



Raman for Bioprocessing Workshop

June 25 & 26, 2019

Grenoble, FRANCE

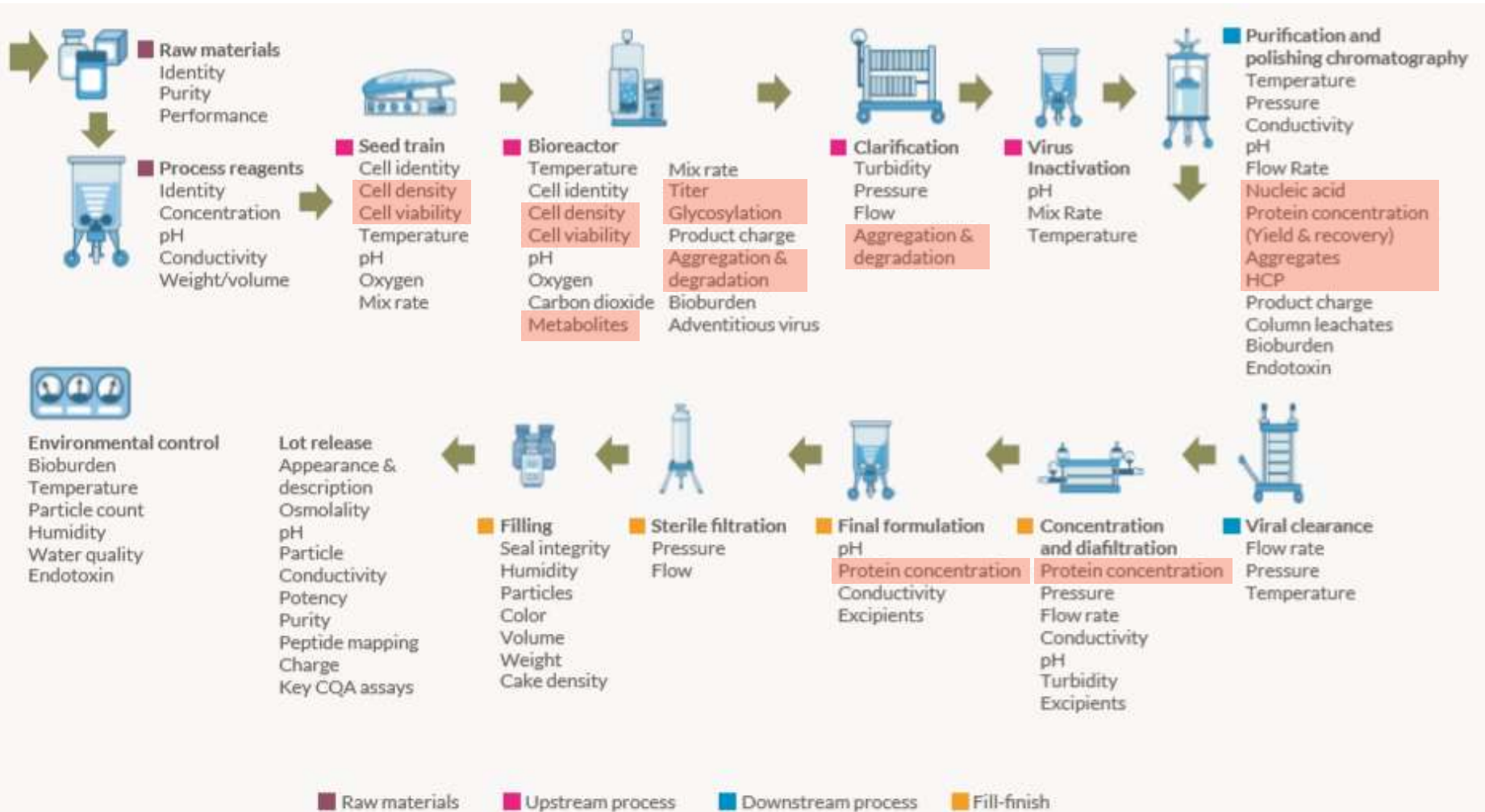
Overview of CQAs Measurement and other Key Applications with Raman

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Raman in biopharma processes



Product and process characterization across the traditional monoclonal antibody manufacturing workflow (from BPOG)



Agenda

1. Raman real-time monitoring of CQAs
 - a) Antibody aggregation
 - b) Antibody glycosylation
2. Raman real-time monitoring of CPPs
 - a) Fermentation
 - b) Perfusion
 - c) Amino acids
3. Conclusion



