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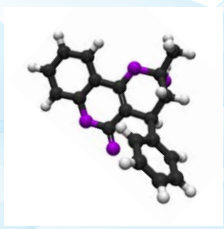
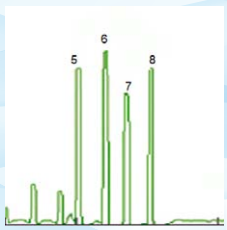
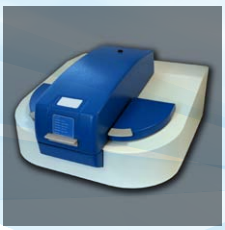
Capillary Electrophoresis







Cell Lysate Analysis

Analysis of *E. Coli* Lysate

with deltaDOT Technology

Abstract

Analytical instruments with the capability of analysing complex mixtures of bio-molecules with high precision, excellent reproducibility and quantitative indications are highly sought-after in fields ranging from routine quality control procedures in bio-molecule production, to biomarker discovery and diagnostics.

This application note describes the high resolution separation and subsequent molecular weight characterisation of four of the most abundant proteins in *E.coli* cell lysate, using the HPCE-512 system. Its ability to produce high quality reproducible data in terms of peak mobility (<1% RSD) and peak area (<5% RSD) was also demonstrated.

Introduction

The analysis of cell lysate samples is required to identify the expression of protein products, their molecular weights, quantity and purity. Another routine procedure is the analysis of up and down regulation of proteins as a result of cells being challenged during growth. Other applications include the identification of potential biomarkers of drug toxicity, disease state or predisposition to disease.

The analysis of complex protein mixtures, such as lysates, often requires the use of fluorescent or chemical labels, requiring in turn dedicated experimental design. The analysis of proteins with these bulky labels can cause changes in their characteristics, such as deformation or mobility, leading to additional errors in the characterisation process.

The experiments demonstrate the capability of the HPCE-512 for label-free analysis of complex protein mixtures from crude cell lysates, using just nanolitres of sample.

Materials & Methods

The protein marker ladder and *E. coli* samples were diluted in Beckman sample buffer, water and ß-mercaptoethanol, heated at 95 °C for 5 minutes and cooled over ice to room temperature. After micro-centrifuging for 2 minutes to de-gas, they were transferred to vials and loaded on the HPCE carousel.

Separation by electrophoresis was performed in a Beckman run buffer. The buffer was passed through a 0.2 mm filter and degassed under vacuum before use. The samples were injected electrokinetically at 5 kV for 15 seconds and then run at a voltage of 16 kV. Separations were carried out in bare fused silica capillaries of 50 µm internal diameter and a separation length of 22 cm. Total length of capillary was 34 cm.

During the separations the instrument was held at 25°C. Between runs the capillary was rinsed with a proprietary methodology, followed by sterile water for 2 minutes and run buffer for 10 minutes. After this, the continuity was checked. All detection was at 214 nm with a ±7 nm bandwidth.

Results & Discussion

Analysis of the separations was performed using the Equiphase Vertexing Algorithm (EVA) and General Separation Transform (GST) algorithm. GST is a method of combining the data from the 512 pixels in a natural way which preserves the peak shape information of the electropherograms while at the same time maximizing the signal-to-noise ratio. An increase in signal-to-noise by a factor of 10 is typically achieved using GST as compared to single electropherograms (RAW). EVA is a technique which optimises resolution and – in an unbiased way – establishes quantification analysis from peak height rather than area, and maximises signal-to-noise.

The single pixel RAW electropherograms, GST- and EVA- processed data for the protein ladder are shown in **Figure 1**. Cross-referencing between EVA, GST and RAW may be used to optimise the interpretation of the data collected from the separation.

