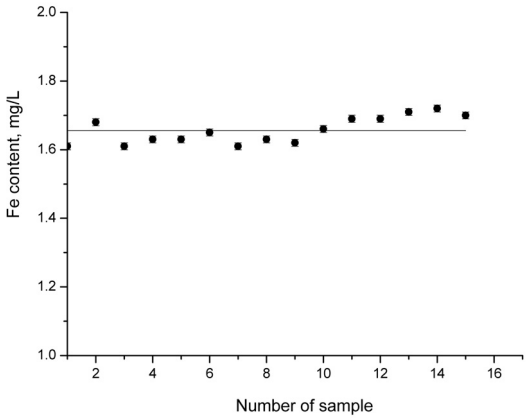

Figure 2. Fe contents of first probe.

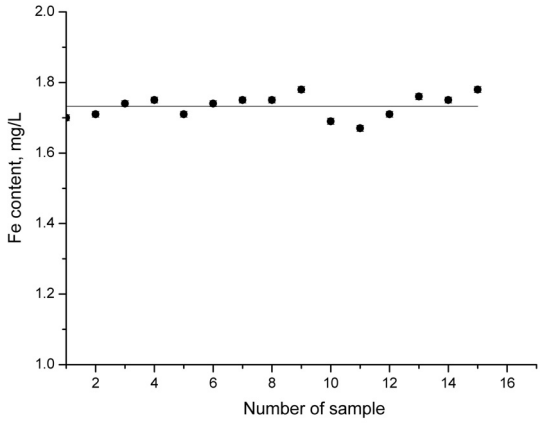

Figure 3. Fe contents of second probe.

Probe 1: (1.656 ± 0.01) mg/L $\times$ (0.1 mL/0.0925 g) $\times$ 12500 = (2.238 ± 0.0135)% (wt/wt)

Probe 2: (1.732 ± 0.008) mg/L $\times$ (0.1 mL/0.1010 g) $\times$ 12500 = (2.144 ± 0.0099)% (wt/wt)

The average from the two parallel assessments gives: (1.694 ± 0.009) mg/L $\times 12500 = (21.175 \pm 0.1125)$ g/L, or (2.191 ± 0.0117)% (wt/wt) iron in the initial solution of ferritin. The corresponding error, imposed by the procedure of manipulation of the probes, turned out to be insignificant in comparison with the error coming from the sensitivity of the apparatus (0.125% according to the manual). So, finally we found the iron content of (2.191 ± 0.125)% in the original solution offered by Merck.

On the other hand, based on the above results, we can calculate the molar concentration of the iron in this solution:

$$\begin{aligned}(1.694 \pm 0.009)\ \text{mg/L} \times 12500/56\ \text{g} &= (21.175 \pm 0.1125)\ \text{g/L}/56\ \text{g} \\ &= (0.378125 \pm 0.002)\ \text{M Fe.}\end{aligned}$$

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

$$100\ \text{gL}^{-1}/474000\ \text{gmol}^{-1} = 0.211\ \text{mM ferritin.}$$

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

$$0.378125\ \text{M Fe}/0.211\ \text{mM ferritin} = 1792.3125\ \text{atoms per molecule.}$$

The obtained result shows that in our case one molecule of ferritin is loaded with aprox. 1800 iron atoms. It should be said that this is well in agreement with the works of other investigators, leading specialists in this area, *e.g.* P.M. Harrison (1730–2000 atoms/molecule) and E. Theil (800–2000 atoms/molecule) [4,5]. This gives us motivation to claim that the use of the Bulgarian standard in the AAS study of iron in the ferritin or other significant proteins is a reliable tool for quantitative evaluations.

The use of the Bulgarian standard for investigation of the iron contents with the aim of AAS is an opportunity to study many other biological objects, connected with the iron processes.

References

[1] J. McKnight, N. White, G.R. Moore (1997) *J. Chem. Soc., Dalton Trans.* 4043-4045.
[2] Kyung S. Kim *et al*. (2000) *Inorg. Chim. Acta* **298** 107-111.
[3] J.L. Beard, J.W. Burton, E.C. Thiel (1996) *J. Nutrition* 154-160.
[4] N.D. Chasteen, E.C. Theil (1982) *J. Biol. Chem.* **257** 7672-7677.
[5] A. Treffry, P.M. Harrison (1978) *Biochem. J.* **171** 313-320.
[6] P.M. Harrison (1977) *Semin. Hematol*. **14** 55-70.
[7] E.C. Theil (2003) *J. Nutr.* **133** 1549S.
[8] X. Liu, E.C. Theil (2005) *Acc. Chem. Res*. **38** 167.
[9] K.J. Hintze, E.C. Theil (2006) *Cell. Mol. Life Sci*. **63** 591.
[10] L. Michaelis, C.D. Coryell, S.J. Granick (1943) *Biol. Chem*. **148** 463-480.
[11] D.W. Rice, G.C. Ford, J.L. White, J.M.A. Smith, P.M. Harrison (1983) *Adv. Inorg. Biochem*. **5** 39.
[12] G.C. Ford, P.M. Harrison, D.W. Rice, J.M.A. Smith, A. Treffry, J.L. White, J. Yariv (1984) *Philos. Trans. R. Soc. London Ser. B* **304** 551.
[13] X. Liu, W. Jin, E.C. Theil (2003) *Proc. Natl. Acad. Sci. USA* **100** 3653.
[14] 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