Real-Time Monitoring of CQAs: Antibody Aggregation

Real-Time Monitoring of CQAs: Antibody Aggregation



- Publication:
 - **Engaging with Raman Spectroscopy to Investigate Antibody Aggregation**
 - Ilokugbe Ettah and Lorna Ashton, Department of Chemistry, Lancaster University, UK
 - Antibodies, 2018
- Challenge:
 - Key issue: control and understanding of aggregation, which can compromise not only product quality but also safety → propensity of aggregates to trigger immune responses
 - **Antibody aggregates occurs in various forms and there is currently no standard definition for an aggregate**

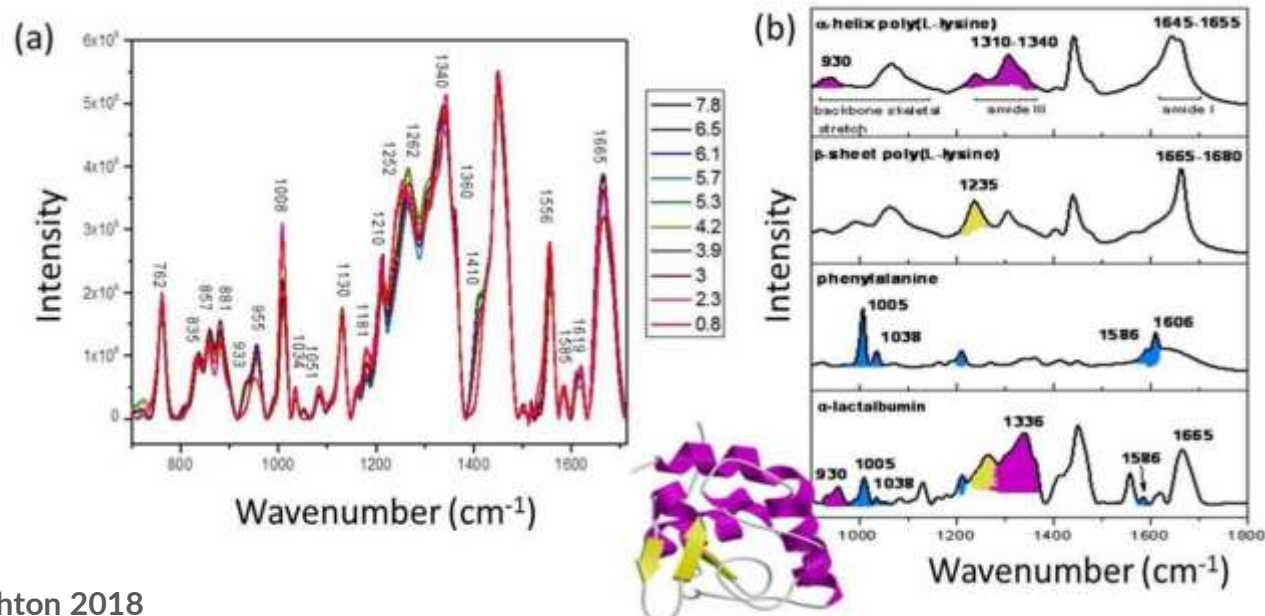
Real-Time Monitoring of CQAs: Antibody

Aggregation

- Aggregation, Secondary and Tertiary structure:
 - Key cause of protein aggregation is the formation of non-native, partially unfolded intermediates with exposed regions capable of inter-molecular interactions
 - changes in physiochemical conditions (e.g., temperature, pressure, pH and excipients)
 - intrinsic conformational properties of the protein (e.g., primary sequence, secondary and tertiary structure)

■ Raman spectroscopy of Proteins

- amide I region (~1630–1700 cm^{-1}) assigned to C=O stretching of carbonyl groups,
- amide III region (~1230–1340 cm^{-1}) assigned to NH bending and Ca-N stretching modes,
- backbone skeletal stretch (~870–1150 cm^{-1}) is assigned to Ca-C, Ca-C β , Ca-N stretches



Real-Time Monitoring of CQAs: Antibody

Aggregation

■ Aggregation, Results:

- Evolution of **aromatic amino acid signal** indicating **aggregation**
- Raman regions of $\sim 760\text{--}770$ and $875\text{--}880\text{ cm}^{-1}$ assigned to tryptophan, displayed the most significant changes

