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Implementation of an Inline Analysis System to Monitor Reactions in Continuous Flow Mode

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AFFIDAVIT

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Abstract

Inline monitoring of processes with robust and versatile analytical tools is inescapable for a successful transition of the pharmaceutical industry towards continuous manufacturing. This thesis details implementation of an inline UV/Vis analysis system in an existing continuous flow "Plug & Play Reactor" setup to monitor two model heterogeneous reactions: (i) the production of acetylsalicylic acid, and (ii) Suzuki-Miyaura cross couplings.

In addition to the UV/Vis flow cell, sized to enable monitoring of yield of both synthesis and coupled with the reactor outlet, the system features chemometric tools using calibration sets within defined temperature conditions. A specially designed helical tube heat exchanger ensured defined temperature conditions for calibration and monitoring. Calibration was performed in flow by analysis of single component mixtures for all analytes present in the reaction mixture. The performance of the system was then validated by comparison of classical least squares method and The method of simultaneous equation to reference data obtained by offline HPLC.

As recently published by our group in Chemie Ingenieur Technik, comparison of the yield obtained by these simple methods showed good agreement with offline HPLC and the system presents a sensitive tool for precise, accurate monitoring of continuous process setups.

Kurzfassung

Die Inline-Prozessüberwachung mit robusten und vielseitigen Prozessmessgeräten ist notwendig für einen erfolgreichen Übergang der Pharmaindustrie zur kontinuierlichen Produktion. In dieser Arbeit wird die Implementierung eines Inline-UV/Vis-Analysesystems in einen bestehenden kontinuierlichen Aufbau, dem sogenannten "Plug & Play Reactor", beschrieben und die Überwachung des Prozesses anhand von zwei heterogenen Modellreaktionen, der Produktion von Acetylsalicylsäure und der Suzuki-Miyaura-Kreuzkupplungen, gezeigt.

Zusätzlich zur UV/Vis-Durchflusszelle, welche so dimensioniert wurde, dass sie die Überwachung der Ausbeute beider Synthesen ermöglicht, während sie direkt an den Reaktorausgang gekoppelt ist, verfügt das System über chemometrische Werkzeuge durch Verwendung von Kalibriersätzen bei festgelegten Temperaturbedingungen. Ein speziell entwickelter spiralförmiger Wärmetauscher sorgt für definierte Temperaturen bei der Kalibration und in der späteren Prozessüberwachung. Die Kalibrierung erfolgt im kontinuierlichen Betrieb durch Analyse von Einzelkomponentenmischungen aller im Reaktionsgemisch vorhandenen Analyten. Die Performanz des Systems wurde mit der Methode der kleinsten Fehlerquadrate und der Methode simultaner Gleichungen durch Vergleich mit Referenzdaten von einer Offline-HPLC Messung validiert.

Wie bereits von unserer Gruppe in Chemie Ingenieur Technik veröffentlicht, zeigte der Vergleich der Ausbeute, welche mit diesen einfachen Methoden erhalten wurde, eine gute Übereinstimmung mit jener der Offline-HPLC. Daher stellt das System ein sensibles Werkzeug für eine präzise und genaue Überwachung kontinuierlicher Prozessaufbauten dar.

Acknowledgement

First and foremost, I would like to thank Professor Heidrun Gruber-Wölfler, who gave me the opportunity to work in her research group. In this group, I had the pleasure of working not only individually but also in an international team work environment which stimulated my intellectual growth as well as the development of my character. Furthermore, she provided me with excellent support and supervision and gave me great freedom regarding the time management in scope of my master's thesis within the Plug & Play reactor project.

Another big thanks go to Georg Johannes Lichtenegger for technical advice, team work in scope of reaction monitoring experiments, and to other team members, Manuel Maier and Markus Hackl, who were of great help during long and labour-intensive experiments and technical discussions on the topic of this thesis. Without their help it would not be possible to perform these long experiments. Additionally, Manuel Maier supervised my translation of the thesis abstract from English to German.

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Table of Contents

List of Figures

List of Tables

List of Abbreviations

Name	Unit	Description
φ	$g \cdot cm^{-3}$	density
ψ	$W \cdot m^{-2}$	light intensity
A	AU	absorbance
c	M (mol/L)	concentration
d	cm	path length
ID	mm	inner diameter
OD	mm	outside diameter
r		dilution factor
T	°C	temperature
ε	$(M \cdot cm)^{-1}$	molar absorption
λ	nm	wavelength
τ		transmission

Nomenclature

AH	acetic anhydride
ASA	acetylsalicylic acid
BM	binary mixture
CIT	Chemie Ingenieur Technik
CLS	classical least squares
DIN	German Institute for Standardization
EA	ethyl acetate
EtOH	ethanol
FDA	Federal Drug Administration
GC	gas chromatography
GMP	Good Manufacturing Practice
HPLC	high-performance liquid chromatography
IPPE	Institute of Process and Particle Engineering
P&ID	process and instrumentation diagram
PAT	Process Analytical Technology
PEEK	polyetheretherketone
PLS	Partial least squares
PR	pressure recording
PTFE	polytetrafluorethylen
QbD	Quality by Design
R&D	research and development
SA	salicylic acid
SCM	single component mixture
SE	The method of simultaneous equation
TC	temperature controller
UV/Vis	ultraviolet-visible spectroscopy

1. Introduction

Until recently, pharmaceutical manufacturing was performed mainly in batches (i.e. conventional processes) [1]. This was done because the industry, aiming for higher profits and rushing new products to marked, invested mainly in discovery of new drugs which were then patented, even though investing in the switch from batch to continuous processes could also yield higher revenue, as well as more predictable processes and enabling of better process control [1]. Additionally, research activity focused on new drug discovery has recently proven more challenging. In order to cope with high costs of those challenges, the current tendency in pharmaceutical industry is towards continuous processing [1], [2], [3]. This transition, initiated by Federal Drug Administration (FDA), includes the implementation of Process Analytical Technology (PAT) into manufacturing [3]. This initiative aims to enable better process control and greater understanding of manufacturing, supported by monitoring of process variables (e.g. raw material properties) and quality parameters (e.g. desired reaction yield) with inline measurement techniques (i.e. sampling device is placed directly into the process apparatus) [3], [9, p. 17]. Enhanced understanding of the process can then be used to predict the quality of the final product and to control the process in order to compensate for variation of process variables to meet the demands for high quality standards of the regulatory agencies [5], [6], [1]. In conclusion, integration of inline monitoring (Figure 4) to improve the understanding of the processes plays a major role in advancing technologies and in compensating for escalating challenges in the pharmaceutical industry [1], [3]. For instance, to increase the understanding of the process, inline analysis can be used to measure reaction progress of a reaction setup. This data can then be used to determine reaction kinetics in order to scale up or optimise reactor performance.

1.1 Opportunities for engineers

Modern chemical engineering professionals posses not only traditional knowledge of manufacturing (e.g. of classical unit operations), but also that obtained through modern study curriculum programmes (e.g. Pharmaceutical process control and analysis), as well

as skills for multidisciplinary assignments. Therefore this profession can play a major role in assuring the best advances in pharmaceutical processing at the laboratory and the manufacturing scale based on the understanding of the process and scientific principles by implementing FDA initiatives (e.g. PAT) [1].

The need for professional chemical engineers as well as the benefits of PAT have been reported in a recent survey [7], conducted by International Society for Pharmaceutical Engineering. This survey reports eleven out of twelve contributing pharmaceutical companies used PAT. The majority of the participants reported an increase in process understanding, however most also lack the skills to fully utilise PAT tools.

1.2 Aim and structure of this work

This work aims to implement an inline UV/Vis analysis system in an existing continuous flow reactor setup. This setup includes a flexible "Plug & Play Reactor" device (See Figure 1) [8]. The setup features flexibility in terms of replaceable HPLC segments used for heterogeneous catalysis, as well as heat exchange and mixing modules [8]. The analysis system should then be used to monitor the reaction progress of different Suzuki-Miyaura reactions and heterogeneous esterification.

The objectives of this work:

1. The development of an appropriate inline analytical method including the comparison of the obtained results with offline HPLC analysis.

2. The implementation of the UV/Vis system in the reaction setup.

3. The application of the inline system to monitor different reactions in continuous flow mode.

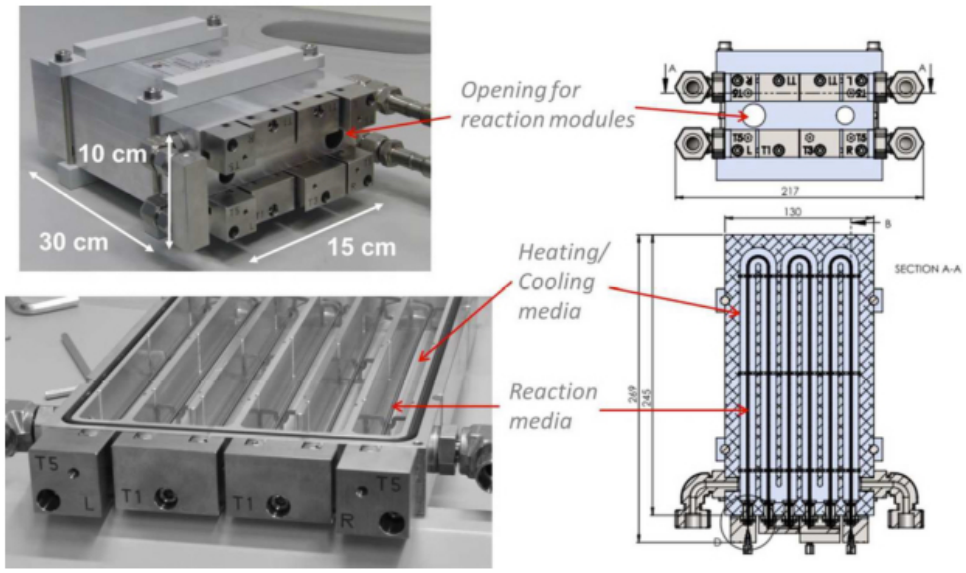

Figure 1: The Plug & Play reactor [8].

Structure of this work

To achieve successful implementation of an inline UV/Vis analysis system to monitor acetylsalicylic acid (ASA) and 4-phenyltoluene production in a Plug & Play reactor setup the following steps were performed:

1. First, an inline sensor for reaction monitoring was chosen from commercially available sensors in order to provide optimal absorbance measurements for both productions (Chapter 3.1).
2. The UV/Vis system to monitor ASA production (Chapter 3.2) and 4-phenyltoluene production (Chapter 3.3) was implemented to the reaction setup. In addition, chapters 3.2, 3.3, and the respective subchapters also describe comparison of different chemometric methods, used for the inline UV/Vis reaction monitoring, with offline HPLC.
3. Finally, the application for evaluation of the inline system in Excel coupled with AVASOFT spectroscopic software, which can be used to monitor different reactions in flow, was developed (Chapter 3.4) and used to monitor 4-phenyltoluene production.

2. State of the art

2.1 UV/Vis spectroscopy

While only a small part of the electromagnetic spectrum (Figure 2) is covered by UV/Vis (ultraviolet/visible) radiation range (i.e. approximately 200 nm - 800 nm), UV/Vis spectroscopy plays an important role as a process analytical technique [4, p. 137].

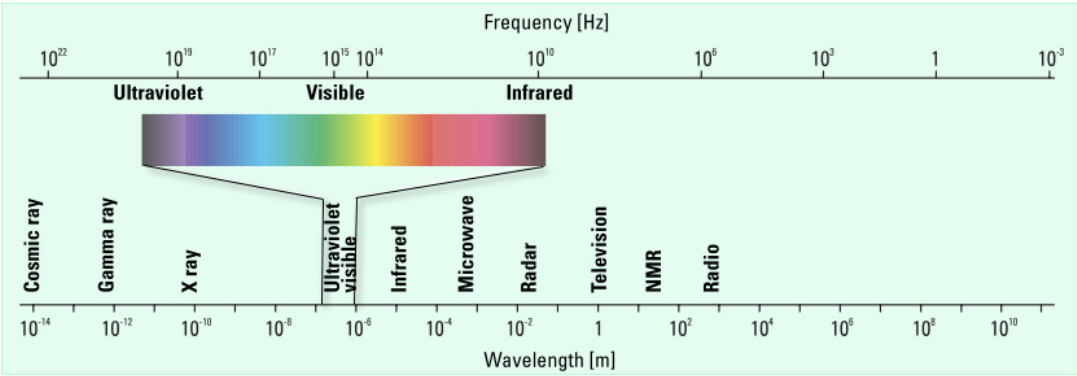

Figure 2: Electromagnetic spectrum [9, p. 2].

The importance of UV/Vis spectroscopy results from [4, p. 137], [9, p. 18]:

- Very high analytical sensitivity and reproducibility.
- Robust instruments, commercially available at low price.
- Applicability of all known chemometric methods.
- Uncomplicated measurement methods.

The major disadvantage of the method is the low selectivity in comparison to IR (infrared) and Raman spectrometry [4, p. 137].

2.1.1 Basic principles

In a UV/Vis measurement, electromagnetic radiation passes through a sample. The interaction between sample ingredient and the electromagnetic source of energy can cause absorption of light. This means that some of the electromagnetic energy is used for an electronic transition to an excited electronic state of that ingredient [10, p. 3,4]. The amount of the energy used for the electronic transition is that of the absorbed wavelength and is reversibly proportional to the wavelength [9, p. 3]. Electronic transitions between pi and pi*, n and pi* orbitals occur in range between approximately 200-800 nm and are thus typical for UV/Vis spectroscopy [10, p. 144]. Such transitions are common for many organic components containing molecular groups with unsaturated molecular bonds, i.e. chromophore(s). [9, p. 10] For instance (Figure 3), in case of formaldehyde, with a molecular group with an unsaturated bond (i.e. ketone), both of this typical transitions at 187 and 285 nm respectfully are observed [9, p. 4]. In the Figure 3 horizontal black lines display electron orbitals (i.e. n, pi, pi*) and each arrow crossing black line presents one electron on that orbital.

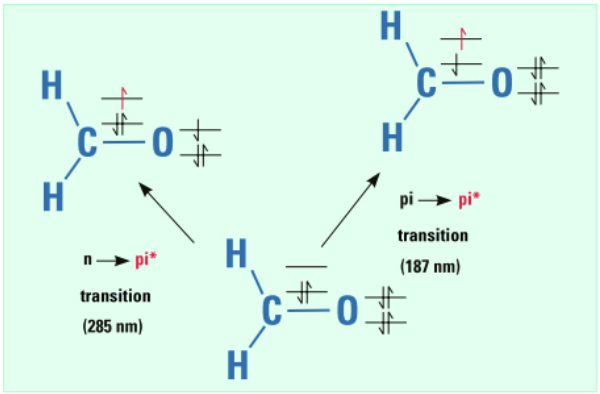

Figure 3: Scheme of the formaldehyde transitions to excited electronic states [9, p. 4].

To use this principle for quantitative analysis, only absorbance should occur while other light interactions with sample and equipment (e.g. reflection) should be minimised [9, p. 3].

Quantitative prediction of single sample component concentration is, according to DIN 1349 [11, p. 279], modelled with Beer–Lambert–Bouguer law (1):

$$A(\lambda) = \varepsilon(\lambda) \cdot c \cdot d \tag{1}$$

where absorption A is a linear function of optical path length d, the concentration of the absorbing component c and the molar absorption, also known as extinction coefficient ε. This linear relationship is only valid in a specific concentration range. For high solution concentrations extinction coefficient ε can vary. Thus the calibration concentration range with valid linearity has to be respected when predicting concentrations.

Absorbance phenomena can also be expressed in terms of transmission τ. This expression (2) of absorbance describes logarithmic ratio of the light intensity proportion introduced to the sample (ψ_{in}) to the light intensity measured after the light has passed through a sample (ψ_{out}) [10, p. 146].

$$A = log\ \tau = log \frac{\psi_{in}}{\psi_{out}} \tag{2}$$

While absorbance can be described with a simple relationship (1) there are several known factors that can influence absorbance spectral measurements. Thus, keeping these factors constant (as shown in Table 1) is essential for accurate quantitative analysis [9, p. 60]. Therefore, any calibration, i.e. determination of extinction coefficient, is only valid under specific conditions (i.e. temperature, pH, solvent used). Likewise, extinction coefficients found in literature can't be directly used for quantitative analysis [9, p. 16].

Table 1: Proposals for control of different factors influencing accuracy of inline UV/Vis quantitative analysis [10, pp. 19, 60-61], [12, p. 172].

Factor	Effect	Example(s)	Proposed control
Temperature	Expansion of solvent which alters molarity of present solutes.	Expansion of several organic solvents.	Thermostated sample holder can be used (e.g. a cell equipped with a heat exchange jacket, Peltier controller).
	Alternating chemical or physical equilibrium.	Changing the original structure of nucleic acids.	
Movement of optical equipment	Minor Baseline offsets	Movement / reinstalling of fibre optic cables.	Derivative methods can be used.
Bubbles	Baseline offsets, light scattering	Bubbles are generated by a pump and are transported downstream to the flow cell.	Debubbler, filtering cell or insertion probe can be used.
Film build-up	Baseline offsets, light scattering and blockage of optical paths	Fermenter particle loaded environment.	Manually cleaning the cell, cell with self cleaning module can be used.
pH value	Alternating form of a chemical substance.	Change of form of a pH indicator.	Buffers controlling pH value can be added to the solution.
Equipment used	Change of optical or other parts of equipment	Changing fibre optics cable with cable having different fibre core diameter.	If calibration gives incorrect predictions new calibration has to be performed.
Solvent polarity	Change in electronic surrounding of the analysed substance.	Absorption spectra shifts up to 8% on the wavelength axis for different solvents.	Same solvent for calibration and measurements must be used.

2.1.2 Inline vs. offline analytics

Conventionally UV/Vis sample analysis has been performed *offlin*e (Figure 4), i.e. in a laboratory positioned away from the process [4, p. 15]. As stated by W. Kessler [4, p. 15], manual sampling results into major part of total analytical mistakes (i.e. > 80%). In contrast, in *Inline analytics* the sampling device is placed directly into the process apparatus (e.g. measurement probe is placed directly into the device) or into the flow, thus avoiding sampling by directly measuring and yielding several other advantages in comparison to offline analytics (Table 2) [9, p. 17].

