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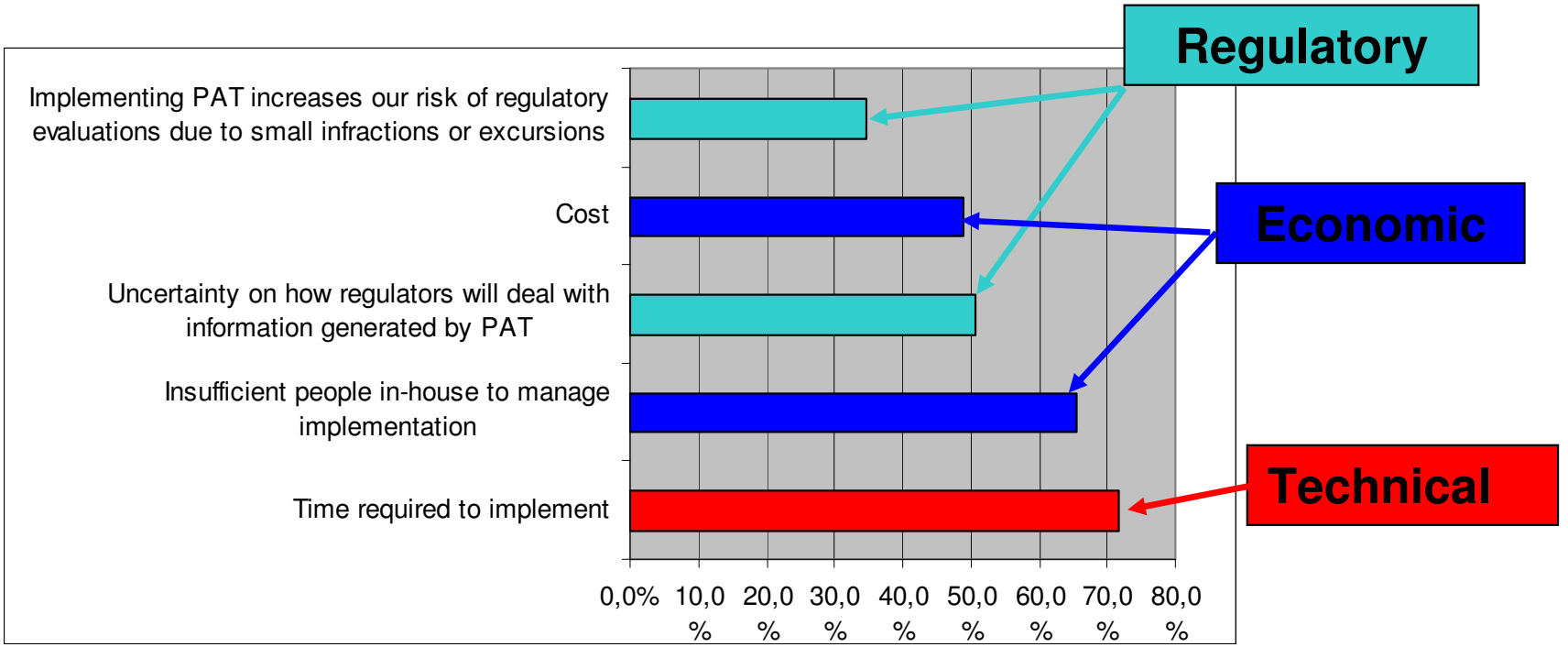
GDCh Colloquium – 1st December 2009
Use of FT-NIR for monitoring of fermentation and lyophilisation processes

PAT in biopharmaceutical manufacturing

Use of PAT in biopharmaceutical industry

BioPlan Associates survey (2008, 434 respondents)

- PAT implementation plans status in biopharmaceutical manufacturing
  - 58% considering PAT for new processes
  - 42.6% considering PAT for existing processes
  - 20% in the process of implementing PAT
  - **3% have implemented PAT initiatives**



Use of PAT in biopharmaceutical industry

General status for biological manufacturing industry – Regulatory aspects

- Exposure to regulatory scrutiny
  - Feeling that more data available means more risks of being challenged
  - Fear that introduction of new analytical technologies in the product development process can be misinterpreted or misunderstood in the submission or during the pre-approval inspection.

  → Current work by FDA: case study with Genentech to see if approval would be faster when using PAT and decrease the overall time to validate a process so that industry will begin adopting PAT in manufacturing.
- Lack of industry-specific guidance
  - PAT currently not required by FDA for biopharmaceutical applications
  - Current PAT guideline adapted to molecular entities but not to biologics → need to be extended with concepts applicable to complex biologics products.
  - Authorities must recognise the fact that PAT tools will have to be more product and process-specific than with traditional drugs.

Use of PAT in biopharmaceutical industry

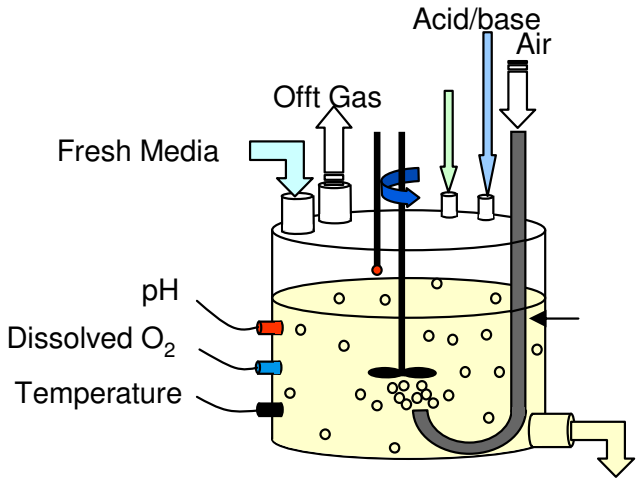
General status for biological manufacturing industry – Economic aspects

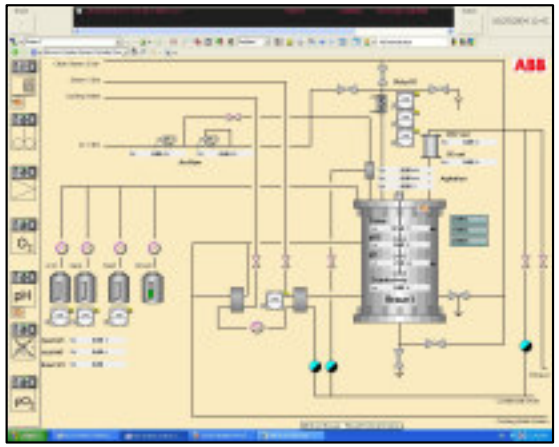
- Complex business case
  - Potential benefits of implementing PAT in the long-term often mitigated by short term uncertainty and risk
  - Trade-off between time and resource investments required from R&D stage and uncertain future for biological drugs in terms of clinical efficacy, regulatory approval, and commercial success
- Process adaptation required
  - Actual benefits of PAT and QbD require an integrated approach to product and process development → Multiple changes needed to allow variability within a process design space while current manufacturing is based on fixed process parameters and end of batch QC tests
  - Scheduling issues between operations if process end-point is based on real-time measurement of a CQA as opposed to pre-specified time (E.g. scheduling of purification steps after fermentation)

