

# Challenges In The Measurement Of Protein Electrophoretic Mobility

Paul Clarke, Mark Potheary, Ciaran Murphy, Stephen Ball, Lisa Newey-Keane

Malvern Instruments Ltd. Grovewood Road, Enigma Business Park, Malvern

## ABSTRACT

Dynamic Light Scattering (DLS) is a widely implemented technique for characterization of proteins and their formulations that allows the determination of molecular size and protein stability, while Electrophoretic Light Scattering (ELS) can be used to measure protein mobility, which has been identified as a promising indicator of formulation stability, viscosity and behaviour.

Measurement of electrophoretic mobility requires the application of an electric field to the sample, which can stimulate aggregation that could reduce the reliability of the measurement. A new DLS/ELS measurement technique, the Diffusion Barrier Method, will be described that protects the protein by isolating it from the cell electrodes, which ensures that the measured electrophoretic mobility is that of the protein of interest rather than aggregates that have formed during the measurement [1].

**Keywords:** Protein mobility, dynamic light scattering, formulations, stability, diffusion barrier method

## THE ZETASIZER NANO ZSP

The Zetasizer Nano ZSP is a new, significantly more sensitive, addition to the highly successful Zetasizer Nano range, designed to measure protein mobility more accurately than ever before. The instrument provides a tenfold increase in sensitivity in the measurement of electrophoretic mobility as well as doubling the sizing sensitivity in backscatter.

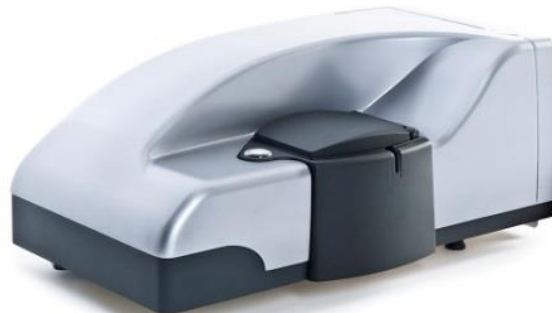


Figure 1. The Zetasizer Nano ZSP

The sample size distribution is monitored as part of the electrophoresis measurement sequence through the same optical detection channel as the electrophoretic mobility, thereby significantly increasing the sensitivity of aggregate detection. A new highly optimised [1] measurement structure is also included in the new software, removing uncertainties associated with Joule heating and sample degradation. These developments, together with Malvern's Diffusion Barrier Technique [2], ensure that the Zetasizer ZSP measures only the mobility of only the protein.

## 'STANDARD' LASER DOPPLER ELECTROPHORESIS OF PROTEINS

During a 'standard' measurement of electrophoresis, the sample fills the cell, the field is activated, and the

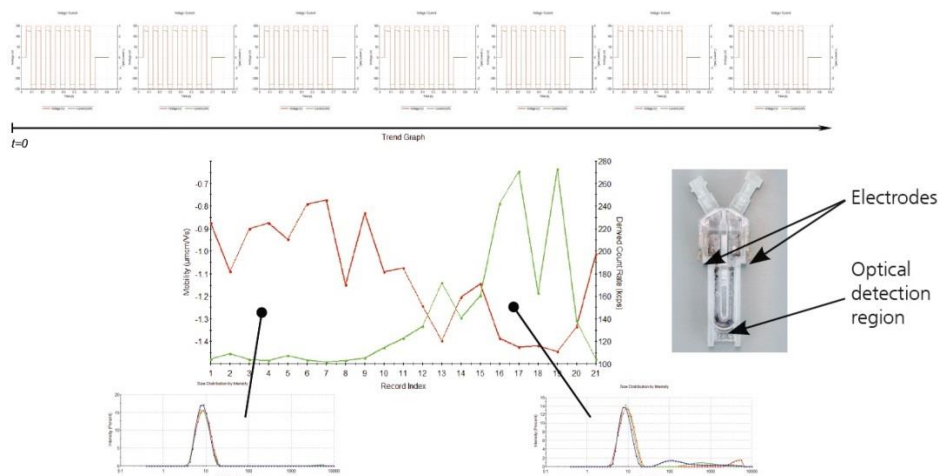


Figure 2: Sample aggregation, detection and the difference in electrophoretic mobility between a protein and its aggregate.

electrophoretic motion of the proteins is measured using PALS [3]. In delicate samples, aggregates are immediately created at the electrodes even at extremely low voltages [1] and due to the integration times required for such weakly scattering particles, aggregates can migrate into the optical detection region of the cell, leading to measurement of the aggregates' electrophoretic mobility, rather than that of the native proteins. As Figure 2 shows, significant differences exist between a protein and its aggregates [1]. Aggregate prevention and/or detection is therefore of central importance.

