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APPLICATION OF PROCESS ANALYTICAL TECHNOLOGY TOOLS IN CHINESE HAMSTER OVARY CELL LINE BASED PROCESSES IN BIOLOGICAL DRUG SUBSTANCE MANUFACTURING

Proceeding of Theses

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1. Introduction and scope of research

Basic goal of my PhD study was to monitor industrially highly relevant monoclonal antibody (mAb) producing Chinese Hamster Ovary (CHO) cell line based cultivations with modern Process Analytical Technology (PAT) tools. Applying state-of-the-art PAT monitoring systems can lead to advanced process control (APC), which increases process reproducibility and robustness.

I intended to investigate the feasibility of CHO cultivation PAT monitoring in three definite and critical areas (Fig. 1.):

- feasibility of viable cell density (*VCD*) monitoring with dielectric spectroscopy (DS);
- feasibility of cellular environment monitoring with near-infrared (NIR) spectroscopy;
- feasibility of the most critical raw material (medium powder) qualification with near-infrared (NIR) spectroscopy.

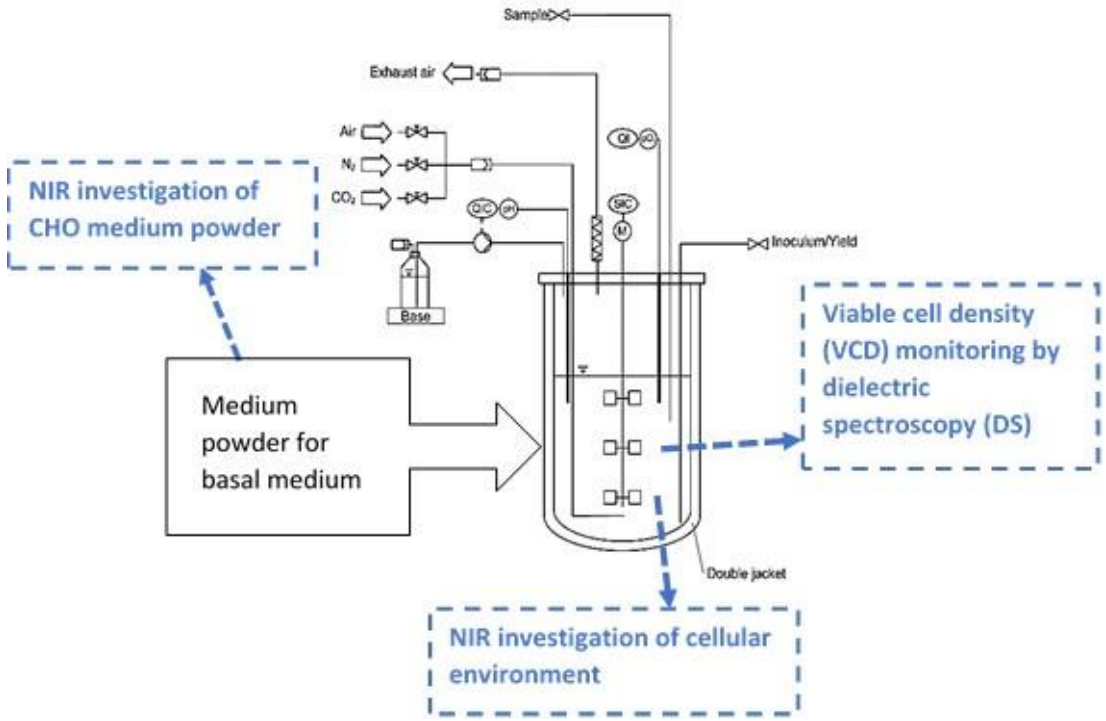

Figure 1. – Investigated PAT areas during my PhD research.

I had the following aims in the three critical areas:

- to investigate the feasibility of dielectric spectroscopy (DS) in the new and extreme ≥ 40-50×10^6 cells/ml viable cell density (*VCD*) range;
- to compare three mathematical procedures (one frequency linear regression, Cole-Cole modelling, partial least squares regression) in *VCD* prediction;

- to investigate the feasibility of directed biochemical and physical change detection with near-infrared (NIR) spectroscopy in shake-flask model system;
- to determine the precision of NIR spectroscopy based glucose concentration prediction in shake-flask model system;

- to investigate the feasibility of directed heat treatment detection with NIR spectroscopy in CHO medium powder formulation;
- to compare the sensitivity of NIR spectroscopy based medium powder qualification and traditional cell culture test;

2. Literature

US Food and Drug Administration (FDA) published its Process Analytical Technology (PAT) guideline in September 2004 in order to make pharmaceutical production more reliable, robust and cost effective.[1] According to the FDA's definition, PAT is a designer, analyzer and controller system, which provides final product quality by the measurement of raw material and process critical parameters. Briefly, PAT measures, analyzes and controls the manufacturing process itself. Accordingly, PAT tools are state-of-the-art process monitoring systems and multivariate data analysis (MVDA) methods.

PAT's state-of-the-art process monitoring systems can be the basis of mammalian cell culture (like CHO) process control as well.[2] It is worth to mention that viable cell density (*VCD*) control has already been established based on dielectric spectroscopy (DS). Amongst automated flow cytometry (AFC) focused beam reflectance method (FBRM) and DS, this latter is the most frequently applied in the industry.