Use of PAT in biopharmaceutical industry

General status for biological manufacturing industry – Technical aspects

- Complexity of biologicals
  - Proteins can be extremely heterogeneous and have complex structure → Many more CQAs than for small molecules, characterization not easy
  - Fermentation media contain multiple ingredients and possible interferents
  - Variability of raw materials and seed inoculum
  - Broad dynamic range in product titers (µg/l to g/l)
  - Biological products are heat sensitive and susceptible to microbial contamination → Need for aseptic equipments and principles from initial manufacturing steps in contrast to most conventional drugs.
  - Culture yield very sensitive to process parameters variations (temperature, aeration, agitation)

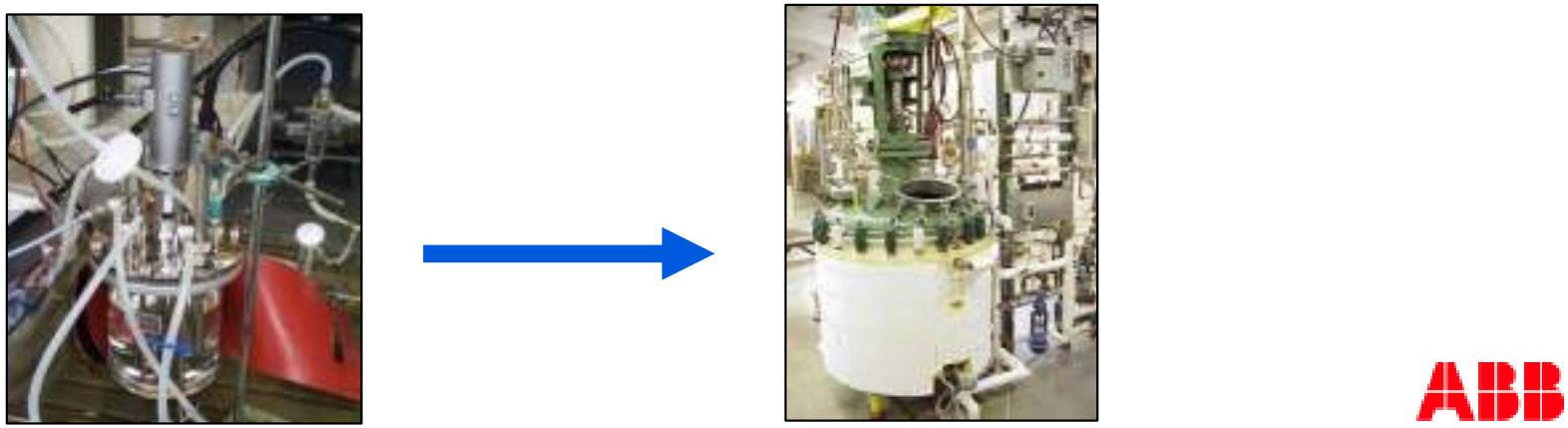




Use of PAT in biopharmaceutical industry

General status for biological manufacturing industry – Technical aspects

- Multiplicity of PAT tools
  - Mass spectrometry, UV, fluorescence, on-line HPLC, chemical imaging, Raman, mid-infrared, near-infrared, …
  - Identification of CPPs and CQAs → Requires tools and experts to handle analytical data overload
- Hardware constraints
  - Availability of instruments that can self-control their operations to ease decisions by manufacturing personnel
  - Most of the current process equipment is not appropriate for use with PAT → Almost impossible to re-qualify and validate legacy equipment
  - Issues associated with transfer of analytics from R&D scale to manufacturing



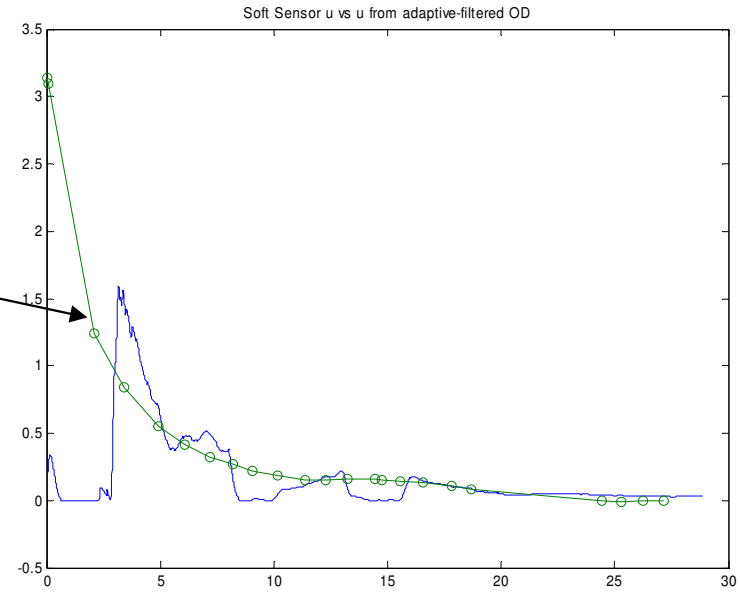
NIR for fermentation and cell culture applications

Use of real-time sensors for fermentation processes

Objective:

- Generation of live data to understand and control fermentation and cell culture processes

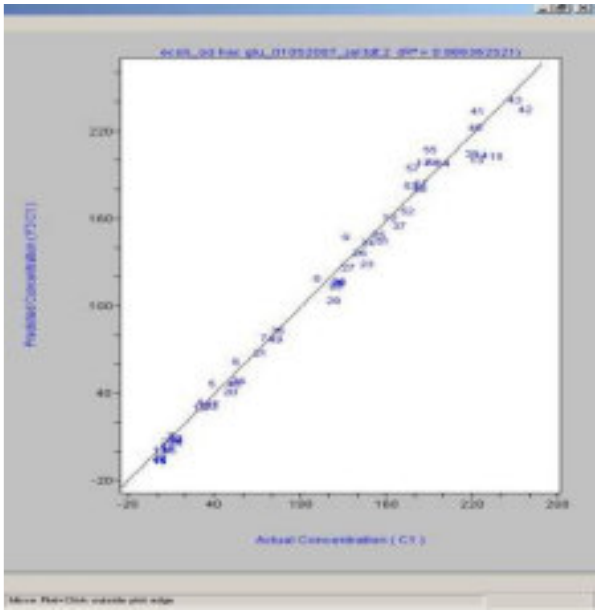
Biomass soft sensor vs OD



Inferential sensors

- pH, temperature, oxygen, agitation, airflow, feed
- Require complex mathematical models to extract process information ("soft sensors")
- Only surrogate markers of product quality

