

# PROCESS PREDICTION IN PROTEIN PROCESSING

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## Part 3. Process prediction

### Membrane ultrafiltration of protein solutions

Membrane ultrafiltration is a pressure-driven process which is capable of separating macromolecules or colloidal particles (of approximate size range 1nm – 0.1mm) from a solvent or smaller solutes.

These particles may be inorganic (metal oxides), polymeric (lattices) or biological (proteins). The ultrafiltration process has become particularly important for concentrating dilute proteinaceous solutions. Examples of commercially important processes

include the concentration of whey proteins in the dairy industry, protein recovery from blood plasma, and protein concentration in the processing of fermentation broths. Ultrafiltration performance is limited, however, due to the build-up of the solutes separated at the membrane surface. This is known as the concentration polarisation effect.

The electrostatic interactions may be calculated if the zeta potential of the proteins is known. Such zeta potentials may be obtained from electrophoretic mobility measurements with a Malvern Zetasizer as described in the preceding section. Figure 6 shows an example of the time course of filtration at different pH values calculated incorporating such information and compared to experimental data. The data and calculations are shown for different pH values and hence cover a wide range of zeta potential. The Figure shows that the rate of filtration is very dependent on zeta potential and that the calculations agree well with the experimental data. There are also substantial variations in zeta potential and the rate of filtration if the salt concentration of the processing solutions is varied. Hence, a knowledge of the zeta potential of proteins can be invaluable in identifying the pH and ionic strength that will give the fastest ultrafiltration of protein solutions.

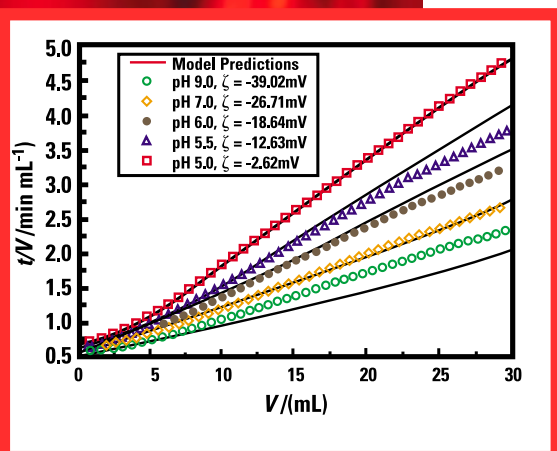


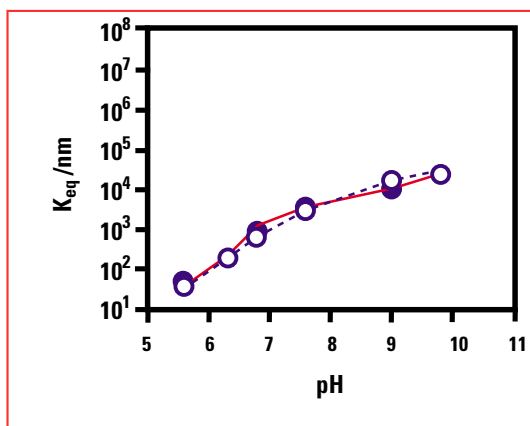
Fig 6

Time of filtration / volume filtered vs. volume filtered for the ultrafiltration of BSA at varying pH at an ionic strength of 0.03M.

Maximisation of rates of ultrafiltration is important for financial reasons. This requires good theoretical descriptions of the effect of the concentration of proteins close to the membrane surface on the rate of filtration. Such descriptions must be based on a good knowledge of the physicochemical interactions between protein molecules. These descriptions have been recently developed in a way which allows them to be used in the prediction of rates of protein filtration from a fundamental knowledge of protein properties (4-6). The predictions take into account electrostatic repulsion, London-van der Waals forces, hydration forces and configurational entropic effects to predict the hydrodynamic resistance of the concentrated layer of proteins which forms close to the membrane surface and controls the rate of filtration.

### Predicting equilibrium constants for ion-exchange of proteins

Ion exchange is one of the most effective and cost efficient means of separating macromolecules in biotechnological applications. It is used, for example, for the large-scale isolation of commercially important proteins. The ability to develop *ab initio* models for predicting adsorption equilibrium constants for protein ion exchange



**Fig 7**  
Comparison of experimental (●) and predicted (○) equilibrium constants for the ion exchange of BSA at an ionic strength of 0.06 M and various pH values.

processes would greatly facilitate their simulation and design.

Ion exchange can be considered as a sorption process and has many similarities with adsorption. The continuum principles of colloid science can be appropriate for characterising the relevant features of protein surface interactions without involving atomic detail. Ion exchange of proteins can then be considered in terms of an electrical double layer interaction between the protein and the ion exchanger. The electrostatic interaction may be computed from the non-linear Poisson-Boltzmann equation using a boundary condition of constant zeta potential for both protein and ion exchanger – it has been shown that a constant zeta potential boundary condition gives a good description of protein-protein interactions. London-van der Waals interactions may be readily included into the overall calculation. Figure 7 shows a comparison of ion exchange equilibrium constants calculated in this way with experimental values for the protein BSA (7). The calculations used zeta potentials for the protein measured by a Malvern Zetasizer and zeta potentials for the ion-exchanger obtained using an electro-osmotic technique.

The zeta potential of the protein varied from  $-7.0\text{mV}$  to  $-32.5\text{mV}$  over the range of pH shown. It may be seen that there is very good agreement between the calculations

and the experimental values confirming the validity of the approach adopted.

### Conclusions

The rapid growth in the biotechnological industries is leading to an increased need for effective methods of processing proteins. Use of photon correlation spectroscopy for protein sizing and electro-phoretic mobility measurements for determination of zeta potentials has an important role to play in optimising such

processing. Such information is often in itself very useful, and even more so when coupled with mathematical descriptions on the individual processes. This note gives just some examples of measurements and techniques that may be applied to most of the processes used to separate and purify proteins and other biological macromolecules.

### References

4. W.R. Bowen and F. Jenner, Dynamic ultrafiltration model for charged colloidal dispersions: a Wigner-Seitz cell approach. *Chemical Engineering Science* 50 (1995) 1707-1736.
5. W.R. Bowen and P.M. Williams, Dynamic ultrafiltration model for proteins a colloidal interaction approach, *Biotechnology and Bioengineering* 50 (1996) 125-135.
6. W.R. Bowen, A Mongruel and P.M. Williams, Prediction of the rate of cross-flow membrane ultrafiltration: a colloidal interaction approach, *Chemical Engineering Science* 51 (1996) 4321 - 4333.
7. W.R. Bowen, L-C Pan and A.O. Sharif, Predicting equilibrium constants for ion exchange of proteins – a fundamental approach, submitted for publication.

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