Peak (cm^{-1})	Spectral Change	Sample (Perturbation)	Structural Implication
510/531/547 (S-S stretch)	Observable peaks at 510,531 & 545 in aggregate spectra vs. 531 for soluble Fc	Fc (unspecified)	GGG, TGG and TGT conformations present in aggregates vs mainly TGG in soluble Fc
	Increased perturbation in (I_{510}/I_{540}) for ND aggregates	Anti-streptavidin IgG1(temperature)	ND aggregation pathway showed greater sensitivity to this marker.
850/830 (Tyr)	No change	rhuMab * (Lyophilisation)	-
	Downshift	IVIG * (temperature)	Change in tertiary structure
	Increased ratio	Rabbit IgG (concentration)	Increased molecular interaction
770/875 (Trp)	Increased/decreased intensity	IgG4 variant (temperature)	Less exposure to solvent/more exposure to solvent
1000 (Phe)	Increased intensity	Rabbit IgG (storage)	Pre-aggregation
1230 (β -sheet)	Broadening and shift to 1245 cm^{-1}	Human IgG1 (pH)	Formation of intermolecular β -sheet
I_{1340}/I_{1360} (Trp)	Decreased ratio	IVIG (temperature)	Reduced hydrophobicity (change in tertiary structure)
1555 (Trp)	Downshift	IVIG/temperature	Change in tertiary structure
	Redshift	Anti-streptavidin IgG1(temperature)	Change in tertiary structure
1630 (β -structure)	Increased/decreased intensity	IgG4 variant (temperature)	Increase in intermolecular H-bonding of β -structure/Loss of bonding or loss of secondary structure
1669 (β -sheet)	Decreased intensity	Human IgG1(pH)	Loss of Intramolecular β -sheet
1686 (β -sheet)	Increased intensity	Human IgG1(pH)	Intermolecular β -sheet formation



Real-Time Monitoring of CQAs: Antibody Aggregation



“Raman spectroscopy is a **powerful technique** for the analysis of antibody aggregates, providing **dynamic information** about secondary structure, tertiary structure, and **aggregation mechanisms**. Raman features can clearly be used to **identify changes**, including solvent exposure of residues, conformation, molecular interaction and hydrogen bonding”

“Spectral markers of aggregation could be used to assure and monitor product quality”

Real-Time monitoring of CQAs: Antibody Glycosylation

Real-Time Monitoring of CQAs: Antibody Glycosylation



- Publication:
 - **Real-time monitoring of antibody glycosylation site occupancy by in situ Raman spectroscopy during bioreactor CHO cell cultures**
 - Meng-Yao Li *et al*, CNRS-Lorraine University, France
 - Biotechnology Progress, 2018
- Context:
 - Glycosylation characterization techniques are time- and labor-intensive analyses
 - Glycosylation plays important role in properties and functionalities (e.g. *in vivo* half-life, immunogenicity,...) of the mAbs produced → Essential to monitor and control mAbs glycosylation
 - Its challenging to distinct glycosylated antibody and non glycosylated with only 2-3% molecular weight
- Challenge:
 - To Raman monitor in real-time the glycosylation state of therapeutic monoclonal antibodies

Real-Time Monitoring of CQAs: Antibody Glycosylation



■ Experimental conditions:

Set-up characteristics

- Bioreactor: **Pierre Guérin Technologies** bioreactors
- Raman analysis: **BioViserion® Raman system**
- Off-line analysis: **photometric analyzer Gallery** (Thermo Fisher Scientific) for mAb concentration and **UHPLC-MS** (Thermo Fisher Scientific) for peptides from mAb hydrolysis

Culture characteristics

- **CHO cell line** (CHO M250-9) producing an anti-Rhesus D IgG
- **Fed batch** cell culture
- **2L** working volume
- VCD max: **9.8 E6 cells/mL**

Data acquisition

- Integration time: **60 seconds**
- Accumulation per acquisition: **6 spectra**
- **92 data points** obtained to perform