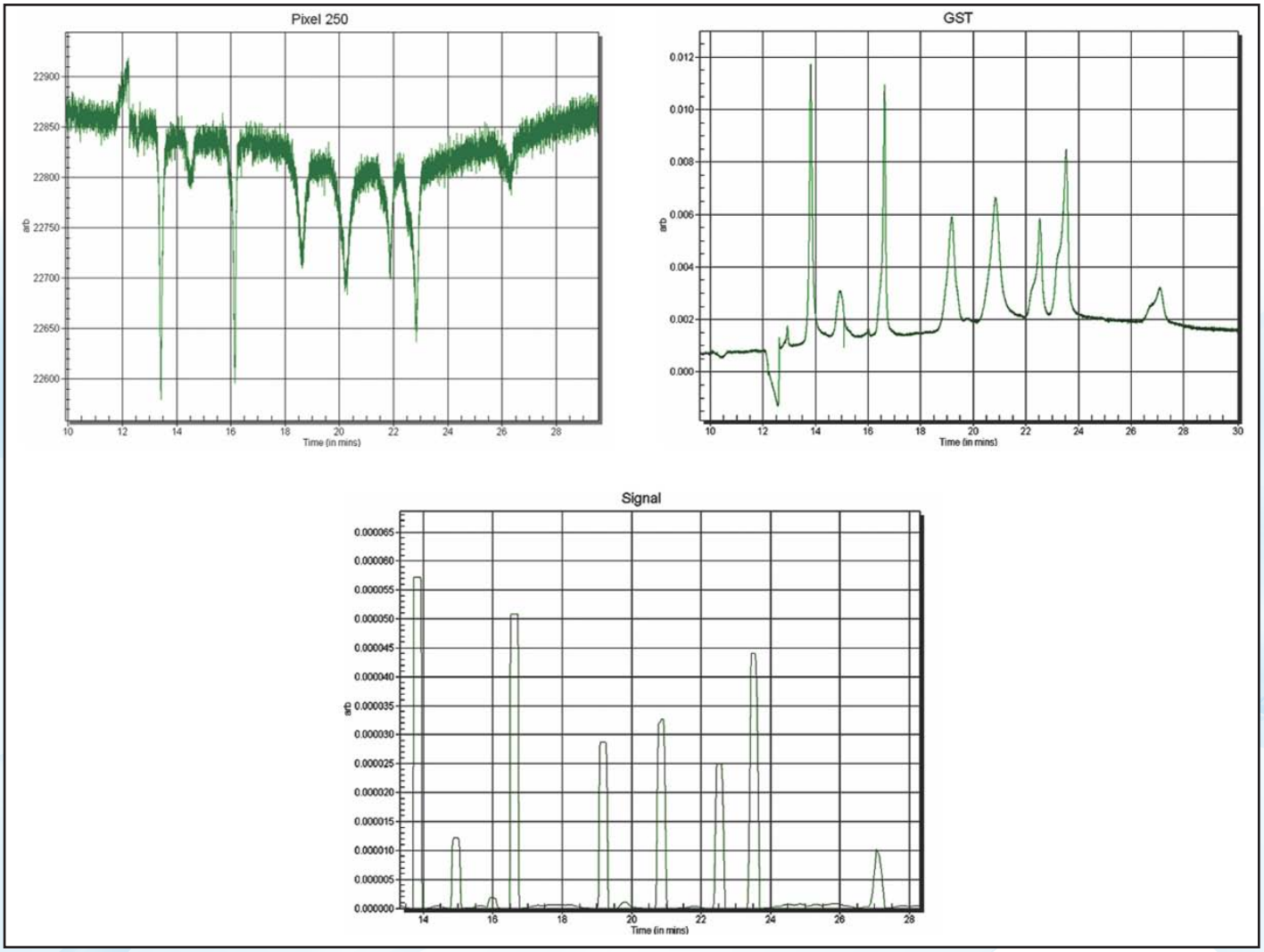


Figure 1: *Comparison of RAW, GST and EVA data for protein marker ladder.*

A standard protein marker ladder consisting of lysozyme (14.4 kDa), trypsin inhibitor (21.5 kDa), carbonic anhydrase (31 kDa), ovalbumin (45 kDa), serum albumin (66.2 kDa), phosphorylase B (97 kDa), ß galactosidase (116 kDa) and myosin (200 kDa) was analysed to show reproducibility of the HPCE system (see **Table 1** & **Table 2**).

	mean	std dev	% RSD
lysozyme	13.84	0.044	0.32
trypsin inhibitor	14.93	0.034	0.23
carbonic anhydrase	16.62	0.058	0.35
ovalbumin	19.18	0.077	0.40
serum albumin	20.84	0.062	0.30
phosphorylase B	22.52	0.108	0.48
ß galactosidase	23.51	0.114	0.49
myosin	27.06	0.156	0.57

Table 1: *Reproducibility of Peak Migration Times for protein marker ladder n=10.*

	mean	std dev	% RSD
lysozyme	0.0079	0.00037	4.6
trypsin inhibitor	0.00182	0.00005	2.8
carbonic anhydrase	0.00734	0.00012	1.7
ovalbumin	0.00416	0.00007	1.7
serum albumin	0.00457	0.00013	3.0
phosphorylase B	0.00363	0.00006	1.7
ß galactosidase	0.00643	0.00014	2.2
myosin	0.00146	0.00003	1.9

Table 2: *Reproducibility of Peak Area Data for protein marker ladder n=10.*

Data from a protein ladder separation was used to create a calibration curve (see **Figure 2**), that was used for the determination of the molecular weights of four of the most abundant proteins in the *E.coli* cell lysate. The excellent reproducibility depends crucially on system stability – which is known to be excellent given the sophisticated thermal management systems in place.

