

Optimization of Stepwise Elution Chromatography on the Basis of Iso-Resolution Curve

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We have proposed a concept “iso-resolution curve” for linear (salt concentration) gradient elution of proteins in electrostatic interaction chromatography (ion-exchange chromatography, IEC).

In this study this concept was extended to stepwise elution chromatography. The distribution coefficient was determined from linear gradient elution experiment by using our model. The iso-resolution curves were similar to those for linear gradient elution. However, compared with the curves for linear gradient elution where no distinct optimum conditions were found, the curves for stepwise elution showed optimum conditions where the separation can be performed with the shortest separation time and the minimum elution volume consumption.

Figs.1-4 show the concept of iso-resolution curve for step-wise elution.

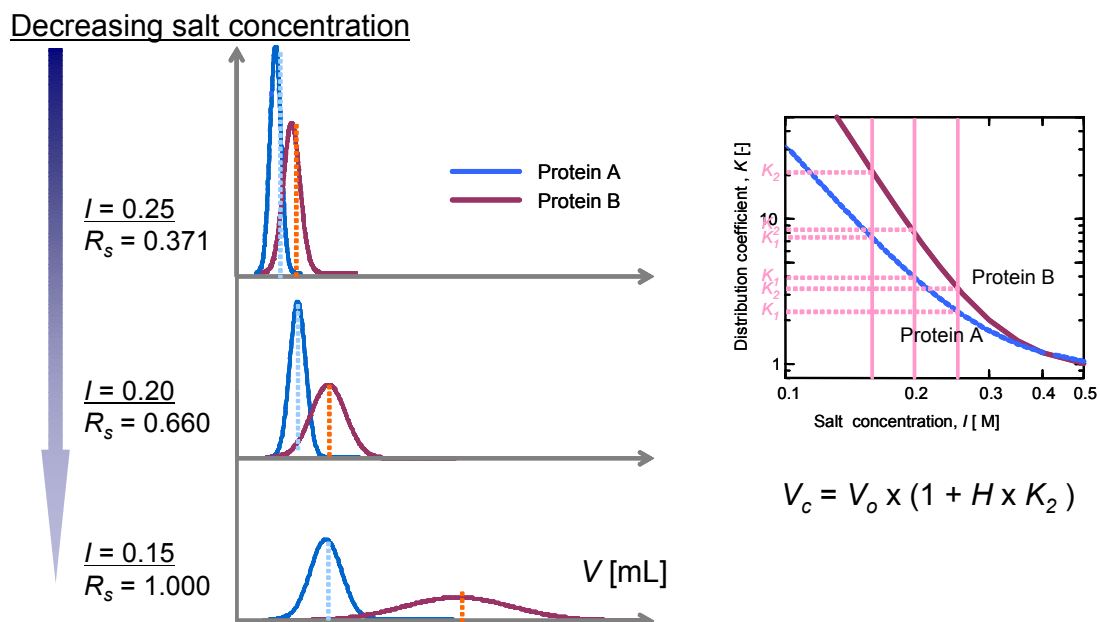


Fig.1 Effect of mobile phase salt concentration on the resolution at a fixed mobile phase velocity;. The right panel shows the distribution coefficient as a function of salt concentration.

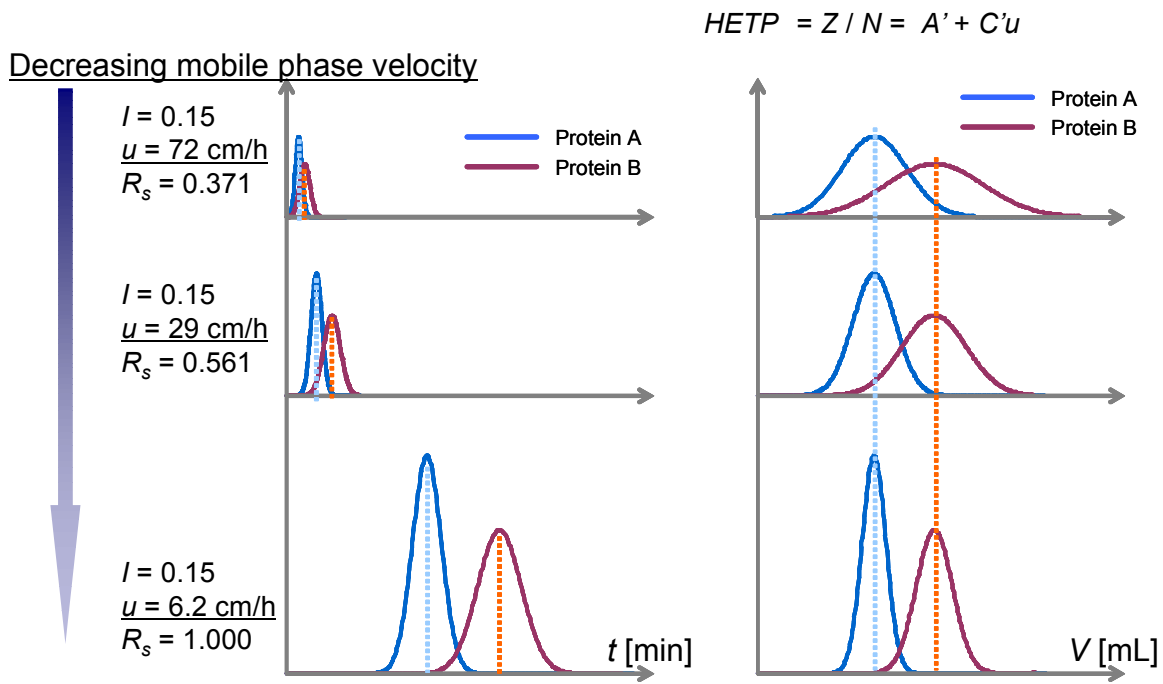


Fig.2 Effect of mobile phase velocity on the resolution at a fixed mobile phase salt concentration.