Biomass via NIR vs OD



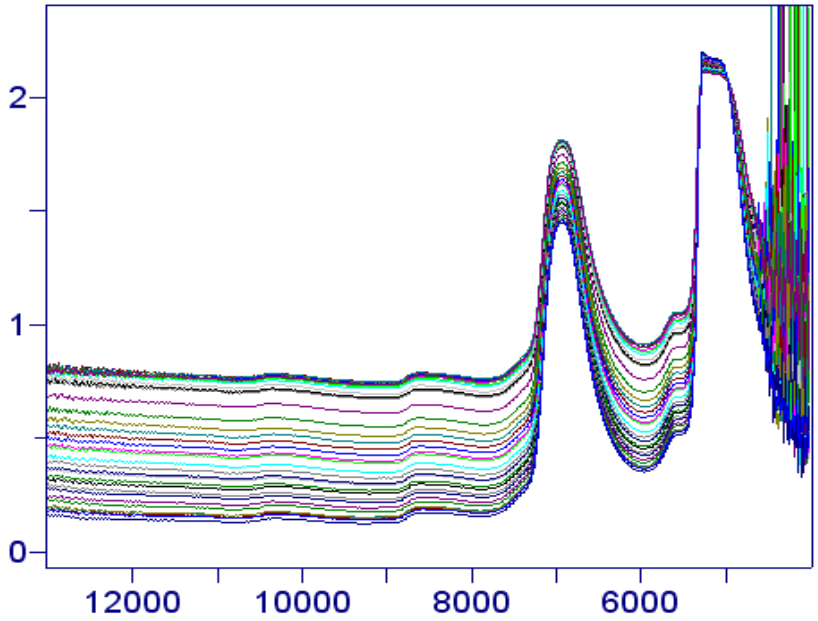
Spectroscopic sensors

- Direct measurement in fermentation broth of nutrients, products and by-products
- Provide status of fermentation matrix condition
- Provide direct product quality information
- Often require multivariate process models

NIR spectroscopy applied to fermentation

Features

- Fast → Allows real-time measurements
- Flexible
  - Fiber optics allows in-situ analysis
  - Different sampling options allow to accommodate different fermentation media (clear, opaque) → Multiplex instruments
  - No sample preparation required
- Accurate → Can be used as an alternative method to laboratory at-line measurements
- Information rich
  - Determination of chemical and physical characteristics of samples → can be used for in-line qualitative and quantitative analysis (nutrients, products, by-products, pH)
  - Can pick-up information on the complete matrix (not chemically characterized)
  - For R&D, can screen a large number of fermentation process attributes
  - For manufacturing can monitor CQAs



FT-NIR spectra: in-line monitoring of Saccharomyces Cerevisiae yeast growth on malt extract (ethanol production)

NIR for fermentation – Technical aspects

Process matrix categorisation

- Classification based on bioprocess fluid and operationnal characteristics of reactor → Determines complexity of NIR modelling for key analytes[1]

Phase/characteristic	Anaerobic	Aerobic		
	(e.g. lactic acid, ethanol production)	Mammalian and insect cell culture (e.g. CHO cells)	Unicellular (bacteria/yeast) (e.g. E.coli, Pichia)	Filamentous bacteria and fungi
Continuous	Water	Water	Water	Water & oil
Discontinuous	Cells	Cells	Cells	Particulates and cells (filaments)
Fluid rheology	Non-viscous Newtonian	Non-viscous Newtonian	Low/moderate viscosity Newtonian	Viscous non-Newtonian
Gas phase	No areation	Low aeration	Highly aerated	Highly aerated
Agitation	Moderate	Moderate	High	High
Cell density	Low	Low	High	High
Optical medium	Clear	Clear	Dark	Dark

Increasing complexity →

- Selection of sampling interface
  - For in-line control of manufacturing processes; in-situ probes are preferred to avoid introduction of manual sampling in sterile processes or sampling loops with potential dead zones

NIR for fermentation – Technical aspects

Probe optimisation

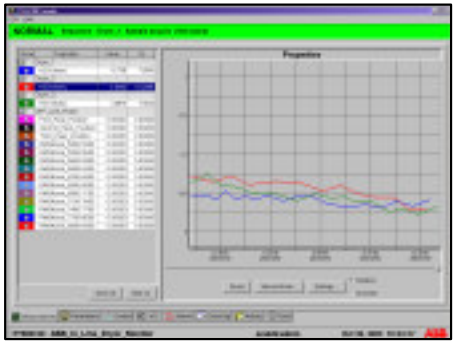
- Transflectance probes
  - Can accommodate clear (transmission) and optically dense (reflectance) media → Useful for exploratory R&D work from lag phase to exponential and stationary phases (alternative: multiplex measurement)
  - Adjustable pathlength useful for method development but issues in manufacturing (cleaning, reproducibility and stray light issues)
- Transmission probes
  - Useful for most fermentation processes except filamentous fermentations (e.g. antibiotic production)
  - Require aseptic design
- Reflectance probes
  - Useful for some filamentous fermentations

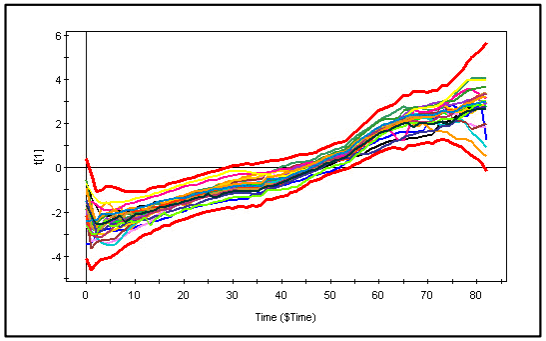
- Important constraints related to sterility regardless of probe type
  - Surface finish of wetted parts
  - Special fiber and connector design if autoclaving required
  - Special window design if CIP/SIP required (temperature gradient)

NIR for fermentation – Technical aspects

Chemometrics[1,2]

- Spectral region optimisation (exclude C-H combination region below 4800 cm^{-1} and strong water band at 5100 cm^{-1})
- Full spectra algorithms preferred to extract complex matrix information
- Incorporate temperature fluctuations in model
- Consider time-segmented calibrations to handle fast matrix changes
- Use of spectral preprocessing
  - Effect of agitation rate, gas flow rates and biomass variations on scattered light variations → baseline shifts
- Use process trajectories to detect culture contaminations
- Use on-the-fly statistical evaluation of model applicability to detect extrapolation conditions where model robustness can be challenged (*e.g.* F-ratios)