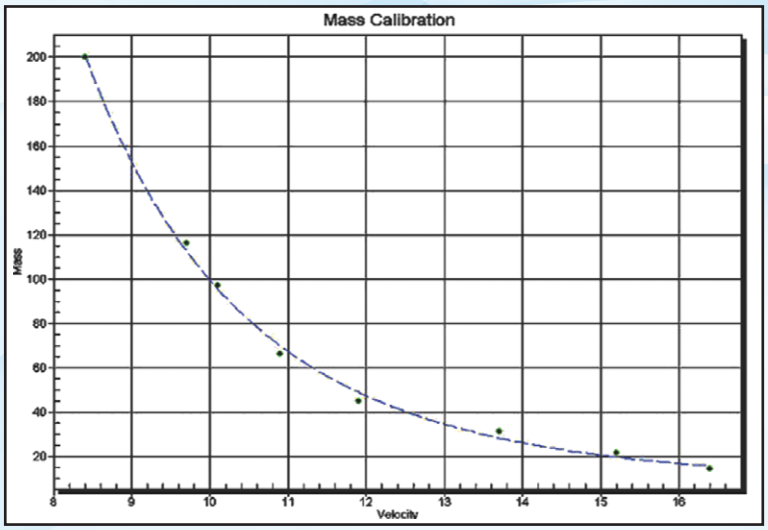


Figure 2: *Protein Marker Ladder Calibration curve.*

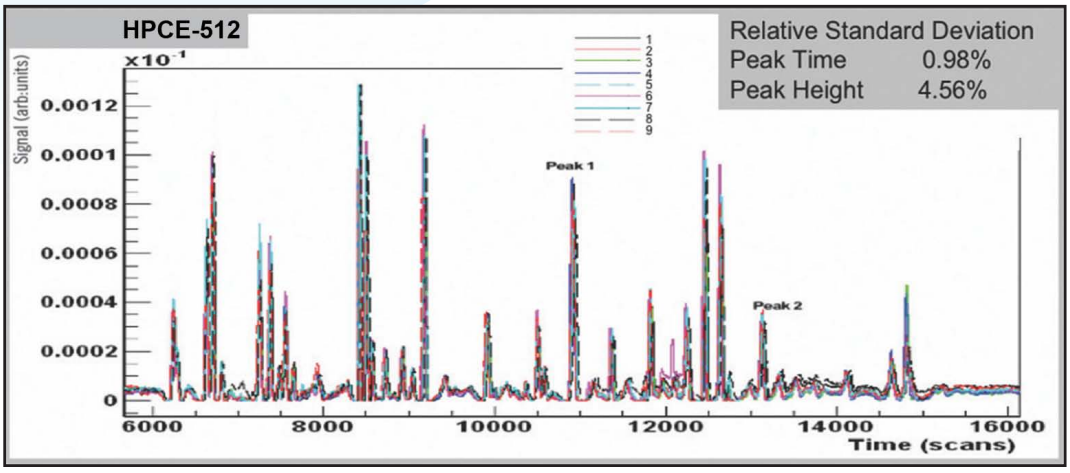


Figure 3: *Nine runs on a cell lysate sample showing excellent HPCE-512 reproducibility.*

The four major peaks chosen at migration times 13.40, 17.19, 19.54 and 19.83 minutes were determined to have molecular masses of 14.4, 32.7, 54.4 and 57.8 kDa.

To determine the reproducibility of the separation the protein ladder and *E.coli* lysates were consecutively separated six times. The analysed data was then represented in a 'mirror plot' (**Figure 4**), which shows the six *E.coli* lysate separations compared to six protein ladder separations.