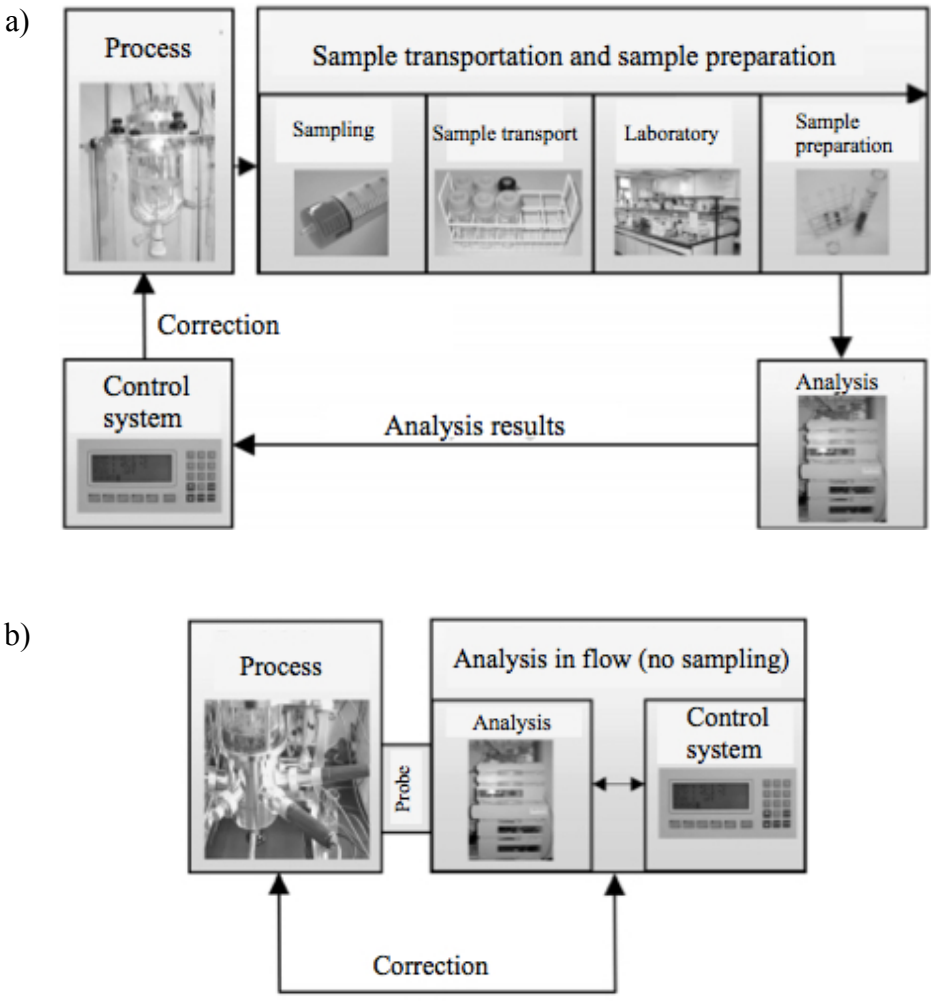

Figure 4: Scheme of offline (a) and inline (b) analytics [9, p. 16, 17].

Table 2: Advantages and disadvantages of Offline and Inline analysis [4, pp. 14–17], [10, pp. 19, 60-61], [12, p. 172], [6]:

	Offline analysis	Inline analysis
Advantages	+ analytic experts are available on-site + flexibility to analyse samples coming from different processes with one device	+ fast (e.g. monitoring processes in real time) + feedback, feedforward control and real-time release can be implemented + increased process understanding used to scale-up equipment + increased product quality + no product loss due to sampling + no error due to sampling procedures and increased reproducibility of sampling + no exposure to toxic samples
Disadvantages	- direct process control is not possible - delayed process control due to sample transport to the laboratory for the analysis - time consuming - requires additional personal	- expensive equipment (i.e. each separate inline analysis require its own equipment) - expensive software packages and calibration - additional factors influencing the measurements can occur (e.g. bubbles and film build up)

To fully utilise all the purposes of inline analytics, the time to obtain information (i.e. sum of measurement, prediction computing and data transfer time) has to be shorter than the time needed for a process variable or final product quality attribute upset to occur [4, p. 15]. Then both, feedforward and feedback control, as well as real time release are possible [3]. The difference between feedforward and feedback control as well as additional pre-requirements for their implementations are shown in Table 3.

To enable future implementation of feedforward and feedback control to the reaction monitoring setup presented in this work (Chapter 5.2.3), measurement time and computing

time needed for prediction of reaction yield were minimised. This was done by choosing simple chemometric methods (Chapter 2.2.1) and by choosing the appropriate UV/Vis equipment (Chapter 3.1) including the flow cell with 0.01 mm optical path length and optical fibres with 400 µm fibre core diameter.

Table 3: Feedforward and feedback control comparison [9, p. 3, 12].

	Feedforward control	Feedback control
Compared values	*Predicted values* (not measured) to a *set point value.*	*Realised* (measured) *values* to a *set point value.*
Action and product quality	A value for manipulated variable is adjusted based on a process model to compensate for a change in process before quality of product can be negatively affected.	When a variation from desired quality of the product is spotted at the product stream, the manipulated variable is adjusted. Because of the "lag" caused by this type of control undesired product quality can occur.
Pre-requirement	Known relation(s) for influence of manipulated variable(s) on the product quality, usually obtained by planned experiments.	Process variable(s) with sufficient influence on the set point have to be determined.

2.2 Chemometrics

Chemometrics is a scientific discipline utilising mathematical and statistical knowledge to produce valid analytical results [4, p. 81]. For instance, the statistical knowledge is used to predict concentrations of multicomponent mixtures based on UV/Vis spectral data. For mixtures, where the absorption behaviour of each present component can be described with *linearity* (1) chemometric methods use *the principle of additivity* [4, p. 102]. This means that at a certain wavelength, total absorbance is the sum of all absorbances of the mixture

components [9, p. 21]. Prediction is then possible by solving the generated equations [4, p. 102]. To solve these equations, minimum of n information are needed for n components present in the mixture (i.e. if two components are present, a minimum two absorbances at their wavelengths are needed for a quantitative prediction) [4, p. 102].

2.2.1 Chemometric methods

This chapter describes two chemometric methods for quantitative prediction of concentrations in multicomponent mixtures, *The method of simultaneous equation* (SE) and *classical least squares* (CLS) method. Pre-requirements for using these two methods for accurate analysis are [10, p. 21-26]:

- Principles of the linearity (i.e. Beer–Lambert–Bouguer law) and the additivity apply.
- Calibration of all compounds analysed that contribute to the UV/Vis spectra.
- Analysed solution should be free of additional components absorbing in the wavelength range of calibrated components.

To find out if the linearity between absorbance and concentration applies to an analysed system, correlation coefficient is a valuable measure (as described in Table 4) [9, p. 82]. This value can be used to confirm applicability of SE and CLS method (i.e. values > 0.999 are expected) [9, p. 83]. Microsoft Excel offers CORREL function which can be used to determine correlation coefficient from two data sets [13].

Table 4: Correlation coefficient specifications [9, pp. 82, 83].

	Optimal	No linear relationship	Expected values
Correlation coefficient	*+1 and -1*	*0*	*> 0.999*

When these pre-requirements are not met, other, more complicated methods for calibration must be used, e.g. Partial least squares (PLS) method [4, p. 102].

SE can be used for quantitative prediction of concentrations in multicomponent mixtures when a low degree of overlapping spectra of the analysed components is present [14], [9, pp. 21–23]. For the quantitative prediction, information obtained from measured absorbance spectra is used in order to solve the system of equations of the following type [10, p. 21-22]:

$$A(\lambda_i) = \sum_{j=1}^{n} A_j(\lambda_i) = \sum_{j=1}^{n} \varepsilon_j \cdot d \cdot c_j \tag{3}$$

where j denotes numbered component and i denotes specific wavelength. Minimum required information is used, i.e. the number of equations equals number of components n [9, p. 21].

In contrast to SE, CLS method can be used to extract more spectral data information. [9, p. 24]. Again the same system of equations (3) is used for prediction [15]. However, the number of equations is not limited by the number of components n. Instead the wavelength range is manually selected and generates a number of equations equal to the number of wavelengths selected in the range [9, pp. 8–9]. To predict concentrations, the solver tool (e.g. Microsoft Excel Solver) can be used. The solver optimises calculated concentration values in the system of equations by minimising the sum of the square of the differences between measured and predicted absorption [16], [17, p. 34]:

$$\sum \left[A(\lambda_i)_{predicted} - A(\lambda_i)_{measured}\right]^2 \tag{4}$$

As reported by Schmidt et. al. (Table 5), CLS method applied to the offline UV/Vis analysis proved to be suitable for the quantitative analysis of the analysed components in the ASA and salicylic acid (SA) mixtures [18]. Further significant applications of the CLS method (Table 5) show the method applies well to a wide range of quantitative multicomponent analysis containing several components [19], [20]. Thus, this method was not only chosen for UV/Vis inline monitoring of ASA production but also for Suzuki-Miyaura reactions in flow. As shown by Palur et. al. (Table 5), SE applied to offline UV/Vis analysis proved accurate for the quantitative prediction of concentrations in the analysed two component mixtures containing ASA and atorvastatin [21]. These mixtures contained similar components to those present in the ASA production (i.e. ASA and salicylic acid). Therefore, SE was chosen to monitor this production.

Table 5: Overview of significant applications of spectroscopy analysis using SE and CLS method.

Authors, year published	Application	Spectrometry	Chemometric methods	Analysed components	Application overview
Schmidt et. al.,1995 [18]	offline	UV/Vis	CLS, PLS	ASA, SA	CLS and PLS methods have proven to be of the same quality in terms of quantitative prediction of analysed components in the studied mixtures.
Owen et. al., 2014 [19]	on-line reaction monitoring (extractive sampling)	Direct liquid sampling mass spectroscopy (DLSMS)	CLS	acetic acid, acetic anhydride, ethyl acetate, butyl acetate, pyridine, ethanol, butan-1-ol	Successful implementation of CLS method to monitor the esterification reaction system yielding comparable results to inline MIR (mid infrared spectrometry).
Edinger et. al, 2016 [20]	inline reaction monitoring	UV/Vis	CLS	naphthalene, benzene, toluene	Inline analysis using CLS method compared to offline GC-FID proved the CLS method is suitable for process monitoring to applied system.
Palur et. al., 2016 [21]	offline	UV/Vis	SE	ASA, atorvastatin	SE has proven accurate for this multicomponent system.

2.2.2 Statistical evaluation of chemometric methods

To determine how well the predicted value (e.g. concentration) of both chemometric methods (i.e. SE and CLS method) fit to a single acceptable reference value, one can calculate accuracy (also called % error):

$$accuracy = \frac{measured\ value - accepted\ reference\ value}{accepted\ reference\ value} \cdot 100\ \% \tag{5}$$

where the accepted reference value is a known value (e.g. known concentration of calibration mixture prepared with chemicals of high purity, also called standards) or a value determined by another analytical method used for comparison [18], [22], [23].

To compare series of predicted and reference values (e.g. concentrations), both methods can be evaluated in terms of individual mean error (%) [24]:

$$individual\ mean\ error = \frac{\sum_{i=1}^{g} |accuracy_i|}{g} \tag{6}$$

where g is number of comparisons for a single component.

Both statistical measures of error, accuracy, and individual mean error were used to optimise the CLS method and to compare the SE and CLS method to different accepted reference values (e.g. accepted reference value determined by HPLC used for comparison) in order to estimate error of prediction of these UV/Vis methods.

2.3 Model reactions

In this work two model reactions were studied. First reaction included synthesis of acetylsalicylic acid (i.e. active pharmaceutical ingredient of Aspirin®) and acetic acid (see Figure 5) from salicylic and acetic anhydride (AH). Acidic catalysts are used to fasten this reaction [8, p. 9, 23]. In the Plug & Play reactor project, a catalytic active fixed bed was used for this purpose (i.e. the ion exchange catalyst Amberlyst 15) [8].

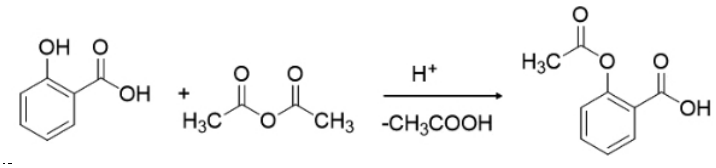

Figure 5: Synthesis of acetylsalicylic acid [8].

The Suzuki-Miyaura reaction of different aryl bromides with phenylboronic acid with a palladium catalyst [8], [26], [27] is shown in Figure 6. Its importance in organic chemistry, for the formation of a C-C bond, was recognised with a Nobel Prize in 2010, awarded to one of its inventors, Akira Suzuki [28]. Furthermore, produced biaryls are of high importance in the pharmaceutical industry [8]. The reaction advantages are low toxicity of the reaction mixture, good thermal stability, insensitivity to water and oxygen, and additionally the reagents are commercially available [28], [26], [29]. In this work, a heterogeneous, palladium-substituted mixed cerium-tin oxide catalyst ($Ce_{0.495}Sn_{0.495}Pd_{0.01}O_{2-\delta}$) was used to speed up the reaction in the presence of a base (i.e. potassium carbonate) which was added to the reaction mixture [8], [30]. $Ce_{0.495}Sn_{0.495}Pd_{0.01}O_{2-\delta}$ was prepared by Dr. Georg Johannes Lichtenegger within Plug & Play reactor project as described in his doctoral dissertation [31, p. 68].

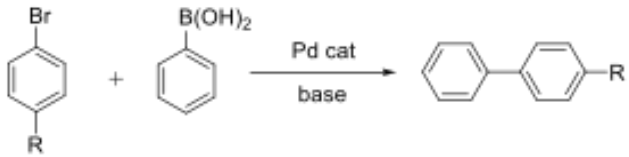

Figure 6: Suzuki-Miyaura reaction [8]. In the formulas, R denotes a functional group.

For both model reactions performed in flow, an inline UV/Vis monitoring was implemented and the results were compared to offline HPLC. Production of ASA was monitored in terms of ASA yield and SA conversion while Suzuki-Miyaura monitoring was performed on a model reaction of the synthesis of 4-phenyltoluene from 4-bromotoluene and phenylboronic acid in terms of yield [8].

3. Results and discussion

3.1 Choice and sizing of the inline sensor for reaction monitoring

The general idea for the sizing of the inline sensor was to perform absorbance measurements of single component mixtures with the UV/Vis equipment already available at the IPPE. These absorbance measurements were then used to scale down the optical path length of the UV/Vis sensor based on Beer–Lambert–Bouguer law. The equipment for these measurements included the insertion probe (FDP-7UV200-2- 2.5) with optical path length of 5 mm connected to the Spectrometer (AvaSpec-ULS2048-USB2-UA-50) and the light source (AvaLight-D-S-DUV). The measurement setup (Figure 26) included the oil bath placed on the magnetic stirrer (IKA C MAG HS7 digital) equipped with a heating plate and a thermostat to provide uniform temperature conditions for measurements and the measuring vessel into which the probe was inserted. The probe provided with necessary information to scale down the sensor optical path length.

To determine the optimal optical path length 0.4 M ASA and SA stock solutions were prepared by dissolving these components in EA (Chapter 5.3.1). The concentrations were chosen based on the planned concentration range (i.e. 0 - 0.4 M SA and ASA) for ASA production in the Plug & Play reactor project. The measurements were performed against an EA blank at 25°C. The integration time was 38.5 ms and 2000 spectra were averaged. Stock solutions analysis resulted in an oversaturated signal as a consequence of oversaturated solutions for absorbance measurements with the insertion probe. Thus, to reduce the saturation of the signal the measurements had to be performed at lower concentrations. The general idea was to prepare up to 500-times more diluted solutions in respect to the 0.4 M single component stock solutions. The factor of 500 corresponds to a 500-times signal reduction according to the Beer–Lambert–Bouguer law. This factor was covered with reduction of an optical path length of a process analyser. The optical path length corresponding to a 500-times signal reduction is 0.01 mm and is to the best of my knowledge the lowest optical path length of a commercially available process analyser (i.e. Starna flow cell 584.4-Q-0.01). Thus, 500-times diluted solutions were prepared (Chapter

5.3.1) and analysed in order to observe absorbance of components with the highest signal reduction. Concentrations of the obtained single component mixtures were 0.80 mM ASA and 0.80 mM SA, both in EA. The UV/Vis Analysis of these solutions resulted in an absorbance measurements ≤ 1.49 AU (Figure 7) for both components in the wavelength range of interest ≥ 260 nm. According to T. Owen [9, p. 52], an absorbance measurement in the range 0.3 - 1 AU results in the lowest theoretical absorbance error due to stray light and spectral noise while this error exponentially increases below ~ 0.01 and above ~ 2 AU. The measured absorbance of ≤ 1.49 AU (Figure 7) at the local absorbance maximum of SA (306.1 nm) is above this optimal range, however the absorbance could be further decreased by decreasing intensity of the light passing through analysed solutions. This could be done not only by increasing the integration time up to a minimum analyser integration time (i.e. 1 ms for the spectrometer setup used) but also by choosing an optical cable with a lower core diameter. Thus, under this consideration, the cell with the lowest commercially available optical path length (0.01 mm) was chosen as the optimal analyser for ASA production.

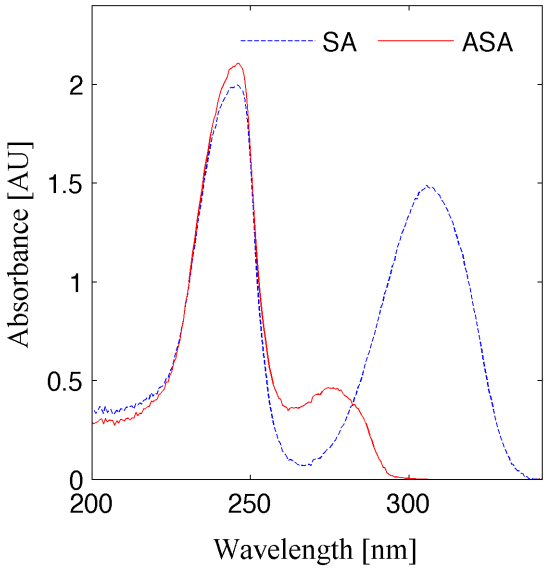

Figure 7: Absorption spectra of 0.80 mM SA and 0.80 mM ASA, both in EA. The measurements were performed at 25°C against an EA blank. The integration time was 38.5 ms and 2000 spectra were averaged.

To see if the optimal optical path length (0.01 mm) chosen for ASA production is suitable to monitor of the 4-phenyltoluene production in flow, single component mixtures (Table 6) of the main products and educts of this reaction (i.e. 4-phenyltoluene, 4-bromotoluene and 4-phenyltoluene) were prepared (Chapter 5.3.1) by 500-times dilution of stock solutions, all in EtOH:H_2O 7:3 (v.v). The stock solution concentrations (Table 6) were chosen based on the planned concentration range of reactants and products for 4-phenyltoluene production in flow.

