Adaptation of Retention Models to Allow Optimization of Peptide and Protein Separations

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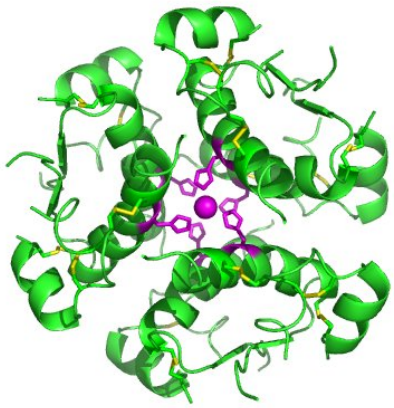
INTRODUCTION



Proteins and peptides are analytes of increasing interest.

CURRENT STATE

Retention modelling has been successfully used for optimization of small molecule separations but not expanded to accurate modelling of protein separations.

GOAL OF STUDY

Create retention models that allow accurate simulation of protein separations .

METHOD

Isocratic retention modes were used in combination with numerical solutions to calculate retention times for gradient conditions.

6 proteins with MW ~25 000 Da were chromatographed and modelled by reverse phase chromatography (RPC) and ion exchange chromatography (IEC).

Software

ACD/LC Simulator software was used for gradient modelling.

Equations Identified for Retention Prediction

RPC/HIC model: $\ln k = a + b x + c x^2$ IEC/HILIC model: $\ln k = d + e \ln(x)$

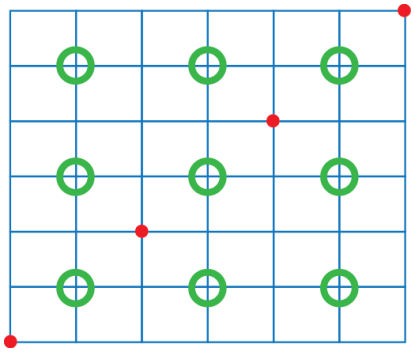
2nd order temperature model $\ln k = f + g / T + h / T^2$

RESULTS

Modelling and evaluating retention prediction

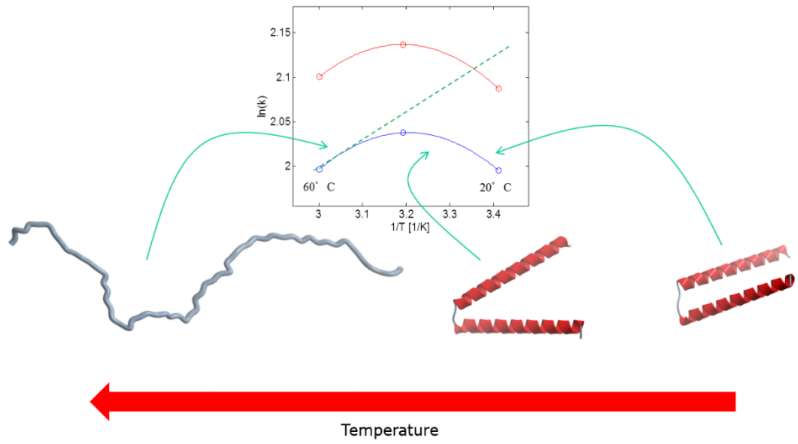
Combined gradient and temperature models were evaluated by reverse phase chromatography and ion exchange chromatography by fitting the models based on 9 experiments (3 gradient times x 3 temperatures).

Predictions were then made for 4 additional combinations of gradient time and temperature as shown.

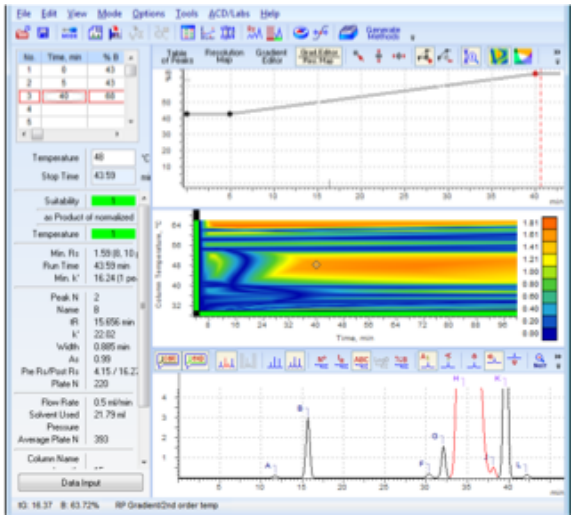


- Experimental conditions used to evaluate predicted retention time & peak width
- Experimental data used to fit the model

Explaining the retention behaviour of proteins at different temperatures



Modelling retention in software



CONCLUSION

RPC and IEC gradient chromatography, at different temperatures, can be modelled with the same accuracy for proteins as for small molecules. A second order temperature model is likely needed due to the changing structure (and therefore retention) of proteins with temperature.

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Read the full poster

www.acdlabs.com/ProteinPoster