Real-Time Monitoring of CQAs: Antibody Glycosylation

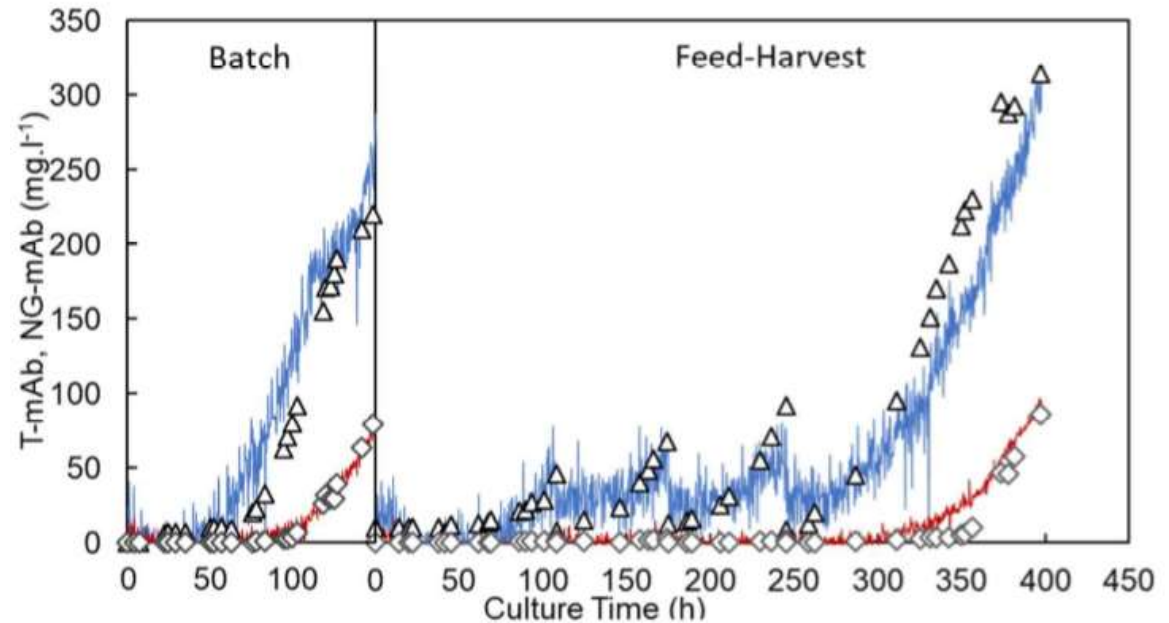


Results:

- Classical data pre-processing and PLS model

RT monitoring of total monoclonal antibodies (T-mAbs) & non glycosylated monoclonal antibodies (NG-mAbs)

T-mAbs: — on-line
 Δ off-line
 NG-mAbs: — on-line
 ◇ off-line



PLS models	Range (mg.l ⁻¹)	LV	R ² _c	RMSEC		R ² _{cv}	RMSECV		R ² _p	RMSEP	
				(mg.l ⁻¹)	%		(mg.l ⁻¹)	%		(mg.l ⁻¹)	%
T-mAbs	0-380	5	0.98	12.9	3	0.88	33.6	9	0.95	17.9	5
NG-mAbs	0-97.6	7	0.99	1.8	2	0.89	7.0	7	0.95	5.7	6

Real-Time Monitoring of CQAs: Antibody Glycosylation



“Raman spectroscopy can thus be considered as a promising PAT tool for feedback process control dedicated to on-line optimization of mAb quality”

“In situ Raman spectroscopy combined with appropriate chemometric methods proved to be a useful tool not only for real-time monitoring of mAb concentration throughout CHO cell culture processes, but also to distinguish between glycosylated and non-glycosylated molecules”

Real-Time monitoring of CPPs: Fermentation

Real-Time Monitoring of CPPs: Fermentation



■ Publication:

- **Raman Spectroscopy and Chemometrics for On-Line Control of Glucose Fermentation by *Saccharomyces cerevisiae***
- Thiago C. Avila *et al.*, Institute of Chemistry, University of Campinas, Brazil
- Biotechnology Progress, 2012

■ Context:

- Fermentation: first historical application of Raman spectroscopy in bioprocesses
- Increased demands on cost reduction while also ensuring the quality of the end product
- Analysis of CPPs by traditional methods, such as HPLC requires pretreatment of samples, analysis by specific equipment and they are time consuming

■ Challenge:

- To use Raman spectroscopy and chemometrics for on-line control of the fermentation process of glucose by *Saccharomyces cerevisiae*

Real-Time Monitoring of CPPs: Fermentation



■ Experimental conditions:

Set-up characteristics

- Raman analysis: **Raman Station 400F Dispersive Spectrometer** (Perkin Elmer)
- Off-line analysis: **HPLC Agilent 1200 Infinity Series LC**.

