Raman Spectroscopy as a Rapid Analytics Tool

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WCBP: Rapid Analytics Session

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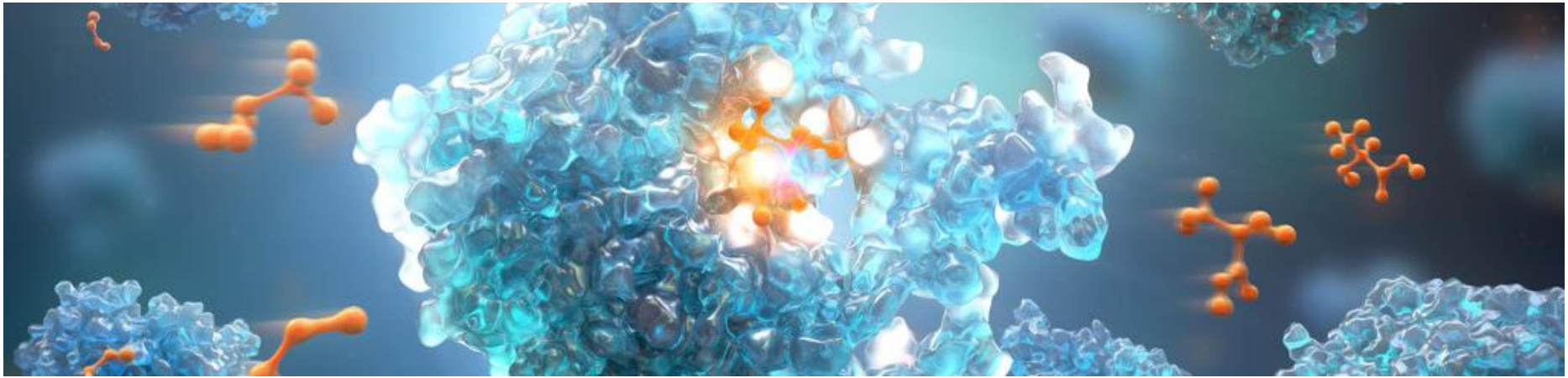


Presentation Overview

1. An Introduction to Raman Spectroscopy
2. Case Study: Bioreactor Performance Monitoring
3. Case Study: High Molecular Weight Species (HMWS) Determination
4. Case Study: Product Identification
5. Conclusions
6. Acknowledgements

An Introduction to Raman Spectroscopy

Introduction to Raman Spectroscopy

- Raman spectroscopy was discovered by C.V. Raman and K.S. Krishnan in 1928[1], it is a type of vibrational spectroscopy much like Infra-Red (IR)
- Raman spectroscopy bands arise from a change in the polarizability of the molecule due to an interaction with light
- The vibrational transitions can be used to identify the molecule and provide a molecular skeleton identified in fingerprint region (400-1900 cm^{-1})

- Advantages of Raman Spectroscopy:
  - **Specificity**
  - **Aqueous analysis**
  - **No sample preparation**
  - **Non-destructive**
  - **Compatible with different container materials**
  - **Short measurement times**

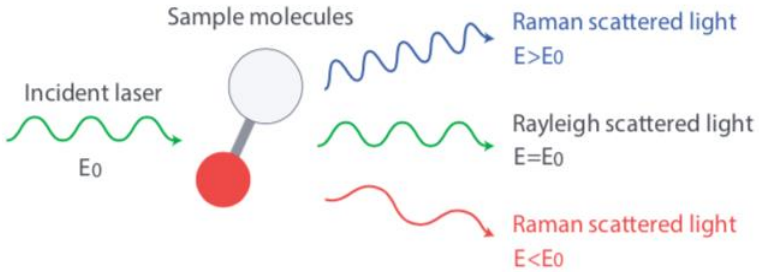


Image courtesy of www.nanophoton.net

[1]Raman, C., Krishnan, K. A New Type of Secondary Radiation. *Nature* **121,** 501–502 (1928)

Chemometrics and Data Processing

Chemometrics uses **multivariate methods** e.g. all variables are considered at the same time, to extract qualitative or quantitative results, **the model fits the data**

Commonly applied tools

- Principle Component Analysis (PCA)
- Partial Least Squares (PLS)

Chemometric data processing comprises of three primary steps:

- Training
- Cross-validation
- Test set validation

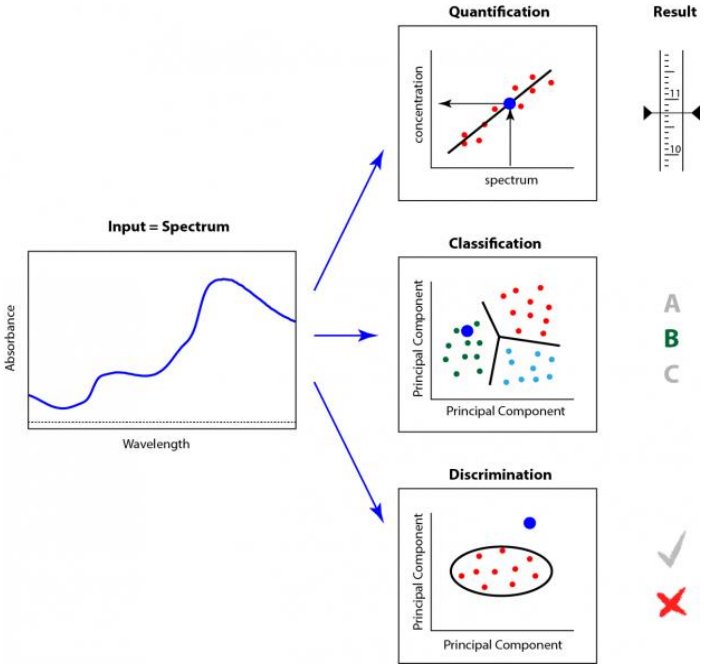


Image courtesy of www.oceanoptics.com

Case Study: Bioreactor Performance Monitoring

Challenges with Bioreactor Performance Monitoring

Most bioreactor monitoring occurs through offline measurements which come with a series of challenges:

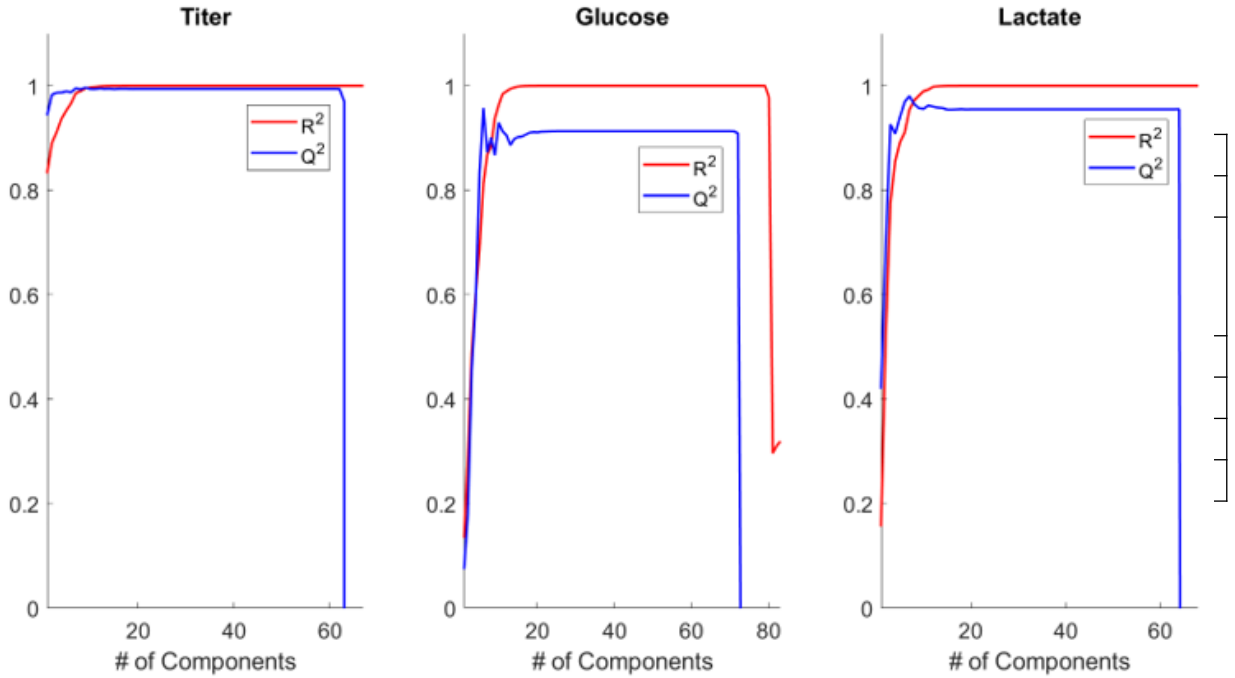
- Time and labor intensive
- Delayed information through single day measurements
- Off-shift sampling
- Potential contamination of the bioreactor

The benefits of in-situ monitoring could be dramatic!

