

Application of Capillary Isoelectrofocusing for Analysis of Protein Antigens

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What is isoelectric focusing (IEF)?

Determines isoelectric point (pI) of amphoteric molecules

Traditionally performed by SDS-PAGE:

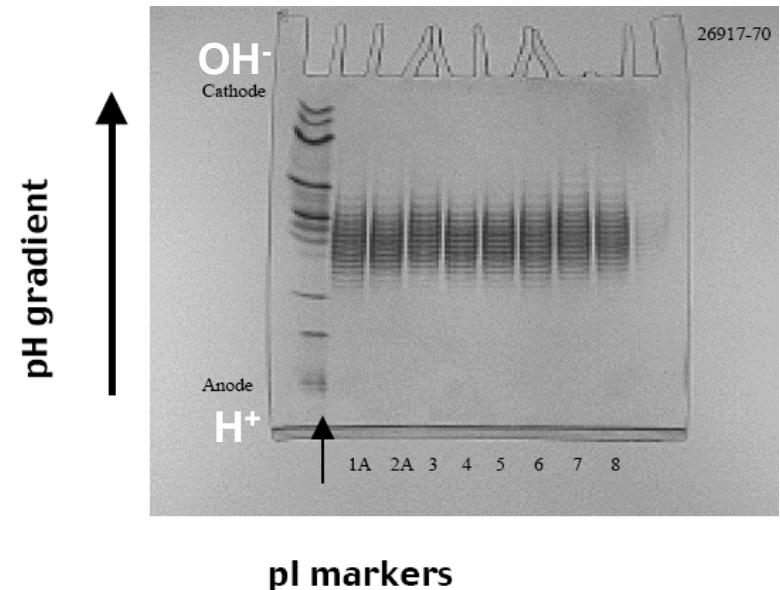
- pH gradient set by carrier ampholytes
- Sample will settle once pH matches pI

IEF profile acts as a “fingerprint”

- Sensitive to changes in surface charge

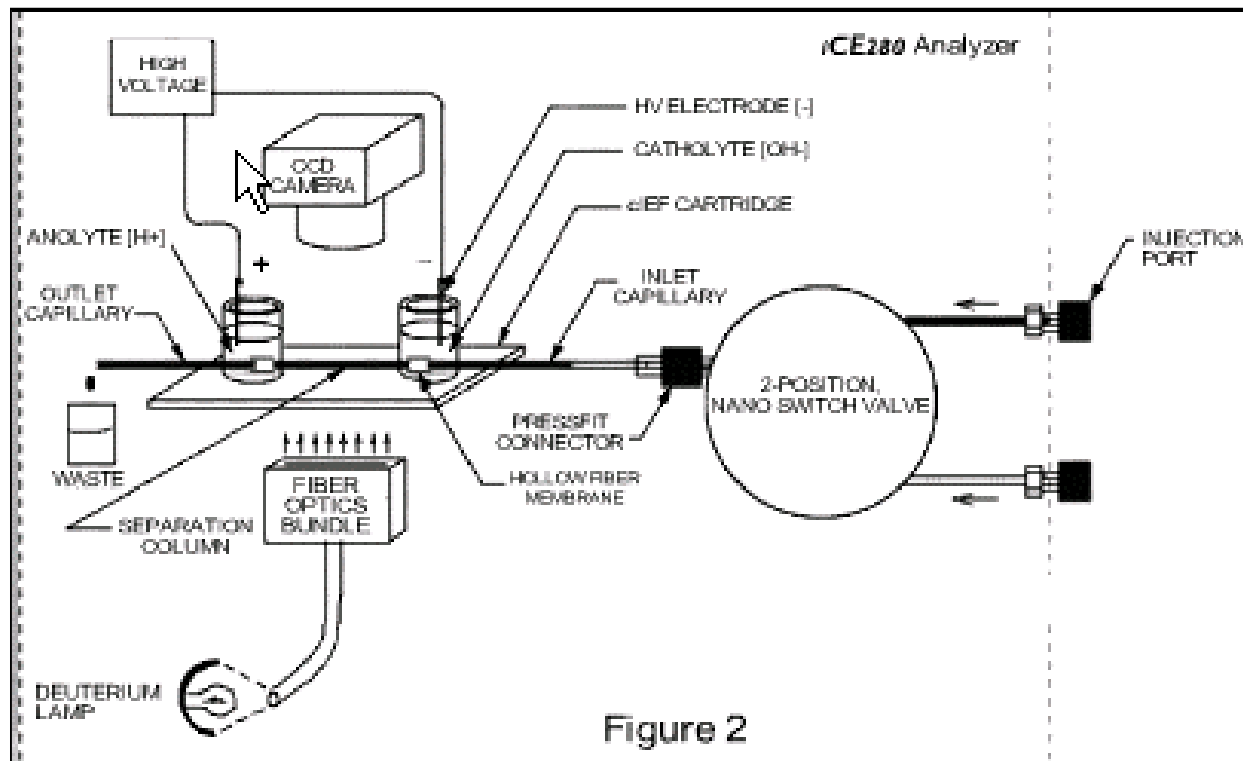
Limitations:

- Pore size
- Single gel takes 16 hours to run



Source: http://www.ciefforum.com/download_file.php?id=pdf/CEpharm05poster.pdf

iCE280 Analyzer schematic



Courtesy of Convergent Bioscience

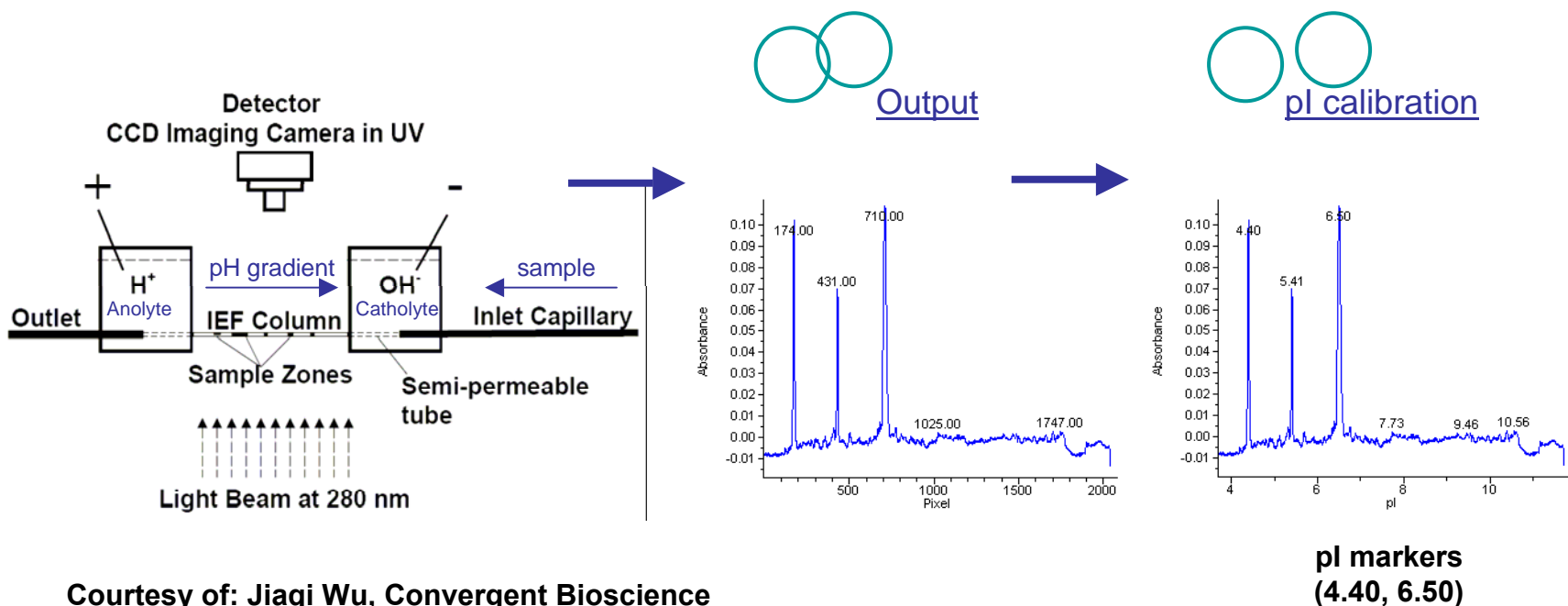
Capillary IEF

iCE 280 Analyzer by Convergent Bioscience

Manufacturer reports:

Standard deviation < 0.03

82% success rate (31% for vaccines)



Courtesy of: Jiaqi Wu, Convergent Bioscience

Capillary IEF (cont.)

Advantages:

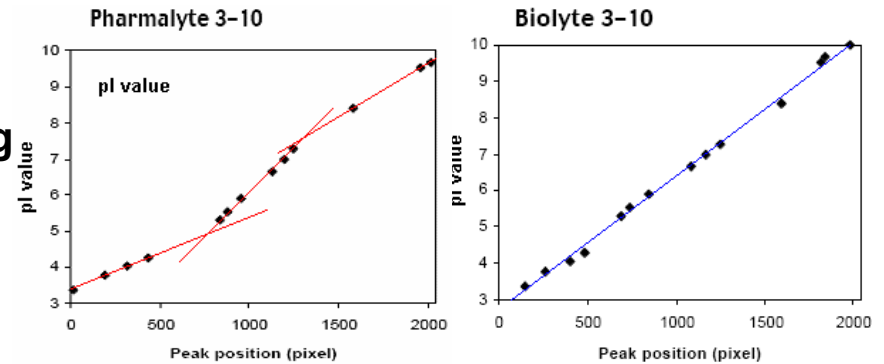
- Larger samples can be analyzed
- High throughput
- Sample preparation is fast
- Easy to operate
- Data analysis is simple and rapid
- Method development can be quickly achieved



Method development for protein analysis

Carrier ampholytes:

- Fixed pH gradient
- Variability in linearity among brands



Focusing time:

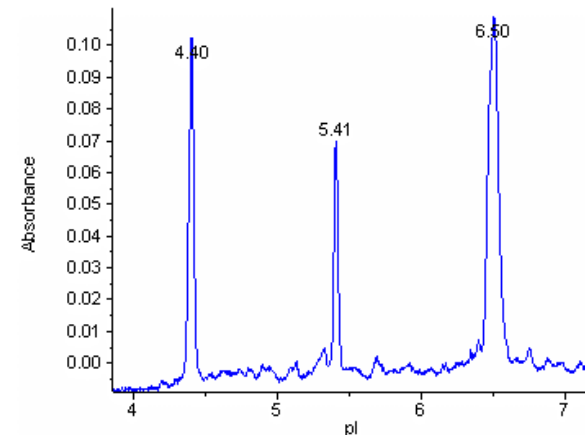
- Too short → non-reproducible peaks
- Too long → aggregation

Source:

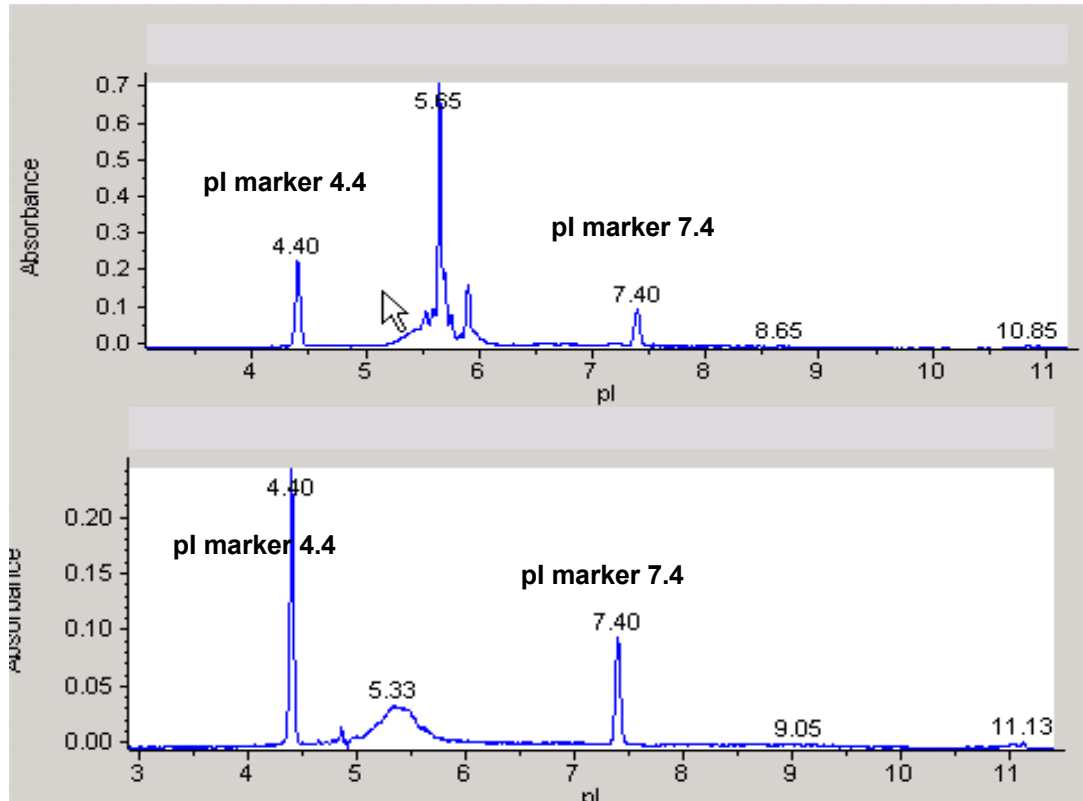
http://www.ciefforum.com/download_file.php?id=pdf/CEpharm05poster.pdf

Desired outcome:

- Reproducible peak distribution
- Good resolution
- Appropriate absorbance intensity