NIR for fermentation and cell culture

Complex raw materials identification and quality analysis

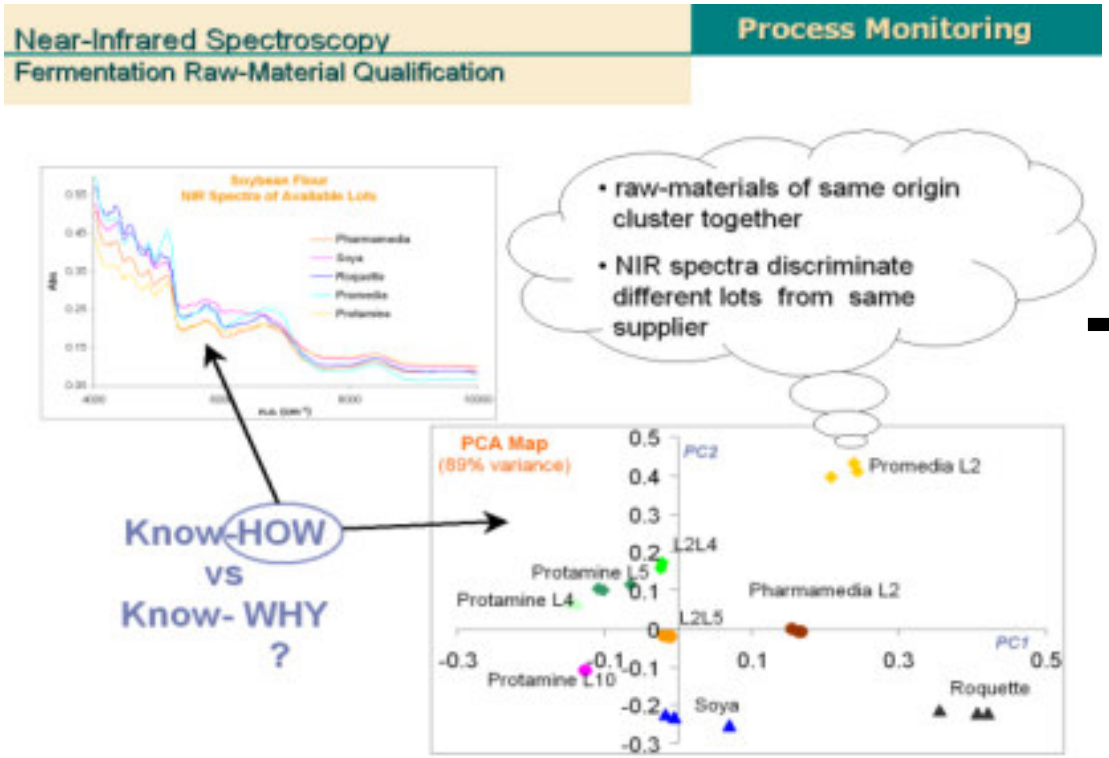
- Qualification of master cells and nutrients for fermentation step
  - Monitoring of inoculum generation and prediction of inoculum quality
  - Selection of the best inoculum out of a number of candidates to reduce process variability
- Qualification of enzymes and solvents for modification step
- Qualification of column packing material for chromatography and purification steps

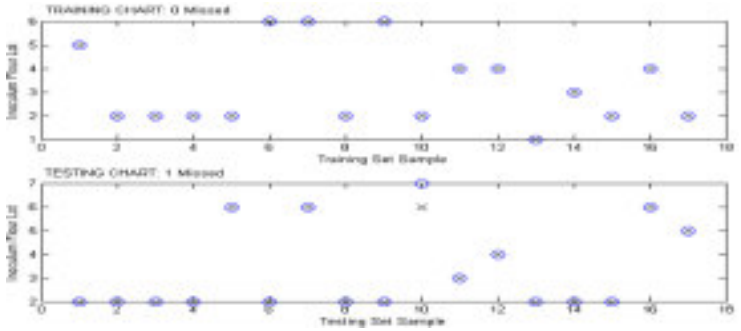
Courtesy J. Cardoso de Menezes (IST Lisbon)

NIR spectroscopy applied to fermentation processes

Complex raw materials identification and quality analysis

- Example: Classification of soybean flours from different suppliers and Kohonen network mapping of inocula quality from FT-NIR spectra of raw materials. Objective: minimise impact of flour quality variations (nitrogen source) on titer of final antibiotic produced by microbial fermentation of strain of mycellial micro-organisms "streptomycetes clavoligerus"[3].