Further to this, since the field needs to be applied for extended periods in order to increase the signal to noise ratio, Joule heating of the sample will also occur. Figure 3 shows the increase in conductivity of phosphate buffered saline at various field strengths and the associated increase in sample temperature. Since the mobility is calculated directly from the sample viscosity this temperature increase, if not monitored or removed, creates a substantial systematic uncertainty in the electrophoretic mobility measurement.

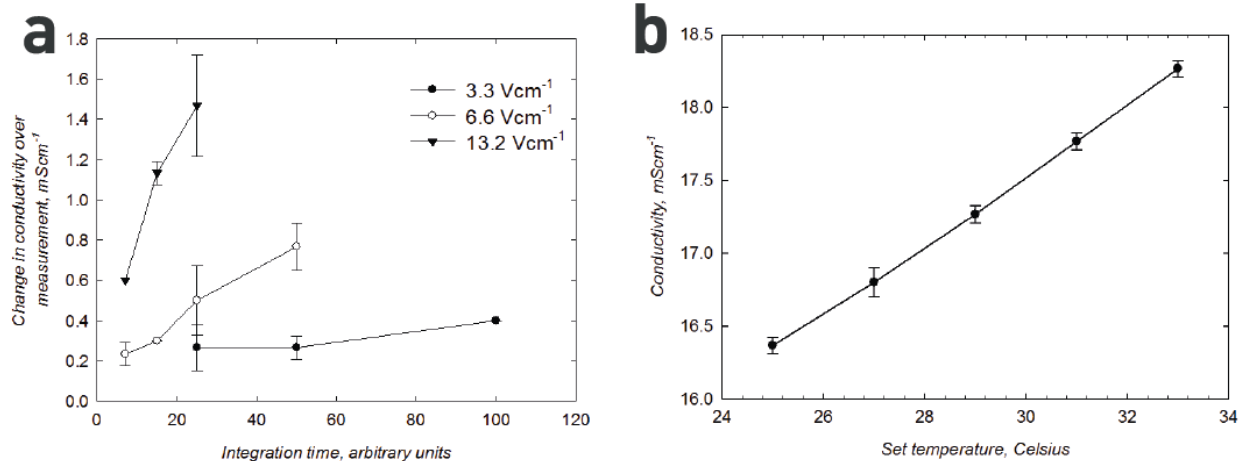


Figure 3: (a) The increase in conductivity of phosphate buffered saline with integration time; (b) Associated increase in sample temperature.

### ZSP: NEW OPTIMISED MEASUREMENT PROTOCOL

The new 'Protein Mobility' measurement sequence is shown in Figure 4. After a short delay to enable thermal equilibration, the particle size distribution is measured in the electrophoresis cell before the mobility measurement takes place, thereby ensuring that mobility of only the native protein is measured. This size distribution measurement is recorded through the same forward detection channel as the electrophoretic mobility and is therefore exquisitely sensitive to the presence of minute fractions of aggregates which could potentially affect the mobility measurement.

The conductivity of the sample is then measured using a minimally invasive, high frequency proprietary method. The resulting value is used to set a predetermined cell voltage and integration time for the measurement, selected to avoid any Joule heating of the sample and to minimise concentration polarisation effects which might otherwise affect the field strength measurement from which the electrophoretic mobility is calculated – see Reference [1] for further details.

When this stage is complete, small groups of electrophoresis measurements with an appropriate delay between groups are collected. The count rate is monitored for each group, and any significant deviation detected in count rate, indicating the presence of aggregates, is removed from the final average – thereby ensuring mobility of only the native protein is reported.