cIEF profiles of Protein Q: native and chemically-treated



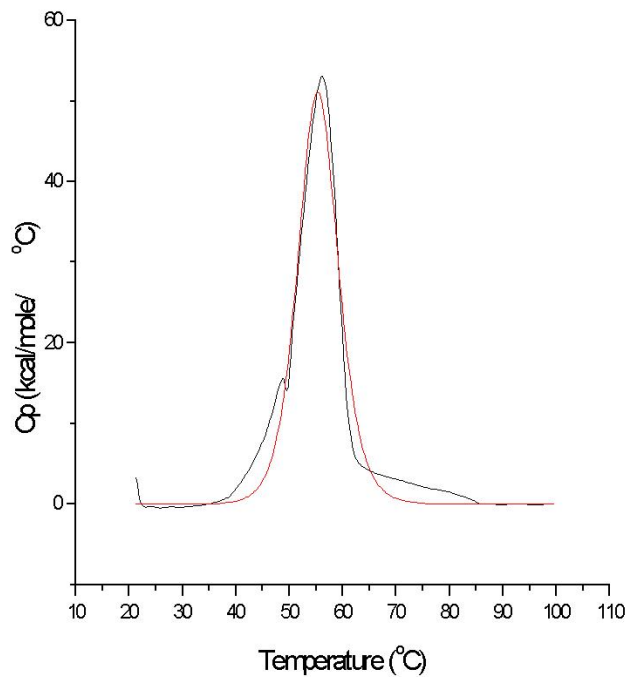
Protein Q

Chemically-treated
Protein Q

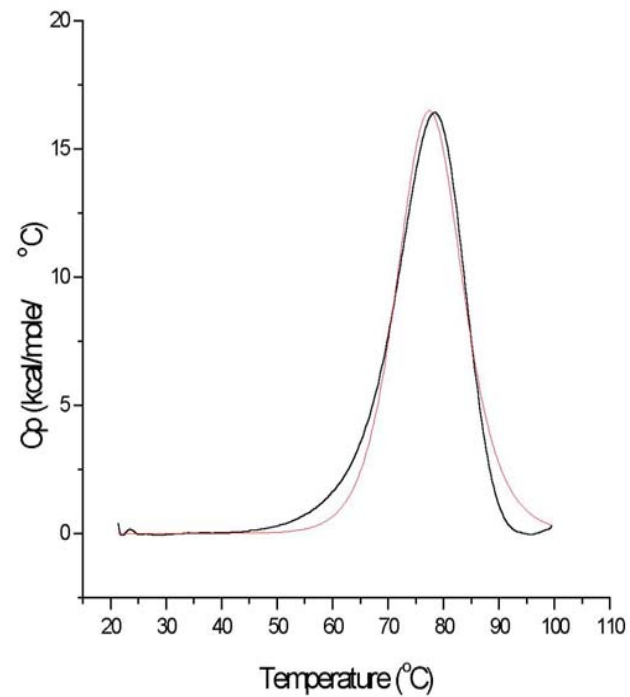
0.3 pI shift was detected

DSC profiles of Protein Q: native and chemically treated

Protein Q

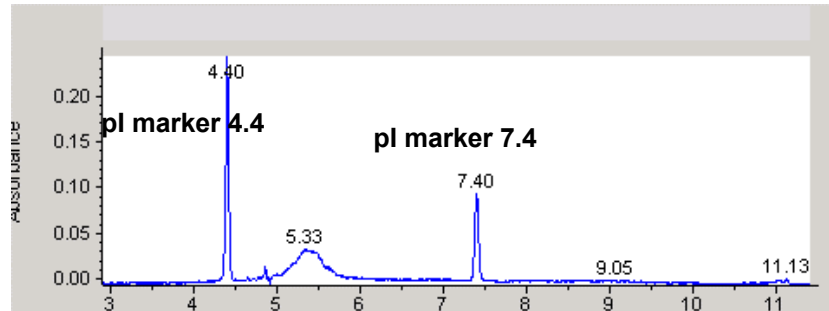


Chemically-treated Protein Q



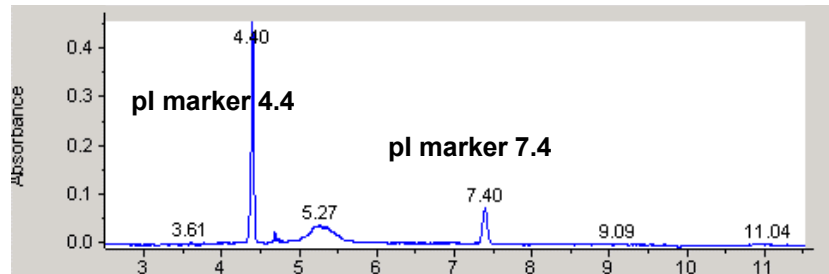
T_m shift of 22°C was detected

cIEF spectra of chemically treated Protein Q



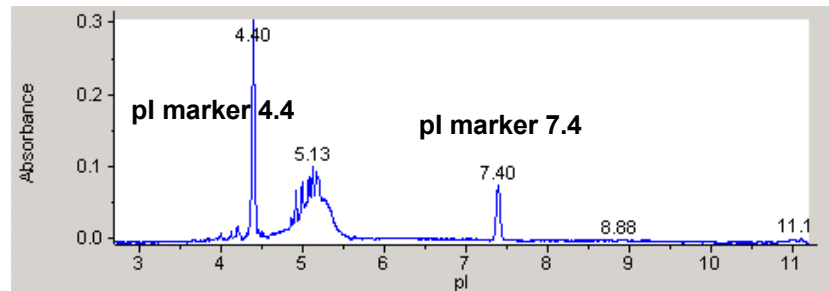
Process 1

0.05 pI difference was detected between 1 and 2



Process 2

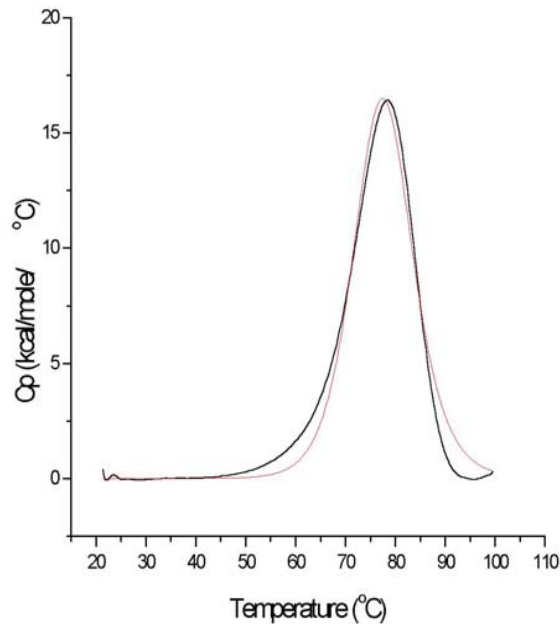
0.15 pI difference was detected between 1 and 3