Concerning glucose concentration control in CHO bioreactor processes, there are Raman spectroscopy based control solutions[3] but only monitoring was established with NIR spectroscopy. Off-gas analysis has been applied in fermentation industry for a long time but feedback control solutions are not well-established yet. Nevertheless oxigen uptake rate (*OUR*) and carbon-dioxide evolution rate (*CER*) can be calculated real-time. *OUR* based specific cell growth rate control was already established[4] and *OUR* based specific productivity control with L-tyrosin feeding was also performed.[5]

1. US Food and Drug Administration (2004). *U.S. Department of Health and Human Services*, 1–16.
2. Gilbert et al. (2014). *Pharmaceutical Bioprocessing*, *2*(6), 519–534.
3. Berry et al. (2016). *Biotechnology Progress*, *32*(1), 224–234.
4. Aehle et. al. (2011). *Cytotechnology*, *63*(1), 41–47.
5. Zalai et al. (2016). *Applied Microbiology and Biotechnology*, *100*(16), 7011–7024.

In dielectric spectroscopy (DS) CHO applications, linear *VCD* prediction using permettivity value measured in one frequency is only reliable and precise under specific conditions. Howerver, multi-frequency scanning solutions provides a more detailed fingerprint of the dielectric behaviour of the cell suspension.[6] There are two possible ways to extract relevant information from dielectric spectra. One solution is to fit a mathematical model onto the measured capacitance spectra, which is called Cole-Cole modelling.[7] The other option is to apply multivariate projection methodology (like principal component analysis, PCA) and multivariate regression (like partial least squares, PLS). Precision of PLS regression based *VCD* prediction was higher compared to the one frequency linear regression or Cole-Cole modelling[8] but this method requires historical data sets for calibration.

First significant *in situ* NIR spectroscopy based CHO cultivation monitoring was performed by Arnold et al.[9] in 2003. They applied on-line dispersive NIR equipment in transflectance measurement mode. During their data handling, variable selection (wavelength selection), standard normal variate (SNV) and second derivative pre-treatment methods were used. They built calibrations for glucose, lactate, glutamine and ammonium concentrations by using reference data. They achieved promising result but limited number of runs were involved and NIR models were probably over-fitted. Nevertheless their results gave an impulse to NIR based cell culture monitoring research. Henriques et al.[10] applied Fourier transform (FT-) NIR spectroscopy and were able to achieve acceptable calibrations for the four metabolites (glucose, lactate, glutamine and ammonium) in CHO bioreactor system. They investigated five, not totally identical fermentation runs and they proposed NIR spectroscopy for overall qualitative fingerprint-like monitoring of fermentation runs.

Clavaud et al.[11] also applied qualitative fingerprint-like monitoring in 10 fermentation runs and could identify different phases of cultivations based on principal component analysis (PCA) of FT-NIR spectra. Besides they built quantitative calibrations for product titer, viable cell density (*VCD*) and glucose concentration. Sandor et al.[12] achieved similar results with diode array (DA) NIR spectrometer for the four metabolites and total cell count (*TCC*). Milligan et al.[13] performed glucose and lactate additions during CHO cultivation in order to eliminate correlation of these two components. Afterwards they built calibrations for glucose based on NIR spectra measured with dispersive equipment.

6. Davey et al. (1993). *Analytica Chimica Acta*, *279*(1), 155–161.
7. Dabros et al. (2009). *Bioprocess and Biosystems Engineering*, *32*(2), 161–173.
8. Opel et al. (2010). *Biotechnology Progress*, *26*(4), 1187–1199.
9. Arnold et al. (2003). *Biotechnology and Bioengineering*, *84*(1), 13–19.
10. Henriques et al. (2009). *Advances in Biochemical Engineering/biotechnology*, *116*(June), 73–97.
11. Clavaud et al. (2013). *Talanta*, *111*, 28–38.
12. Sandor et al. (2013). *Journal of Biotechnology*, *168*(4), 636–645.
13. Milligan et al. (2014). *Biotechnology and Bioengineering*, *111*(5), 896–903.

Nowadays CHO medium powder qualification is performed based on simple measurements (like solubility test), expensive and time-consuming cell culture tests and liquid chromatography (HPLC) quantification of some critical components.[2] Therewith PAT applications (spectroscopy techniques and MVDA tools) are more and more frequently applied in this field as well.[14,15] Feasibility of NIR[16,17], fluorescent[18,19] and Raman[20] spectroscopy were investigated in mammalian cell culture medium powder formulations. Additionally, some research groups combined the latter techniques and achieved promising results[21-23]. The basic reason of this activity is the fact that different spectroscopic applications are sensitive for different material parameters. Nevertheless in current industrial applications the NIR based test methods are widespread in raw material supplier and medium powder qualification due to robust spectrometers, relatively low investment cost and general pharmaceutical experience.[17,22,23]

3. Methods

3.1. CHO cultivations investigated with PAT tools

Monoclonal antibody (mAb) producing recombinant CHO-S cell line was applied and the content of cell bank vials was further cultivated in shake-flask cultures for my experiments. Shake-flasks were incubated at 37 °C and 5% CO_2 in the incubator with 110 rpm shaking.