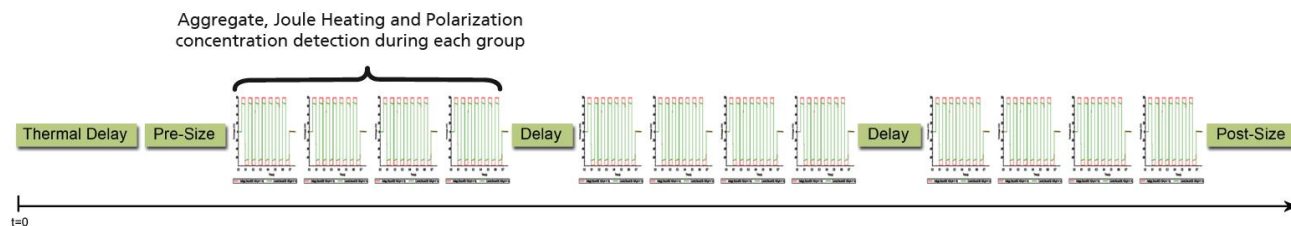


Figure 4: The optimised protein measurement structure

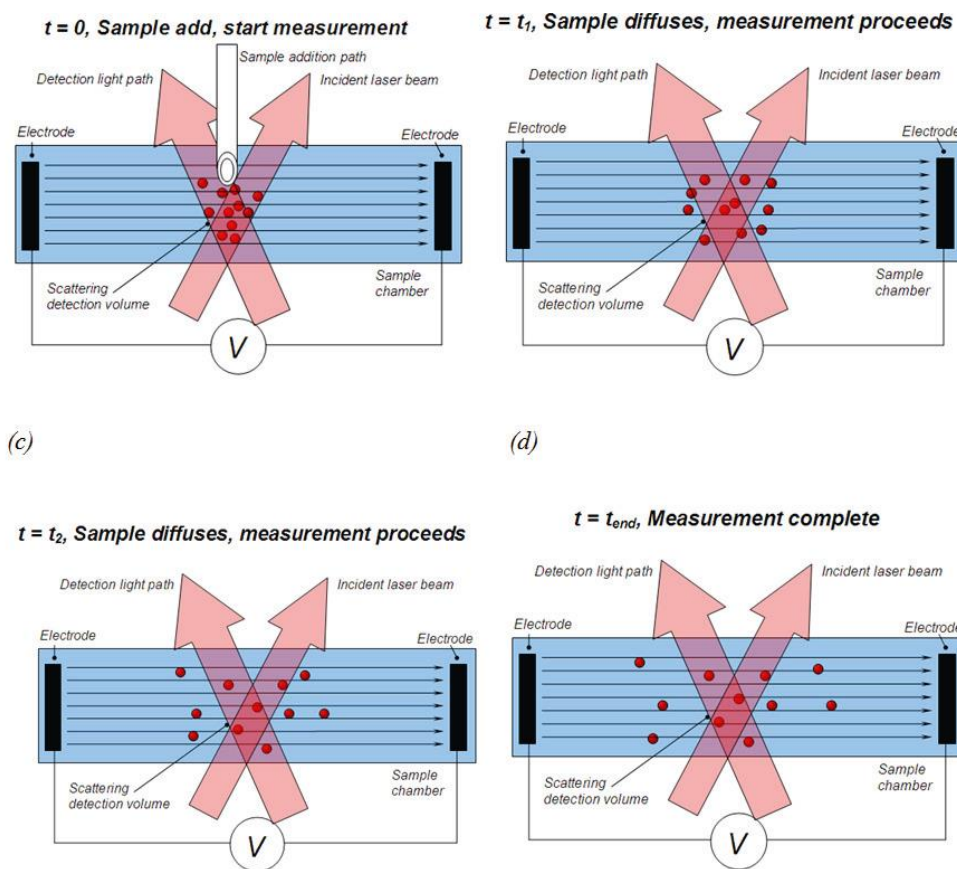


Figure 5: Schematic of the Diffusion Barrier technique.

## DIFFUSION BARRIER TECHNIQUE

Alongside improvements to the mobility measurement structure, Malvern's Diffusion Barrier method [2] (Figures 5 and 6) can now be used to further improve measurement quality, by preventing the protein from direct contact with the electrodes, eliminating associated aggregation events. This method is illustrated practically in Figure 6: the cell is filled with buffer, and the sample (dispersed in the same buffer) is loaded directly into the optical detection region at

the bottom of the cell only, using a readily available Gel-loader pipette tip. This method also enables retrieval of the sample from the cell following electrophoresis.

As Figure 6(b) shows, the integration time is increased by approximately one order of magnitude before protein aggregates are detected by the software, significantly increasing the signal to noise ratio without requiring an increase in voltage. Sample volume required is reduced to approximately 20 $\mu$ l.

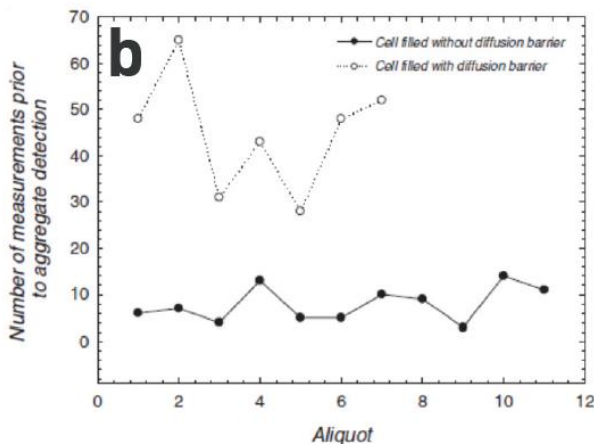


Figure 6: (a) Cells filled without (left) and with (right) a Diffusion Barrier; (b) The number of groups recorded prior to aggregate detection in 100mM KCl.

