Capillary Electrophoresis as a Powerful Tool in Therapeutic Protein Production Analytics

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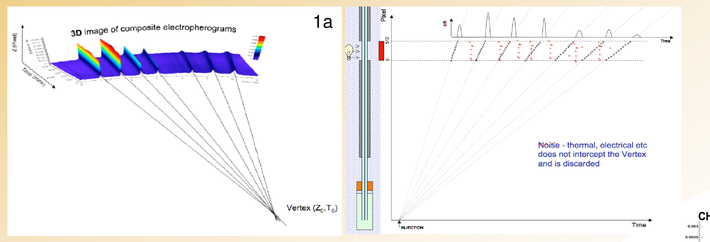
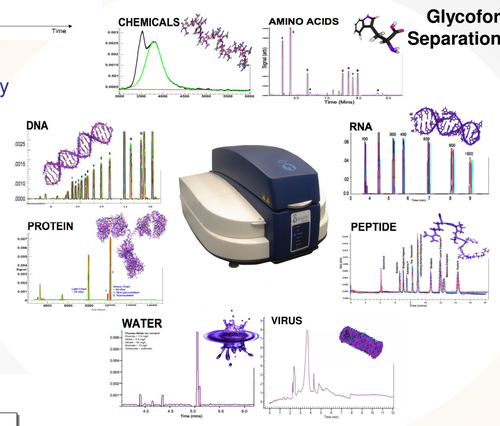
Capillary Electrophoresis is a powerful and versatile analytical tool that can be used to address many aspects of bio-processing analytics. deltaDOT's High Performance Capillary Electrophoresis (HPCE) system benefits from its core Label Free Intrinsic Imaging technology (LFII® system), which allows improved resolution, quantification and reproducibility.

Label Free Intrinsic Imaging (LFII®) is a form of analysis which removes a whole class of systematic errors. It is our ability to track an entity across multiple pixel detectors, in the form of a photo diode array, and correlate the space/time data that gives LFII® its power. As the analytes move across the detection window, a three dimensional view of the separation, where the axes are distance, time and absorption, is generated. The 3D image tracks the analyte as it migrates past the detector. The slope of the track generated is used to calculate the velocity of the analyte.. Extrapolation of analyte bands back to their injection point is described in Figures 1a and b.

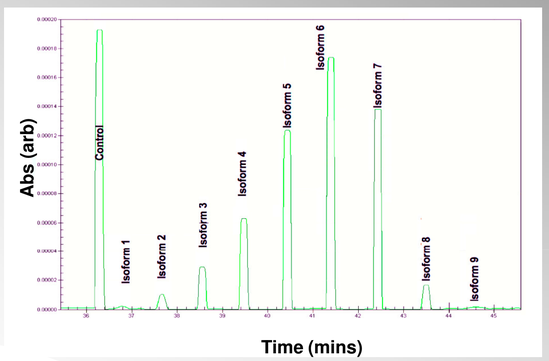
Any noise in the system, such as lamp fluctuations or bubbles will not hit the vertex (or injection time) and be ignored. The height or intensity of the peak directly correlates to the amount of analyte in the band(Figure 1a), allowing very accurate quantification.



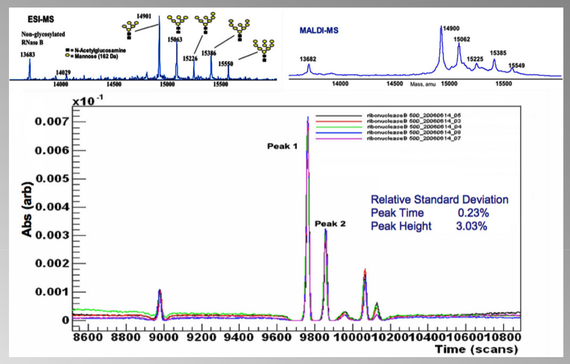
Advantages of LFII® in Capillary Electrophoresis over traditional techniques include:
Accuracy and Precision / **Efficient** separation / High **resolution** / **Rapid** analysis / direct on-capillary **Quantitation** of **unlabelled** molecules/ **Economy** (small sample and reagents requirement) and **Automation**.
It also provides a **Versatile** analytical platform where multiple parameters of the biopharmaceutical can be analyzed.



