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Fluorescence

Protein quantification at low concentration using fluorescence and absorbance

Application Note
Biology/ Biochemistry
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Introduction

Transferrin is a glycoprotein with a molecular weight (MW) of 80 kDa, synthesized in the liver. Each transferrin protein can fix up to two iron atoms and distribute them to cells. The half-life of transferrin in humans is around eight days, while iron delivery via the transferrin/transferrin receptor cycle can be completed in approximately 5–20 min, depending on the cell type. In other words, each transferrin molecule may accomplish hundreds of cycles of iron binding and delivery to cells during its life span[1].

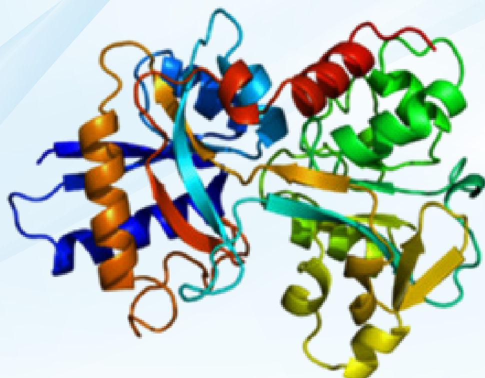

Figure 1: Transferrin 3D structure (PDB, RCSB)[2]

Measuring transferrin iron levels in serum is an important part of iron metabolism exploration. Abnormal levels of transferring in serum are also related to several diseases such as some cancers and other inflammatory syndromes[3].

In this application note, we highlight the high sensitivity of the Duetta for measuring transferrin at very low concentrations. The Duetta spectrofluorometer combines both fluorescence and absorbance measurements simultaneously, thus providing key advantages for protein analysis.

In parallel, we compare the fluorescence results obtained using the Duetta with absorbance results obtained with a dedicated low-volume, benchtop spectrophotometer.

Methods and results

Transferrin was prepared according to a concentration range from 50 µg/mL to 0.75 µg/mL in 10 mM PBS for fluorescence and absorbance measurements. The same buffer (without the protein) was used as blank.

The excitation wavelength was set to 250nm and the emission collected from 265 nm to 550 nm with 2 s integration time on the CCD and 10 nm slits.

Transferrin solutions from 0.75 µg/mL to 50 µg/mL were measured in absorbance mode. Below a comparison between absorbance spectra obtained on both systems for the highest concentrations.

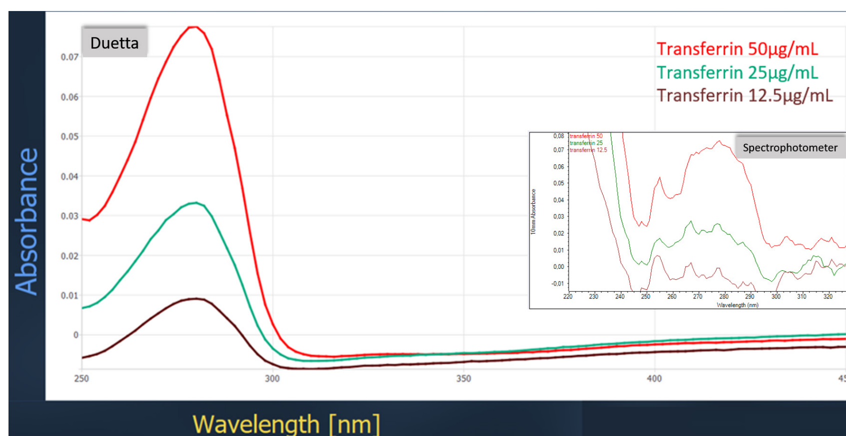

Figure 2: Comparison of Absorbance spectra between the instruments

On the Duetta, transferrin is detected at 12.5 µg/mL and the signal increases proportionally to the concentration. On the tested spectrophotometer, the limit of detection is around 25µg/mL with very noisy curves.

In parallel, fluorescence spectra of transferrin solutions were acquired according to the defined method. Figure 3 shows the overlaid fluorescence spectra.

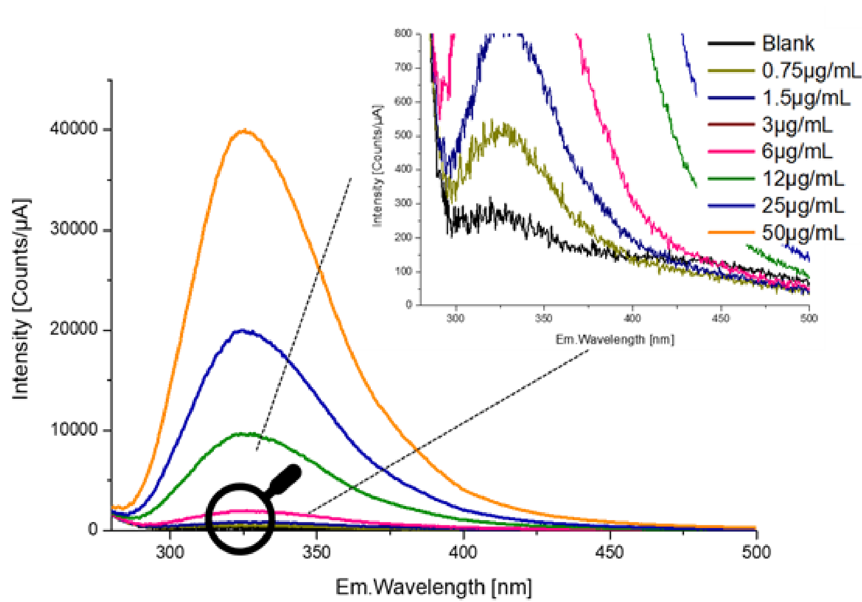

Figure 3: Fluorescence Emission spectra of transferrin at increasing concentrations (from 0.75 µg/ml to 50 µg/ml)

The zoom on the lowest concentrations points out a limit of detection around 0.75 µg/mL. Although the Duetta shows a twice higher sensitivity in measuring protein concentration through UV absorption, its unique performance is highlighted through fluorescence as shown below.