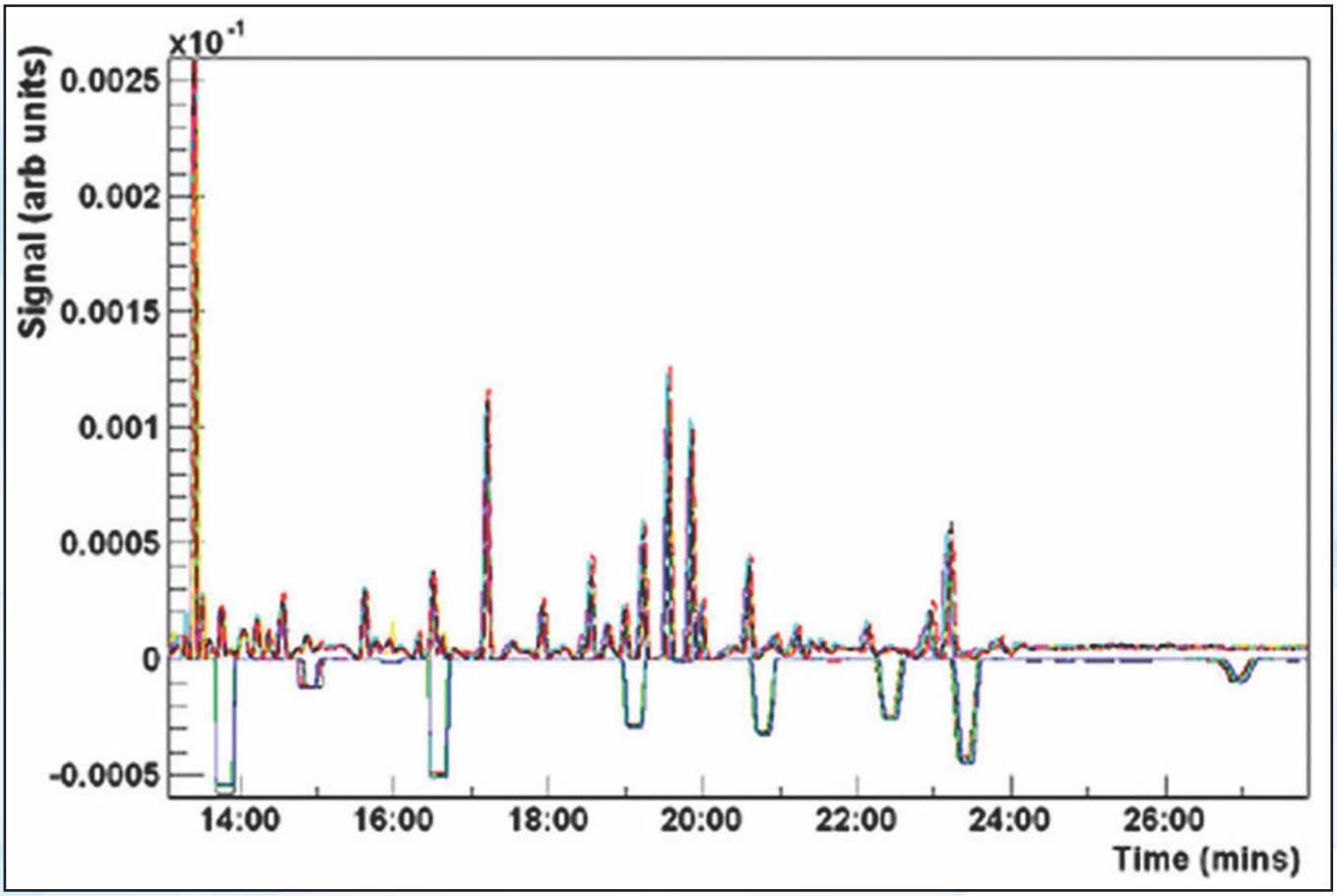


Figure 3: *Overlay of EVA processed data for recombinant human EPO (N=10). The peaks migrating in front of the EPO peaks are solvent fronts associated with the buffer.*

Further analysis of the six runs (**Table 3** and **Table 4**) determined that the peak migration times vary <0.1 % and the peak area varies <3%.

Mass/kDa	Mean migration (minutes)	std dev	% RSD
14.4	13.41	0.05272	0.03932
32.6	17.2	0.01045	0.06078
54.4	19.86	0.01497	0.07538

Table 3: *Reproducibility of migrations times of selected* E.coli *lysate peaks n=6.*

Mass/kDa	Mean peak area	std dev	% RSD
14.4	0.003924	8.82E-05	2.247
32.6	0.00425	4.78E-05	1.125
56.1	0.004043	6.53E-05	1.616

Table 4: *Reproducibility of peak heights of selected* E.coli *lysate peaks n=6.*

Conclusion

The HPCE-512 was successfully used to determine the molecular weights of three of the most abundant proteins in *E.coli* lysate (14.4, 32.6 and 56.1 kDa) and the corresponding quantities being expressed.

The HPCE showed robust, high quality reproducible data in terms of molecular weight determination (<1% RSD) and quantitation (<3% RSD) making it an ideal instrument for the analysis of cell lysates.

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