Dielectric spectroscopy measurements were performed in six concentrated fed-batch (CFB) fermentation runs (Run1-6) in 1 liter maximal working volume bench-top Biostat B plus (Sartorius-Stedim Biotech, Göttingen, Germany) bioreactors. Experiments were initiated in batch mode and continuous feeding was started on the second day of the culture.

In NIR spectroscopy based cell culture monitoring experiments shake-flask cultures were investigated. Eight shake-flask cultures were performed (SF1-8), which were relevant models of bioreactor conditions (e.g. biochemical composition and cell density). Initial culture volume was 430 ml and initial viable cell density was adjusted to $0{,}3\times10^6$ cells/ml.

In NIR spectroscopy based medium qualification experiments powder quantity according to the protocol of the supplier was dissolved in purified water (PW) and cell culture test was performed in 125 ml shake-flask with 30 ml working volume and $0{,}3\times10^6$ cells/ml initial cell density. After 116 hours of incubation, the actual viable cell density (*VCD*) was determined.

14. Read et al. (2010). *Biotechnology and Bioengineering*, *105*(2), 276–284.
15. Read et al. (2010). *Biotechnology and Bioengineering*, *105*(2), 285–295.
16. Kirdar et al. (2009. *Biotechnology Progress*, *26*(2), 527.
17. Lee et al. (2012). *Biotechnology Progress*, *28*(3), 824–832.
18. Li et al. (2011). *Applied Spectroscopy*, *65*(11), 1240–1249.
19. Ryan et al. (2010). *Analytical Chemistry*, *82*(4), 1311–1317.
20. Li et al. (2010). *Biotechnology and Bioengineering*, *107*(2), 290–301.
21. Hakemeyer et al. (2013). *Biotechnology Journal*, *8*(7), 835–846.
22. Lee et al. (2012). *Biotechnology and Bioengineering*, *109*(11), 2819–2828.
23. Jose et al. (2011). *Biotechnology Progress*, 27(5), 1339–1346.

3.2. Equipment and data handling of dielectric spectroscopy (DS)

For dielectric measurements Biomass Monitor 220 (Aber Instruments, Aberystwyth, UK) equipment was applied with 12 mm diameter annular probes. Permittivity spectra was collected in 25 frequencies (100, 120, 160, 190, 240, 300, 370, 470, 580, 720, 900, 1120, 1400, 1740, 2170, 2700, 3360, 4190, 5220, 6500, 8100, 10090, 12560, 15650 and 19490 kHz) in every 8 minutes automatically.

Acquired data were stored in a PC and were further analyzed (Cole-Cole modelling) with AberScan Beta 4.2 software (Aber Instruments, Aberystwyth, UK). Spectral data could be reached in "txt" format for PCA, for PLS regression and for one frequency linear regression.

3.3. NIR spectra acquisition from CHO cultivations and data handling

From all samples three NIR spectra were measured with Bruker Matrix-F (Bruker Optics, Ettlingen, Germany) FT-NIR spectrometer. The equipment had InGaAs detector with a measurement range of 834.2-2327.3 nm (11988-4297 cm^{-1}). Measurements were performed with INGOLD IN271P type transflectance probe with 2 mm optical path length and were managed by OPUS 6.5 (Bruker Optics, Ettlingen, Germany) software. Probe was connected to the spectrometer with 10 meter long fiber optic bundle. Each spectrum was the average of 256 records with 16 cm^{-1} resolution. I applied this on-line *in-situ* measurement set-up in order to be closer to the representative modelling of bioreactor monitoring.

Data alignment was executed in Microsoft Office Excel 2010 (Microsoft, Redmond, WA, USA) while data analysis, pre-treatment, calibration and predictive regression was done with Unscrambler X 10.3 software (CAMO Software, Oslo, Norway).

3.4. NIR spectra acquisition from CHO medium powder and data handling

NIR spectra were measured with Foss NIRSystems 6500 (Foss NIRSystems, Silver Spring, MD, USA) spectrometer. The equipment had PbS detector with a measurement range of 1100-2498 nm. Each spectrum was the average of 32 records with 2 nm resolution. NIR spectra acquisition was managed by Vision 3.20 software (Foss NIRSystems, Silver Spring, MD, USA).

FT-NIR spectra were measured with PerkinElmer Spectrum 400 (PerkinElmer, Waltham, MA, USA) spectrometer. The equipment had InGaAs detector with a measurement range of 10000-4000 cm^{-1}. Each spectrum was the average of 32 records with 2 cm^{-1} resolution. FT-NIR spectra acquisition was managed by Spectrum 6.3.2 software (PerkinElmer, Waltham, MA, USA).

Data alignment was executed in Microsoft Office Excel 2010 (Microsoft, Redmond, WA, USA) while data analysis and pre-treatment was done with Unscrambler X 10.3 software (CAMO Software, Oslo, Norway).

Central composite face-centered (CCF) heat treatment design of experiment (DoE) was analyzed for *VCD* (as response variable) with MODDE 11 software (Umetrics, Umeå, Sweden).

4. Results

4.1. VCD monitoring of CHO cultivations with dielectric spectroscopy (DS)

In the six alternating tangential flow (ATF) based CHO cultivations (Run1-6) the basic and original aim could be reached: viable cell density (*VCD*) increased above 40-50×10^6 cells/ml. This *VCD* range has not been investigated with dielectric spectroscopy (DS) before.