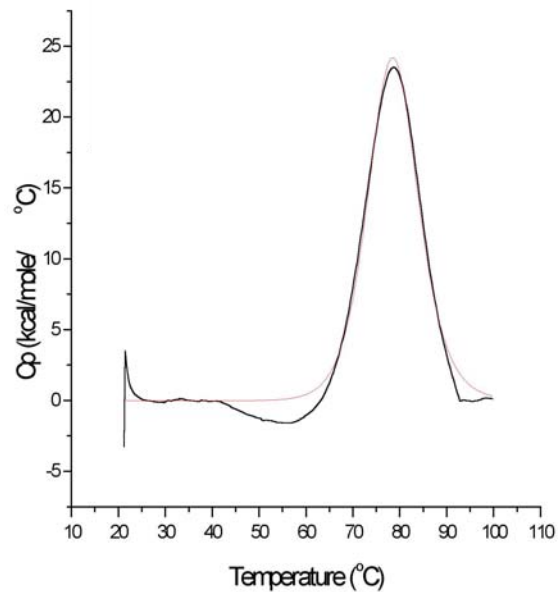
Process 3

DSC profiles of chemically-treated Protein Q

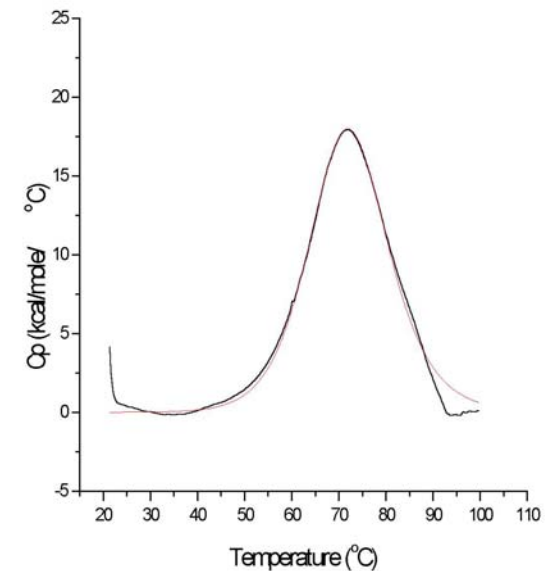
Process 1



Process 2



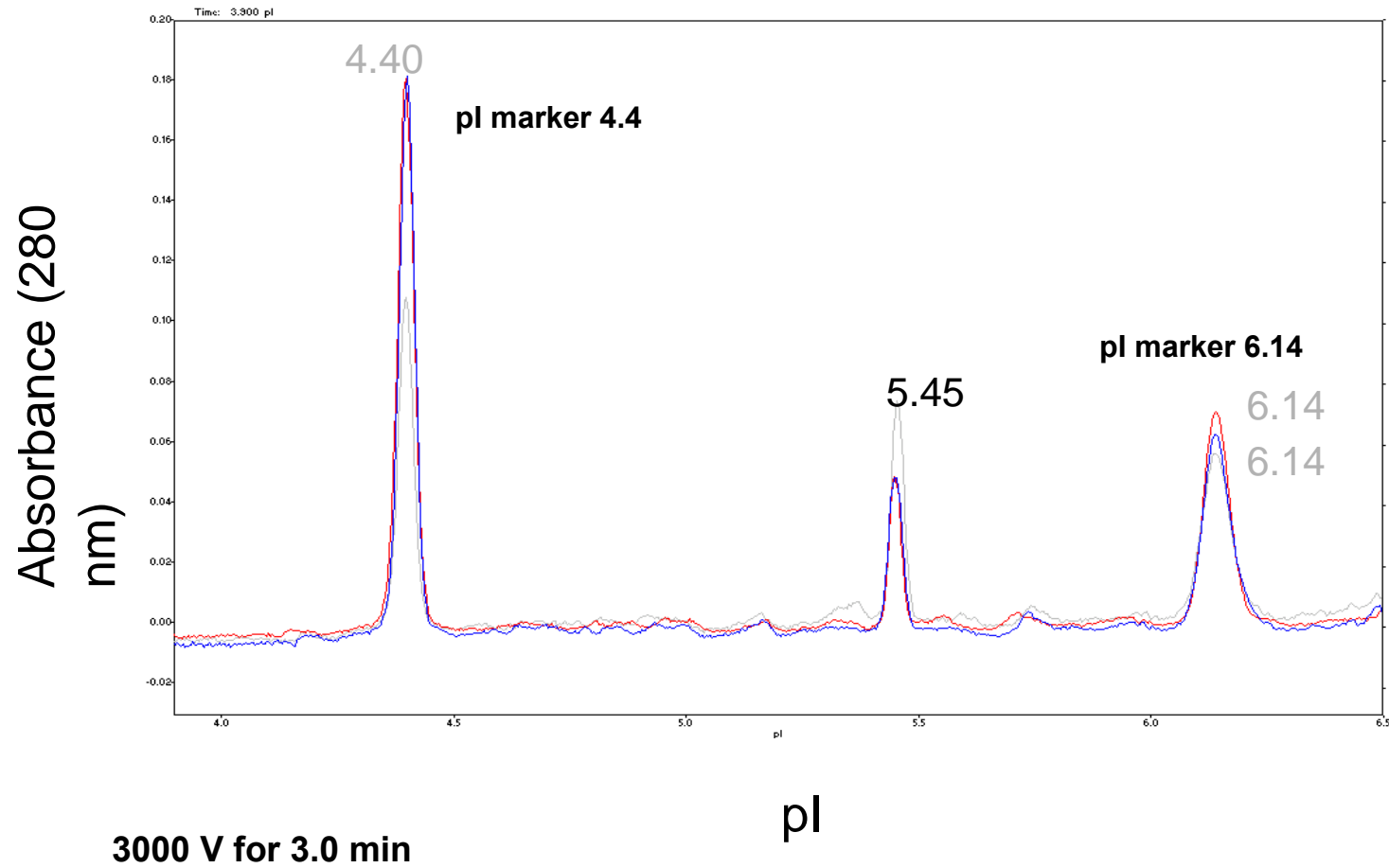
Process 3



T_m shift of 1°C was detected between 1 and 2
 T_m shift of 6°C was detected between 1 and 3

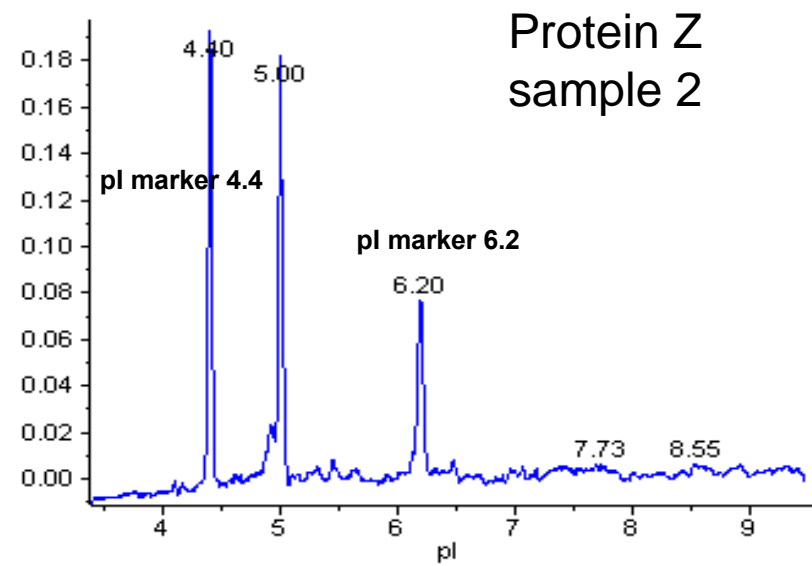
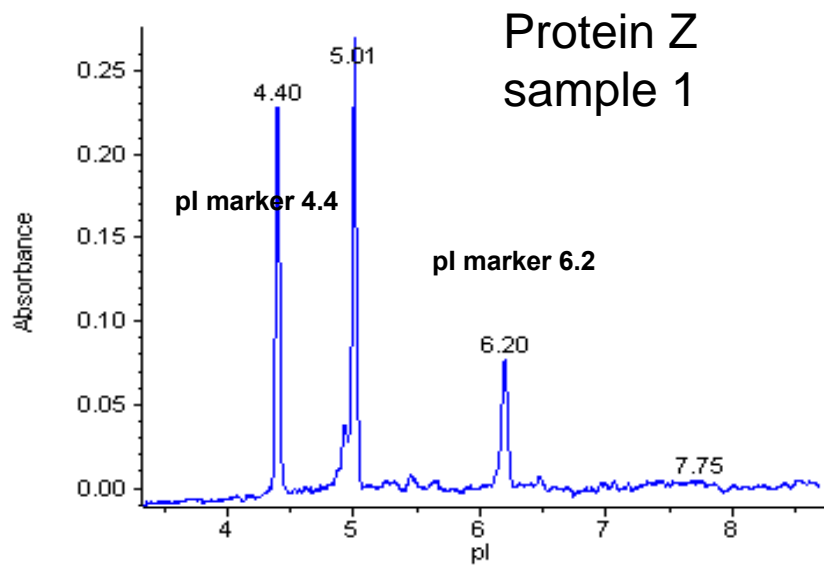
Protein N