- Earlier detection of process deviations
- Enhanced process control and understanding
- Reduced risk of contamination
- More automatable processes

Bioreactor Performance Monitoring

Some of the key components we want to measure in our bioreactors are product titer, glucose and lactate concentration as these are key indicators of bioreactor performance



We were able to successfully generate robust models for all three components from a single Raman measurement

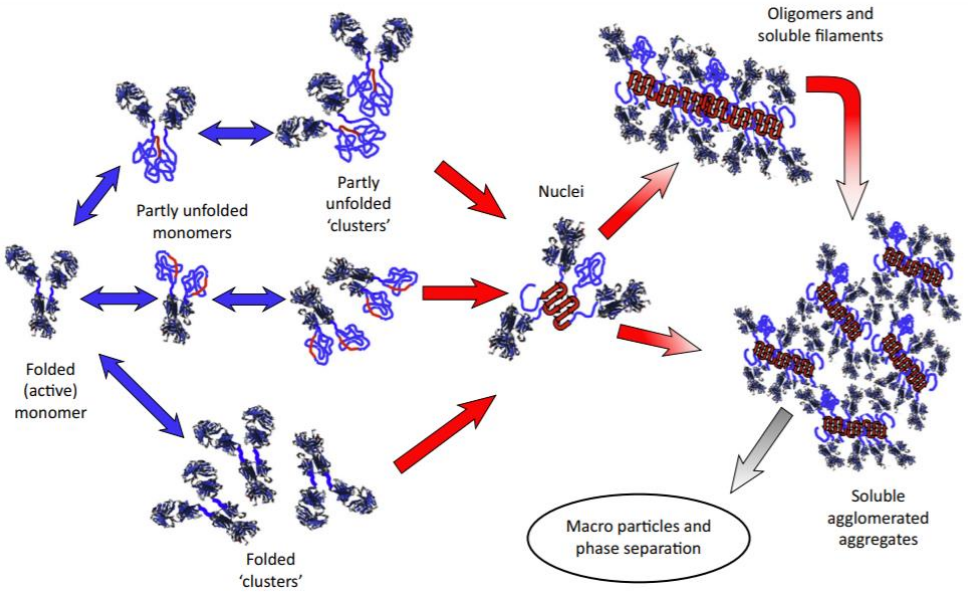
Case Study: HMWS Determination

Why are High Molecular Weight Species (HMWS) Important?

Characterization of protein aggregation is of particular significance, as aggregates may lose the intrinsic pharmaceutical properties as well as engage with the immune system instigating undesirable downstream immunogenicity

Therefore, the monitoring of HMWS is critical to ensure the safety of the product to the patient

In most cases the current HMWS monitoring techniques are not amenable to in-line monitoring applications

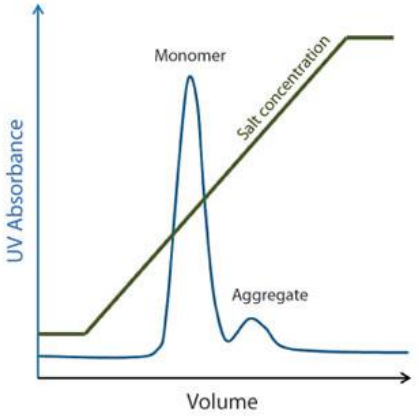


Roberts, C., *Trends in Biotechnology* **32(7)**, 372-380 (2014)

HMWS monitoring during protein purification

Classically two ways to perform column purifications

- Bind and Elute
  - Batch elution
  - Fraction elution
- Flow through



Evans, W. Pharmaceutical Technology **39(3)**, 72-74 (2015)

When performing bind and elute purifications we want to analyze the eluate to ensure the desired product quality[2,3]

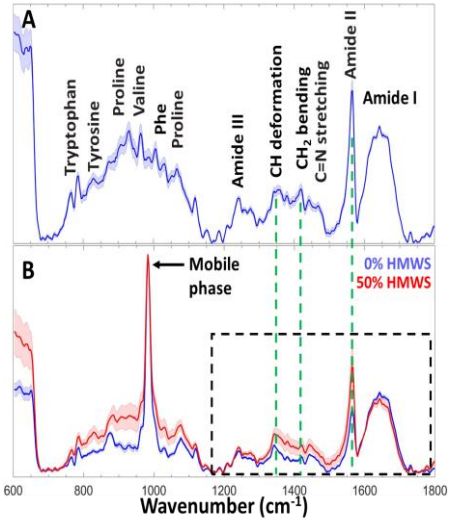
In-line monitoring is highly desirable for this scenario

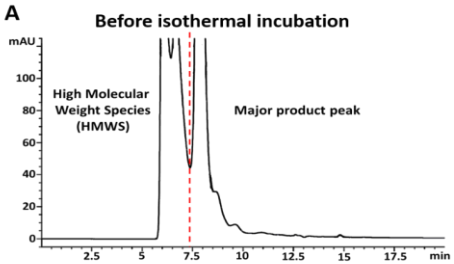
- Reduced in-process hold times
- Reduced requirements for product stability
- Faster processing times
- Less scientist time