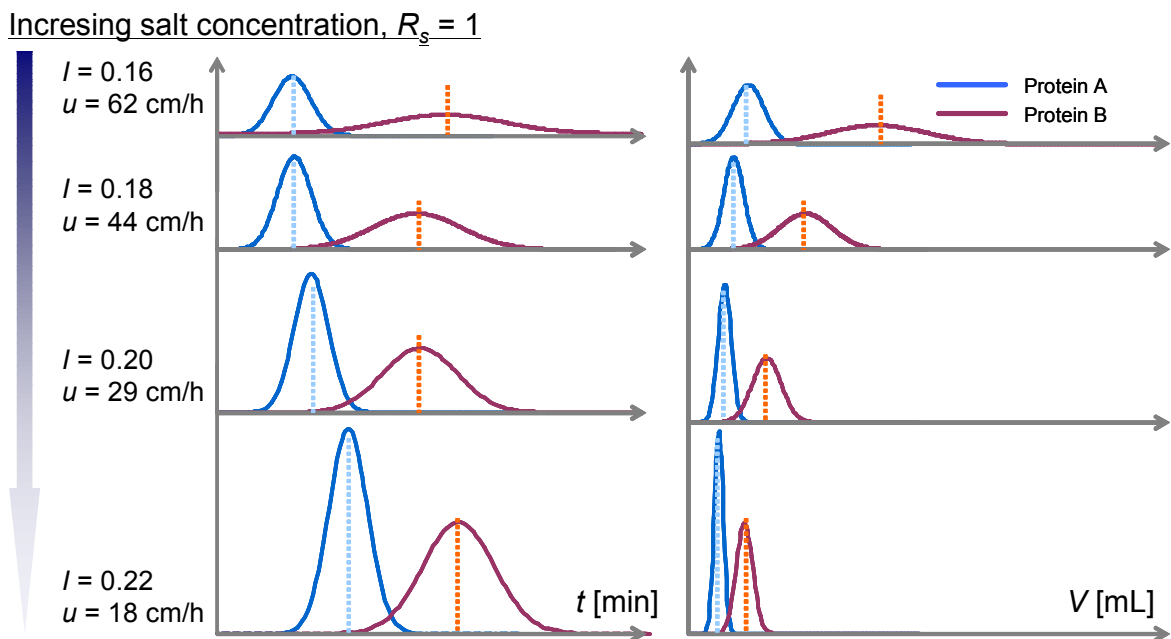


Fig.3 Separation behavior at a fixed resolution ($R_s=1$) at different combinations of mobile phase salt concentration and velocity.

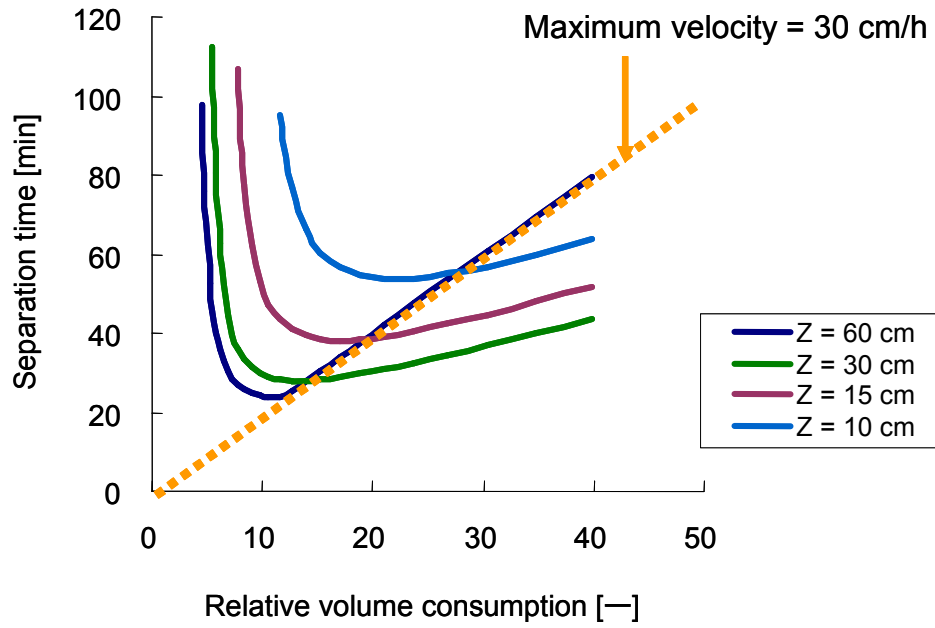


Fig. 4 Iso-resolution curves – Separation time vs. relative volume consumption.

References

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Theoretical background of short chromatographic layers. Optimization of gradient elution in short columns,

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