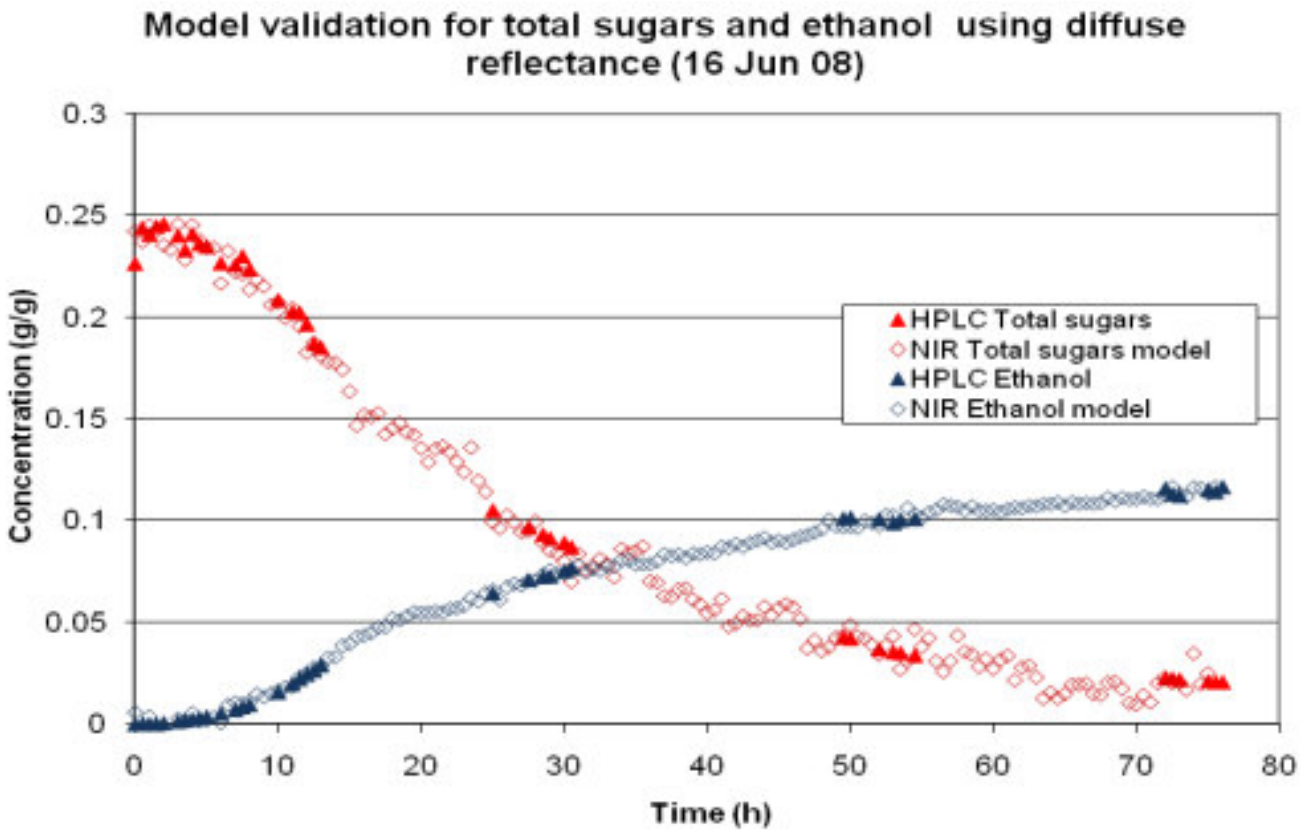


Predictive determination of inoculum spectra quality from flour spectra. Calibration + test set. (IST-Cipan)

NIR spectroscopy applied to fermentation processes

Monitoring of the fermentation process

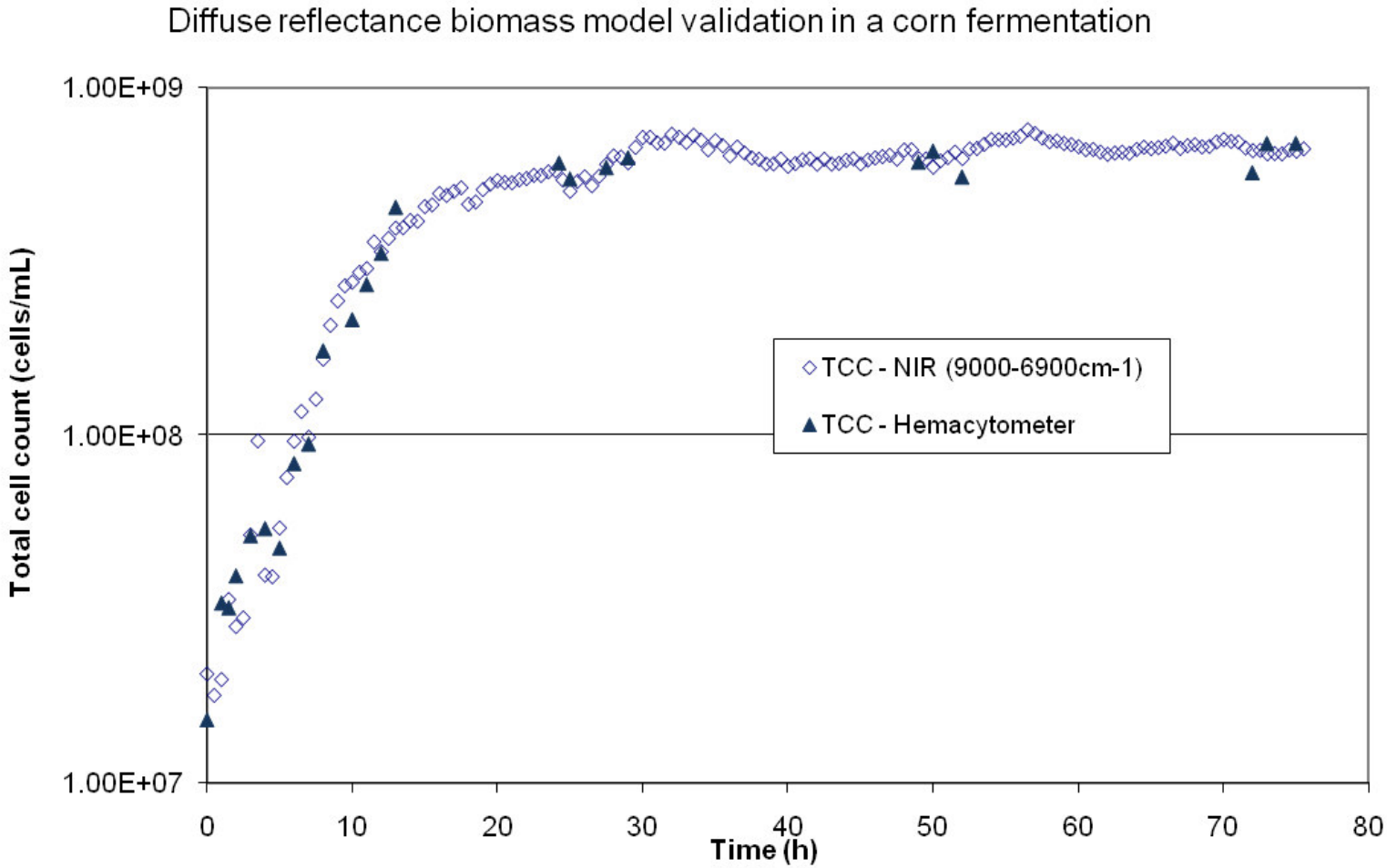
- Determination of fermentation analytes concentration (nutrients, metabolites, by-products)
- Example: monitoring of total sugars and ethanol from FT-NIR spectra during very-high-gravity corn mash fermentation (NCSU-BTEC)