Culture characteristics

- ***Saccharomyces cerevisiae* JAY270** (Laboratory of Genomic and Expression of the Institute of Biology of the University of Campinas)
- **Fed batch** cell culture
- **2L** working volume

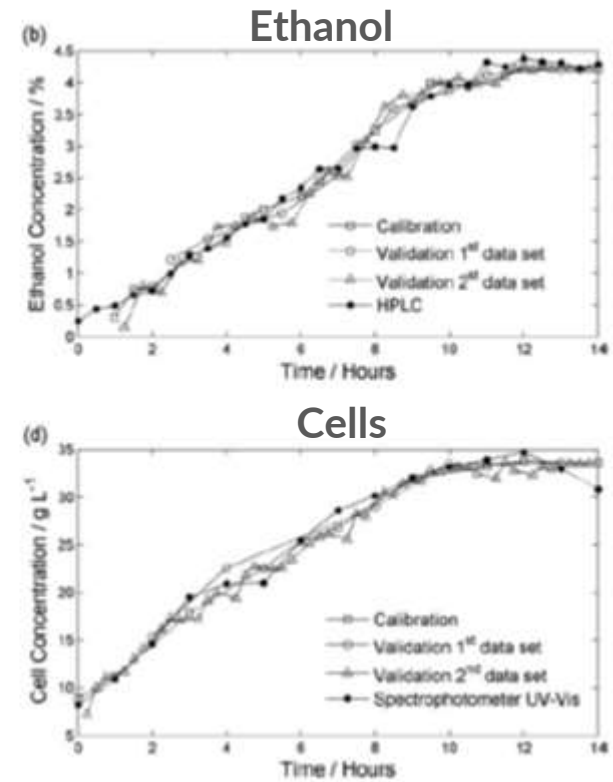
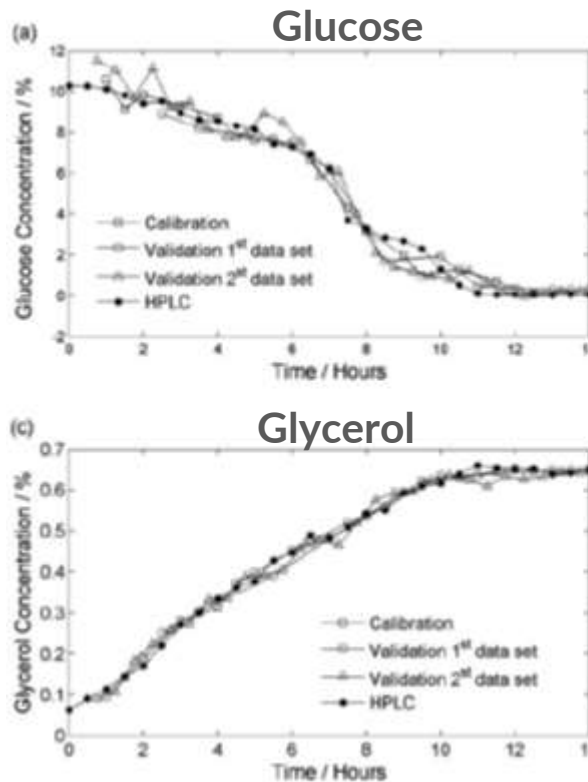
Data acquisition

- Integration time: **1 seconds**
- Accumulation per acquisition: **100 spectra**
- **57 data points** obtained for each batch

Real-Time Monitoring of CPPs: Fermentation



- Treatments, Results:
 - Classical data pre-processing and PLS model
 - Glucose, Ethanol, Glycerol concentrations and Cells densities are predicted with a good accuracy



PLS Model	Sample Number		No. Latent Variables	% of Variance Explained	R ²	RMSEC*	RMSECV*	RMSEP*	Range of the Calibration*
	Calibration	Validation							
Glucose	18	9	2	97.93	0.980	0.55	0.74	0.54	10.12–0.06
Ethanol	18	9	2	99.07	0.981	0.13	0.18	0.25	0.48–4.28
Glycerol	18	9	2	99.57	0.992	0.01	0.02	0.02	0.11–0.65
Cell	10	5	2	97.74	0.979	1.34	2.06	1.02	8.19–30.84

* % for the glucose, ethanol, and glycerol and g L⁻¹ for the cell.