I concluded that different feeding strategies applied amongst the six runs and (as a consequence) minor differences in process parameters did not influence dielectric behavior of the cultivations significantly. The measurement system was robust from this perspective.

On the other hand, measurement errors due to technical reasons could be identified during thorough data analysis. With the ease of PCA and deeper spectra analysis baseline shift could be detected in case of Run5-6 in the 2.7-20 MHz (high frequency) range. This error could not be compensated with standard normal variate (SNV) pre-treatment but could be eliminated with Savitzky–Golay- (SG) smoothing combined with first derivative mathematical transformation (Fig. 2.).

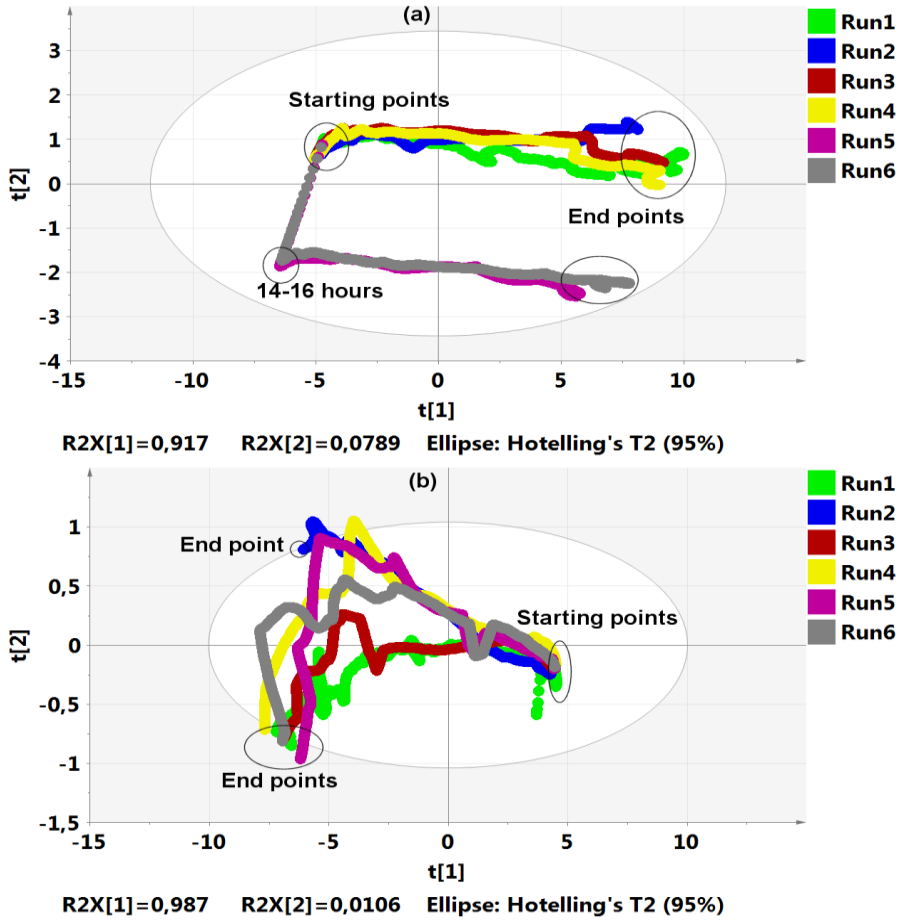

Figure 2. – PCA score plots of dielectric spectroscopy runs:
Run1-6 raw spectra score plot (a),
Run 1-6 spectra after Savitzky–Golay- (SG) smoothing combined with first derivative pre-treatment score plot (b).
(Grey ellipse represents 95% confidence intervall based on Hotteling T^2 statistics.)

The applied SG-smoothing combined with first derivative pre-treatment increased the accuracy PLS regression based *VCD* prediction when calibration-set did not contain baseline shift but the prediction-set was hardly influenced by this measurement error. Additionally, with the ease of PCA in Run2 data measurement error caused by electrode polarization could be detected in the 0-300 kHz (low frequency) range, which was also compensated by the mathematical pre-treatment applied. In case of pre-treatment, *VCD* prediction error (*RMSEP*) of PLS regression was 6.1-10.6×10^6 cells/ml.

Similarly to PLS regression, Cole-Cole modelling was also sensitive to the measurement errors detected (baseline shift and electrode polarization). In case of Cole-Cole modelling low frequency error could be compensated with low frequency cut-off (*LFC*) adjustment while high frequency error could be eliminated with changing the membrane capacitance (C_m) set-up. In this way 3.9-7.4×10^6 cells/ml *RMSEP* could be achieved.

Comparing PLS regression and Cole-Cole modelling results, the latter turned out to be more accurate. Nevertheless the SG-smoothing combined with first derivative mathematical transformation and PLS method can be easily inserted to an on-line and real-time PAT system while real-time adaptive *LFC* and C_m adjustment is challenging.

Accurate *VCD* prediction could be achieved with one frequency linear regression in the first, so called cell growth phase (under 50×10^6 cells/ml *VCD*) with an *RMSEP* of 2.9-3.7×10^6 cells/ml. However, in the second, so called plateau phase (when cell growth is finished) the linear model resulted in an inaccurate prediction with 17.2×10^6 cells/ml *RMSEP* value (Fig. 3.). In contrast with PLS and Cole-Cole modelling, linear regression was not influenced by measurement errors detected.