Table 6: Concentrations of prepared stock solutions and single component mixtures.

	Concentration [mM] in EtOH:H_2O 7:3 (v.v)		
	Phenylboronic acid	4-Bromotoluene	4-Phenyltoluene
Stock solution	54.37	35.97	35.27
Single component mixture	0.11	0.07	0.07

Then the analysis of 4-phenyltoluene at different integration times ranging from 15-300 ms were performed against an EtOH:H_2O 7:3 (v.v) blank at 30°C. These measurements resulted into absorbance spectra (Figure 8) with absorbance maximum at 0.71 AU.

Spectral loss (Figure 8) was observed with the increasing integration time. For instance, at integration time of 15 ms spectra in range 200-310 nm is observed while at 300 ms this range is reduced below 257 nm. Thus, the optimal integration time of 15 ms and averaging 100 spectra were chosen for further spectral measurements (Figure 8) of phenylboronic acid and 4-bromotoluene single component mixtures (Table 6). Absorbance spectra (Figure 8) of these two components reaches maximum at 0.07 AU. This shows that absorbance of these two components presents only a minor contribution to the total absorbance expected for 4-phenyltoleuene production (i.e. for yields > 50%, expected for the production of 4-phenyltoluene in flow in scope of the Plug & Play reactor project). Thus, for the expected yields one could approximate that absorbance maximum (254 nm) of 4-phenyltoluene will reach values of at least 0.35 AU for the inline measurements with a flow cell with 0.01 mm optical path length. This approximation is based on the observation of the absorbance

measurement maximum (0.71 AU) of the 4-phenyltoluene single component mixture (Figure 8), perfromed with integration time of 15 ms and corresponding to 100% yield. It is important to notice that the expected absorbance values (i.e. approx. 0.35-0.71 AU) at absorbance max. of 4-phenyltoluene lay in the optimal absorbance range. Thus, the Starna flow cell with 0.01 mm optical path length was proven to be the suitable choice for the ASA production and also for 4-phenyltoluene production inline reaction monitoring of these synthesis in flow.

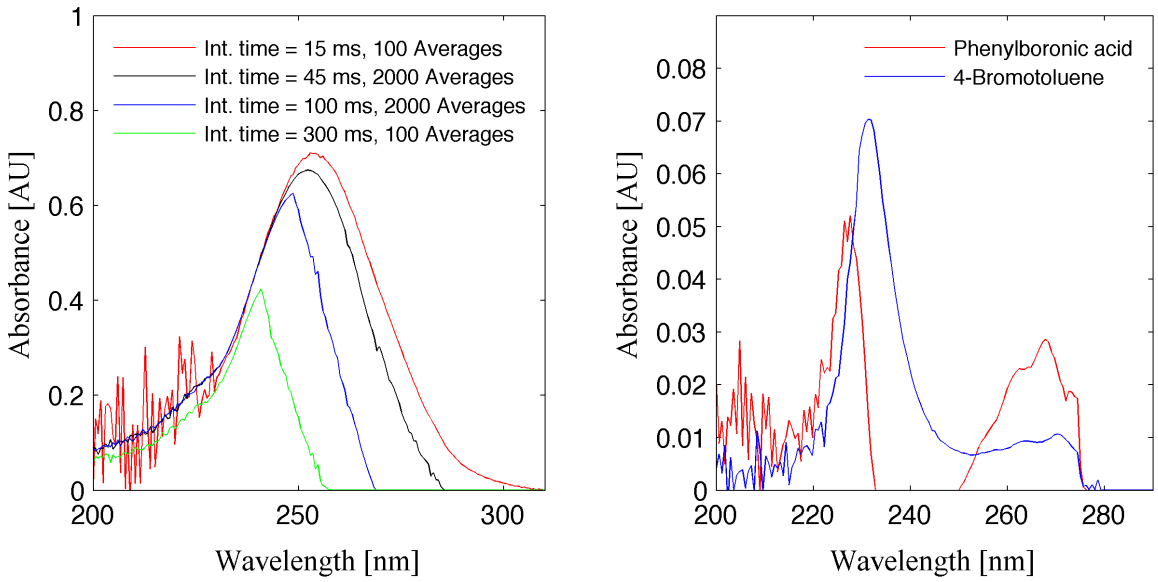

Figure 8: Absorption spectra of 0.07 mM 4-phenyltoluene in EtOH:H_2O 7:3 (v.v) at different integration times and different number of averages (left). Absorbance spectra of 0.11 mM phenylboronic acid, 0.07 mM 4-bromotoluene at integration time of 15 ms and 100 averages. All measurements were performed at 30°C against an EtOH:H_2O 7:3 (v.v) blank [8], [31].

The UV/Vis flow cell with 0.01 mm optical path length was then ordered together with the Avantes cuvette sample holder and 2 fibre-optic cables with 400 µm optical fibre diameter used to connect the holder to Avantes spectrometer already available at IPPE. Furthermore, the equipment supplier provided an option to test fibre-optic cables with different optical fibre core diameters (200-800 µm) that would enable further manipulation of the signal intensity.

The fibre-optic cables with 400 μm optical fibre diameter and the UV/Vis flow cell placed in the cell holder were then used for implementation of ASA (Chapter 3.2) and 4-phenyltoluene production monitoring (Chapter 3.3) in flow. The chosen optical path length allowed for accurate, precise and rapid (i.e. every 2 - 3.4 seconds depending on the model reaction) measurements of yield in the absorbance range below ~ 1.2 AU for ASA (i.e. for 0.4 M SA in the educt mixture) and below ~ 0.6 AU (i.e. for 40.05 mM 4-bromotoluene in the educt mixture) for 4-phenyltoluene production.

3.2 Implementation of inline UV/Vis analysis to monitor production of acetylsalicylic acid in flow [8], [31]

Monitoring acetylsalicylic acid production in flow included quantitative analysis with inline UV/Vis to give information on ASA yield and SA conversion. First, two chemometric methods, SE and CLS method were chosen for the implementation of quantitative analysis. These simple chemometric methods were chosen to minimise computation time needed for prediction of yield and conversion which in combination with low measurement time resulted in almost real time ASA production monitoring. It is important to notice that for ASA and SA successful quantitative predictions with offline UV/Vis using SE and CLS method have been previously reported in literature (Chapter 2.2.1) [18], [21]. Thus, good results were expected for implementation of these two methods to the inline ASA production monitoring. The general idea (Figure 9) was to perform calibration at simulated reactor outlet conditions (i.e. at the same temperature and flow conditions). The UV/Vis setup (Chapter 5.2.3) was coupled with Microsoft Excel via spectrometer software AVASOFT (version 7.7.2, available at the IPPE).

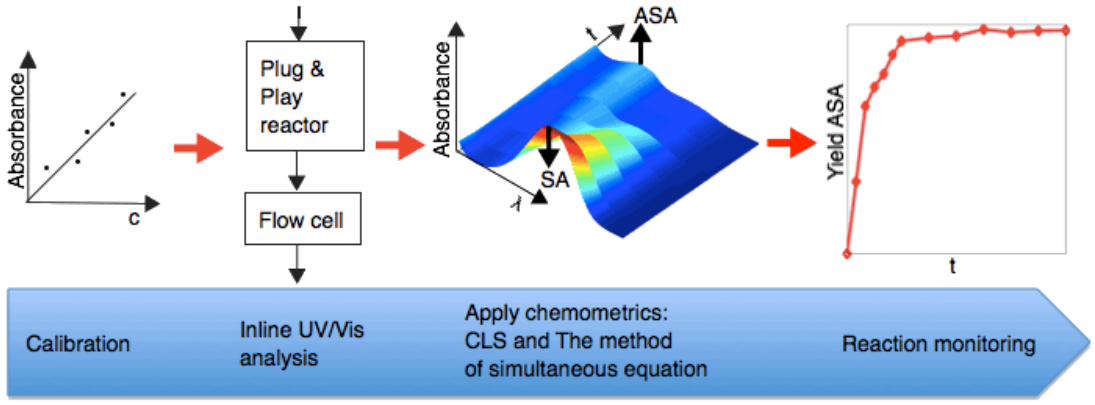

Figure 9: Scheme of the general idea for the implementation of the inline monitoring to monitor ASA production in continuous flow mode.

This entire chapter including subchapters 3.2.1-3.2.3 are based on the results and the ideas presented in the publications:
[8] G. J. Lichtenegger, V. Tursic, H. Kitzler, K. Obermaier, J. G. Khinast, and H. Gruber-Wölfler, "The Plug & Play Reactor: A Highly Flexible Device for Heterogeneous Reactions in Continuous Flow," *Chemie Ing. Tech.*, vol. 88, no. 10, pp. 1518–1523, 2016.
[31] G. J. Lichtenegger, "Continuous Processes for the Synthesis and Isolation of Functionalized Biphenyls via Suzuki-Miyaura Cross-Coupling Reactions," Doctoral dissertation, Technische Universität Graz, 2016.

Then Microsoft Excel was used for performing model predictions of yield and concentration in order to monitor the reaction progress. This agenda (Figure 9) resulted in a successive implementation of the inline UV/Vis analysis to monitor ASA production in continuous flow mode. It proved simple and successful in terms of accuracy and precision of the inline UV/Vis analysis in comparison to offline HPLC (Chapter 3.2.3).

3.2.1 Calibration with single component mixtures [8], [31]

Prior to calibration, the upper concentration limit for calibration, determined by crystallisation of ASA in EA at 25°C, was determined. At this temperature, a concentration of 0.4 M ASA in EA resulted into fully dissolved mixture. In contrast, at the higher concentration (i.e. 0.5 M) crystallisation was observed (i.e. visible crystals of ASA in EA on the bottom of the testing flask). Thus, only mixtures of ASA and EA with concentrations < 0.4 M were prepared for the calibration.

To determine extinction coefficients of SA and ASA (Figure 10), required for the application of chemometric methods, calibration in continuous flow mode was performed. The calibration and ASA production monitoring (Chapter 3.2.3) were done at same flow (i.e. 1ml/min) and temperature conditions (the water bath temperature was 25 °C). This was done to avoid influence of temperature and flow pattern on the quality of the quantitative prediction. To provide uniform temperature conditions a helical tube heat exchanger (Chapter 5.2.2) was implemented to the UV/Vis system which was also used to prevent crystallisation. For the calibration 24 fully dissolved single component mixtures with concentrations ranging from 0.0052 - 0.399 M ASA and 0.0089 - 1.303 M SA (both in EA) were analysed against an EA blank. The linearity of the regression was then optimised by selection of 18 mixtures. The optimisation yielded high linearity. This is shown in terms of the correlation coefficients in range from 240 to 340 nm (Figure 10) that approach values of 1. For the selection of 18 mixtures, correlation coefficients of SA and ASA were calculated at each wavelength in this range using Microsoft Excel CORREL function. Data sets of absorbance values obtained from calibration measurements and of corresponding known concentrations were selected for this calculation. The final result of the calibration is the series of obtained extinction coefficients (Figure 10) resulting from

the optimised selection of mixtures. This series was then used for the application of chemometric methods.

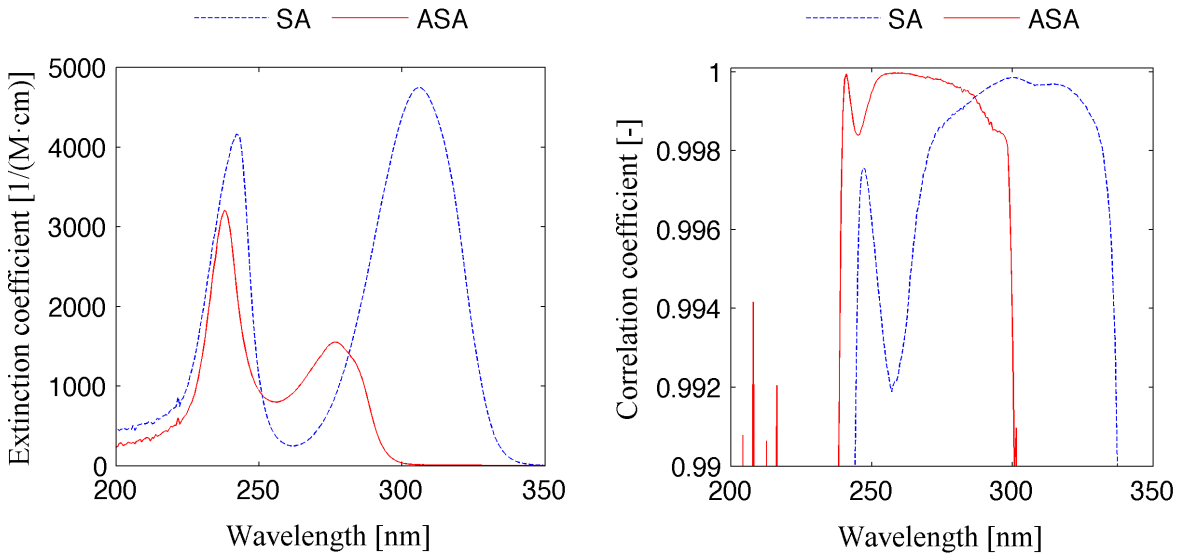

Figure 10: Plot of the SA and ASA extinction coefficient (left) and plot of the SA and ASA correlation coefficient (right), both in respect to wavelength. The water bath temperature of the helical tube heat exchanger was 25°C and the flow through the calibration setup 1 ml/min.

3.2.2 Selection of the wavelengths and validation of the calibration by using binary mixtures [8], [31]

For validation purposes and to determine the optimal wavelength range for CLS method, 6 binary mixtures were studied. For SE wavelengths at the SA maximum (λ=306.103 nm) and ASA maximum (λ=276.218 nm) were selected. Optimal wavelength range for CLS method (257.051 - 312.071 nm) was determined by manually adjusting the range and throughout comparison of the resulting SA and ASA prediction accuracy.

Comparing known concentrations and predicted values yielded poor prediction of ASA in terms of individual mean error (Table 7) for both methods. Individual mean error was calculated from accuracy (see chapter 2.2.2 for the theoretical background) which was

estimated based on known concentrations and predicted concentration. In contrast, the same comparison shows that the predictions for SA were significant more accurate for both methods. Further inspection of the individual mean error shows CLS performed better for prediction of ASA. However, better performance of SE is shown for predicting SA concentrations.

Both of the statistical measures of error, accuracy and individual mean error, were used to optimise the CLS method and to compare the SE and CLS method to different accepted reference values (e.g. accepted reference value determined by HPLC used for comparison) in order to estimate error of prediction of these UV/Vis methods.

Table 7: Comparison of SE and CLS method in terms of individual mean error.

Method	Compound	Individual mean error [%]
CLS	SA	3.35
CLS	ASA	13.66
SE	SA	2.32
SE	ASA	21.26

Furthermore, comparison of the accuracy of methods (Figure 11) shows that the high individual mean error for ASA mainly results from the poor accuracy at low concentrations where both of the methods gave incorrect predictions. However, above 50.72 mM ASA, the worst accuracy with the CLS method was + 1.60 % and with SE - 4.66 %. The same pattern of prediction accuracy was observed for SA as can be seen by the offset of the accuracy below 81.43 mM. Above this concentration the worst accuracy with the CLS method was + 3.35 % and with SE - 3.25 %.

It is of importance notice that the binary mixtures were prepared from the remaining amount of single component mixtures used for calibration. This was done to utilise the solutions that remained unused in calibration with single component mixtures in order to save resources. Thus only a small part of the calibration concentration range was inspected.

This is for ASA at concentrations < 0.078 M, while calibration concentrations ranged up to 0.400 M ASA and for SA at concentrations < 0.200 M, while the calibration concentrations ranged up to 1.303 M SA. Based on the accuracy study, much better prediction capabilities of both methods were expected for SA production monitoring. This is because the reactive mixtures were to be in range up to 0.4 M ASA and SA. Nevertheless, the study of binary mixtures showed the outlined limits for the desirable accuracy of the chemometric methods.

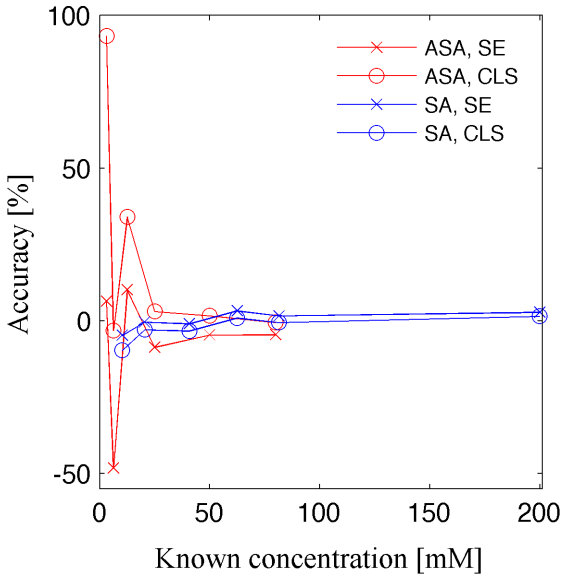

Figure 11: Comparison of the accuracy of SE and CLS method for quantitative prediction of ASA and SA in binary mixtures.