NIR spectroscopy applied to fermentation processes

Monitoring of the fermentation process

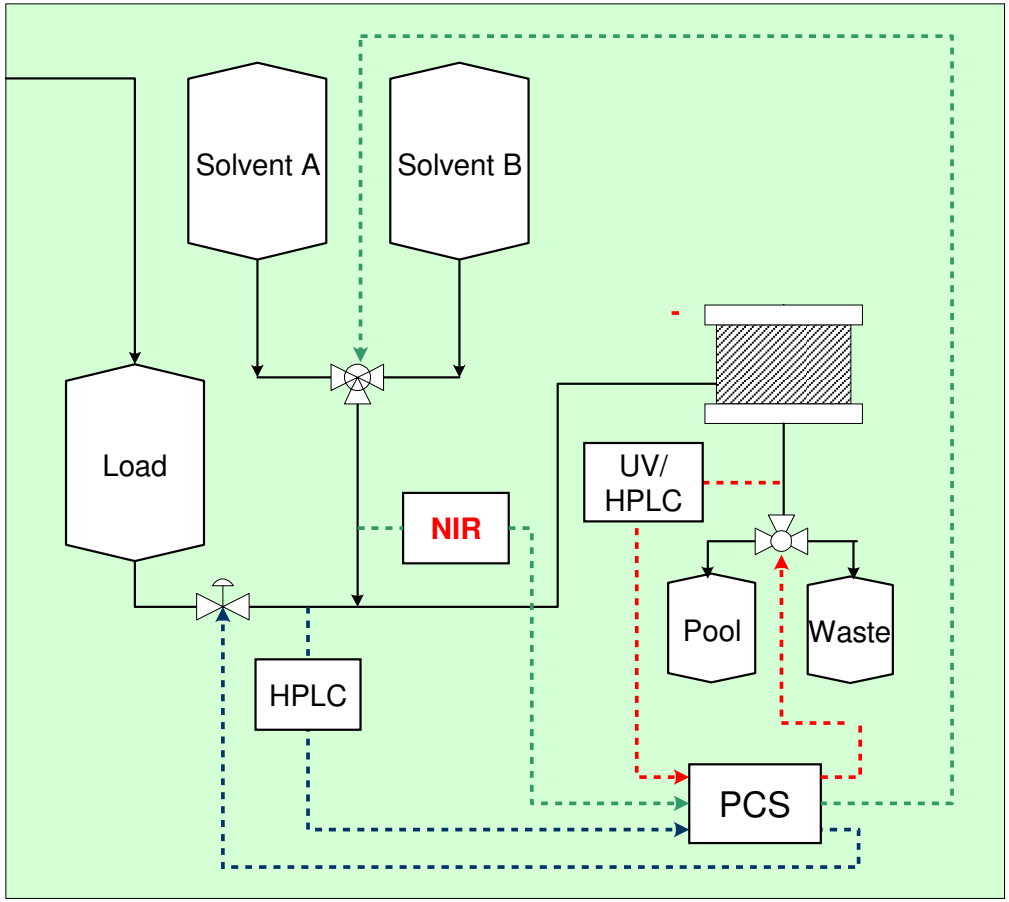
- Cell density and biomass content
- Example: determination of biomass growth by correlation of FT-NIR spectra with cell density during very-high-gravity corn mash fermentation (NCSU-BTEC)



NIR spectroscopy applied to fermentation processes

Monitoring of downstream purification process

- Monitor the elution process and fraction collection to reduce loss of API through ionic exchange column (alternative to assay that takes 10 minutes)[3]
- Example: Use of FT-NIR to control chromatography column gradient mixing in a protein purification process (NNE Pharmaplan)

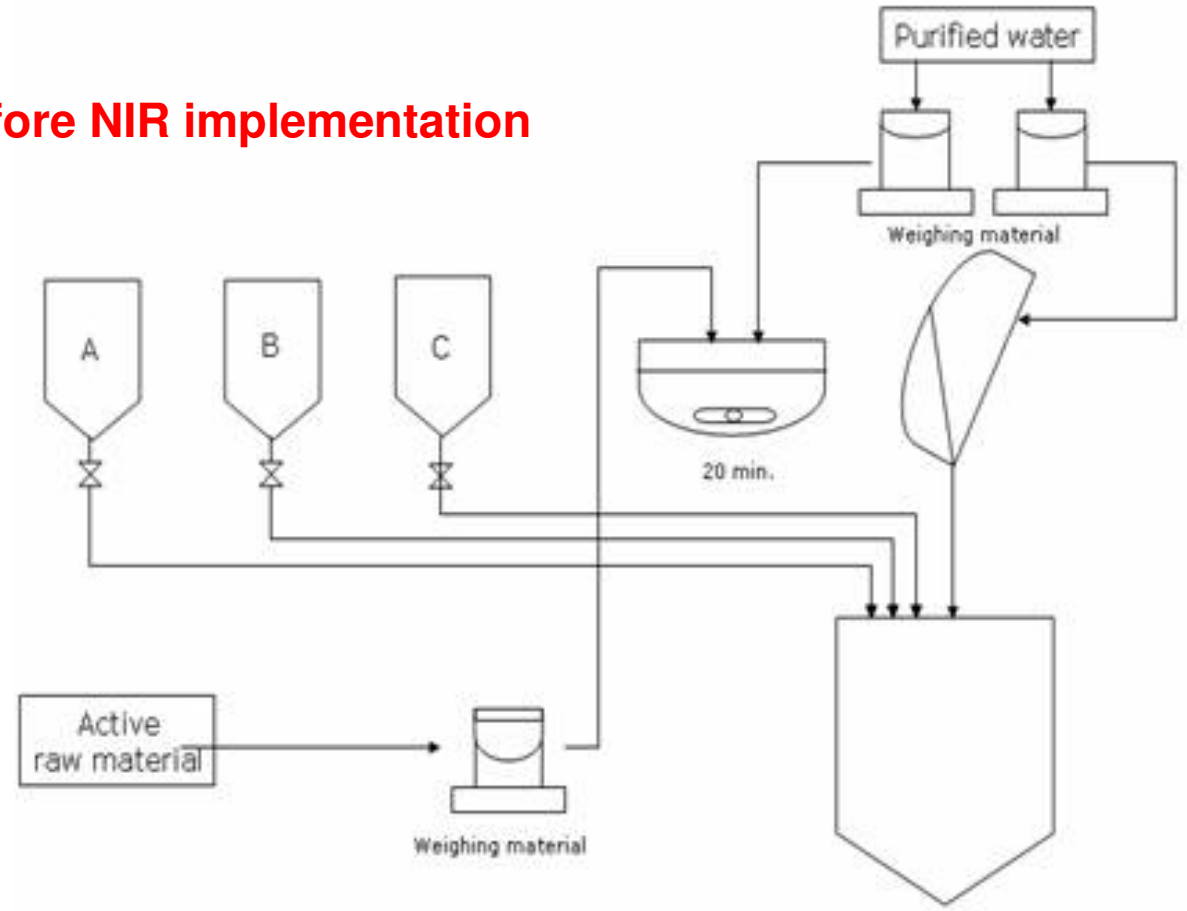


NIR spectroscopy applied to fermentation processes

Monitoring of downstream modification process

- Control of matrix condition to improve selectivity of modification process
- Example: on-line water/organic solvent ratio to optimise selectivity of fatty acid addition to a protein (NNE Pharmaplan)