PTMs / Glycoform Analysis

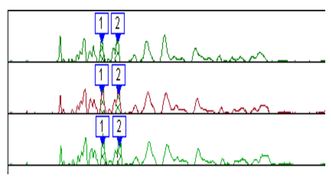


Glycoform analysis of Human Erythropoietin. Separation of the clinical significant 8 glycoforms are shown.



Ribonuclease B Glycoform analysis. Resolution of deltaDOT's data is comparable to mass spectrometry data

Yield / Batch Consistency / Stability Analysis -

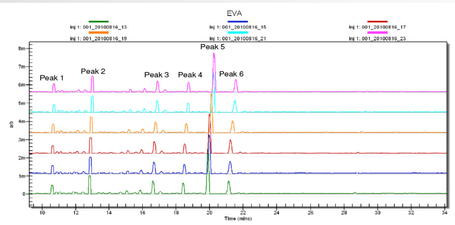


Precision ~ 30% RSD *
Poor precision leads to poor quality control

Quantification accuracy affected by coomasie dye-protein binding dynamics.

* http://www.bio-rad.com/webroot/web/pdf/lsr/literature/Bulletin_5782B.pdf

Traditional SDS-PAGE Densitometry data suffers from poor resolution and precision



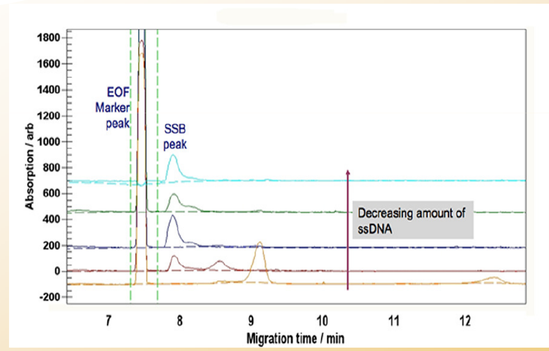
	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6
Relative Std (%) – Migration time	0.43	0.52	0.64	0.69	0.73	0.78
Relative Std (%) – peak area	6.34	5.75	6.95	6.04	6.86	3.03

Precision ~ 5% RSD% ensures thorough quality control

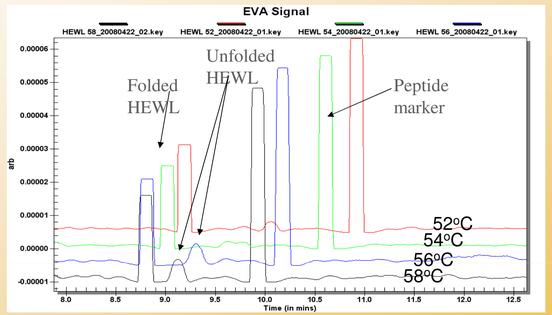
deltaDOT SDS-CGE data allows direct quantification

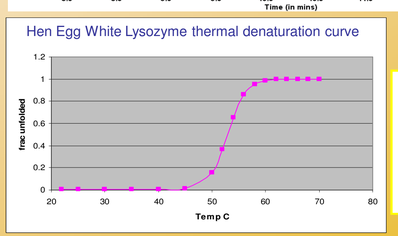
Batch-to-batch consistency and stability can be assessed based on peak area of interest

Denaturation / Affinity Analysis



Single-stranded DNA binding protein (SSB) – DNA binding data . Kinetic parameters of the binding event can be derived





Based on this thermal unfolding curve,
Tm is estimated to be 53.0˚C at pH2
ΔH = 118.4kCal/Mol
ΔS = 363cal/K.mol

Thermal denaturation data of Hen Egg White Lysozyme (HEWL) . Thermodynamic parameters of the unfolding event can be derived