3.2.3 Comparison of the inline UV/Vis analysis with offline HPLC [8], [31]

In total, 4 reaction monitoring experiments for ASA production were performed to compare the UV/Vis SE and CLS method results to the data obtained with offline HPLC. The synthesis and the inline analysis were done in a typical reaction monitoring setup (Figure 28) connected to the Plug & Play reactor setup (Figure 30). All information about these setups are summarised in chapter 5.2.3. Analyses were performed without internal standard to reach higher purity of the final product without a further separation step. Two HPLC sample preparation methods (Chapter 5.3.8) were tested in order to obtain acceptable reference values from the HPLC method. First method included filling a sample into a HPLC vial. Then 1 mL of the eluent was added. In contrast, for method 2, a GC vial was filled with a sample and 10 mL of the eluent was added. Then 1 mL of the obtained solution was pipetted to a HPLC vial. For weighting samples prepared with method 2, the more accurate analytical scale (Mettler HK 60) was used in contrast to the simple scale (AND GR 120) used for weighting samples prepared with method 1. Once the acceptable reference values were obtained from the HPLC, the quantitative evaluation of the UV/Vis methods was possible. Wavelengths chosen for SE were the same as for the analysis of the binary mixtures. All reactions were carried at 60°C and with a flow rate of 1 ml/min. Reaction mixtures varied in molarity of SA (i.e. 0.108 - 0.403 M). The amount of Amberlyst 15 catalyst (~ 1 g) was fixed limited with the volume of the reactor (1 HPLC column). After the first reaction monitoring experiment, wavelength range (257.051 - 312.103 nm) for the CLS method, determined with the validation in flow with binary mixtures (Chapter 3.2.2) was optimised. This was done due to the poor performance of the wavelength range selected by analysing binary mixtures. For the optimisation of the wavelength range the one point baseline corrected spectra (Figure 12) of the first reaction monitoring experiment was used for the wavelength optimisation. The range was optimised in the same way as it was done in a study of the binary mixtures to obtain best fit (Figure 12) of the predicted and HPLC values. Optimisation (Figure 12) resulted in the CLS method individual mean error of 18.07 % for SA and 6.53 % for ASA for the wavelength range selected (i.e. 269.034 - 295.95 nm). This experiment also showed it is possible to measure the reaction progress with inline UV/Vis analysis. Principally the reaction progress (Figure 12) is well seen in graph of absorbance in respect to wavelength and time, as well as in the graph of concentration in respect to time. In particular, for absorbance

measurements, variations in the absorbance spectra in the wavelength range 260-350 nm, where ASA (λ_{max}=276.218 nm) and SA (λ_{max}=306.103 nm) strongly absorb the emitted light, present the consumption of SA and production of ASA. Resulting individual mean errors for ASA of 8.93 and 6.53 % for SE and CLS respectively were calculated from the resulting concentrations obtained with UV/Vis and HPLC analyses. For SA, higher individual mean errors were obtained (i.e. 10.65 and 18.08 % for SE and CLS respectively). The optimised wavelength range of the CLS method was then used for further monitoring. Better results were expected for higher reaction solution concentrations (~ 0.4 M SA) for the reasons same to the results shown in Chapter 3.2.2 which showed high dependency of the UV/Vis accuracy on concentration of analysed components.

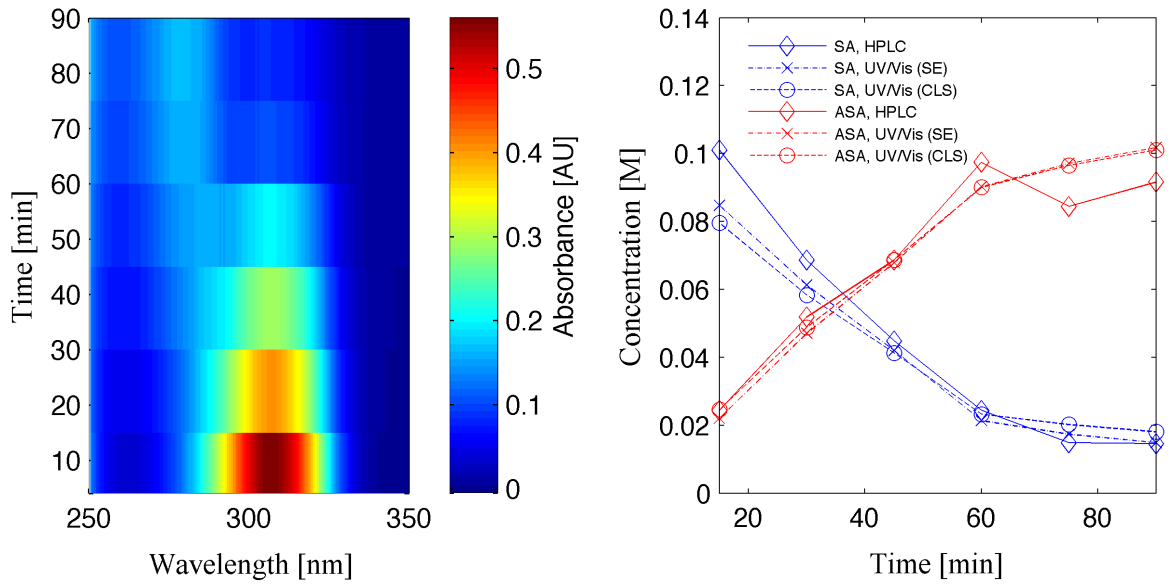

Figure 12: Absorbance after single point (354.35 nm) correction (left). Comparison of the concentrations obtained by the inline UV/Vis CLS (269.034 - 295.95 nm) and SE with offline HPLC (right). Reaction was carried out with 0.108 M SA, 0159 M AH, both in EA, 1.111 g Amberlyst 15, at 60°C and with a flow rate of 1 ml/min.

Reaction monitoring experiments showed dependence of the HPLC accuracy on the HPLC sample preparation method (Chapter 5.3.8). In particular sample amount was the main influence factor influencing the accuracy and precision level observed (Table 8). Principally the individual mean error greatly decreased with the increased sample volume.

Table 8: Impact of the sample volume and of the different sample preparation methods on the individual mean error of the concentration prediction for SE and CLS method in comparison to a reference HPLC [8], [31].

Reaction monitoring experiment #	Sample preparation method	Sample volume [µL]	Individual mean error [%]			
			SA, SE	SA, CLS	ASA, SE	ASA, CLS
3	1	40	45.14	97.34	40.58	40.06
2	1	75	8.34	45.07	7.98	6.67
1	1	125	10.65	18.08	8.93	6.53
4	2	375	13.75	51.10	1.93	1.03

For instance, in reaction monitoring experiment 3, the sample volume was the smallest (i.e. 0.45 µL) and HPLC gave worst predictions for SA and ASA. An example (Figure 13) of such incorrect prediction of ASA (i.e. at 20 minutes) is approx. 2 fold of the stoichiometric amount of the 0.401 M SA in the reaction mixture. Additionally, in the steady state (i.e. at times > 40 min) it was possible to make qualitative approximation of accuracy for the HPLC measurement. This was possible because, the absorbance spectra obtained from the measurements displays good precision in the steady state. In particular no variations in the absorbance spectra in the wavelength range 260-350 nm are seen. However, in contrast to the steady state displayed with the absorbance spectra, the HPLC measurements showed deviations from the steady state observing slopes of the concentration profile (e.g. at 60 min for both components).

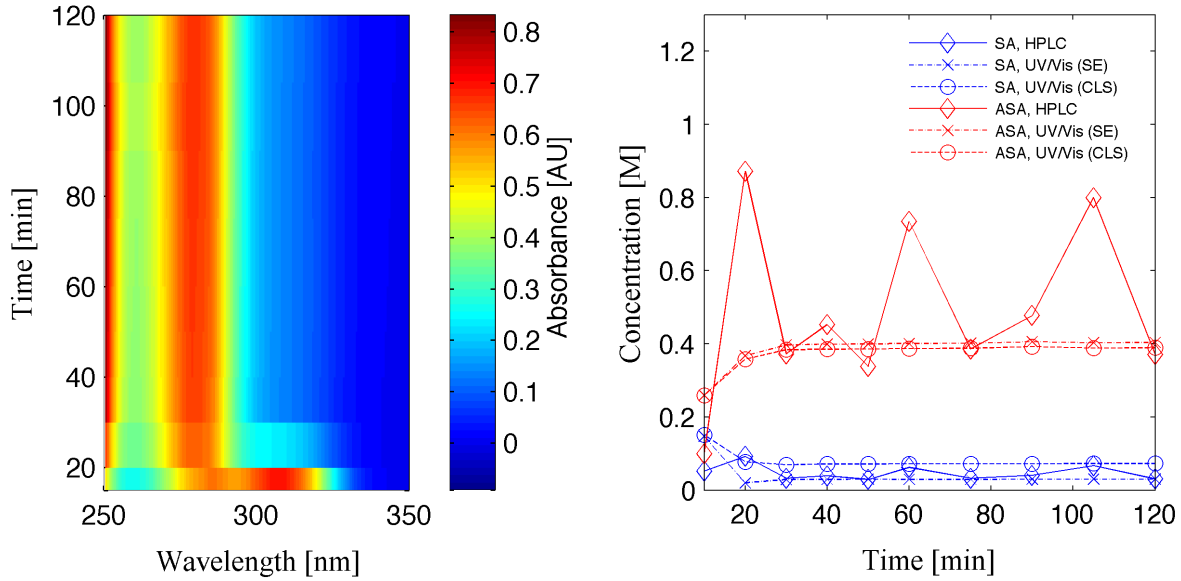

Figure 13: Absorbance after single point (354.35 nm) correction (left). Comparison of the concentrations obtained by the inline UV/Vis CLS (269.034 - 295.95 nm) and SE with offline HPLC (right). Reaction was carried out with 0.401 M SA, 0.688 M AH, both in EA, 1.106 g Amberlyst 15, at 60°C and with a flow rate of 1 ml/min.

Similar qualitative observations, showing poor prediction of ASA and SA of the HPLC sample preparation method 1, can also be made in case of the reaction monitoring experiment 2 (Appendix I). For this experiment, at the steady state displayed with the absorbance spectra obtained with inline UV/Vis analysis, the HPLC measurements showed deviations from the steady state observing slopes of the concentration profile at times > 60 min for ASA (e.g. at 120 min approx. 1.25 fold of the stoichiometric amount of the 0.200 M SA in the reaction mixture). Though provoking, this was not observed when analysing HPLC reference samples of reaction mixtures (i.e. for reaction monitoring experiment 1, 2, 3). For these experiments accuracy of ~ 0 % for SA was observed. Thus, in order to decrease the error of UV/Vis analysis, amount of SA in reaction mixture was increased with every further experiment based on observations of accuracy of binary mixtures (Chapter 3.2.2) that showed accuracy decreases with increasing molarity of compounds analysed. The HPLC method error was ignored till experiment 3 where errors became too often observed (i.e. at 50% of the values used for comparison). Thus, for experiment 4, sample preparation 2 was used. For this method higher eluent volume (10 mL) was used,

as well as the more accurate analytical scale (Mettler HK 60) for weighting samples. Thus, lower error due to scale weighting accuracy was expected. The sample preparation method 2 and the resulting HPLC analysis was validated with the reference sample of the reaction solution with accuracy of 0.04%. Additionally, (Figure 14), with the same type of the qualitative observation as previously used to discuss the incorrect prediction performed by HPLC, constant absorbance of both observed components corresponds well to the constant concentration profile of both components for the steady state (i.e. at times > 45 min).

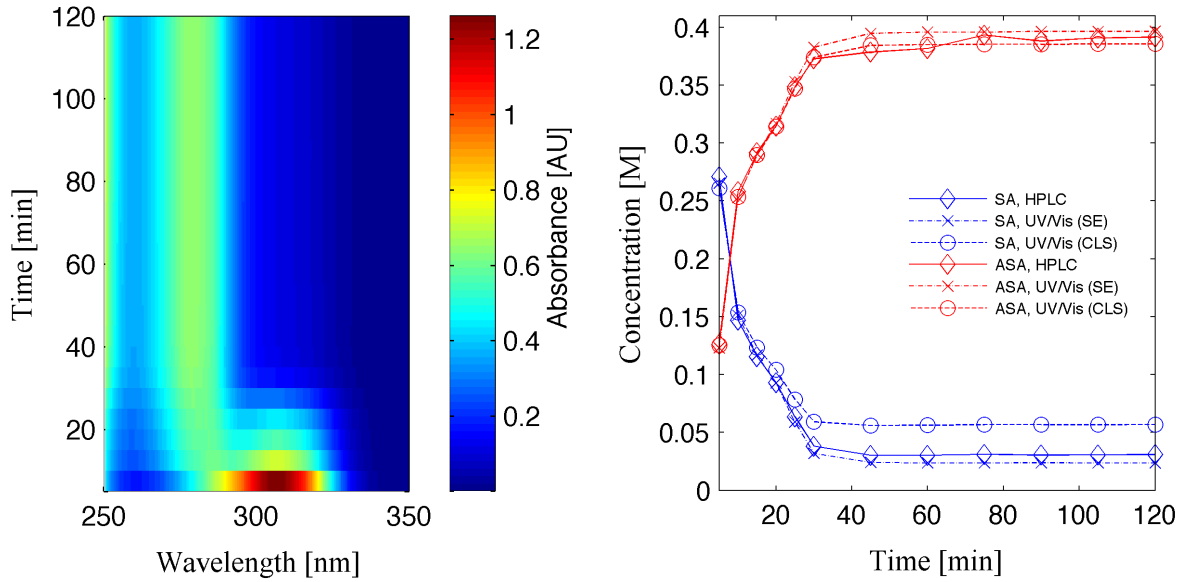

Figure 14: Absorbance after single point (354.35 nm) correction (left). Comparison of concentrations obtained by the inline UV/Vis CLS (269.034 - 295.95 nm) and SE with offline HPLC (right). Reaction was carried out with 0.403 M SA, 0.608 M AH, both in EA, 1.008 g Amberlyst 15, at 60°C and with a flow rate of 1 ml/min [8], [31].

This corresponds to no visible variations of spectra observed in the wavelength range 260-350 nm and in the time range 40 - 120 min (Figure 14). To support this observation quantitatively (Figure 15), absolute value of the relative concentration change for SA and ASA detected by HPLC in a time interval was plotted against the absolute value of the relative change of absorbance at absorbance maximums of each component in the time interval. The comparison shows precise and accurate response of this HPLC method to the changes in absorption measured with the inline UV/Vis. In particular the linear fit resulted

into R^2 = 0.982 for SA and R^2=0.972 for ASA where it is of important notice that actual changes of concentrations measured by HPLC varied in average only 0.020 M for SA and 0.05 M for ASA.

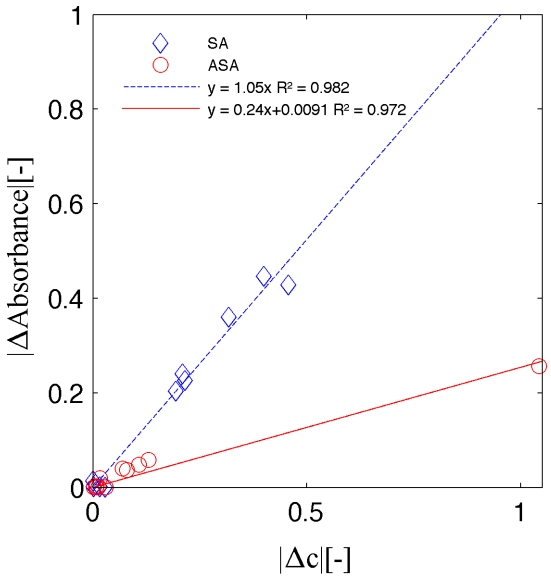

Figure 15: Comparison of the relative absorbance change of SA and ASA measured with inline UV/Vis in comparison to relative concentration change of the components measured by HPLC. Reaction was carried out with 0.403 M SA, 0.608 M AH, both in EA, 1.008 g Amberlyst 15, at 60°C and with a flow rate of 1 ml/min.

A valid quantitative comparison of the inline UV/Vis methods with this offline HPLC method was then possible. HPLC concentration values were considered acceptable measured value for further statistical measures (i.e. for individual mean error) for comparison of the methods.

The comparison in terms of ASA yield of the final experiment (Figure 16) for the CLS method generated individual mean error of 1.04 % while individual mean error of SE was slightly higher (i.e. 1.91 %). In contrast, comparing predicted conversion determined by offline HPLC to the UV/Vis SE yielded individual mean error of 1.73 %, while the UV/Vis CLS method showed poor precision for predicting conversion values with the lowest accuracy of -7.00% and also significant greater individual mean error of 5.69 %. It is

important to notice that yield and conversion (Figure 16) were calculated from the predicted concentration of ASA and SA (Figure 14), respectively. The concentrations of ASA and SA (Figure 14) were predicted with varying accuracy. Thus, the obtained yield and conversion (Figure 16) results vary. Nevertheless, these simple methods showed accurate and precise to predict yield while for SE this is also true for predicting conversion. As measurements with the UV/Vis system are rapid (i.e. every 2) seconds, almost real time monitoring is possible. Thus feedforward and feedback control to the reaction system could be implemented.

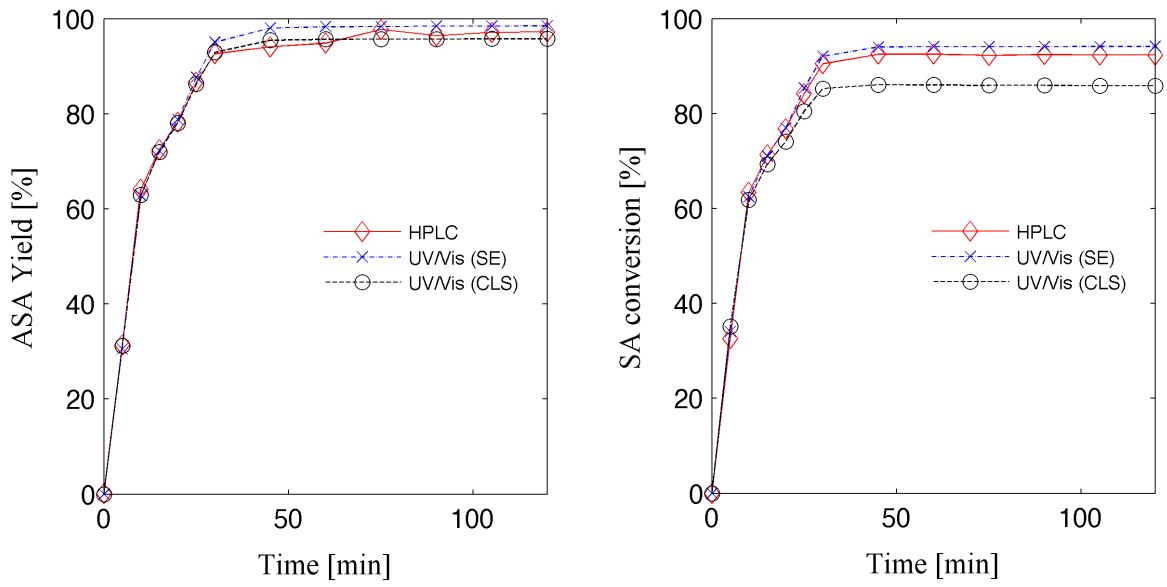

Figure 16: Comparison of the inline UV/Vis SE (269.034 - 295.95 nm) and CLS method with the offline HPLC in terms of ASA Yield (left) and SA conversion (right). Reaction was carried out with 0.403 M SA, 0.608 M AH, both in EA, 1.008 g Amberlyst 15, at 60°C and with a flow rate of 1 ml/min. [8], [31].