[2] *Zhang, Y., Wang, Y., Li, L. Protein Expression and Purification* **158**, 65-73 (2019) [3] Gagon, P. *Journal of Immunological Methods* **336,** 222-228 (2008)

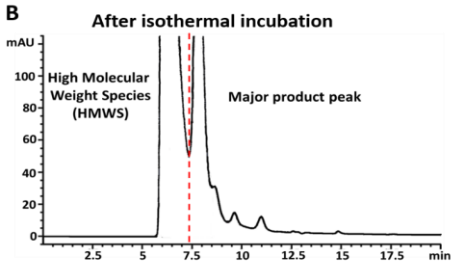
HMWS Determination using Raman Spectroscopy

We built a model using a HMWS standard curve and then trained the model on the data generated

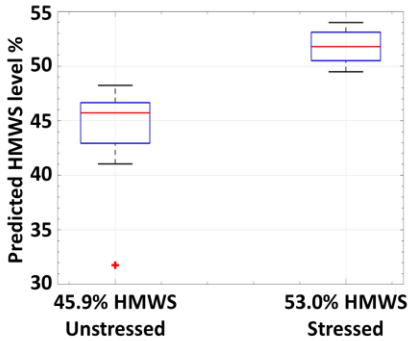




Peak	Assignment	Area %
1	High molecular weight species	45.9
2	Monomer	54.0
3	Low molecular weight species	0.1



Peak	Assignment	Area %
1	High molecular weight species	53.0
2	Monomer	42.1
3	Low molecular weight species	4.9



When presented with two samples of unknown HMWS the model was accurately able to determine the amount of HMWS which was confirmed by SEC

Zhang, C., Springall, J.S., Wang, X., Barman, I. *Analytica Chimica Acta* **1081,** 138-145 (2019)

Case Study: Product Identification

Why is Product Identification Important?

Regulatory agencies **re** release of the drug product.

Product identity is also os:

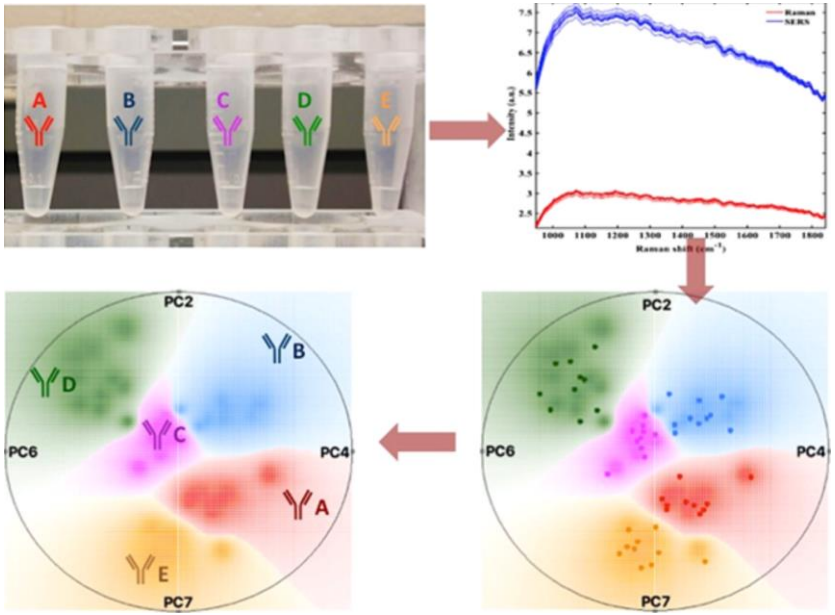
- Product transfers e
- Investigations agai

Proteins can be challe n their amino acid sequences

Common techniques u nd immunoassays

Both can be challengi to execute and requiring reagents specific to the

Product Identification



22 different products were used to construct the sample set for model generation. The concentrations varied from 1-150 mg/mL with the majority in the 50-100 mg/mL range.

The leave-m-out decision algorithms yielded an overall accuracy of classification of ≥ 99.0%

A correct classification rate of ≥ 98% was achieved

Misclassification occurred due to samples belonging to the same isotype and species, some of the samples tested differ by only 3 amino acids

Paidi, S,K., Siddhanta, S., Strouse, R., McGiveny, J.B., Larkin, C., Barman, I. *Analytical Chemistry* **88**, 4361-4368 (2016)

Conclusions and Acknowledgements

Conclusions

- We have successfully demonstrated the applicability of Raman Spectroscopy for in-line monitoring applications throughout the manufacturing process of protein therapeutics

- We have built reliable and robust models for a number of product quality attributes, HMWS and identification and critical process parameters, protein titer, glucose and lactate concentration

- We are looking to implement these models and technologies into our current and next generation manufacturing processes

- We are on the look out for other potential opportunities for expansion of our Raman and other spectroscopy work

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