Real-Time Monitoring of CPPs: Fermentation



“The concentration profiles obtained by **PLS models** calibrated against the references values revealed the **ability of the model** to satisfactorily **predict the concentrations** of the species involved during course of **fermentation**”

“These developments can be useful to **industrial bioprocesses** to help in the **control of additions of substrates**, amount, and **purity** of products formed and the presence of **contaminants**”

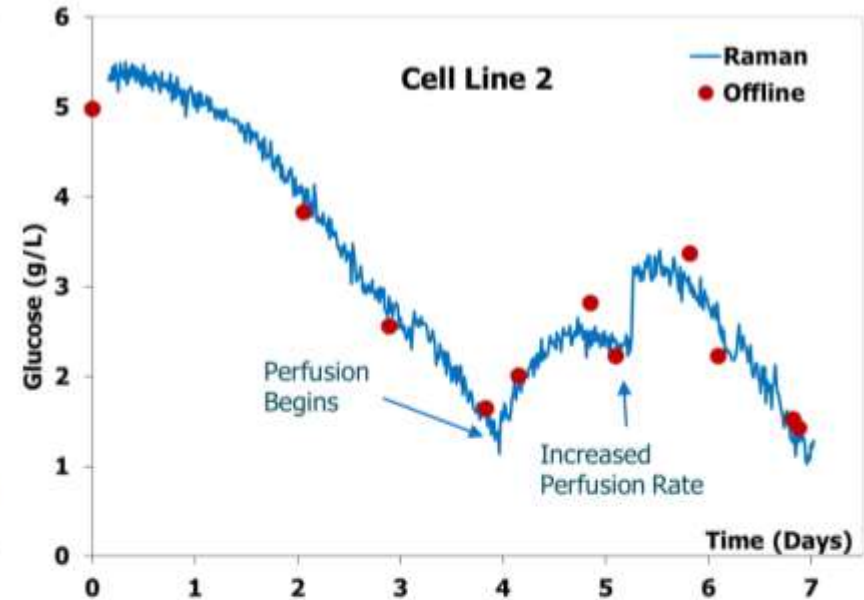
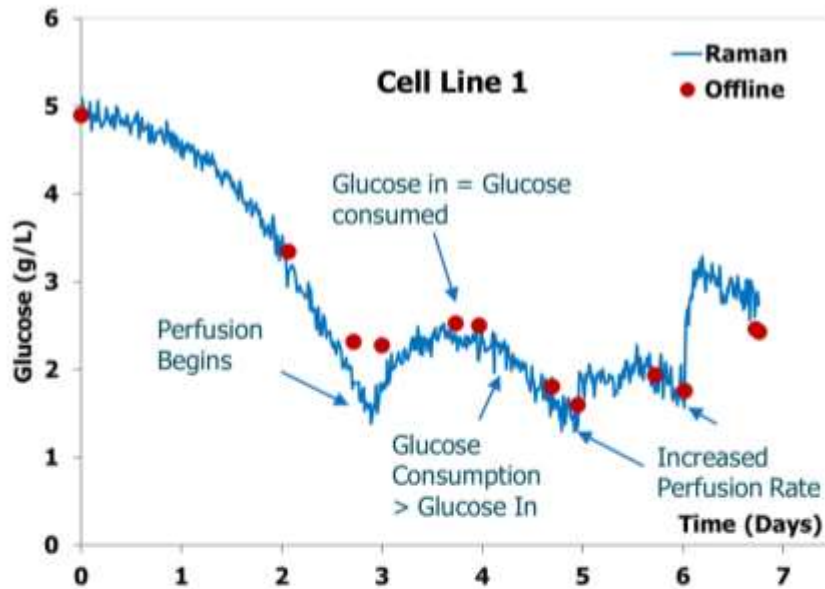
Real-Time Monitoring of CPPs: Perfusion

Real-Time Monitoring of CPPs: Perfusion



- Presentation:
 - **Raman Spectroscopy for Real-Time Process Monitoring in Upstream Bioprocessing**
 - Nobel Vale, BMS
 - Presented at BioProcess International (BPI) West 2019
- Context:
 - Synthetic models built with 15 different samples varying from 0-8 g/L glucose range and 0-3 g/L lactate range, without cells
 - Synthetic models used in prediction in perfusion batches
- Challenge:
 - To Raman monitor glucose and lactate in perfusion from synthetic models in real-time