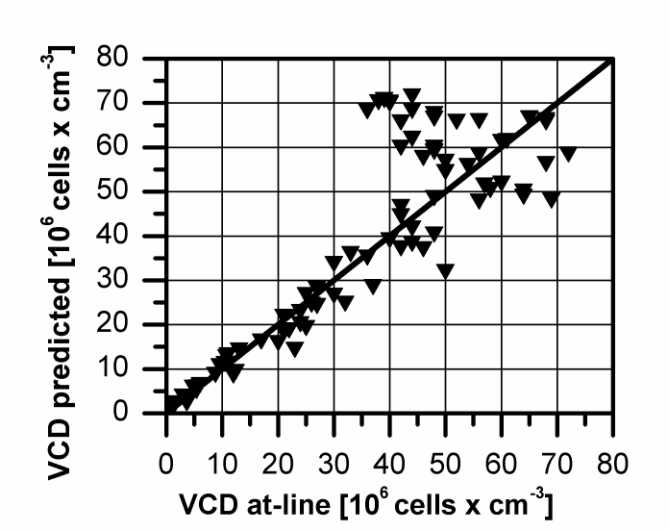

Figure 3. – *VCD* prediction with linear modelling from Run1-6 data.
At-line measured (reference) *VCD* values *vs. VCD* values predicted by the modell.
Linear equation applied: $VCD = 414{,}492 \times C_{580} + 1{,}187 \times 10^6$,
C_{580} – permettivity measured at 580 kHz frequency.
A 45°line represents the theoretical case when measured and pedicted values are the same.

With PLS and Cole-Cole approach acceptable *VCD* prediction accuracy could be achieved for both phases after compensating the effects of measurement errors. Dielectric spectroscopy is a reliable and robust tool for *VCD* prediction even in the high (≥ 40-50×10^6 cells/ml) range as well. The most robust approach was the PLS regression with mathematical pre-treatment.

4.2. Monitoring of CHO cultivations with NIR spectroscopy

In the investigated CHO shake-flask cultures directed biochemical changes (due to different feeding strategies) and directed physical changes (due to vortex treatment, Fig. 4.) could be detected with PCA of NIR spectra. As a consequence, NIR spectroscopy based monitoring has the proper sensitivity for CHO cultivation supervision.

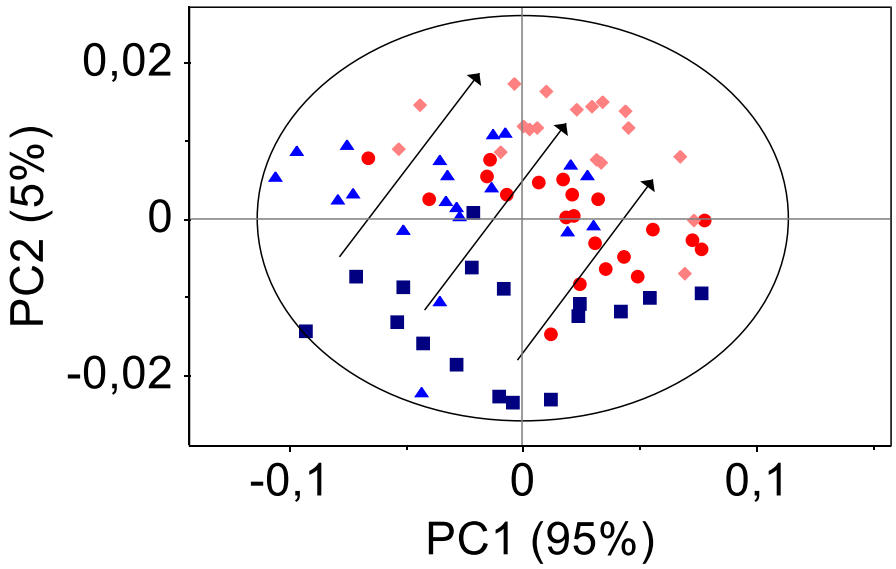

Figure 4. – PCA score plot of NIR spectra from SF2 and SF5 shake-flask samples with and without vortex treatment. SF2 without vortex (*dark blue square*), SF2 with (*red circle*), SF5 without vortex (*light blue trianlge*), SF5 with votex (*pink diamond*). PCA was perfomed for the 6534-5678 cm^{-1} range. Arrows show the direction of change due to vortex treatment. Ellipse represents 95% confidence interval.

The NIR spectroscopy based system was capable of predicting glucose concentration from CHO shake-flask cultivations with 3.1-5.5 mM precision (Fig. 5.), which is a border-line precision for the CHO process control.