3.3 Implementation of inline UV/Vis analysis to monitor Suzuki-Miyaura cross coupling reactions in continuous flow [8], [31]

Monitoring Suzuki-Miyaura cross coupling reactions in flow was performed on a model reaction of 4-phenyltoluene synthesis from 4-bromotoluene and phenylboronic acid. Inline UV/Vis was used to give information on 4-phenyltoluene yield. For the analysis, the CLS method was chosen, due to its simplicity, and to minimise computation time needed for prediction of yield which in combination with low measurement time resulted in almost real time monitoring of the reaction progress (Chapter 3.3.5). The general idea (Figure 17) was to first perform calibration at simulated reactor outlet conditions (i.e. at the same temperature and similar flow conditions). To analyse the absorbance spectra obtained, Microsoft Excel was coupled with AVASOFT software and used to perform calculations of yield (Chapter 3.4). First calibration (Chapter 3.3.1) was performed with a helical heat tube exchanger water bath temperature of 30°C.

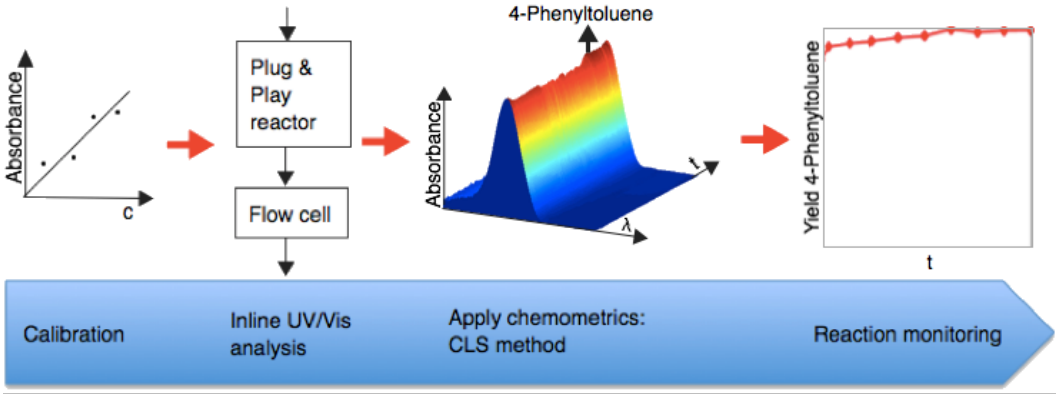

Figure 17: Scheme of the general idea for the implementation of the inline monitoring to monitor 4-phenyltoluene production in continuous flow mode.

This entire chapter including subchapters 3.3.1-3.3.5 are based on the results and the ideas presented in the publications:
[8] G. J. Lichtenegger, V. Tursic, H. Kitzler, K. Obermaier, J. G. Khinast, and H. Gruber-Wölfler, "The Plug & Play Reactor: A Highly Flexible Device for Heterogeneous Reactions in Continuous Flow," *Chemie Ing. Tech.*, vol. 88, no. 10, pp. 1518–1523, 2016.
[31] G. J. Lichtenegger, "Continuous Processes for the Synthesis and Isolation of Functionalized Biphenyls via Suzuki-Miyaura Cross-Coupling Reactions," Doctoral dissertation, Technische Universität Graz, 2016.

Purpose of this heat exchanger (Chapter 5.2.2) was to provide uniform temperature conditions of both calibration and monitoring setup and to prevent crystallisation. At this temperature, crystallisation occurred inside the flow cell when monitoring reaction in flow (Chapter 3.3.2). To prevent crystallisation, calibration was repeated at 84°C and a special isolated sample holder (Chapter 5.2.4) was used to prevent heat loss. The implementation of this isolated sample holder was essential to obtain valid predictions. This is because the increased temperature alone was not sufficient to prevent crystallisation (Chapter 3.3.2). This agenda (Figure 17) resulted in a successive implementation of the inline UV/Vis analysis to monitor 4-phenyltoluene production in flow. At the temperature of 84°C, the inline UV/Vis with the applied CLS method proved simple and successful in terms of precision and accuracy to monitor the process upsets (Chapter 3.3.5).

3.3.1 Calibration at 30°C with single component mixtures [8], [31]

For the calibration, 30 single component mixtures (Appendix C) were prepared with the following stock solutions: 70.17 mM phenylboronic acid, 1.42 mM 4-4'-Dimethylbiphenyl, 29.14 mM biphenyl, 35.14 mM 4-bromotoluene, 35.13 mM 4-phenyltoluene, all in EtOH:H_2O 60:40 (v.v.).

To determine extinction coefficients of these components (Figure 18), calibration in continuous flow mode was performed at the same flow (i.e. 0.5 ml/min) and temperature conditions (the water bath temperature of 30 °C) as the 4-phenyltoluene production monitoring. This was done for the same reasons as in the case of the single component calibration for ASA production monitoring (i.e. to provide uniform temperature and flow conditions for calibration and reaction monitoring). To provide uniform temperature conditions, a helical tube heat exchanger (water bath temperature was 30°C) and the same setup as used for the calibration in flow for ASA production (Figure 32) were used. For the calibration, 30 fully dissolved single component mixtures and 3 of the stock solutions were analysed against an EtOH:H_2O 60/40 (v.v.) blank.

Single point baseline correction at wavelength 319.228 nm was applied to the measured spectra. The linearity of the regression obtained from these mixtures and stock solutions analysed is shown in Figure 18 in terms of correlation coefficient in respect to wavelength

and was determined with Microsoft Excel CORREL function, in the same way as described previously in Chapter 3.2.1. The obtained correlation coefficients of all components analysed were > 0.99 in the wavelength range 239.653-279.808 nm. This shows a high degree of linearity as correlation coefficients approach value of 1. This value could be further improved by increasing the number of analysed single component mixtures and then additionally by manual selection of the resulting spectra in order to obtain the best linearity. However, in order to save chemicals and time, the resulting extinction coefficients (Figure 18) were considered acceptable and were then used for the application of the CLS method.

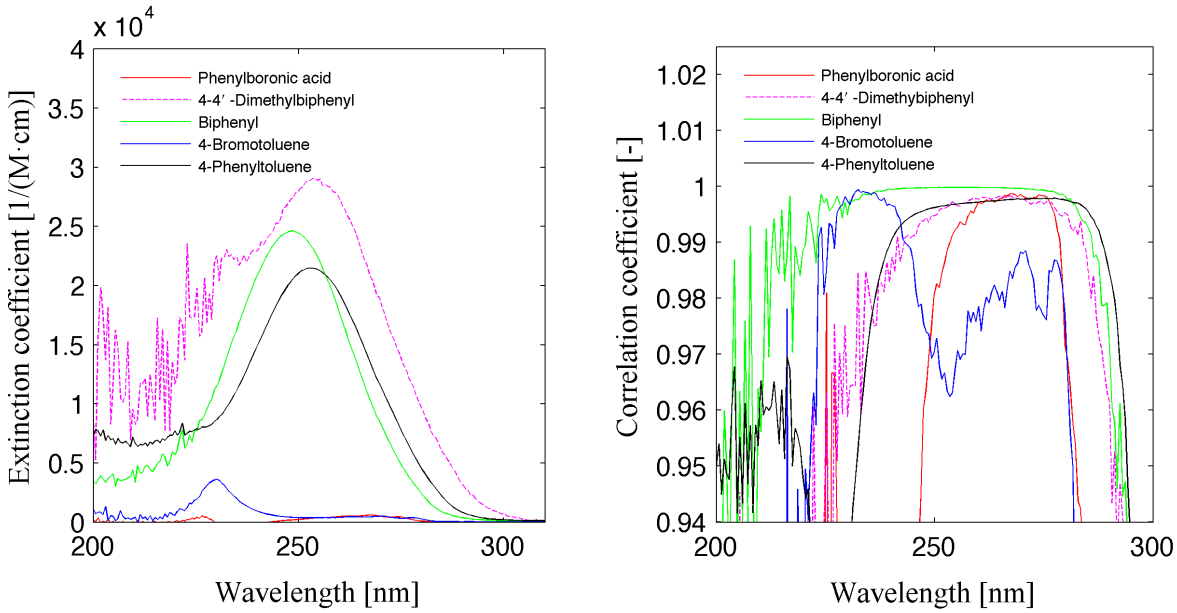

Figure 18: Plot of the extinction coefficient (left) and plot of the correlation coefficient (right), both in respect to wavelength. The water bath temperature of the helical tube heat exchanger was 30°C and the flow through the calibration setup 0.5 ml/min [8], [31].

3.3.2 **Comparison of the inline UV/Vis analysis with offline HPLC** [8], [31]

The extinction coefficients determined through calibration with helical tube heat exchanger water bath temperature of 30°C (Figure 18) were used for quantitative prediction of 4-phenyltolune yield of reaction monitoring experiment 5 and 6. For these experiments, the

yield of 4-phenyltoluene obtained with the inline UV/Vis CLS method and HPLC analysis was compared (description of the HPLC method can be found in chapter 5.3.8). For the inline UV/Vis analysis of these experiments, the same setup (Figure 30) as for the inline UV/Vis analysis of ASA production was used. Additionally, a filter (Whatman TM 52 filter papers with pore size 7 µm) was used to hold back the catalyst bed and to remove the dust and other impurities from the reactor product stream in order to avoid contamination of the UV/Vis cell. Analyses were performed without internal standard to reach higher purity of the final product without a further separation step. Both syntheses in these experiments were carried out at 91°C and with a flow rate of 0.5 ml/min.

After reaction monitoring experiment 5, the wavelength range (239.228 - 306.700 nm) for the CLS method was determined. Further production specifications for this experiment are gathered in description of Figure 19.

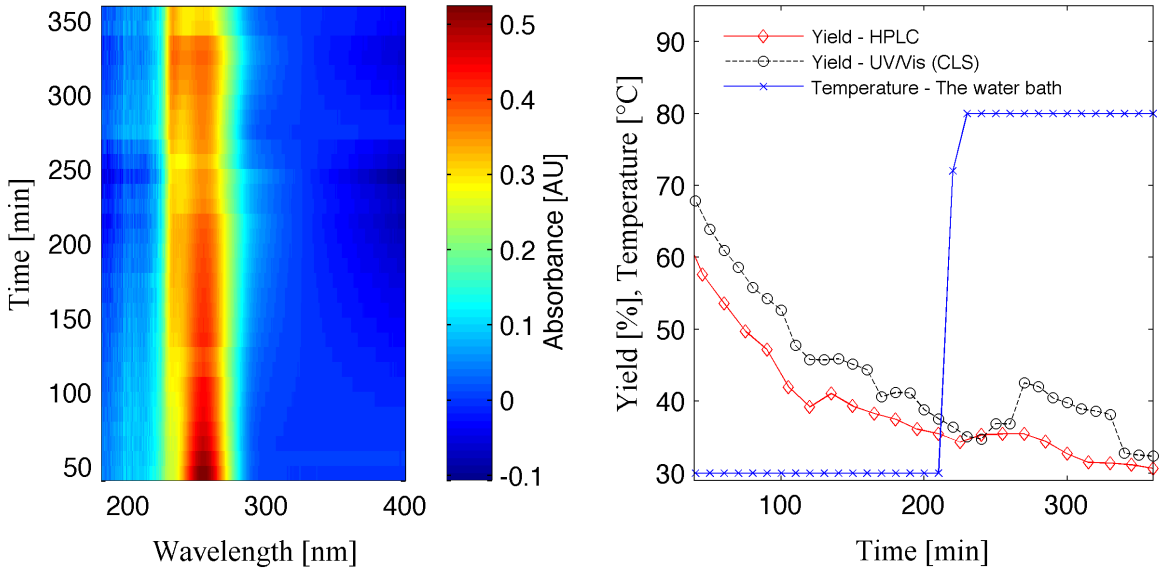

Figure 19: Absorbance after single point (319.228 nm) correction (left). Comparison of the yield obtained by the inline UV/Vis CLS (239.653 - 306.700 nm) with offline HPLC and the temperature profile of the helical heat tube exchanger water bath (right). Reaction was carried out with 35.37 mM 4-bromotoluene, 52.90 mM phenylboronic acid and 52.80 mM potassium carbonate, all in EtOH:H_2O 6:4 (v.v), 1.36 g catalyst ($Ce_{0.495}Sn_{0.495}Pd_{0.01}O_{2-\delta}$), at 91°C and with a flow rate of 0.5 ml/min.

The wavelength range was chosen based on the comparison of yield values obtained with UV/Vis CLS method and offline HPLC. The range was adjusted manually to obtain the best fit (Figure 19) between the yield values obtained with the UV/Vis CLS method and offline HPLC. This best fit (Figure 19) resulted in individual mean error of 13.06 %. It is important to notice that only yields at times > 40 min were compared. This is because the UV/Vis flow cell was first plugged into the reactor outlet stream after any traces of leaching catalyst were no longer visibly seen in the outlet stream. During this experiment, a high amount of undesired spectral drift (Figure 19), reaching maximum at -0.103 AU, was observed at times > 150 min and at wavelengths > 334 nm. The spectral drift was first spotted at 200 min during performing the experiment and immediately the flow cell was inspected. With inspection of the interior of the flow cell crystals were spotted. Thus, in order to check if crystallisation could be avoided at higher temperatures, the temperature of the helical heat tube exchanger water bath was set to 80°C. As can be seen in Figure 19, the increase in temperature was insufficient because when 80°C were reached at 230 min, the spectral drift due to crystallisation at times ≥ 230 min is still seen at wavelengths > 334 nm and reaches its maximum (-0.058 AU) at 330 min. Nevertheless, this experiment provided an important finding that the inline UV/Vis analysis of 4-phenyltoluene production has to be performed at higher temperatures (>80°C) to avoid crystallisation and incorrect predictions of the yield.

To inspect the effect of higher temperatures (i.e. 84, 90 °C) of the helical heat tube exchanger water bath, the reaction monitoring experiment 6 was performed. For the CLS method, used for predictions of yield in this experiment, the wavelength range (239.228 - 306.700 nm) determined in reaction monitoring experiment 5 was used. Production specifications for this experiment are gathered in description of Figure 20. During the entirety of the experiment, the comparison of the yield obtained with the inline UV/Vis CLS method and offline HPLC (Figure 20) showed that the UV/Vis CLS gives incorrect results. The individual mean error resulting from these yields was 9.70%. During the entirety of the experiment, the spectral drift (Figure 20) resulting from crystallisation inside the flow cell, regardless of the temperature of the water bath, was observed at wavelengths > 330 nm with a maximum value of -0.041 AU (at 90 min and 389.9 nm).

Because temperatures of the water bath chosen in experiments 5, 6 were not sufficient to avoid crystallisation, heat losses through the system boundaries had to be minimised, as discussed in chapter 5.3.6 in order to increase the actual temperature inside the flow cell.

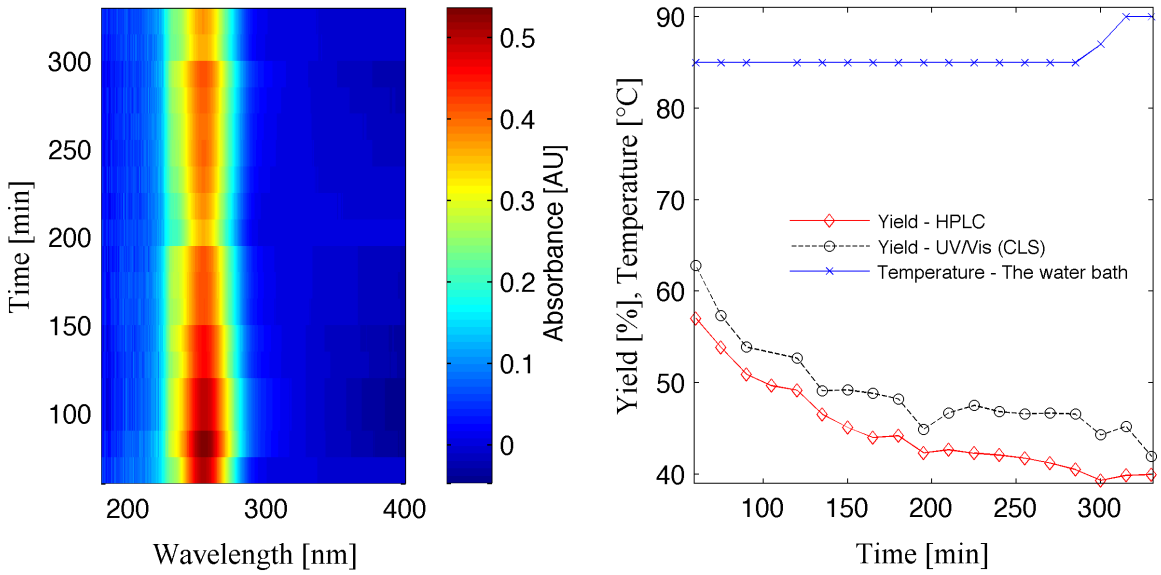

Figure 20: Absorbance after single point (319.228 nm) correction (left). Comparison of the yield obtained by the inline UV/Vis CLS (239.653 - 306.700 nm) with offline HPLC and the temperature profile of the helical heat tube exchanger water bath (right). Reaction was carried out with 35.08 mM 4-bromotoluene, 52.49 mM phenylboronic acid and 52.82 mM potassium carbonate, all in EtOH:H_2O 6:4 (v.v), 1.20 g catalyst ($Ce_{0.495}Sn_{0.495}Pd_{0.01}O_{2-\delta}$), at 91°C and with a flow rate of 0.5 ml/min.

3.3.3 Isolated sample holder design and performance [8], [31]

As the water bath temperatures ≤ 90°C were not sufficient to avoid crystallisation inside the flow cell (Chapter 3.3.2), the heat losses through the flow cell holder and other units of the UV/Vis system connected to the Plug & Play reactor system (Figure 31) had to be avoided in order to conserve more heat. Thus, the Avantes cell holder was isolated with 1 cm thick polystyrene plates that were cut to fit the shape of the holder (Figure 31). The same plates were used to isolate the tube connecting the helical tube heat exchanger and the reactor outlet. Moreover, the water vapours were used to provide additional heat to the

Avantes cell holder. Further description of the isolated sample holder design can be found in chapter 5.2.4.

The Plug & Play reactor connected to the UV/Vis system with the aforementioned adjustments (Figure 31) was then tested to see if the adjustments made will be sufficient to prevent crystallisation inside the flow cell during the inline measurements. For this purpose the reaction monitoring experiment 7 was performed. During this experiment, the synthesis was done at 91°C and with a flow rate of 0.5 ml/min. Further reaction parameters for this experiment are listed in description of Figure 21. The helical tube heat exchanger water temperature was 84°C throughout the experiment. Before the experiment, the UV/Vis system was preheated for 3 hours prior to the start of the experiment. This was the time needed to reach the steady state in terms of temperatures of the air inside the cell holder and on its outer surface. Temperature profiles in the steady state are shown in Figure 21. No spectral drift was visibly seen from the absorbance measurements at wavelengths > 300 nm (Figure 21) and no crystals were visible when inspecting the flow cell. Thus, the adjustments made were considered sufficient to prevent crystallisation and provide isothermal conditions inside the flow cell.