Figure 4 shows a comparison between the maximum intensities of emission spectra (Duetta) and the concentrations measured (spectrophotometer).

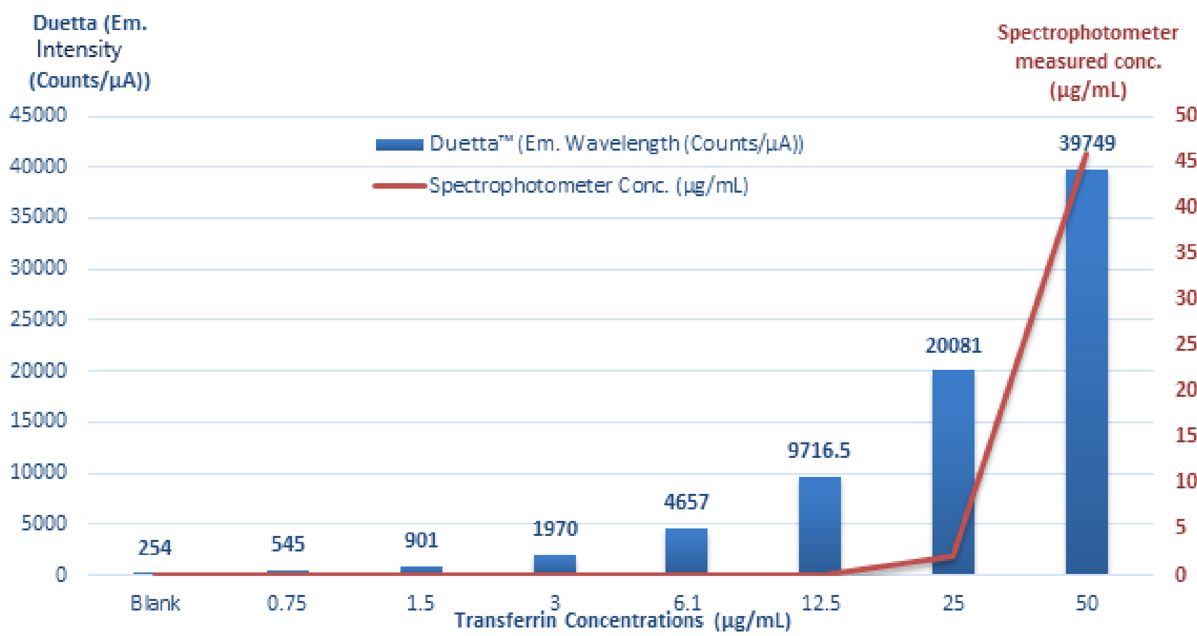

Figure 4: Comparison of Transferrin concentration measurements obtained with the spectrophotometer and Duetta instruments.

The graph shows a limit of detection of transferrin around 0.75µg/mL with the Duetta. The signal amplitude is directly proportional to the measured concentrations. In parallel, the lowest concentration detected using the spectrophotometer is 25 µg/mL, which is 33 times higher than the LOD observed with the Duetta. This confirms the sensitivity of fluorescence spectroscopy using Duetta.

Conclusion

Fluorescence spectroscopy has many applications in life sciences from cell biology to genomics. In protein characterization, intrinsic fluorescence of some amino acids (tryptophan, tyrosine, and phenylalanine) is a valuable feature that allows protein study without labelling.

In this application note, we show the high sensitivity of the Duetta through fluorescence measurements compared to classical UV-absorbance approach. While the Duetta's limit of detection is around 25 µg/mL using absorbance, the Duetta was able to detect transferrin from 0.75 µg/mL using fluorescence, which is 33 times lower LOD with the applied parameters. However, this LOD can be further improved by optimizing some parameters: increasing the integration time, opening the slits and increasing the Emission increment (binning). In summary, Duetta is capable of measuring both UV-visible absorbance spectra and fluorescence spectra and is more sensitive than other benchtop spectrophotometers which are solely used for absorbance measurements. The dual purpose and optimal optical layout are ideal for protein spectroscopy and low concentration solutions.

References

[1] Encyclopedia of Signaling Molecules, 2018 Edition Editors: Sangdun Choi, https://doi.org/10.1007/978-3-319-67199-4_101940

[2] Two high-resolution crystal structures of the recombinant N-lobe of human transferrin reveal a structural change implicated in iron release. *MacGillivray, R.T., Moore, S.A., Chen, J., Anderson, B.F., Baker, H., Luo, Y., Bewley, M., Smith, C.A., Murphy, M.E., Wang, Y., Mason, A.B., Woodworth, R.C., Brayer, G.D., Baker, E.N.*

[3] Gkouvatsos, K. (2012). Regulation of iron transport and the role of transferrin. Biochimica et Biophysica Acta, 1820 (2012) 188–202

JOBIN YVON Technology

info.sci@horiba.com www.horiba.com/scientific

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USA: +1 732 494 8660 **France:** +33 (0)1 69 74 72 00 **Germany:** +49 (0) 6251 8475 20
UK: +44 (0)1604 542 500 **Italy:** +39 06 51 59 22 1 **Japan:** +81 (0)3 6206 4721
China: +86 (0)21 6289 6060 **Brazil:** + 55 (0)11 2923 5400 **Other:** +1 732 494 8660