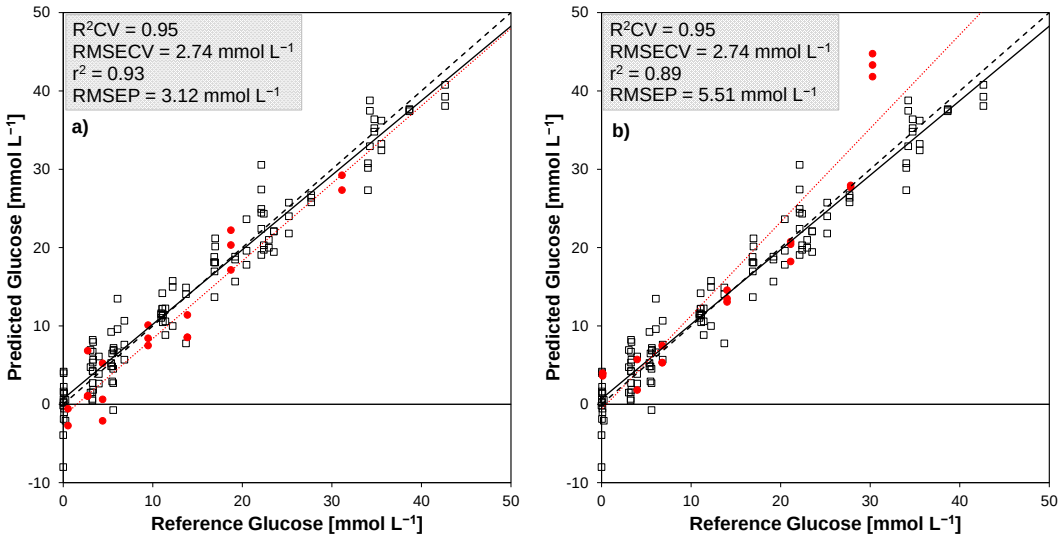

Figure 5. – Calibrations for glucose concentration from SF1, SF4, SF5, SF6, SF7 és SF8 shake-flask NIR spectra (n = 126). Before PLS calibration baseline offset – deresolve mathematical pre-treatment was performed. At-line measured (reference) glucose concentration values are shown vesus the values according to the calibration model (*empty squares*.) Real calibration line is *continous black*, theoretical calibration line is *dotted black*. Independent prediction of SF2 samples (*red circle, red dotted line*) *(a).* Independent prediction of SF3 samples (*red circle, red dotted line*) *(b). R^2CV* – Squares of Pearson's correlation coefficient of cross-validation at calibration; *RMSECV* – error of cross-validation; *r^2* – Squares of Pearson's correlation coefficient of prediction; *RMSEP* – error of prediction.

4.3. Qualification of CHO medium powder with NIR spectroscopy

In case of heat treated and control medium powder spectra measured with both dispersive and Fourier transform (FT-) spectrometer baseline shifts could be compensated with second derivative mathematical pre-treatment.

As a consequence of heat treatments (30, 50, 70 °C) weight changes could be detected, which occurred due to moisture-content changes. At 30 °C moisture-content increased, at 70 °C it decreased while at 50 °C there was no significant difference compared to the control. Direct effect of moisture-content changes on NIR spectra could be eliminated with variable selection (usage of 2000-2500 nm range only).

In PCA of mathematically pre-treated (2000-2500 nm, second derivative) spectra measured with both dispersive and FT-NIR spectrometer definite clustering could be detected according to the temperature levels (control, 30 °C, 50 °C and 70 °C) of heat treatments (Fig. 6.). In case of dispersive equipment spectra a more definite clustering occurred. In PCA there was no separation according to the duration (1, 7, 13 hours) of heat treatments.

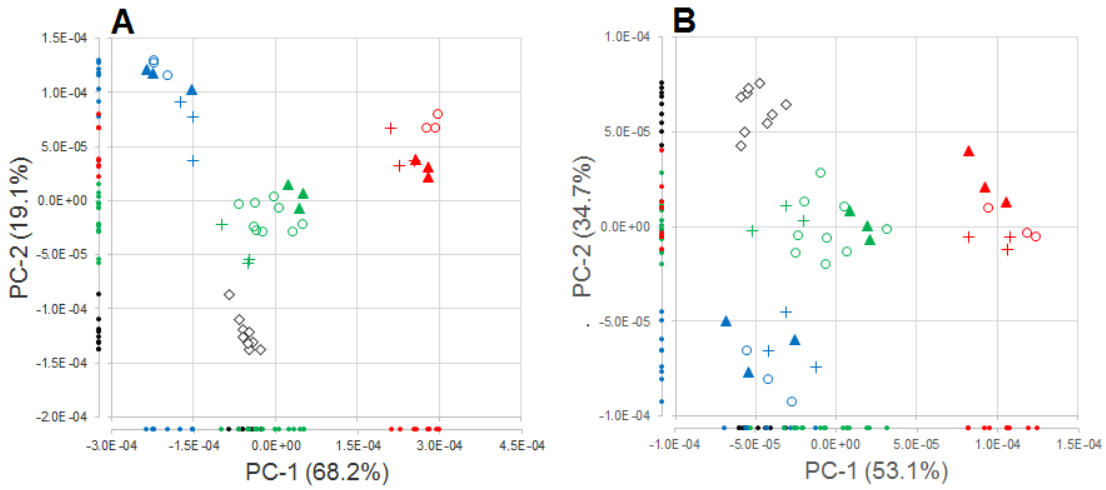

Figure 6. – Second derivative NIR *(a)* and FT-NIR *(b)* spectra.
PCA score plot of 2000-2500 nm (5000-4000 cm^{-1}) range.
Black empty diamond – control samples;
blue cross – 30 °C, 1 hour; *blue empty circle* – 30 °C, 7 hours; *blue triangle* – 30 °C, 13 hours;
green cross – 50 °C, 1 hour; *green empty circle* – 50 °C, 7 hours; *green triangle* – 50 °C, 13 hours;
red cross – 70 °C, 1 hour; *red empty circle* – 70 °C, 7 hours; *red triangle* – 70 °C, 13 hours.
Projection of points is shown on both axis.