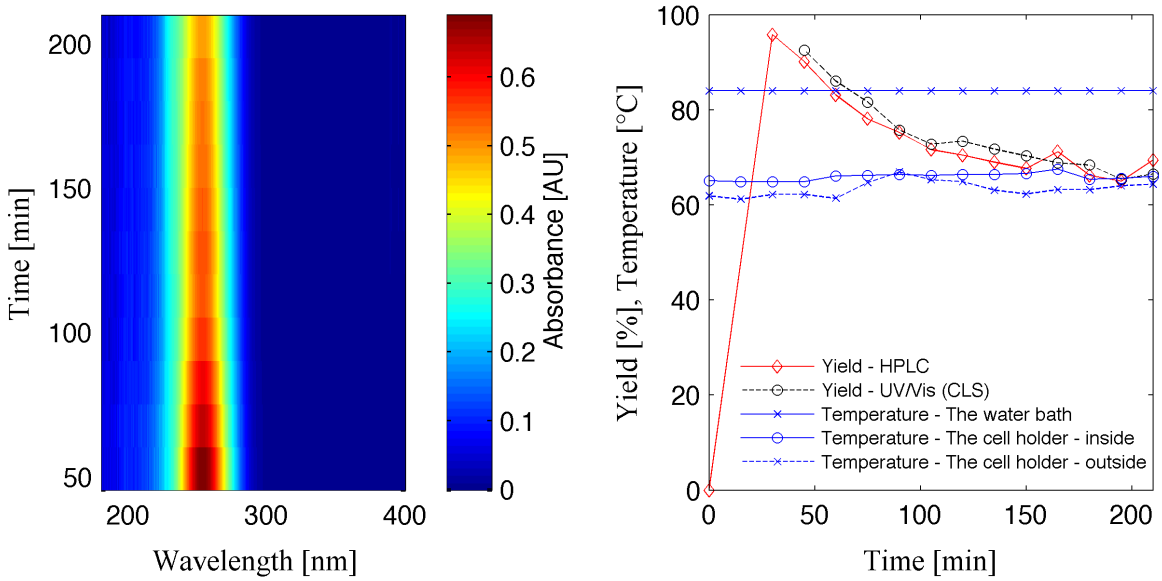

Figure 21: Absorbance after single point (319.228 nm) correction (left). Comparison of the yield obtained by the inline UV/Vis CLS (239.653 - 279.808 nm) with offline HPLC and the temperature profiles of the helical heat tube exchanger water bath, of the air inside the Avantes cell holder and of its outer surface (right). Reaction was carried out with 35.66 mM 4-bromotoluene, 52.49 mM phenylboronic acid and 52.82 mM potassium carbonate, all in EtOH:H_2O 6:4 (v.v), 1.20 g catalyst ($Ce_{0.495}Sn_{0.495}Pd_{0.01}O_{2-\delta}$), at 91°C and with a flow rate of 0.5 ml/min.

After the experiment was performed, single component mixtures calibration at the water bath temperature of 84°C was performed (Chapter 3.3.4). This was needed because the calibration at 30°C resulted in incorrect predictions of yield. Extinction coefficients (Figure 22) determined with the calibration at the water bath temperature of 84°C were then used for application of UV/Vis CLS method. Best fit (Figure 21) between yield obtained with UV/Vis CLS and offline HPLC was determined by manual adjustments of the wavelength range (239.653 - 279.808 nm) and comparison of the resulting individual mean errors. This fit resulted in individual mean error of 3.05 % with a minimum accuracy of 4.51 %.

After the experiment, the flow cell was severely contaminated with particular matter. Thus, higher accuracy was expected for further monitoring experiments with the cleaned flow cell. Nevertheless, this experiment showed that the adjustments made to equipment used

were sufficient to avoid crystallisation. In addition, the wavelength range (239.653 - 279.808 nm) for application of CLS method that was used for all further reaction monitoring experiments (i.e. experiment 8,9), was determined.

3.3.4 Calibration at 84°C with single component mixtures [8], [31]

For the calibration, 24 single component mixtures (Appendix C) were prepared with the following stock solutions: 67.32 mM phenylboronic acid, 23.55 mM biphenyl, 51.99 mM 4-bromotoluene, 35.39 mM 4-phenyltoluene, all in EtOH:H_2O 60/40 (v.v.).

4-4'-dimethylbiphenyl was not included in the calibration. This was acceptable because, as shown previously for synthesis of 4-phenyltoluene in the Plug & Play reactor, high selectivity of 99.5 % can be reached, as well as only a small amount of 4-4'-dimethylbiphenyl (i.e. < 0.2 % yield). This was also true for the concentrations of this by-product obtained with the inline UV/Vis analysis at reaction monitoring experiment 5 and 6 where none of the by-product was detected.

To determine the extinction coefficients of these components (Figure 22), calibration in continuous flow mode was performed at same flow (i.e. 0.5 ml/min) and temperature conditions (the water bath temperature of 84 °C) as for the 4-phenyltoluene production monitoring. The typical calibration setup (Figure 32) was additionally equipped with the isolated sample holder placed on the helical tube heat exchanger (Figure 31). This was done for the same reasons as discussed in Chapter 3.3.1. For the calibration, all stock solutions and single component mixtures were analysed against an EtOH:H_2O 60/40 (v.v.) blank and used for the determination of the extinction coefficients (Figure 22). Single point baseline correction at wavelength 319.228 nm was applied to the measured spectra. The linearity of the regression obtained from these mixtures and the stock solutions is shown in Figure 22 in terms of correlation coefficient in respect to wavelength and was determined with Microsoft Excel CORREL function in the same way as described previously in Chapter 3.2.1. Correlation coefficients of 4-bromotoluene, 4-phenyltoluene and biphenyl with > 0.999 in the wavelength range 220-280 nm and slightly lower correlation coefficients of phenylboronic acid with a maximum value of 0.998 were obtained. Nevertheless, this shows a high degree of linearity as correlation coefficients approach

almost 1. The resulting extinction coefficients (Figure 22) were considered acceptable and were then used for the application of the CLS method.

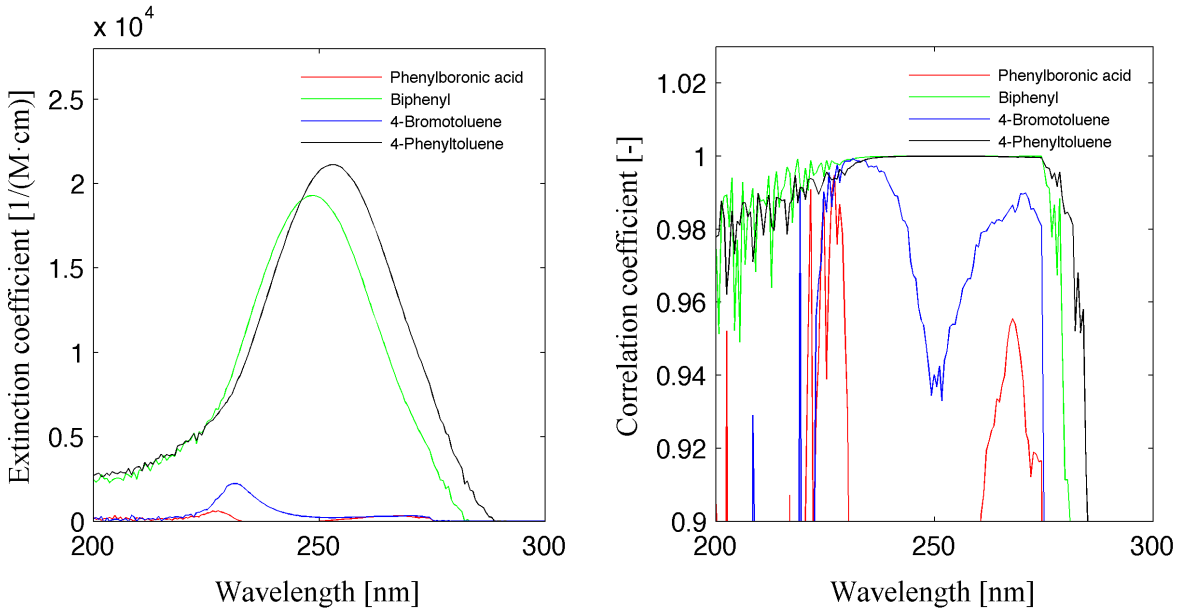

Figure 22: Plot of the extinction coefficient (left) and plot of the correlation coefficient (right), both in respect to wavelength. The water bath temperature of the helical tube heat exchanger was 84°C and the flow through the calibration setup 0.5 ml/min [8], [31].

3.3.5 Comparison of the inline UV/Vis analysis at 84°C with offline HPLC [8], [31]

For the final validation of the inline monitoring of 4-phenytoluene production, the flow cell was cleaned. This was done because after the reaction monitoring experiment 7 (Chapter 3.3.3), contamination of the cell with particle matter was spotted. Thus, better results were expected in comparison to those obtained with the reaction monitoring experiment 7. In total, 2 reaction monitoring experiments were performed for validation purposes. Again, the inline UV/Vis CLS method was compared with offline HPLC. For these experiments (i.e. reaction monitoring experiment 8,9), temperature of the helical tube heat exchanger water bath was 84°C and the same setup as for the reaction monitoring experiment 7 was used. The wavelength range used for application of CLS method was that obtained in the reaction experiment 7.

Reaction monitoring experiment 8, performed at flow rate of 0.5 ml/min and other parameters listed in the description of Figure 23, showed high degree of accuracy and precision (Figure 23) of the inline UV/Vis CLS method in comparison to offline HPLC. The individual mean error and the lowest accuracy, obtained from comparison of the yields obtained by these techniques, were 1.55 % and 4.55 %, respectfully. The absorbance measurements (Figure 23) as well as yields obtained with the UV/Vis CLS method, show good response of the yield measured with the inline UV/Vis analysis to the process upsets in terms of yield measured with offline HPLC (Figure 23). Two such upsets (Figure 23) are well seen at 135 and 165 min. It is of importance to notice that good results of this experiment were expected as both calibration and production analyses were performed at the same flow rate (0.5 ml/min) and at the same temperature conditions (84°C). To observe performance of the inline UV/Vis at different flow rates and with induced process upsets caused by the change of flow (Figure 24), experiment 9 was performed at flow rates ranging from 0.5 - 1 ml/min. This experiment showed slightly lower degree of accuracy and precision of the inline UV/Vis CLS method in comparison to offline HPLC in terms of individual mean error (1.91%) and lowest accuracy (5.59%), both obtained from the comparison of the yield obtained by these techniques (Figure 24). Nevertheless, from the same comparison, good response to the process upsets (Figure 24) was observed. For instance (Figure 24), at times 1065-1320 min, when highest yields were measured with both, the inline UV/Vis and offline HPLC, also the highest absorbances at 4-phenyltoluene absorption maximum (253 nm) were measured. Because of the flow behaviour changes induced by variations in the flow rate, slightly higher error was expected. This is because at higher flow rates a higher number of air bubbles was spotted in the reactor outlet flow. As the number of bubbles increased the possibility of the bubble disturbing absorbance measurement increased. These disturbances of the absorbance measurements were observed as rapid changes in the absorbance spectra while performing the experiment (i.e. every few seconds). Such disturbances are well seen in graphs of both yield and absorbance (Figure 24) for the steady state at increased flow rate between approx. 450 and 750 min. As no debubbler was installed this led to a higher degree of error. Nevertheless, comparison of yield obtained by inline UV/Vis CLS method with offline HPLC showed high accuracy and precision with the individual mean error < 1.91 % as well as good measurement response to changes in process setups (i.e. flow rate and amount of the catalyst) for both experiments. As measurements with the UV/Vis system are rapid (i.e.

every 3.4 seconds), almost real time monitoring was possible. Thus feedforward and feedback control to the reaction system could be implemented.

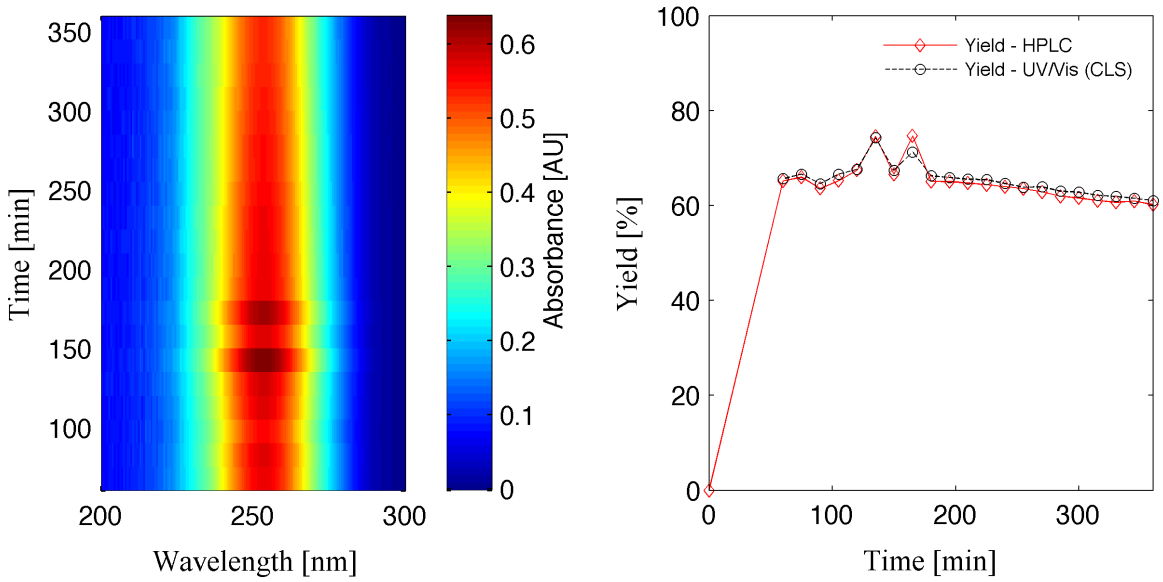

Figure 23: Absorbance after single point (319.228 nm) correction (left). Comparison of the yield obtained by the inline UV/Vis CLS (239.653 - 279.808 nm) with offline HPLC (right). Reaction was carried out with 40.05 mM 4-bromotoluene, 52.49 mM phenylboronic acid and 52.46 mM potassium carbonate, all in EtOH:H_2O 6:4 (v.v), 1.20 g catalyst ($Ce_{0.495}Sn_{0.495}Pd_{0.01}O_{2-\delta}$), at 91°C and with a flow rate of 0.5 ml/min.

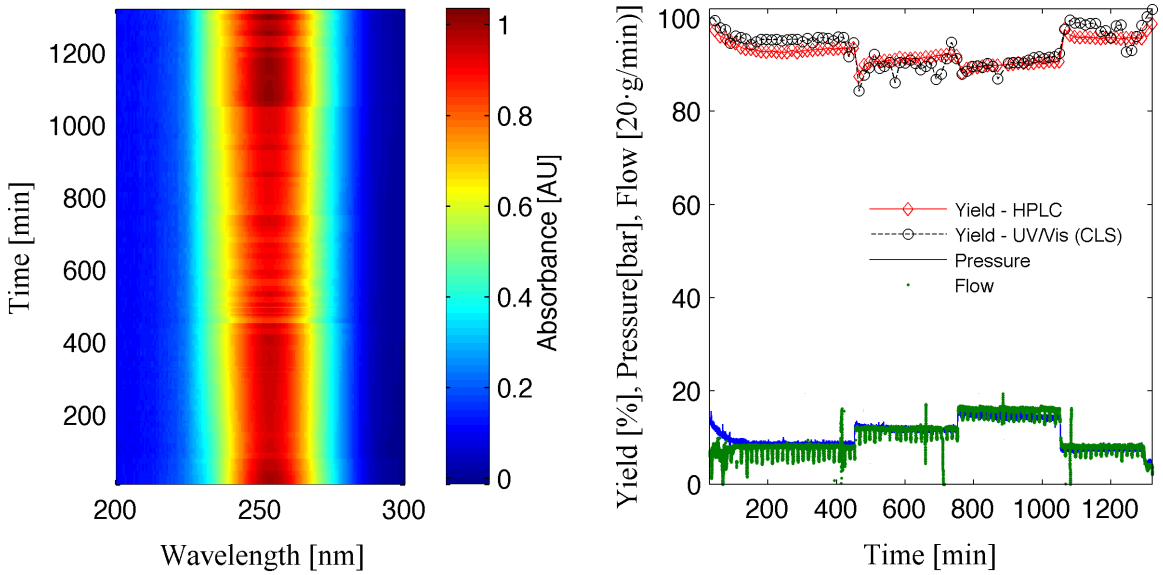

Figure 24: Absorbance after single point (319.228 nm) correction (left). Comparison of the yield obtained by the inline UV/Vis CLS (239.653 - 279.808 nm) with offline HPLC, pressure and flow profile (right). Reaction was carried out with 46.95 mM 4-bromotoluene, 70.20 mM phenylboronic acid and 70.04 mM potassium carbonate, all in EtOH:H_2O 6:4 (v.v), 3.67 g catalyst ($Ce_{0.495}Sn_{0.495}Pd_{0.01}O_{2-\delta}$) and at 91°C [8], [31].

3.4 Application of the inline system to monitor reactions in flow

The general idea for the application of the inline system to monitor reactions in flow was to couple Microsoft Excel with the AVASOFT software (version 7.7.2, available at the IPPE) in order to continuously measure yield of different reactions in flow. For this purpose an application in Microsoft Excel (In-line.xlsm), included on the CD attached to this thesis was developed and used for continuous measurements of absorbance spectra with AVASOFT while the Excel file was used to perform automated calculation of 4-phenyltoluene yield from the 4-phenyltoluene concentration obtained with CLS method. Reader can find the exact instructions for using this application in Chapter 5.3.10.

