FLOW INDUCED DISPERSION ANALYSIS (FIDA) AS A NOVEL TECHNOLOGY FOR PROTEIN QUANTIFICATION IN PLASMA AND FOR PROBING IMMUNE RESPONSES IN PATIENTS

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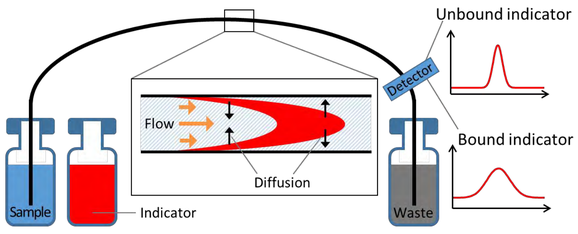
Introduction

Flow Induced Dispersion Analysis (FIDA) is a new method for quantification of proteins and affinity constants. In the FIDA assay the size of a small ligand (indicator) known to bind the protein (analyte) is measured. When the indicator is bound by the analyte the apparent size increases and this change in size can be used to estimate the concentration of protein in the sample.

Method

The automated FIDA protocol is performed using dedicated equipment. The inlet vials contain sample, indicator solution and washing solutions. A hydrodynamic flow is employed giving rise to a parabolic flow profile (insert). The dispersion of the indicator measured at the detector increases if antibodies in the sample have bound the indicator.



The FIDA assay is fully automated with the use of dedicated equipment. Fluorescence detection is used due to the high sensitivity as well as the ability to selectively detect the analyte even in complex sample matrices such as human blood plasma.

Aknowledgements

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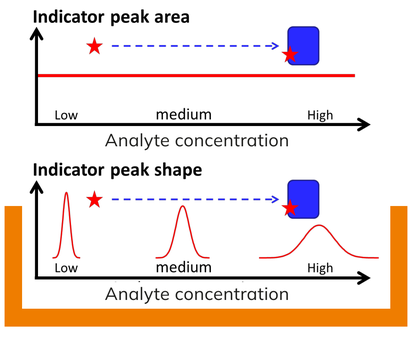
References

Poulsen NN, Andersen NZ, Østergaard J, Zhuang G, Petersen NJ, Jensen H. Flow Induced Dispersion Analysis Rapidly Quantifies Proteins in Human Plasma Samples. The Analyst. 2015;140(13):4365–9

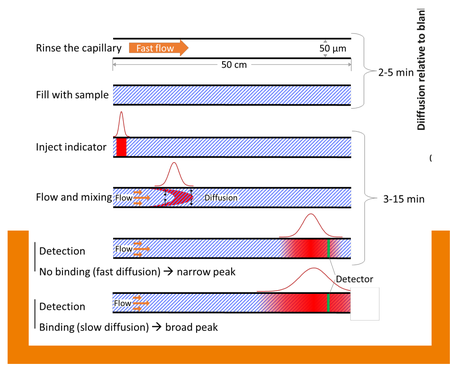
Jensen H, Østergaard J. Flow Induced Dispersion Analysis Quantifies Noncovalent Interactions in Nanoliter Samples. J Am Chem Soc. 2010 Mar 31;132(12):4070–1.

Results

1 In the automated FIDA assay the same indicator peak area is always detected at all analyte concentrations. The indicator peak shape does vary with analyte concentration. This is due to a change in apparent size and thus diffusivity of the indicator when it is bound by the analyte.



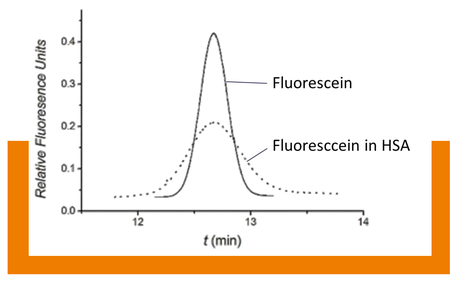
2 The steps of the FIDA assay are given here. Initially he capillary is rinsed with buffer at high speed in order to clean the inner capillary surface. Next the sample is introduced. The a narrow indicator zone is injected. A parabolic flow profile is obtained by a pressure gradient. The indicator is spread over a broader area due to the parabolic flow profile and the radial diffusivity. Finally the indicator is detected at the detector. The width of the peak holds information on the analyte concentration in the sample.



3 Two sample Taylorgrams showing fluorescein in buffer and HSA solution respectively. In this example mixing is done inside the capillary following injection due to the radial diffusion. The apparent diffusivity of fluorescein is calculated from the simple TDA equation:

$$D = \frac{a^2}{24\sigma^2} t_R$$

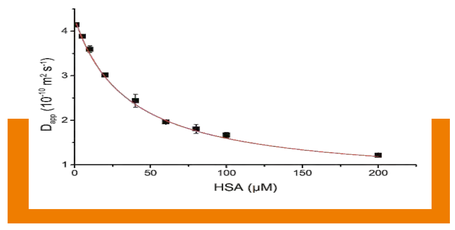
where D is diffusivity, a is capillary radius, t_R is the peak appearence time and σ^2 is the peak variance. Using this method diffusivities can be determined with a few percent deviation.



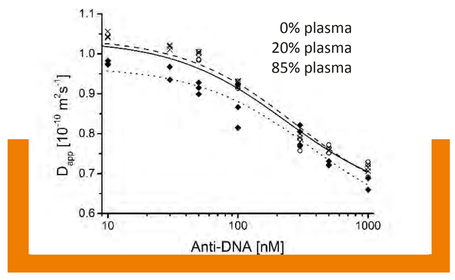
4 Diffusivity of fluorescein as a function of HSA concentration. This standard curve has been used to estimate he concentration of HSA in human blood plasma samples with a standard deviation below 7%. The standard curve has been fitted by the following binding isotherm:

$$D_{app} = \frac{D_I^- - D_{IA}}{[A]K + 1} + D_{IA}^-$$

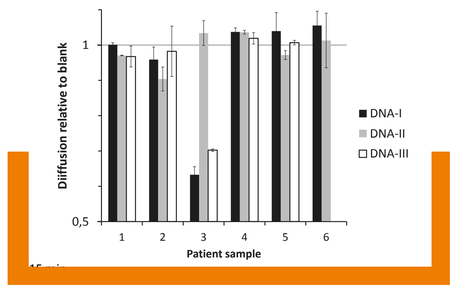
where D_{app} is the apparent indicator diffusivity, D_I is indicator diffusivity, D_{IA} is the diffusivity of the indicator-analyte complex, and K is the binding constant.



5 Apparent diffusivity of a 32-bp DNA sequence as a function of anti-DNA antibody concentration. Data have been obtained in 0%, 20% and 85% human blood plasma spiked with the model antibody. After correcting the observed diffusivities for changes in the viscosity only minor deviation is observed between the standard curves.



6 Relative diffusivity of three short DNA sequences in plasma samples from six Systemic Lupus Erythematous (SLE) patients. SLE is an autoimmune disease characterized by development of antibodies against double stranded DNA among other things. These results indicate that the antibodies developed by the patients are directed against specific DNA sequences rather than double stranded DNA in general.



Conclusions

The FIDA based methodology is a new approach for autoantibody detection. In SLE it holds promise for being used for patient stratification and monitoring of disease activity. Future applications include therapeutic drug monitoring and immunogenicity testing.