Before NIR implementation



NIR spectroscopy applied to fermentation processes

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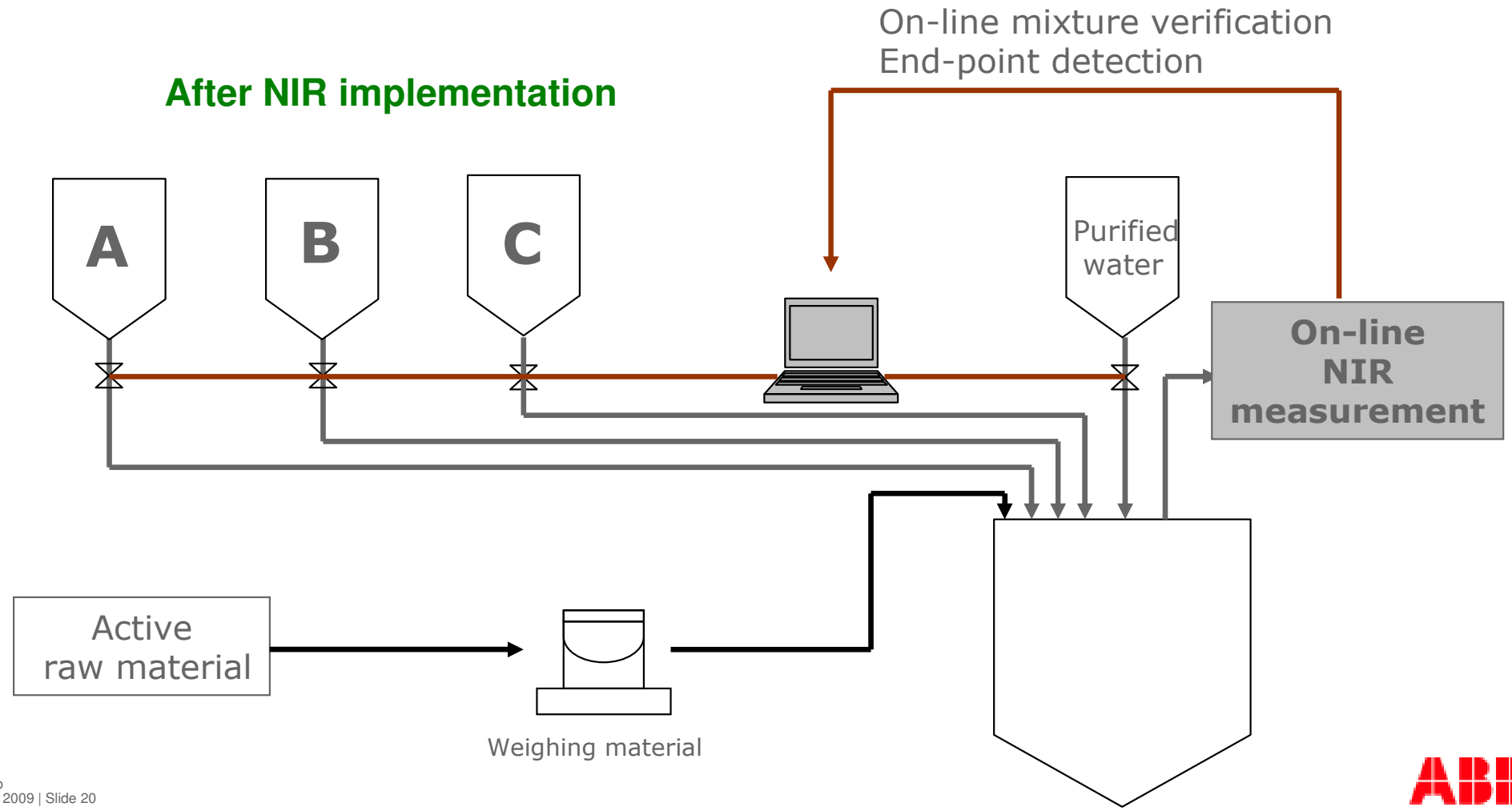


NIR for lyophilisation applications

Use of PAT sensors for lyophilisation processes

Objectives:

- Real-time monitoring of moisture content for end-point determination of freeze-drying process
- Achieve real time feedback control to achieve “right first rime” quality[4]

At-line methods

- Loss on drying (IR) → lack of repeatability, destructive, can not distinguish free/bonded water
- Karl-Fischer titration → destructive, slow, requires skilled operators

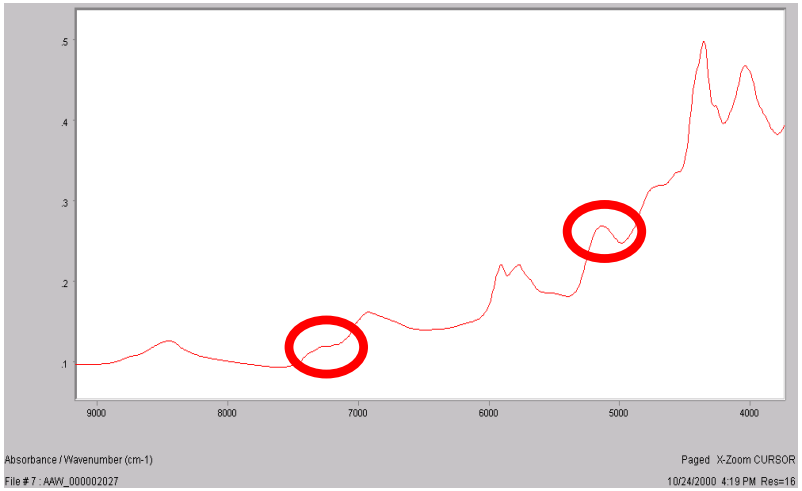
Some PAT sensors

- Temperature / conductivity probes inserted in vials:
  - Potential crystallisation points during freeze-drying
  - Sterility issues
  - Non-representative information
- Mass spectroscopy (Headspace measurement on vaporised water):
  - No process interference
  - Provides overall process end-point information
  - No direct measurement of CQA
  - Requires constant leak rate → frequent leak tests required

NIR spectroscopy applied to lyophilisation processes

Features

- Fast → Allows real-time measurements
- Flexible
  - Fiber optics allows in-situ analysis
  - Non-contact diffuse reflectance probes allow non-invasive measurements through glass vials
  - No sample preparation required
- Strong signature of water of NIR → accuracy comparable to reference methods and better precision (SEP<0.2% w/w)
- Non destructive
- Multiplex instruments provide information on several points → “mapping” of lyophiliser
- Information rich
  - Determination of moisture level, crystallinity and polymorph variations