The final application included the UV/Vis reaction monitoring system (Chapter 5.2.3), coupled with the AVASOFT software to monitor 4-phenyltoluene production yield. The

spectral measurements (every 3.4 seconds) were overwritten continuously to a fixed position in the workbook (In-line.xlsm) and were displayed in the final Excel application (Figure 25). Then a single point (319.228 nm) spectral correction was applied to the spectra (Figure 25), concentrations of 4-phenyltoluene were predicted, yield was calculated and plotted (Figure 25). To calculate 4-phenyltoluene yield with CLS method, the automated Excel solver optimised the calculated concentration values by minimising the sum of the square of the differences between measured and predicted absorption. The yield was then calculated from the predicted concentration of 4-phenyltoluene and the concentration of 4-bromotoluene in the educt solution. Additionally, the final application included the display of elapsed time (Figure 25) in respect to time since start of the 4-phenyltoluene production experiment and the spectral data used for prediction of yield were saved to the Excel file to enable further analysis of obtained spectra (e.g. to enable testing of other chemometric methods).

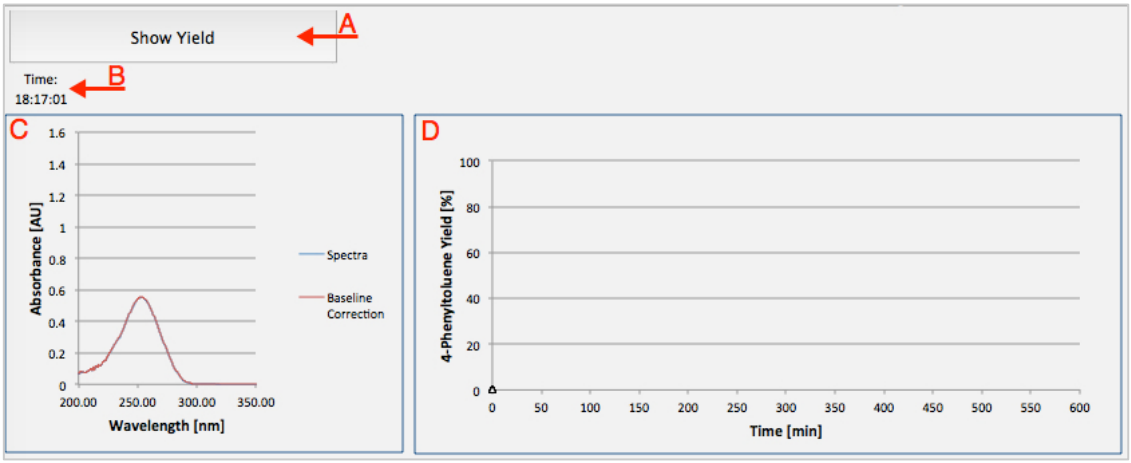

Figure 25: Interface of the Excel application used to monitor 4-phenyltoluene production yield. The interface included (A) 'Show Yield' button, (B) elapsed time, (C) continuously updated graph of absorbance in respect to wavelength displaying last absorbance measurement spectra and the spectra with the one point baseline correction, (D) graph of 4-phenyltoluene yield in respect to time.

While spectral measurements were overwritten continuously, prediction of concentrations with CLS method and further calculation of yield had to be executed manually by pressing on the 'Show Yield' button. The manual execution was necessary as the Avantes software

and the automated Excel solver which were both ran in a loop at the same time resulted in a crash of the automated Excel solver. The execution was only possible after the Avantes software overwrote the spectral data and had to be done before the next overwriting of spectral data. Nevertheless, the application with manual execution by a single press of the button provided the automated prediction of 4-phenyltoluene yield. The application can be easily adjusted to monitor different reactions with CLS method. The adjustment needed to monitor another reaction could be made by simply entering new extinction coefficients obtained with a single component calibration of the chemical compounds involved in the reaction. To automate the execution of yield prediction, automated clicker software could be used (e.g. Speed-auto-clicker ®) with adjustable time between performing automated clicks.

4. Conclusion and outlook

The aim of this work was to implement an inline UV/Vis analysis system in an existing continuous flow reactor setup (i.e. Plug & Play reaction setup). The essay of thesis objectives was accomplished. In particular, (i) the UV/Vis system was implemented to monitor 4-phenyltoluene and ASA production in continuous flow mode, (ii) appropriate inline analytical methods were developed and the results obtained were compared to offline HPLC and (iii) the application of the inline system to monitor these reactions was successfully developed and tested for 4-phenyltoluene production [8].

To achieve these objectives, first the literature of UV/Vis analysis basic principles, inline analysis, chemometrics and model reactions was reviewed. Prior to reaction monitoring in flow, the inline UV/Vis sensor was sized according to the literature reviewed and chosen to fit into the reaction setup. The sizing focused on scaling a sensor to allow absorbance measurements for both ASA and 4-phenyltoluene production in an optimal absorbance measurement range based on single component measurements performed with an insertion probe. Production monitoring experiments and calibration were then planned following the suggestions from the literature. Specifically, a special helical tube heat exchanger was designed, coupled with the chosen inline sensor (The Starna flow cell with 0.01 mm optical path length). Additionally, to monitor 4-phenyltoluene production, an inline filter was employed to the setup and the flow cell holder was isolated [8]. This allowed us not only to control temperature but also prevented undesired crystallisation and film build-up, all in the interior of the flow cell for both production monitoring and calibration [8]. The analyses were performed without internal standard to reach higher purity of the final product without a further separation step.

In summary, the comparison of offline HPLC with inline UV/Vis SE and CLS method to monitor ASA production yield and CLS method to monitor 4-phenyltoluene yield showed high accuracy and precision of these simple methods and demonstrated them to be capable of monitoring process upsets [8]. As measurements with the UV/Vis system are rapid, almost real time monitoring is possible [8]. Thus, feedforward and feedback control to both reaction systems could be implemented [8]. The implementation of the inline system to

monitor different reactions was also shown to be economical. Specifically, to monitor different reactions, only one UV/Vis sensor was needed, while the software and the remaining hardware was already available at the IPPE. Calibration of both methods was performed by simulating similar flow and temperature conditions to reaction monitoring and by determining extinction coefficients from single component mixtures [8], [31].

The application of the inline system to monitor reactions in flow included the application in Microsoft Excel used to manually execute automated yield predictions with a single press of a button. This application was coupled with the AVASOFT software used for fully automated continuous measurements of absorbance. Full automation of the application in Excel was not possible as running both AVASOFT software spectral measurements and Excel yield predictions in a loop resulted in the crash of the Excel solver used to predict yield. Nevertheless, in the future, executing yield predictions in Excel could be fully automated using automated clicker software. Finally, the application was successfully tested for 4-phenyltoluene production and could also be used to monitor different reactions with UV/Vis CLS method (e.g. ASA production) independent of the UV/Vis inline process analyser used.

5. Experimental

5.1 Chemicals

Chemicals used (Table 9) are commercially and commonly available.

Table 9: Chemicals used.

Compound	Purity	Company
Amberlyst 15		Aldrich
Salicyclic acid	≥99 %	Sigma - Aldrich
4,4'-Dimethylbiphenyl	97 %	Aldrich
Acetic anhydride	≥99 %	Roth
Biphenyl	99.5 %	Sigma
Sulphoric acid	> 95 %	Roth
Orthophosphoric acid	> 85 %	Roth
Phenylboronic acid	>98 %	Alfa Aesar
4-Phenyltoluene	≥98 %	SAFC
Hydrochloric acid	37 %	Roth
Acetylsalicylic acid	>99 %	Sigma
Ethyl acetate	≥99.5 %	Roth
4-Bromotoluene	98 %	Aldrich
Phenylboronic acid	>97 %	Fluka
4-Acetylbiphenyl	98 %	Sigma - Aldrich
Methanol	>99.9 %	Roth
Potassium carbonate	99.9 %	Roth
Absolute EtOH	>99.8 %	VWR

5.2 Equipment used

For offline analysis, HPLC Agilent 1100 Series with Poroshell 120 EC-C18 column (Agilent Technologies) was used. Exact description of the column and of the offline analysis can be found in chapter 5.3.8. Density measurements were performed with Anton Paar DSA 500 M digital density meter. Temperature measurements on the outer surface of the Avantes cell holder were performed with Mastech MS6520C infrared thermometer, while the measurements of the air inside the cell holder with thermometer TESTO 110. The rest of the equipment is listed and described in sub-chapters 5.2.1 - 5.2.4.

5.2.1 Preliminary setup for absorbance measurements

The preliminary UV/Vis spectroscopy setup for absorbance measurements included (Figure 26) reflection probe (FDP-7UV200-2- 2.5) with 5 mm optical path length connected via reading fibre-optic cables to the Spectrometer (AvaSpec-ULS2048-USB2-UA-50) and the light source (AvaLight-D-S-DUV).

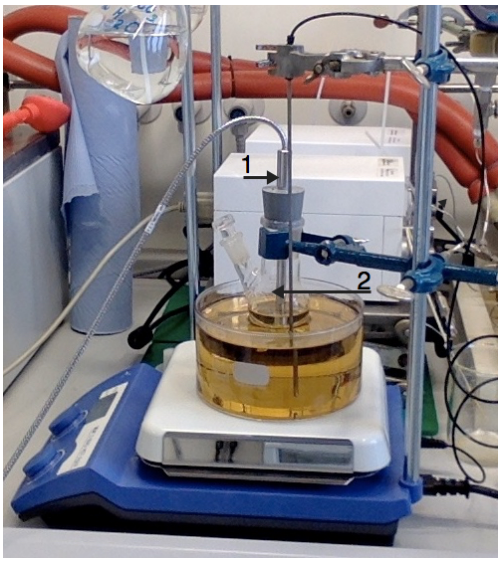

Figure 26: The preliminary UV/Vis spectroscopy setup for absorption measurements including the reflection probe (1) inserted into the measuring vessel (2) and placed into the oil water bath.

The probe was inserted into the measuring vessel that was placed in an oil bath. A magnetic stirrer (IKA C MAG HS7 digital) equipped with heating plate and thermostat was used to guarantee isothermal conditions during measurements and to stir both, the measuring vessel and the oil bath.

5.2.2 Heating thermostat

For calibration in continuous flow mode and inline analysis, a heating thermostat equipped with a helical tube heat exchanger was designed (shown in Figure 27).

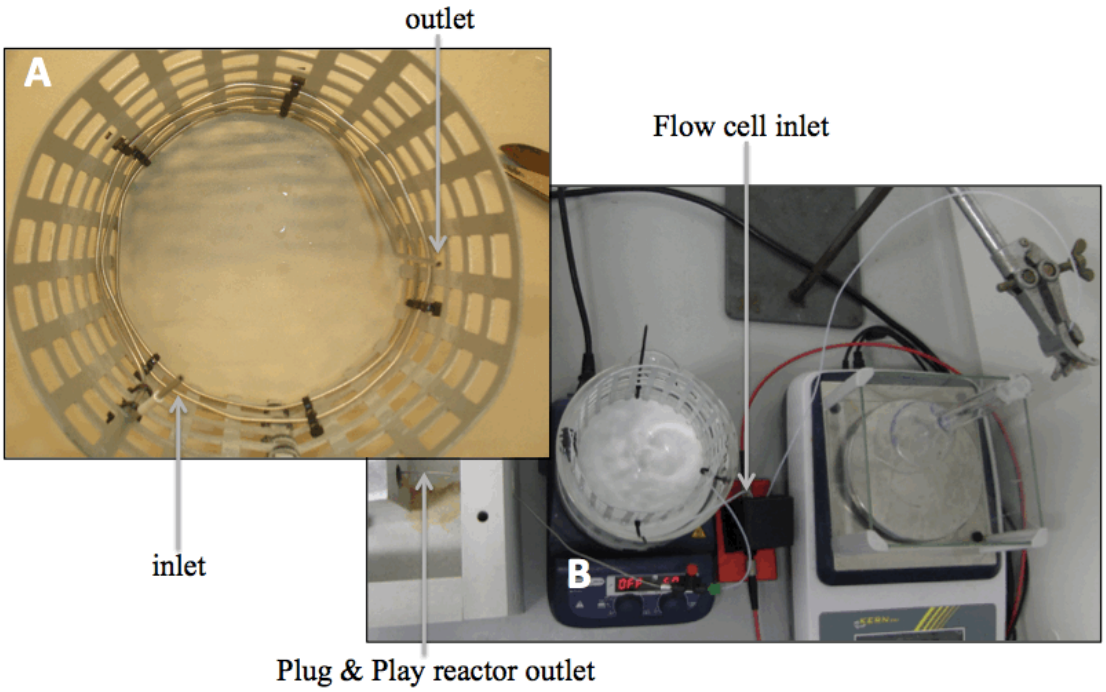

Figure 27: Picture (A) of the inlet and outlet of the helical tube heat exchanger mounted to the aluminium perforated plate and picture (B) of the heating thermostat connected to the Plug & Play reactor outlet and the flow cell inlet.

This design included a helical tube (ID x 0.030, length 1 m) heat exchanger attached to an aluminium cylindrically shaped perforated plate (OD=10.7 cm, height 5.8 cm) to fit into thermostated deionised water bath (OD=14 cm, height 7.3 cm, water height 5.7 cm).

Deionised water bath was heated and stirred with a magnetic stirrer (IKA C MAG HS7 Digital).

5.2.3 Reaction monitoring setup

The typical UV/Vis spectroscopy setup for reaction monitoring included (Figure 28):

- Starna flow cell (584.4-Q-0.01) with 0.01 mm optical path length.
- Avantes cuvette sample holder connected via 2 fibre-optic cables to the Spectrometer (AvaSpec-ULS2048-USB2-UA-50) and the light source (AvaLight-D-S-DUV).
- Heating device with thermostat (IKA C MAG HS7 digital) to guarantee isothermal conditions during measurements and to prevent crystallization of the products.

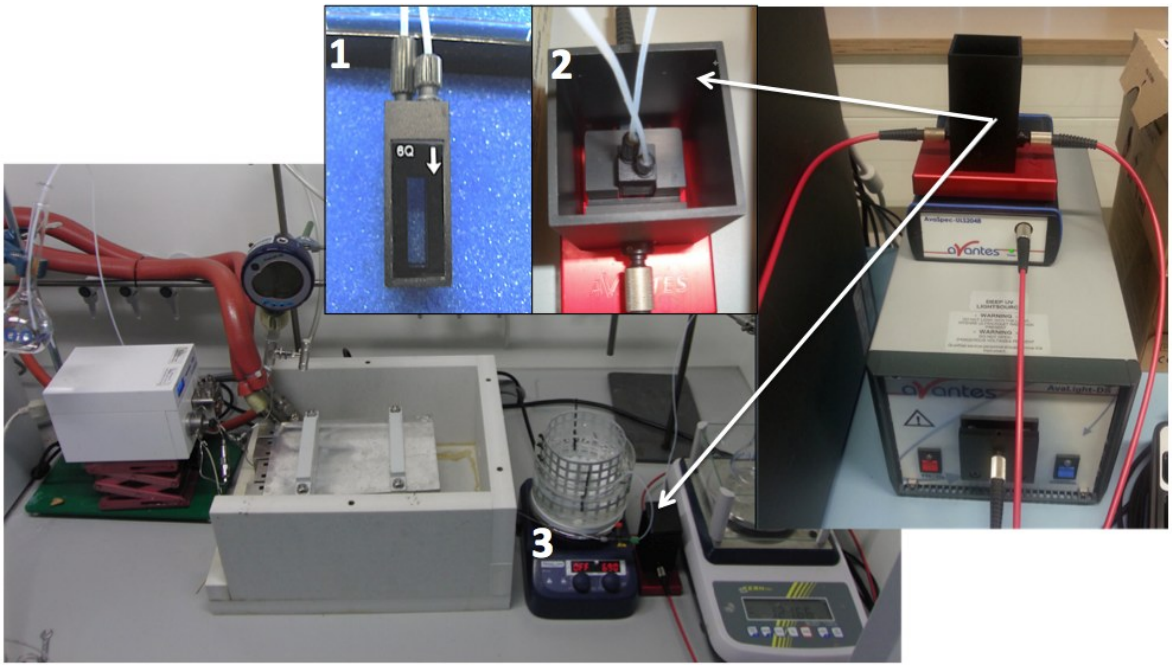

Figure 28: The Plug & Play reactor setup linked to the inline UV/Vis setup including the Starna flow cell (1), the cuvette sample holder (2) and a heating device with thermostat (3) [8], [31].

Reader can find the relevant data about the UV/Vis setup in Appendix F. The UV/Vis reaction setup was connected to the Plug & Play reactor setup (Figure 30). Commercially available HPLC columns filled with catalyst (Figure 29) were used as a fixed bed reactor.

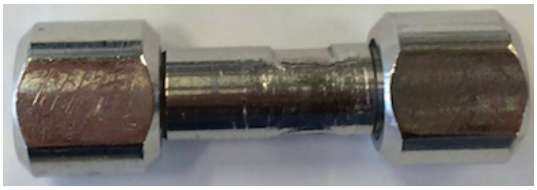

Figure 29: The HPLC column.

HPLC pump (Knauer, Azura P4.1 S) was used to pump the reaction solution through the setup. Mass flow was monitored with Kern balance (EWJ 600 2M) connected to a PC with a RS-232. Reactor was heated with Lauda P18 thermostat and was connected to heating helical tube heat exchanger equipped with thermostat with a PEEK union (Vici Jour union JR-1061). The same union was used to connect the UV/Vis flow cell with the helical tube heat exchanger. Labview software was used to monitor pressure and mass flow. Variations of equipment for this setup for each reaction monitoring experiment can be found in Appendix E.

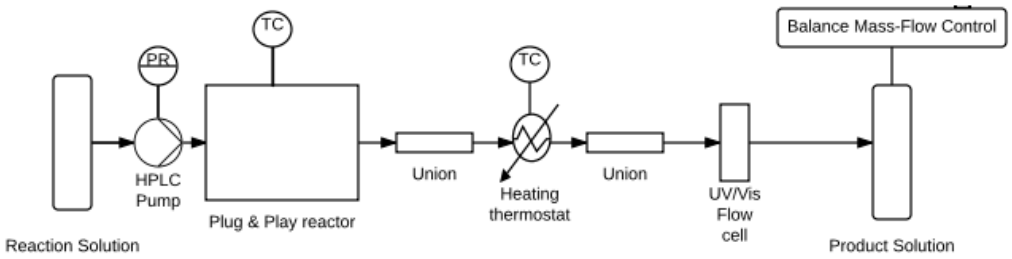

Figure 30: P&ID diagram of the Plug & Play reactor including the UV/Vis setup.