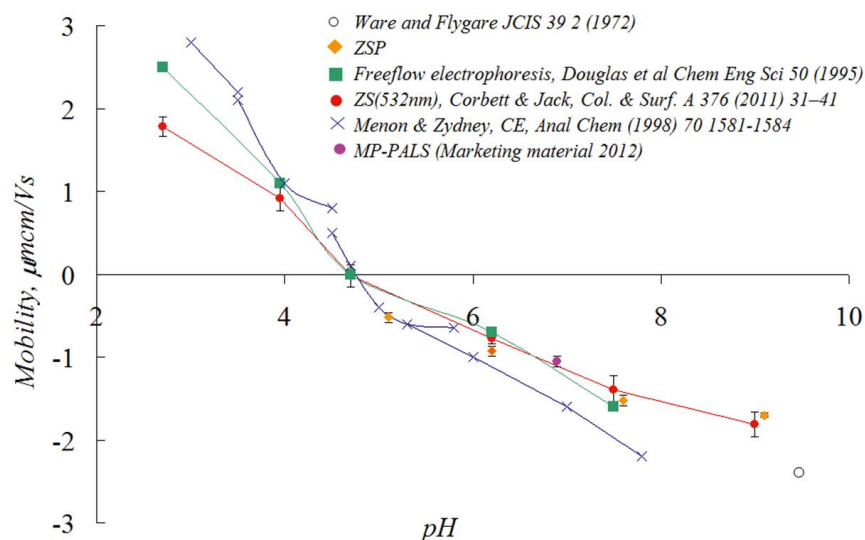


Figure 7: pH titration of BSA in 10mM NaCl at 1mg/ml

Figure 7 shows a pH titration of Bovine Serum Albumin (Sigma-Aldrich UK), recorded using a Zetasizer ZSP. The results are in excellent agreement with literature values. Note the very small error bars, with RSDs for this sample in the order of 6%.

Figure 8 shows a measurement of 5mg/ml Lysozyme (Sigma-Aldrich UK) during a titration of KCl at molar strengths between 10mM and 1M. The electrical double layer is suppressed at higher molarities and we therefore expect the hydrodynamic size and the electrophoretic mobility to remain constant with increasing salt concentration.

These measurements were only possible with use of the Diffusion Barrier technique and the protein's lack of contact with the electrodes [4]. No special cell was required; a standard semi-disposable folded capillary cell (Figures 2 and 6) was used.

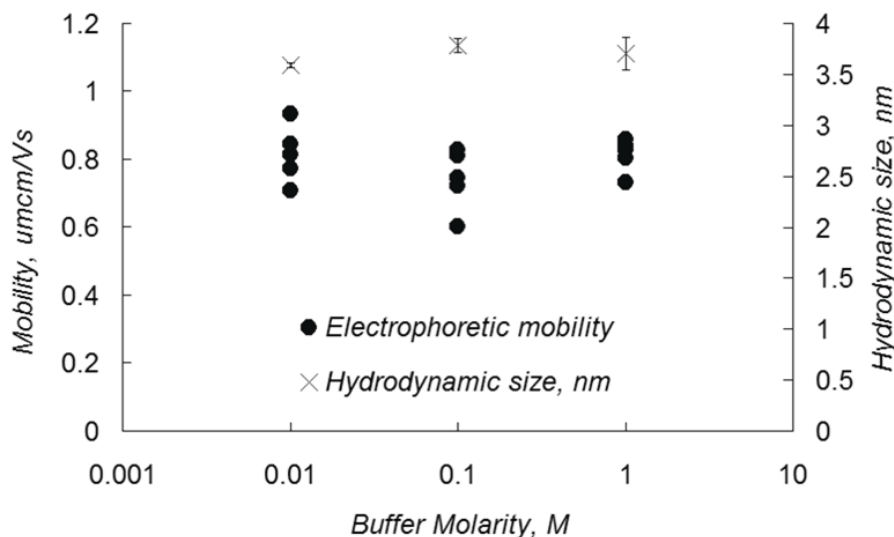


Figure 8: Measurements of Lysozyme at 5mg/ml up to 1M KCl using Diffusion Barrier method.

## REFERENCES

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