lot-to-lot consistency



Protein Z

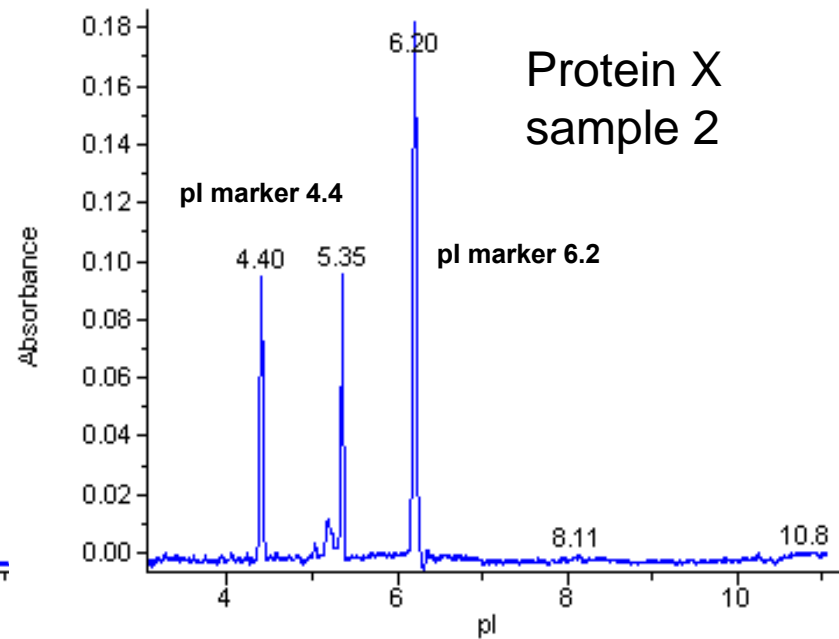
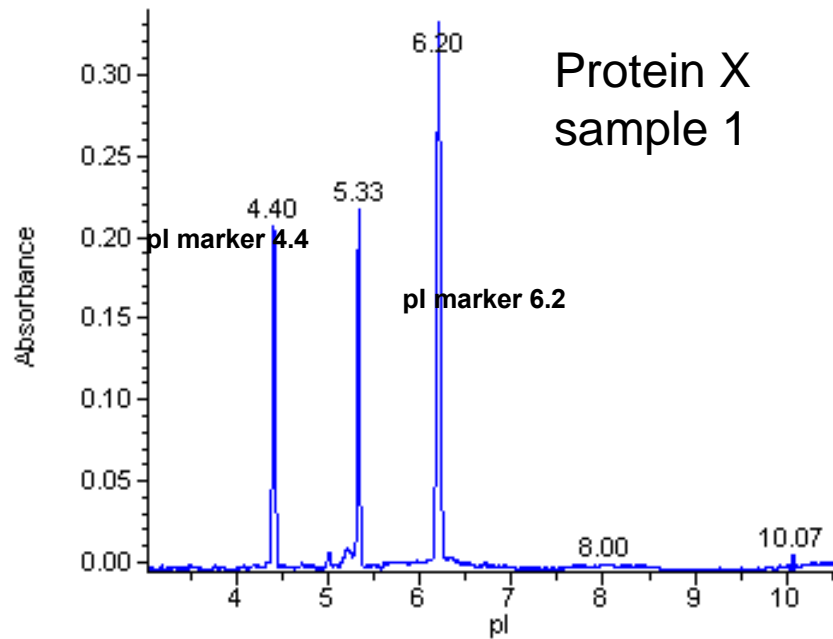
lot-to-lot consistency



