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Application note

Quantitation of low Tryptophan and Tyrosine peptides using the Fluidity One

UV absorbance is commonly used as a fast method for protein and peptide quantitation, however samples must contain tryptophan (Trp) or tyrosine (Tyr) residues to be detected. Here we demonstrate the ability of the Fluidity One to assess the concentration of a range of samples which have low or no Trp and Tyr content. We show that the results obtained on the Fluidity One are reliable to low concentrations and are consistent with UV absorbance and amino acid analysis.

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Introduction

While UV absorbance presents a quick and simple method for the quantitation of proteins and peptides, the sequence must contain Trp or Tyr residues for detection at 280 nm. Although the A205 method presents an alternative, this has its own limitations in terms of buffer absorbance (1).

The Fluidity One offers an alternative method for measuring concentration in a simple benchtop test. Following Microfluidic Diffusional Sizing (MDS), an OPA based fluorogenic dye is used for detection (2). The dye is amine reactive, labelling lysine residues and unprotected N-termini, making it suitable for samples with low or no Trp and Tyr content.

Here we assess the ability of the Fluidity One to accurately quantify samples down to low concentrations by MDS and compare the results to UV absorbance (Nanodrop A205) and amino acid analysis (AAA).

Methods

Four peptides with low or no Trp content were prepared and quantified by AAA, A205 and MDS on the Fluidity One. The sequences and buffer conditions for each species are detailed in the appendix.

The original (highest) concentration of each peptide was assessed by all three methods. Concentration series were then prepared by serial 2-fold dilutions, giving six samples of decreasing concentration for each peptide. These dilutions were each assessed by Nanodrop A205 and Fluidity One only.

To benchmark the results from the other methods against AAA, the highest concentration result of each peptide was normalized to equal the AAA result, then the further five dilution results were corrected by the same factor. For the MDS results obtained on the Fluidity One this calibration factor is stored once in the instrument and is then available for repeat use indefinitely.

Results

Figure 1 displays the results from MDS and A205 analysis for each peptide across the dilution series.
The linear $x = y$ relationship shows the “ideal” response, where the loaded concentration (according to AAA) is exactly equal to the measured concentration.

In the α-synuclein data we see that both techniques provide a linear and comparable response to each other and AAA. However for the other three peptides we observe that the MDS results remain closer to the ideal response to lower concentrations than the A205 analysis.

We also observe smaller standard deviations throughout the MDS data. This suggests that, for the samples measured, MDS offers a more precise method of analysis than A205 – especially at low concentrations.

It should be noted that MDS analysis on the Fluidity One also measures size (hydrodynamic radius, R_h) from the same 5 µL sample during the same 8-minute test. The sizes of each peptide as measured by MDS are shown in Figure 2.