Soft independent modeling of class analogy (SIMCA) was also applied for the same spectra, which separated heat treated samples from the controls similarly to PCA. In case of dispersive NIR equipment higher level of sensitivity occurred.

In traditional cell culture test both temperature and heat treatment duration were significant factors. However, this test distinguished only [50 °C, 7 hours], [50 °C, 13 hours], [70 °C, 7 hours] and [70 °C, 13 hours] samples from the controls significantly (Fig. 7.).

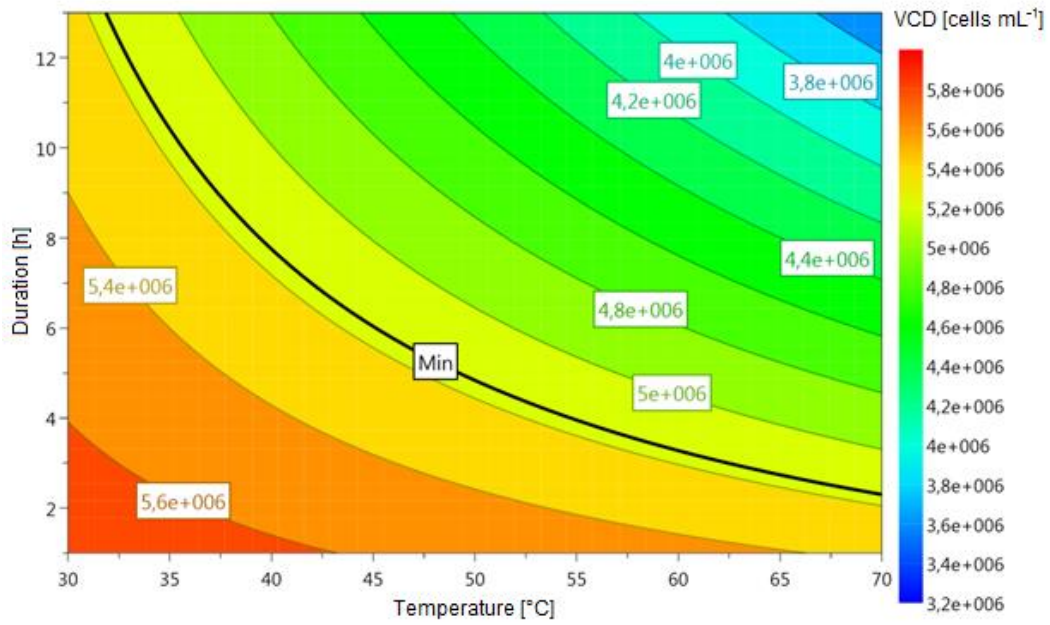

Figure 7. – Contour plot of heat treatment temperature and duration effects on VCD at 116 hours of cultivation.
Black line (*Min*) represents the lower limit of *VCD* achieved with contol medium powder at 95% confidence level (5.16×10^6 cells/ml).

5. Theses

1) In industrial practice frequently applied one frequency (580 kHz) **linear regression** resulted in accurate VCD prediction only in the cell growth phase (under 50×10^6 cells/ml). In the later **plateau phase** (when cell growth is finished) **the linear model resulted in inaccurate VCD prediction**. [3]

2) In case of **Cole-Cole modelling** low frequency noise could be compensated with the change of low frequency cut-off (*LFC*) value, while high frequency measurement error could be eliminated with membrane capacitance (C_m) value adjustment. In this way **excellent VCD prediction accuracy** could be achieved in both growth and plateau phase. [3]

3) Based on my results **the most robust dielectric spectroscopic VCD prediction could be achieved with Savitzky–Golay-smoothing combined with first derivative** mathematical pre-treatment and **partial least squares (PLS)** regression. The applied mathematical transformation could compensate measurement errors (identified with PCA) without eliminating relevant information from spectra. [3]

4) With **near-infrared (NIR) spectroscopic** monitoring of CHO shake-flask cultures directed **biochemical changes** (due to different feeding strategies) and directed **physical changes** (due to vortex treatment) could be detected with **PCA of FT-NIR spectra**. I demonstrated that NIR spectroscopy based CHO cultivation process monitoring can sensitively **detect relevant changes** like altering cell metabolism (biochemical change) or cell degradation (physical change). [2]

5) In case of CHO cultivations with high level of **physical and biochemical variability** beyond standard normal variate (SNV) and multiplicative scatter correction (MSC) mathematical transformations the newly applied **baseline-offset – deresolve pre-treatment combination is also effective** before PLS regression in order to increase the precision of FT-NIR spectra based glucose concentration prediction**.** Based on my results, the **achievable precision of glucose concentration prediction is a border-line precision for the CHO cultivation process on-line control.** [2] [5]

6) Effect of **relevant heat-treatments**, which can occur at **CHO medium powder** storage and transport, could be detected both with **principal component analysis (PCA) and with soft independent modeling of class analogy (SIMCA) investigation of NIR spectra** measured with dispersive and Fourier transform (FT-) spectrometers. NIR spectra of heat treated medium powder samples separated from the control samples and from each other based on **the temperature of heat treatments**. [1]