Glucose Monitoring

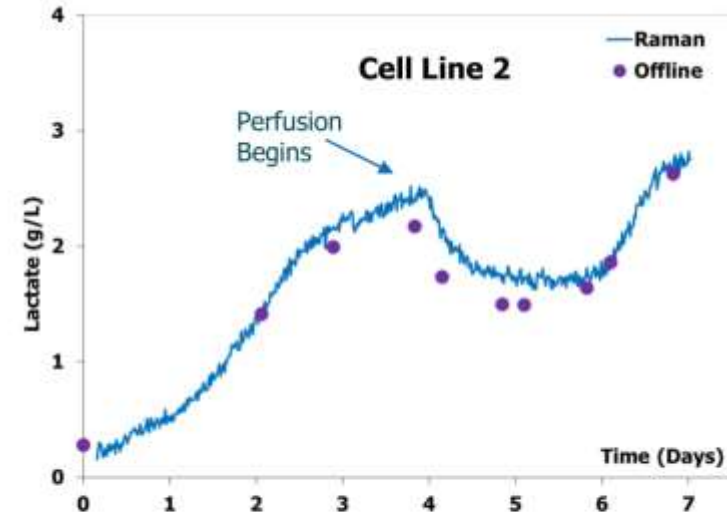
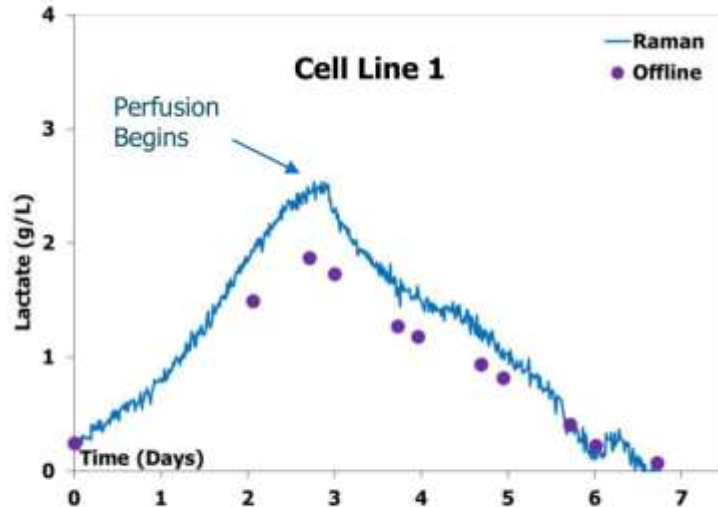


- ▶ Synthetic Model is displayed
- ▶ Raman trending on two different cell-lines - predicted vs offline → good agreement
- ▶ Glucose feedback control is essential as VCD increases ($> 70 \times 10^6$ cells/mL) to prevent depletion
- ▶ Glucose feedback can be used to control the perfusion rate and ensure adequate glucose levels

Real-Time Monitoring of CPPs: Perfusion



Lactate Monitoring



- ▶ Synthetic model displayed - predicts more accurately at high viability
- ▶ Raman Lactate prediction vs offline - correlates well with offline data

Real-Time Monitoring of CPPs: Perfusion



Synthetic Models allow to real-time Raman monitor glucose and lactate in perfusion

The main benefit is the ability to assess perfusion parameters (e.g. perfusion rate) and to adapt them in real-time

Real-Time Monitoring of CPPs: Amino Acids

Real-Time Monitoring of CPPs: Amino Acids



■ Publication:

- **In-Line Monitoring of Amino Acids in Mammalian Cell Cultures using Raman Spectroscopy and Multivariate Chemometrics Models**
- Hemlata Bhatia - Department of Biomedical Engineering and Biotechnology, University of Massachusetts
- Hamidreza Mehdizadeh - Advanced Manufacturing Technology, Global Technology Services, Pfizer Global Supplies
- Denis Drapeau - Bioprocess R&D, Pfizer-Inc.
- Engineering in Life Sciences, 2017

■ Challenge:

- Developing more robust and consistent processes
- **Monitoring four amino acids important for cell growth and production: tyrosine, tryptophan, phenylalanine and methionine.**

Real-Time Monitoring of CPPs: Amino Acids



■ Experimental conditions:

Set-up characteristics

- Bioreactor: **Applikon bioreactors** (Applikon, Inc., Schiedam, Netherlands)
- Raman analysis: **Kaiser RXN2 Raman system** (Kaiser Optics, MI)
- Off-line analysis: Acquity **UPLC-H** class bio-system

Culture characteristics

- **CHO** monoclonal antibody (mAb) producing cell lines
- **Fed batch** cell culture
- **5L** working volume
- VCD range: **2.5-28 E6 cells/mL**

Data acquisition

- Integration time: **10 seconds**
- Accumulation per acquisition: **75 spectra**
- **57 data points** obtained to perform the model

Real-Time Monitoring of CPPs: Amino Acids

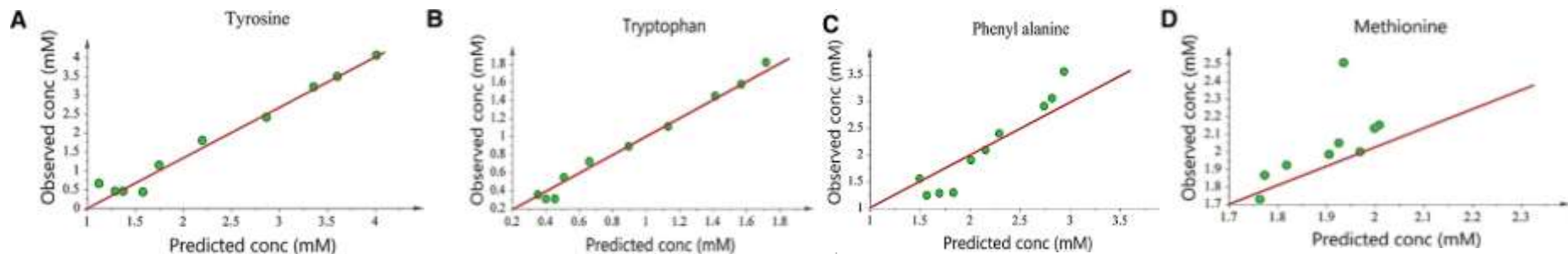


Results:

- Classical data pre-processing and PLS model
- Tyrosine, tryptophan and phenylalanine are predicted with **acceptable accuracy**, while the developed model for measurement of **methionine has lower performance** than the other three models

Data set/AA	Tyrosine	Tryptophan	Phenylalanine	Methionine
Calibration	0.19 - 4.97	0.19 - 2.20	1.20 - 4.35	0.59 - 3.80
Validation	0.28 - 4.05	0.29 - 1.81	1.23 - 3.05	1.70 - 2.50

All the amino acid concentrations are in mM



A.A.	N	# Factors	R ² Y	Q ²	RMSEE	RMSEcv	RMSEP	% error on max. value
Tyrosine	56	2	0.90	0.86	0.41	0.53	0.35	8.6
Tryptophan	55	2	0.82	0.78	0.24	0.27	0.07	3.8
Phenylalanine	56	2	0.79	0.64	0.35	0.47	0.32	10.5
Methionine	57	2	0.21	0.01	0.27	0.29	0.68	27.2

Real-Time Monitoring of CPPs: Amino Acids



“Accurate Raman-based calibration models eliminate the need for offline sampling and manual control in manufacturing processes and render more robust and automated processes”

“This study show that the combination of Raman spectroscopy with chemometrics models is a suitable method for real-time measurement of amino acids in cell culture processes”

Conclusion

Raman benefits



Promising PAT tool for:

- Real-time monitoring of **CPPs and CQAs**
- Real-time **process intervention** (early detection of deviation)
- Real-time assessment of **product quality**
- Enabling a **total automation** and moving to **Pharma 4.0**
- Leading from end product testing to **real-time release testing (RTRT)**

Quality **Pharma 4.0** Critical Process Parameters

Speed **Patient Safety** Real-time

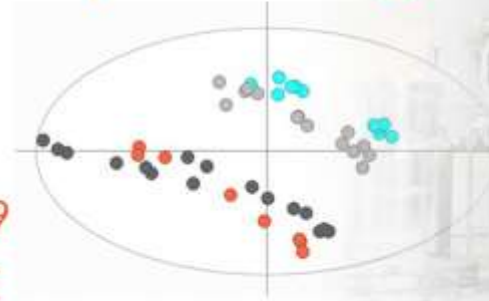
Critical Quality Attributes **Automation**

Cost Reduction

Real-Time Release Testing

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Thank you!

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