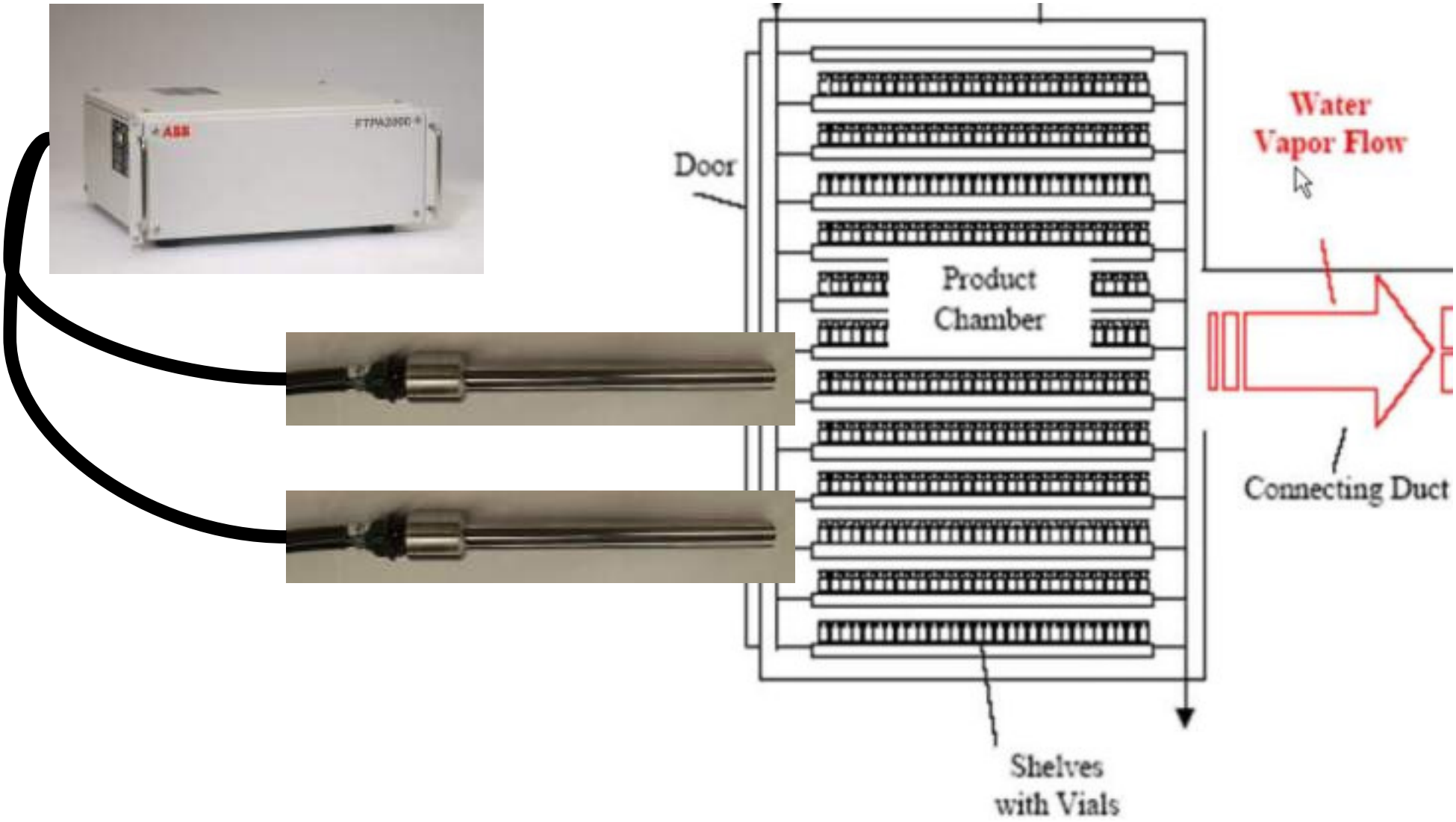


Water absorption (combination bands) in FT-NIR spectra

NIR spectroscopy applied to lyophilisation processes

Possible setup

- Process FT-NIR analyser with non-contact probes inserted in lyophiliser wall



NIR spectroscopy applied to lyophilisation processes

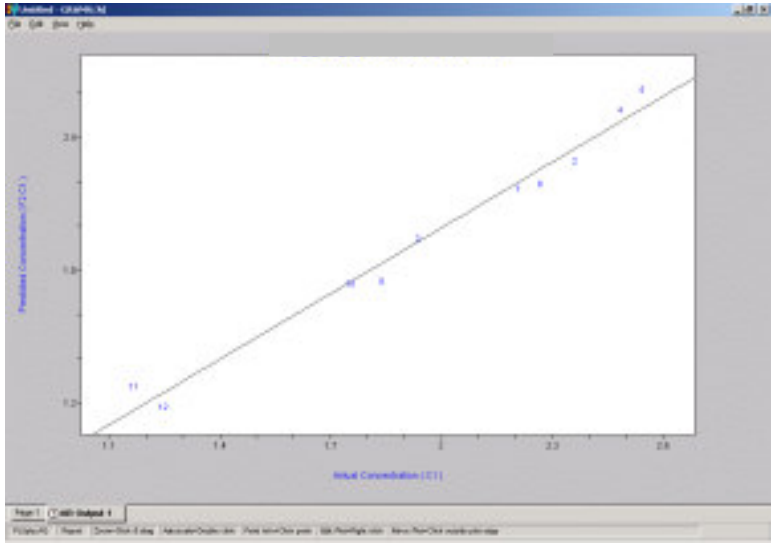
Technical challenges – Probe-related aspects

- Availability of sampling points
  - Not possible to retrofit legacy equipment
  - Collaboration with freeze dryer supplier required early in project
  - Ideally have sampling points coupled with windows
- Positioning of sampling points
  - Usually only external vials accessible due to tray design with silicon oil circulation between shelves
    - Monitoring only (cycle-time reduction) → OK
    - Feedback control or parametric release → Requires careful validation to demonstrate representativity of measurements (drying usually not homogeneous)
  - Reproducibility of probe alignment in front of vial can be challenging (automated tray loading, possibly different vial diameters depending on products) → Possibility to visually preposition retractable non-contact probe after tray loading and manually fine tune probe positioning by using XYZ 3-D stage and maximising reflectance signal after water sublimated (powder), or early in process by focusing beam on stopper.
- Specifications
  - Probe with aseptic design that sustain P and T conditions, including SIP cycle temperature gradient
  - Vacuum-tight flange seal

NIR spectroscopy applied to lyophilisation processes

Technical challenges – Chemometrics-related aspects

- Stable measurements needed
  - Freeze drying cycles can reach up to several weeks → Requires stable instruments that can run during that period with the same background
- Calibration model type
  - Monitoring only: qualitative trending of peak height
  - Feedback control or parametric release
    - Matrix-specific PLS model
    - Spectral peak area linear regression[5]
    - Adaptive process software for real-time prediction (automatic switch between high moisture range model (free water) and low moisture range model (bonded water)



Conclusions

NIR for fermentation and lyophilisation processes

Fermentation and cell culture applications

- NIR useful at multiple stages of fermentation-related processes
- Upstream: raw materials qualification
- In-process: real-time monitoring of matrix condition and product titer
- Downstream:
  - Qualitative analysis of column material + real time monitoring of the purification process
  - Real-time monitoring of modification process
- Key aspects:
  - Proper preliminary matrix categorisation
  - Increasing availability of papers related to manufacturing implementations

Lyophilisation

- Technology potential: achieve true QbD as alternative to QC testing
- Main challenge: in-line analyser interfacing to freeze-dryer
- Expected that first advances in manufacturing will be in the field of process monitoring rather than control

Use of PAT in biopharmaceutical industry

References

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