7) **In traditional cell culture test of heat treated CHO medium powder** samples both temperature and duration of heat treatment were significant factors. This test only separated samples with at least 50 °C temperature and at least 7 hours duration of heat treatments from the controls. In order to avoid causeless raw material rejection (type II error) **combined, hybrid decision strategy is suggested**. I could verify that cell culture test was only needed if sample had not passed NIR based qualification. [1]

6. Potential implementation

My results in CHO based drug substance manufacturing process monitoring have already been implemented in Gedeon Richter (GR) Plc.'s biotechnological development and production procedures: dielectric and near-infrared (NIR) spectra became input data of the current process supervisory system. In case of CHO medium powder qualification, NIR spectra acquisition has already been initiated in parallel with cell culture test at GR. Hopefully my result will facilitate further development of raw material qualification strategy.

7. Publications

7.1. Publications related to PhD thesis

[1] Szabó, É., Párta, L., Zalai, D., Gergely, S., & Salgó, A. (2016). Investigation of heat-treated cultivation medium for mammalian cells with near infrared spectroscopy. *Journal of Near Infrared Spectroscopy*, *24*(4), 373–385. https://doi.org/10.1255/jnirs.1222 (IF: 1,48)

[2] Kozma, B., Párta, L., Zalai, D., Gergely, S., & Salgó, A. (2014). A model system and chemometrics to develop near infrared spectroscopic monitoring for Chinese hamster ovary cell cultivations. *Journal of Near Infrared Spectroscopy*, *22*(6), 401–410. https://doi.org/10.1255/jnirs.1133 (IF: 1,57)

[3] Párta, L., Zalai, D., Borbély, S., & Putics, Á. (2013). Application of dielectric spectroscopy for monitoring high cell density in monoclonal antibody producing CHO cell cultivations. *Bioprocess and Biosystems Engineering*, *37*(2), 311–323. https://doi.org/10.1007/s00449-013-0998-z (IF: 1,95, C: 4)

[4] Gergely, S., Párta, L., & Salgó A. (2013). Közeli infravörös spektroszkópia/mikroszkópia: roncsolásmentes kutakodás. *Magyar Kémiai Folyóirat – Kémiai Közlemények,119*(1), 40–45. http://www.mkf.mke.org.hu/images/stories/docs/2013_1/MKF_2013_40.pdf

7.2. Publications in international conference proceedings related to PhD thesis

[5] Kozma, B., Párta, L., Gergely, S., & Salgó, A. (2013). Developing FT-NIR calibrations to measure glucose level of mammalian cell cultivation broths. In *Bellon-Maurel, V., Williams, P., & Downey, G. (editors) Proceedings of the 16th International Conference on Near Infrared Spectroscopy, NIR 2013*. 02-07. 06. 2013, La Grande-Motte, France (pp. 343–348). IRSTEA – France Institut National de recherche en sciences et technologies pour l'environnement et l'agriculture, 2013, 757 p.

[6] Párta, L., Gergely, S., & Salgó, A. (2012). Pioneer experiences on PAT implementation of pharmaceutical biotech process development. In *Manley, M., McGoverin, C.M., Thomas, D.B., & Downey, G. (editors) Proceedings of the 15th International Conference on Near Infrared Spectroscopy, NIR 2011*. 13-20. 05. 2011, Cape Town, South African Republic (pp. 145–149). 2012, 493 p.

[7] Gergely, S., Párta, L., Salgó, A. (2009). Monitoring of fermentation broths operated with E. coli cells – what is measured by NIR spectroscopy? In *Saranwong, S., Kasemsumran, S., Thanapase, W., & Williams P. (editors) Proceedings of the 14th International Conference on Near Infrared Spectroscopy, NIR 2009*. 07-16. 11. 2009, Bangkok, Thailand (pp. 735–739). Chichester: IM Publications LLP, 2010., 1209 p. (ISBN:978-1-906715-03-8)

7.3. Further publications

[8] Hirsch, E., Pataki, H., Farkas, A., Bata, H., Vass, P., Fehér, C., Barta, Z., Párta, L., … & Marosi, G. J. (2016). Raman-Based Feedback Control of the Enzymatic Hydrolysis of Lactose. *Organic Process Research and Development*, *20*(10), 1721–1727. https://doi.org/10.1021/acs.oprd.6b00212 (IF: 2,54, C: 1)

[9] Zalai, D., Hevér, H., Lovász, K., Molnár, D., Wechselberger, P., Hofer, A., Párta, L.,… & Herwig, C. (2016). A control strategy to investigate the relationship between specific productivity and high-mannose glycoforms in CHO cells. *Applied Microbiology and Biotechnology*, *100*(16), 7011–7024. https://doi.org/10.1007/s00253-016-7380-4 (IF: 3,43, C: 1)

[10] Zalai, D., Koczka, K., Párta, L., Wechselberger, P., Klein, T., & Herwig, C. (2015). Combining mechanistic and data-driven approaches to gain process knowledge on the control of the metabolic shift to lactate uptake in a fed-batch CHO process. *Biotechnology Progress*, *31*(6), 1657–1668. https://doi.org/10.1002/btpr.2179 (IF: 2,07, C: 2)