MEASURING PROTEIN ISOELECTRIC POINT USING THE ZETASIZER NANO ZSP

Method and Comparison with other Techniques

ZETA POTENTIAL

Introduction

Despite measurements having been carried out for over 70 years, protein net-charge under specific solution conditions remains an elusive parameter. Contributing factors to this elusiveness include the range of techniques that purport to measure this parameter and the alterations to protein conformation that such measurements can induce. Many such techniques involve separation of the solution components, solution pre-treatments, and interaction of the protein with a mobile phase, all of which serve to cloud the meaning of the information generated.

Protein mobility measurements, on the other hand, can be made in the absence of a mobile phase and without pre-treatment. The non-separating nature of this cuvette-based technique makes it highly applicable to fields where protein must be characterised under defined conditions, such as biopharmaceutical formulation and comparability analysis, and protein crystallisation.

This application note demonstrates the use of the Zetasizer Nano ZSP in the calculation of protein isoelectric point, using BSA as an example, and compares the data acquired with that obtained using other charge-measuring techniques.

Experimental

Figure 1: A: Zetasizer Nano ZSP; B: Cell entirely filled with blue dextran in 10 mM NaCl and cell filled with 10 mM NaCl loaded with 50 µl blue dextran to demonstrate the diffusion barrier technique.

The electrophoretic mobility of 5.0 mg/ml BSA was measured, using a Zetasizer Nano ZSP, in 10 mM HEPES over a pH-range of 3 – 10. These measurements were performed using the diffusion barrier technique (Figure 1), developed and patented by Malvern

Instruments and detailed in other application notes [1,2], in order to minimise the potentially denaturing effect of contact between the protein and the cell electrodes.

Results

The data acquired for the present work, shown in Figure 2, indicate that the isoelectric point of BSA is ~ 4.5. The net charge of the protein declines as the pH of the solution approaches this point, with the electrophoretic mobility becoming less positive as the pH increases from 3 to 4, and less negative as pH decreases from 7 to 5.

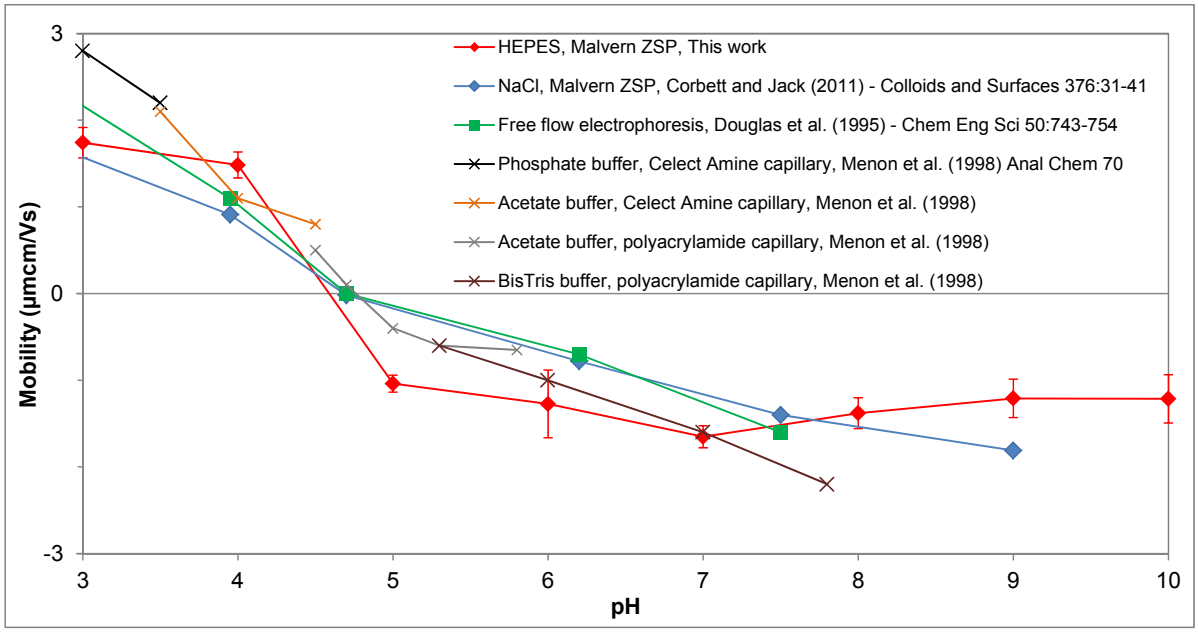

Figure 2: Protein mobility measurements of BSA in 10 mM HEPES buffer compared with literature values acquired using other methods

The microelectrophoresis results of the present work compares well with data acquired previously, both using other techniques (such as free flow electrophoresis and capillary electrophoresis) and using the same technique and instrument with a different, NaCl-based BSA formulation. All techniques indicate an isoelectric point of 4.5 – 5.

Between pH 7 and 10, the protein mobility values acquired for BSA in the presence of HEPES buffer using the Zetasizer Nano ZSP become less negative, in contrast with the results acquired in the presence of NaCl. This contrast is possibly the result of BSA stability varying between the 2 buffers, protein conformational changes often leading to changes in net protein charge. In addition, the Zetasizer Nano ZSP protein mobility measurements of BSA in HEPES buffer are larger in magnitude between pH 3 and 7 than those acquired in NaCl. This is consistent with inorganic NaCl having a greater charge screening effect than organic HEPES, the former having been demonstrated previously to have a strong screening effect on protein ionic interactions [3].

Conclusions

The Zetasizer Nano ZSP allows measurement of the global charge state of a protein, being highly sensitive to changes in protein environment and conformation. In contrast with other charge-measuring techniques, a protein mobility measurement is not a separating technique and does not involve a mobile phase. The cuvette-based method that the Zetasizer Nano ZSP uses places it alongside other biophysical methods, such as fluorescence spectroscopy and circular dichroism, in terms of simplicity of use and relevance of measurements to applications such as biopharmaceutical characterisation and protein crystallisation.

References

[1] The Diffusion Barrier Technique, Practical Aspects and Data interpretation. Malvern Application Note MRK1651-02.

[2] Improving Protein Zeta Potential Measurements Using a Novel Diffusion Barrier Technique. Malvern Application Note MRK1650-01.

[3] B.N. Dominy et al. (2002) J. Mol. Biol. 319, 541-554.

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