

# THE RELEVANCE OF ZETA POTENTIAL IN PROTEIN PROCESSING

## Part 2. Zeta potential and the surface properties of proteins

The surface properties of proteins and especially the surface electrical properties have an important influence on the performance of most of the separation processes identified in the Introduction in part one. The surface charge of proteins arises firstly from ionisation of surface groups, that is the acidic and basic side chains of the component amino acids. These ionisation reactions are acid base equilibria and thus depend on the pH of the solution.

The amino acid sequence of the protein which in many cases will be known, gives the number of such groups. However, not all may be available for charge generation as some may be located within the three-dimensional structure of the protein rather than at the surface.

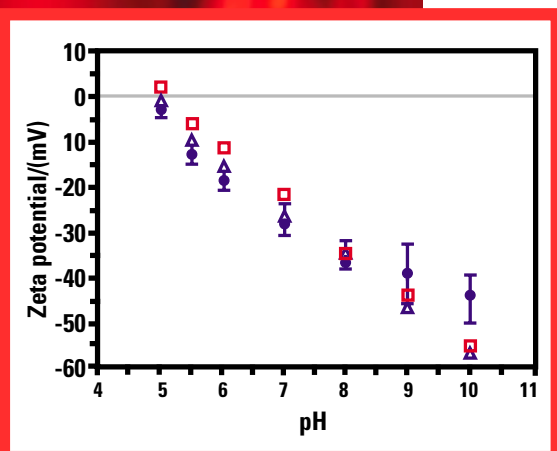
Further, other ions from the solution may bind to the surface of the protein and hence modify the surface charge. There is thus a need for an independent method of assessing the surface electrical properties of proteins in a way which is useful for process operation and prediction. Such a method would also provide a test for fundamental models which describe the surface properties of proteins.

Measurement of electrophoretic mobility is a very useful method of characterising the surface electrical properties of proteins. Such measurements may be made in free solution over a wide range of ionic strength and pH by using an instrument such as the Malvern Zetasizer.

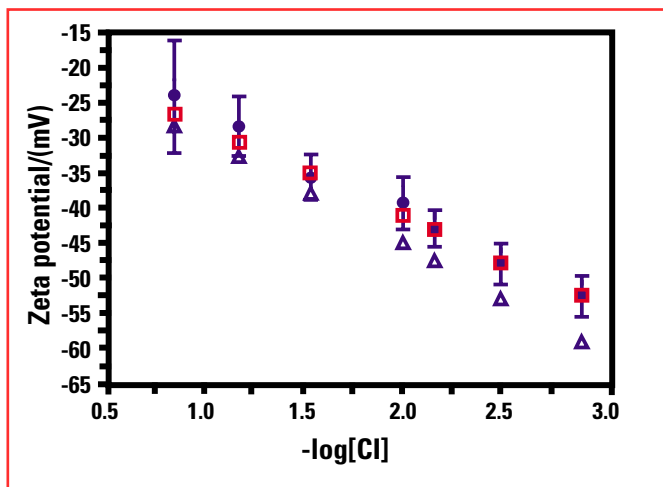
Zeta potentials may be calculated from electrophoretic mobility measurements. For solutes as small as proteins, care is needed in the conversion of electrophoretic mobility measurements to zeta-potentials. However, this is readily achieved by using commercially available software such as the WinMobil programme (Department of Mathematics, University of Melbourne) which is an extended implementation of an advanced theory of electrophoresis. Such equipment has been used to test theoretical descriptions of the surface properties of the protein

bovine serum albumin (BSA) (3) which has a molecular weight of 66,500. The theoretical description took into account the amino acid sequence of the protein and specific binding of chloride ions from the solution. Figure 4 shows a comparison of experimental zeta potential values compared to the predictions of the so-called "charge regulation model" over a range of pH.

Figure 5 shows a comparison of experimental zeta potential values compared to the prediction of the model over a range of ionic strength. In both cases there is good agreement between the experiment and the theory hence validating the theoretical description of the protein surface. The graphs also show good agreement with an alternative basis for determining the number of charge-bearing amino groups from titration data. Such validation of the model allows its use for process prediction over a wide range of solutions conditions. See part 3, (MRK384a)



**Fig4.** Comparison of experimental zeta potential values (●) with values predicted by a charge regulation model at an ionic strength of 0.03M and varying pH values; using number of amino acids groups from sequence (□) and using number of amino acid groups from titration (△)



**Fig 5.**  
Comparison of experimental zeta potential values (●) with values predicted by a charge regulation model at a pH of 8.0 and varying ionic strength; using number of amino acid groups from sequence (□) and using number of amino acid groups from titration (△)

## References

3. W.R. Bowen and P.M. Williams, The osmotic pressure of electrostatically stabilised colloid dispersions, *Journal of Colloid and Interface Science* 184 (1996) 241-250.

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