3000 V for 3.0 min

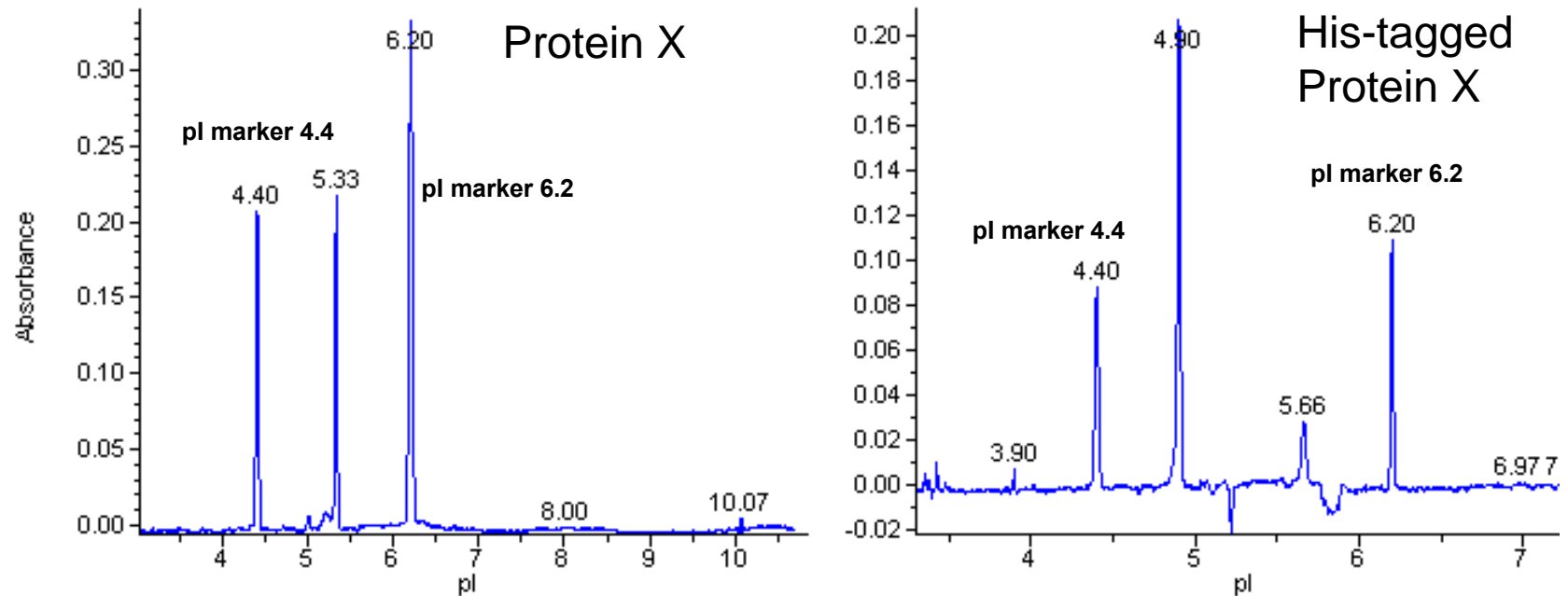
Protein X

lot-to-lot consistency



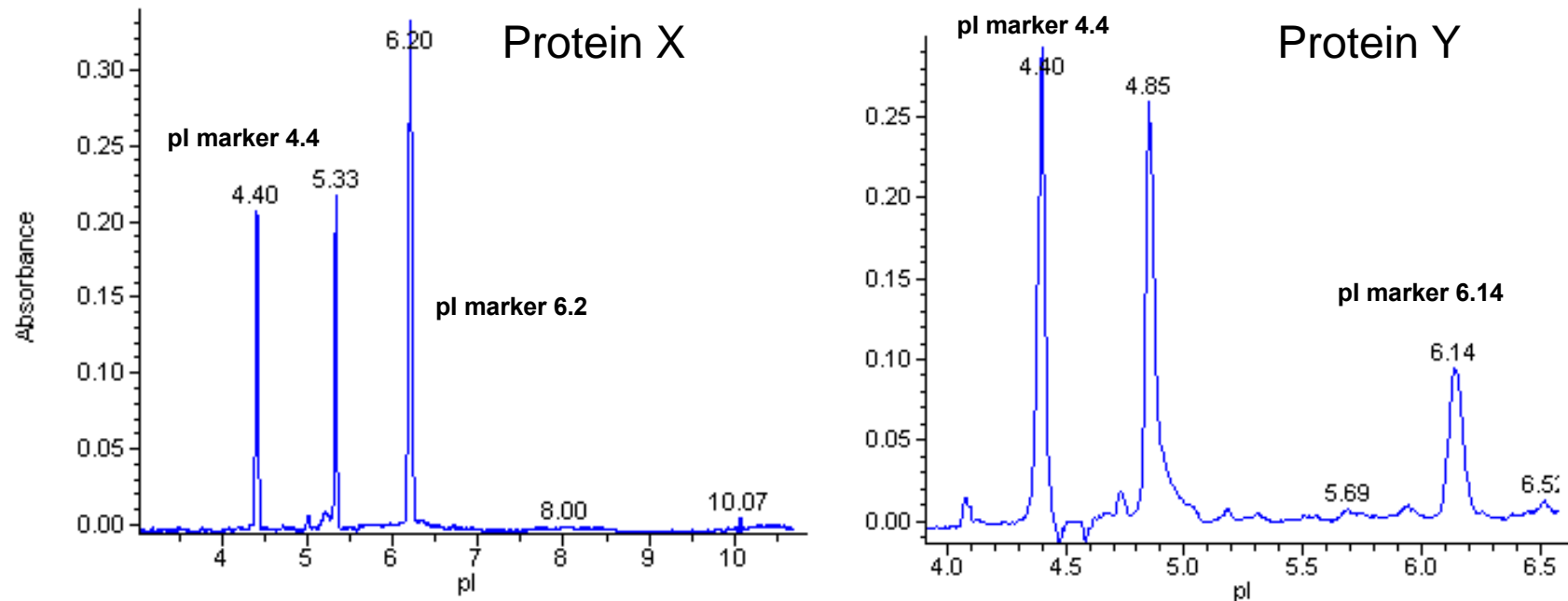
3000 V for 3.0 min

Protein X non-tagged and his-tagged



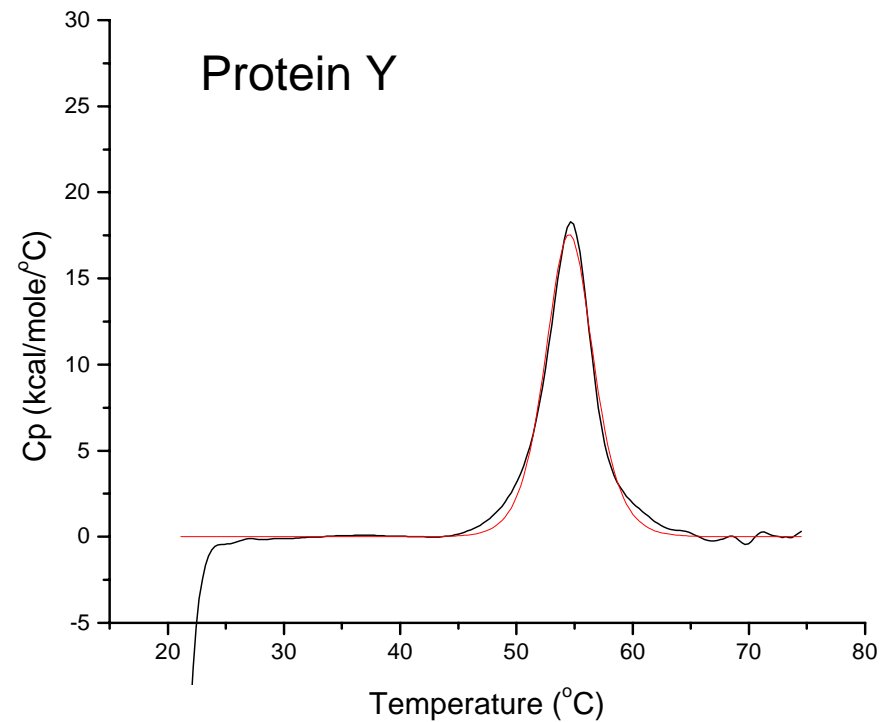
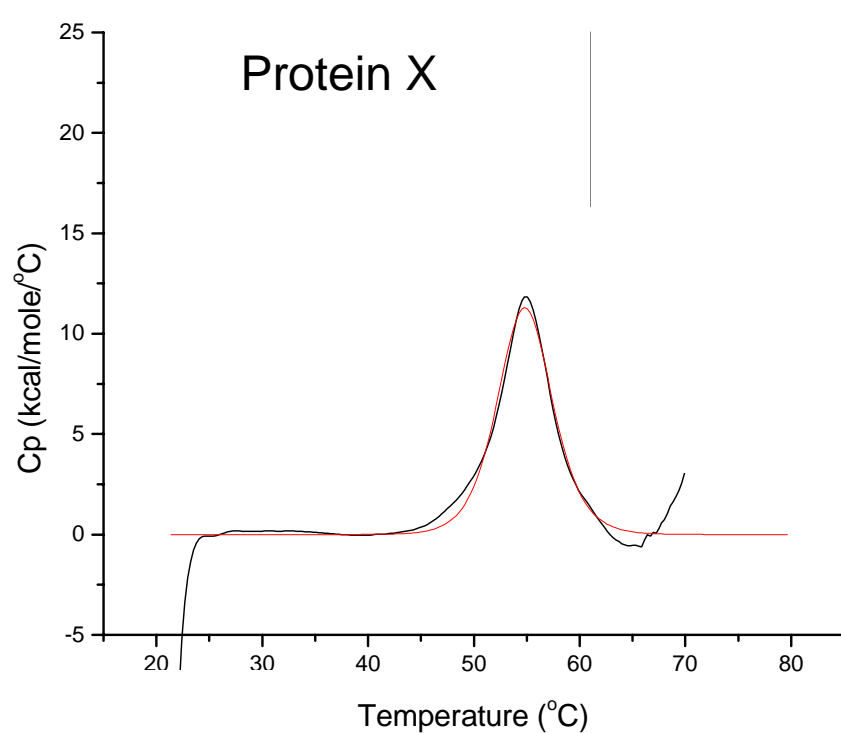
3000 V for 3.0 min

cIEF distinguishes between homologous proteins



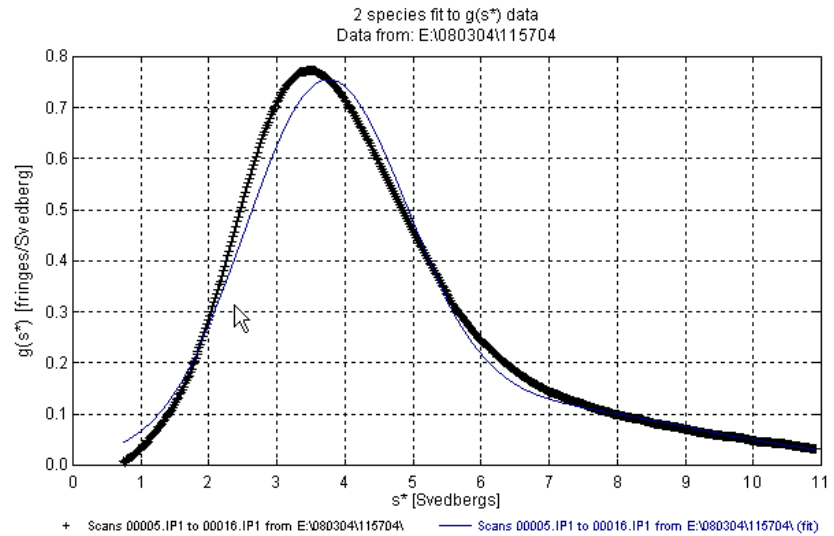
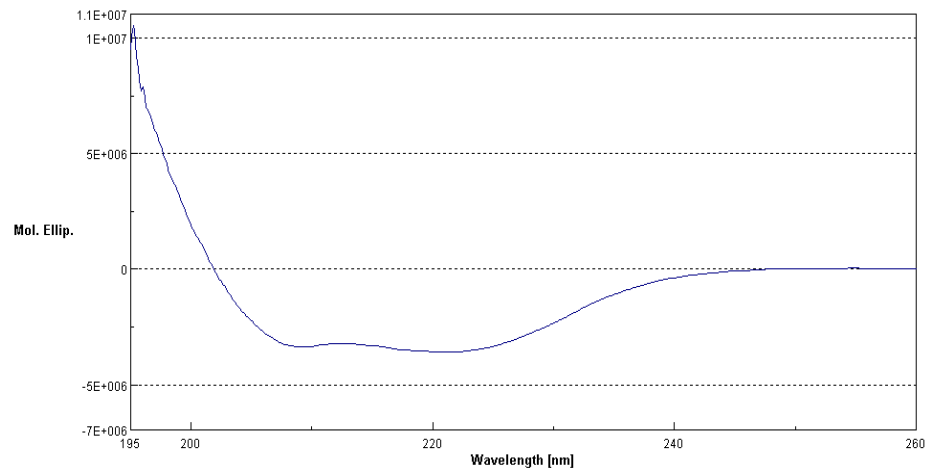
3000 V for 3.0 min

DSC analysis of Proteins X and Y



Protein X and Y have same T_m , but different pI

CD and AUC analysis of Proteins X and Y



Protein X and Y have helical secondary structure and similar sedimentation coefficient

Conclusions

cIEF allows to:

Determine pI of protein in solution

Distinguish between homologous proteins

Monitor process consistency and stability

Obtain signature profile for complex mixtures

Acknowledgements

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