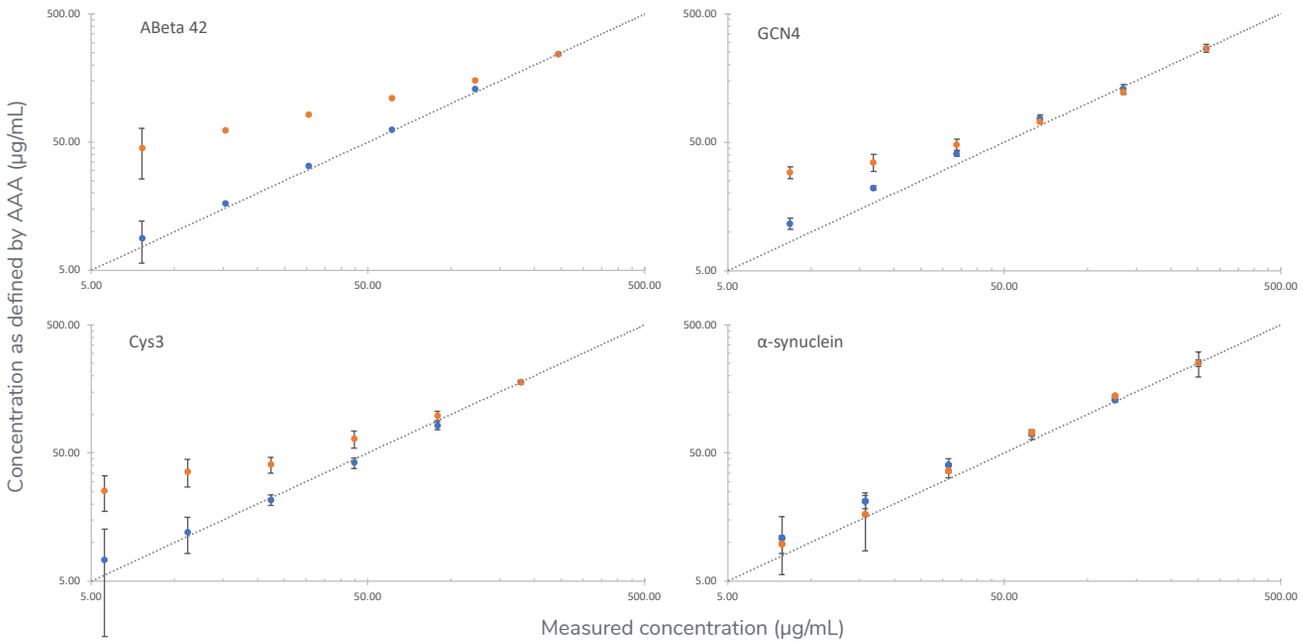
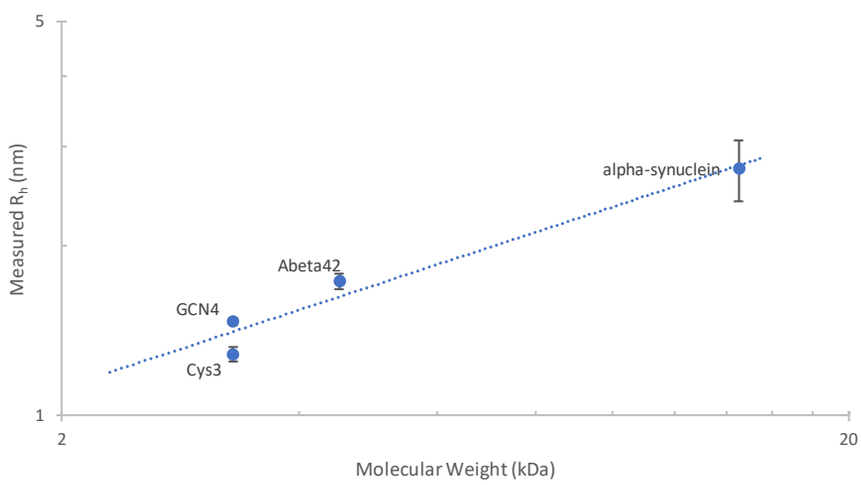


Figure 1: Results of MDS and A205 analysis on low Tyr and Trp peptide dilution series.
Blue points – MDS Fluidity One data, **Orange** points – A205 Nanodrop data, dotted line = ideal response ($x = y$)

Figure 2: Hydrodynamic Radius (R_h) as measured during the MDS Fluidity One analyses.

The dotted line shows the empirically observed relationship between molecular weight and hydrodynamic radius described in our previous work (3).

The proximity of these peptides to the line indicates that they are well folded in solution.



Conclusion

MDS and UV A205 analysis were each assessed for their ability to accurately quantify low Trp and Tyr peptides. The results obtained were benchmarked against the “gold standard” technique of AAA.

We observe that MDS analysis on a Fluidity One provides concentration data significantly closer to the predicted than A205 analysis, with both methods calibrated against an AAA tested sample. We also find lower variation across repeat measurements in the MDS Fluidity One data.

Alongside the higher precision quantification produced by MDS, the quality measure of hydrodynamic radius is also measured. This enables researchers to rapidly and with little sample loss have confidence in both the concentration and quality of samples prior to subsequent downstream analysis.

This shows that MDS analysis offers a compelling alternative to A205, providing quantification at higher accuracy and precision, as well as providing size measurement in the same test.

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References

1. Thermo Fisher Scientific Ltd, “Using the NanoDrop One to Quantify Protein and Peptide Preparations at 205nm”, Application note 52774

2. Fluidic Analytics Ltd. “What is Microfluidic Diffusional Sizing?” [online] https://www.fluidic.com/resources/faq/what-microfluidic-diffusional-sizing/ - accessed October 2018

3. Fluidic Analytics Ltd. “Protein Size as an Indication of Structure” document AP006-1 available online https://www.fluidic.com/resources/protein-size-indication-structure/

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Appendix

Peptide Name	Buffer Conditions	Sequence
ABeta 42	20 mM sodium phosphate, pH 8 with 0.2 mM EDTA	MDAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGV-VIA
Alpha-synuclein	PBS, pH 7.4	MDVFMKGLSKAKEGVVAAAEKTKQGVAEAAGKTKEG-VLYVGSKTKEGVVHGVATVAEKTKEQVTNVGGAVVTG-VTAVAQKTVEGAGSIAAATGFVKKDQLGKNEEGAPQEGILED-MPVDPDNEAYEMPSEEGYQDYEPEA
GCN4	MES, pH 6.4 with 150 mM NaCl	DPAALKRARNTEAARRSRARKLQRMKQL
Cys3	MES, pH 6.4 with 150 mM NaCl	AAEEDKRKRNTAASARFRIKKKQREQAL

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