5.2.4 Heating device with an isolated sample holder

The purpose of the isolated sample holder equipped with the isolated flow cell inlet (Figure 31) was to perform UV/Vis measurements at high temperature settings of the thermostat (i.e. 84°C) without severe heat loss in order to provide uniform, high temperature conditions needed to prevent crystallisation for both, calibration and monitoring purposes of the 4-phenyltoluene production. This sample holder included all components of the typical UV/Vis spectroscopy setup (Figure 28) described in Chapter 5.2.3. Additionally, the heating device was equipped with a cylindrical module on which the Avantes isolated sample holder was placed. This module had an opening below this cell holder which allowed the water vapours to condensate on the bottom outer surface of the holder and provide additional heating.

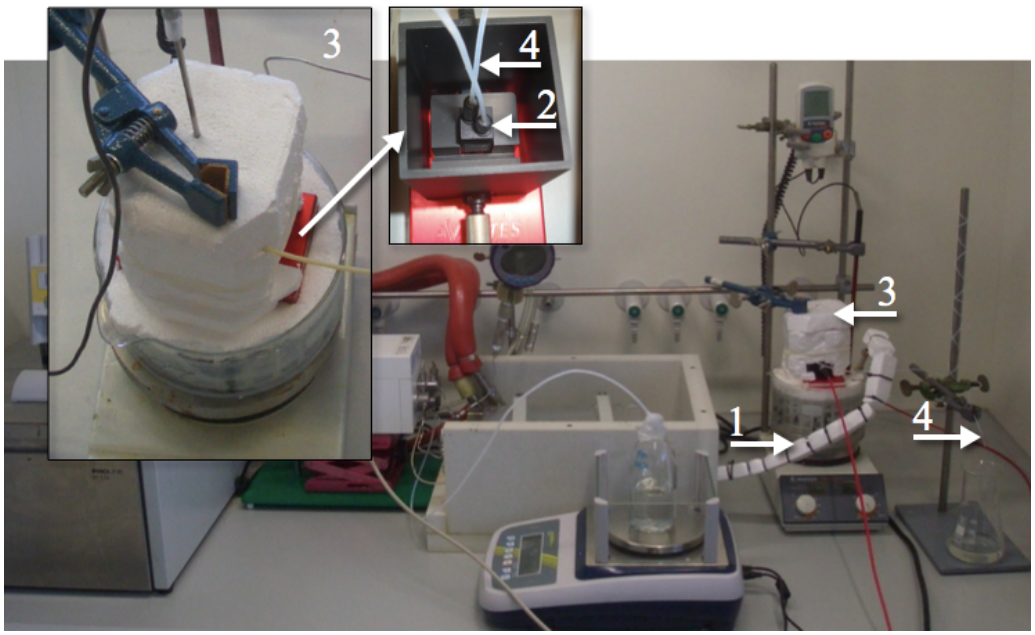

Figure 31: Picture of a typical Plug & Play reactor setup to monitor 4-phenyltoluene production including isolated outlet (1) of the Plug & Play reactor connected to the helical tube heat exchanger and the Starna Flow cell (2) placed inside the isolated sample holder (3) with the flow cell outlet (4) leading to the product solution collected in the Erlenmeyer flask.

Both, the cylindrical module and the isolation for the sample holder were cut out of a 1 cm thick polystyrene plate to fit the shape of the holder. The Starna flow cell inlet and the outlet tubes were placed between the Avantes holder's outside wall and the polystyrene isolation to prevent heat loss. Furthermore, the reactor outlet tube, connected to the helical tube heat exchanger (Figure 31) with a PEEK union (Vici Jour union JR-1061), was isolated with the same material. A magnetic stirrer (IKA C MAG HS7 digital) equipped with heating plate and thermostat was used to guarantee isothermal conditions of the water bath during spectral measurements.

5.3 Methods

5.3.1 Preparation of stock solutions and single component mixtures for sizing of the inline sensor for reaction monitoring

Stock solutions (Appendix A) were prepared by dissolving x amount of a chemical and diluted with the appropriate solvent to the mark of a 20-ml volumetric flask. Solvent used for all SA and ASA mixtures (Appendix A) was EA and for 4-bromotoluene, phenylboronic acid, 4-phenyltoluene EtOH:H_2O 6:4 (v.v.). Single component mixtures (Appendix A) were then prepared from these stock solutions. For instance 0.78 mM SA single component mixture was prepared by pipetting 1 ml of the 15.96 mM stock solution 3 into a 20 ml flask and volume adjusted with EA. Further concentrations and weighted masses of prepared stock solutions as well as concentrations of the prepared single component mixtures are listed in Appendix A.

5.3.2 Acetylsalycilic acid solubility test

At room temperature (i.e. 24.9 °C), 1.4413 g ASA (i.e. 0.4 M) was weighted and 19 ml EA was added to the volumetric flask, shaken by hand for approx. 1 minute and filled up to the mark of 20 ml. Exact same procedure was repeated with 1.8016 g (i.e. 0.5M) ASA. Visibly clear solution without solid crystals was considered as fully dissolved mixture.

5.3.3 Calibration in flow for acetlysaliclylic acid production

Following stock solutions were prepared:

- ASA (0.399 M) by dissolving 7.1946 g ASA in approx. 95 ml of EA and diluted to the mark of a 100-ml volumetric flask,
- SA (0.9993 M) by dissolving 6.9034 g SA in 50 ml of EA in approx. 45 ml of EA and diluted to the mark of a 50-ml volumetric flask,
- SA (1.3028 M) by dissolving 17.9947 g SA in 100 ml of EA in approx. 95 ml of EA and diluted to the mark of a 100-ml volumetric flask.

These stock solutions were then used to prepare 24 single component mixtures. For instance, a single component mixture 1 (SCM 1) was prepared by pipetting 25 ml of the stock solution SA (0.9993M) into a 50 ml flask and volume adjusted with EA to obtain a 0.4997 M solution. Specifications of single component mixtures obtained are gathered in Appendix A.

Calibration mixtures were then pumped through the calibration setup (Figure 32) at conditions similar to the inline (the water bath temperature was 25°C) analysis setup. Acquisition of spectra was performed against an EA blank after a solution was pumped through the flow cell for 15 minutes. This was the time needed for absorbance spectra to become constant (i.e. to wash out previous calibration solution) in the wavelength range of interest (λ = 210-360 nm). After the measurements were done, the UV/Vis flow cell was flushed with injecting 5 ml EA followed by 5 ml absolute EtOH into the cell inlet tube with a 5 ml syringe. Further calibration setup operating specifications, exact calibration equipment, and spectrometer settings used are specified in Appendix D.

The setup was positioned next to Plug & Play reactor in order to avoid plugging the optical fibre cables inlet and outlet when repositioning equipment inside the IPPE laboratory which can cause minor baseline drifts [10, pp. 19, 60-61].

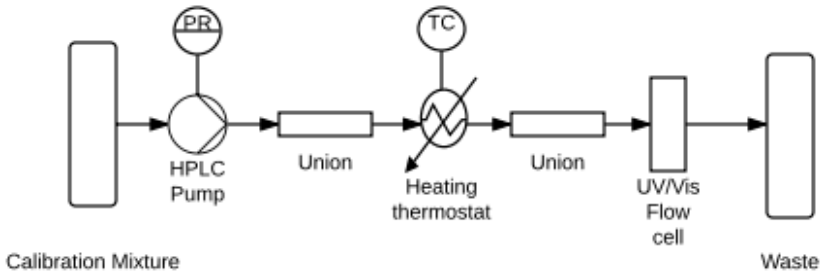

Figure 32: P&ID diagram of a typical calibration setup.

5.3.4 Validation of calibration in flow for acetylsalicylic acid production using binary mixtures

Six binary mixtures were prepared for spectral measurements in flow with different ASA-SA ratios. To save chemicals, unused single component mixtures from calibration in flow (described in chapter 5.3.3) were used. Binary mixtures (BM) were prepared by pipetting 10 ml of a certain SCM containing SA and 10 ml of a certain SCM containing ASA and mixing them in a 20 ml volumetric flask. Then the volume was adjusted to 20 ml by adding EA. Exact prepared solutions are listed in Appendix A. Spectral measurements of BM were performed in exact same way and with the same setup as described in chapter 5.3.3. For quantitative prediction SE and CLS method were tested. For SE wavelengths at the SA maximum (λ=306.103 nm) and ASA maximum (λ=276.218 nm) were selected. For the CLS method, wavelength range was manually varied to see which wavelength range gives best quantitative prediction. Statistical evaluation of fit was performed in terms of accuracy and individual mean error.

5.3.5 Monitoring of acetylsalicylic acid production

ASA monitoring was performed in a series of 4 inline monitoring experiments that included production of acetylsalicylic acid in Plug & Play reactor setup combined with UV/Vis setup for inline analysis (Figure 30). Those experiments were executed to optimise the wavelength range of UV/Vis analysis and to validate the inline analysis by comparing the obtained results with offline HPLC. This chapter describes the typical procedure including variations in the reaction monitoring experiments.

Prior to reaction monitoring of ASA production in flow, the HPLC column (Figure 29) was filled with ion exchange catalysts Amberlyst 15 and mounted into Plug & Play reactor. The reaction solutions for acetylsalicylic production were prepared as follows:

1) SA was weighted to the volumetric flask and approximately half of the flask was filled with EA.
2) Approximately 1.5 equimolar amount of AH with respect to the amount of SA was added to the solution. Ultrasonic bath was then used to fully dissolve the components.
3) The volumetric flask was filled up to the mark with EA, sealed and shaken by hand few times. Sample for HPLC analysis was taken from the solution.
4) Dust was removed from the solution by gravity filtration in order to hinder a film formation in the UV/Vis flow cell. This was done by folding the filter paper (MN 614 1/4) which was then placed into a glass funnel seated into another volumetric flask. Solution was carefully poured onto the paper until the solution was filtered.

Catalyst was filled into the HPLC reaction colom. The essay of prepared educt solutions for each reaction monitoring experiment and the amounts of catalyst are listed in Table 10.

Table 10: The essay of the amount of the catalyst (Amberlyst 15) and the concentrations of SA and AH of the educt solutions prepared for series of reaction monitoring experiments.

Reaction monitoring experiment #	1	2	3	4
Mass of catalyst [g]	1.111	1.095	1.106	1.082
SA concentration [M]	0.108	0.200	0.401	0.403
AH concentration [M]	0.159	0.317	0.688	0.608

Before the synthesis in flow took place, the reactor and the UV/Vis helical tube heat exchanger system were preheated to their operating temperatures (i.e. 25 °C for the UV/Vis heat exchanger and 60°C for the reactor). The temperature conditions of the heat

exchanger and the reactor were kept constant throughout the experiment with the help of the thermostats installed at the devices. The stirring setting of the helical tube heat exchanger was set to 690 rpm. The UV/Vis light source was turned on for a minimum of 30 minutes prior to a monitoring experiment in order to warm up.

When all the conditions prior to the experiment were met, the experiment was started. HPLC samples were collected from the product stream in time intervals ranging from 5 - 15 minutes, while simultaneously, UV/Vis inline analyses were executed. The time interval between sampling varies among experiments in order to obtain the desired data (e.g. measure unsteady state at the start of the synthesis). All UV/Vis measurements were performed against an EA blank. Integration time was 1 ms and 2000 spectra were averaged for each absorbance measurement. Single point (i.e. at wavelength 354.35 nm) baseline correction was applied to the measured spectra. After the experiment, the UV/Vis flow cell was flushed by injecting 5 ml EA, followed by a 5 ml absolute EtOH injection into the cell inlet tube with a 5 ml syringe. Then it was checked for impurities (e.g. small dust particles on the inner Quartz surface) and further cleaned on demand (i.e. after reaction monitoring experiment 2, 3, 4, 5) as described in Chapter 5.3.9. The Plug & Play reactor setup was cleaned by pumping EA through the setup for approx. 30 minutes.

5.3.6 Calibration in flow for 4-phenyltoluene production

Calibration in flow for 4-phenyltoluene production was performed at two different temperature conditions using two different calibration setups. For the *first calibration*, the water bath temperature of the helical tube heat exchanger was 30°C. For this calibration the same setup as it was used for the calibration in flow for ASA production (Figure 32) was used. For the *second calibration*, for which the water bath temperature was 84°C, this calibration setup was equipped with the isolated sample holder (Figure 31).

Stock solutions for both calibrations were prepared by dissolving pure component (e.g. 0.8556 g phenylboronic acid) in approx. 95 ml solvent, i.e. EtOH:H2O 6:4 (v.v), and diluted to the mark of a 100-ml volumetric flask (i.e. to obtain 70.17 mM phenylboronic acid stock solution). For the first calibration, phenylboronic acid, 4-4'-dimethylbiphenyl, biphenyl, 4-bromotoluene and 4-phenyltoluene stock solutions were prepared while for the

second calibration, 4-4'dimethylbiphenyl was not inspected. Stock solutions were filtered with a 30 ml syringe through a syringe filter unit (Spartan 13 / 0.2 RC with 2 μm pores) in order to remove dust particles and other impurities. The stock solutions were then used to prepare 30 and 24 single component mixtures for the first and the second calibration, respectively. For instance, single component mixture 1 (SCM 1) was prepared by pipetting 40 ml of the 70.17 mM phenylboronic stock solution into a 50 ml volumetric flask and volume adjusted with the solvent to obtain 56.14 mM solution. Further specifications of the single component mixtures are gathered in Appendix C.

Calibration mixtures were then pumped through the calibration setup at conditions similar to the inline (i.e. at 30°C and 84°C for first and second calibration respectfully) analysis setup with a flow rate of 0.5 ml/min. Acquisition of spectra was performed against a solvent blank after a solution was pumped through the flow cell for 20 minutes. This was the time needed for absorbance spectra to become constant (i.e. to wash out previous calibration solution) in the wavelength range of interest (λ = 200-300 nm). Single point (i.e. at wavelength 319.228 nm) baseline correction was applied to the measured spectra. After the measurements were done, the UV/Vis flow cell was flushed with injecting 5 ml of the solvent followed by 5 ml absolute EtOH into the cell inlet tube with a 5 ml syringe. Further calibration setup operating specifications, exact calibration equipment, and spectrometer settings are specified in Appendix D.

5.3.7 Monitoring of 4-phenyltoluene production

Monitoring of 4-phenyltoluene production was performed in series of 5 inline monitoring experiments that included production of 4-phenyltoluene in the Plug & Play reactor setup combined with the UV/Vis setup for inline analysis (Figure 30). To monitor the 4-phenyltoluene production an inline filter (Whatman TM 52 filter papers with pore size 7 μm) was used to hold back the catalyst particles and to remove the dust and other impurities from the reactor product stream in order to avoid contamination of the UV/Vis cell. The exact equipment for each reaction monitoring experiment is listed in Appendix E. This filter was installed at the outlet of the reactor. Firstly, the reaction column (Figure 29) was filled with catalyst ($Ce_{0.495}Sn_{0.495}Pd_{0.01}O_{2-\delta}$), the filter was put on the catalyst particles, then the column was sealed and mounted into Plug & Play reactor setup. The amount of

catalyst and number of used reaction columns varied among reaction monitoring experiments. For reaction monitoring experiments 7 and 8, the catalyst was reused from the reaction monitoring experiment 6. This was done to save the catalyst as these experiments were performed only for validation purposes and determination of optimal wavelength range of CLS method. The essay of amounts of catalyst used and the number of HPLC columns for each reaction monitoring experiment are listed in Table 11.

Table 11: The essay of the number of the columns used, of the sum of the amount of the catalyst ($Ce_{0.495}Sn_{0.495}Pd_{0.01}O_{2-\delta}$) in columns used, and of the concentrations of 4-bromotoluene, phenylboronic acid, and potassium carbonate of the educt solutions prepared, all for a series of reaction monitoring experiments.

Reaction monitoring experiment #	5	6	7	8	9
Mass of catalyst [g]	1.36	1.20	1.20	1.20	3.67
Number of HPLC reaction modules	1	1	1	1	3
4-Bromotoluene [mM]	35.37	35.08	35.66	40.05	46.95
Phenylboronic acid [mM]	52.90	52.49	52.49	52.49	70.20
Potassium carbonate [mM]	52.80	52.82	52.82	52.46	70.04

The reaction solutions for 4-phenyltoluene production were prepared as follows:

1) 4-Bromotoluene was pipetted to the volumetric flask and the weight was noted.
2) Approx. 1.5 equimolar amounts of potassium carbonate and phenylboronic acid to the amount of 4-bromotoluene were added to the volumetric flask.
3) Approx. 95% of the volumetric flask was filled with the solvent. Solvent used for all monitoring experiments was EtOH:H_2O 6:4 (v.v). The flask was then sealed and shaken by hand few times. Then the flask was placed into an ultrasonic bath until all components were fully dissolved (approx. 5 minutes).
4) The volumetric flask was filled up to the mark with the solvent. A sample for the HPLC analysis was taken from the solution.

5) Dust was removed from the solution by gravity filtration in the exact same way as it was previously described for the preparation of reaction solutions for ASA production (Chapter 5.3.5).

The essay of prepared educt solutions for each reaction monitoring experiment is listed in Table 11.

Before the synthesis in flow took place, the reactor and the UV/Vis helical tube heat exchanger system were preheated to their operating temperatures (i.e. 91°C for the reactor). The water bath of the UV/Vis helical tube heat exchanger was preheated to 30 or 84°C, depending on the reaction monitoring experiment, to provide the same temperature conditions for calibration and monitoring. For the reaction monitoring experiment 5, the water bath temperature was preheated to 30°C and for the reaction monitoring experiments 6-9, to 84°C. The temperature conditions of the water bath were kept constant throughout the experiments except for inline monitoring experiments 5 and 6. For these experiments the temperature adjustments are noted in Chapter 3.3.2. These temperature adjustments were done to observe crystallisation behaviour inside the flow cell. The stirring setting of the helical tube heat exchanger water bath was set to 690 rpm. The UV/Vis light source was turned on for a minimum of 30 minutes prior to monitoring experiment in order to warm up. The reaction monitoring setup was connected to the Plug & Play reactor setup after no more impurities (i.e. leaching catalyst particles) were seen at the outlet stream from the reactor. This was usually approx. 20 minutes after starting the synthesis in flow. When all the conditions prior to the experiment were met, the experiment was started. HPLC samples were collected from the product stream in 15 minutes time intervals. All UV/Vis measurements were performed against an EtOH:H_2O 6:4 (v.v) blank. Integration time (Table 12) for UV/Vis analysis of a reaction monitoring experiment was the same as the one chosen for the calibration of which extinction coefficients were used for application of CLS method for this experiment. 2000 specra were averaged for each absorbance measurement and single point baseline correction (i.e. at wavelength 319.228 nm) was applied to the measured spectra.

Table 12: Integration times and the corresponding calibrations used for quantitative prediction with UV/Vis for 4-phenyltoluene reaction monitoring experiments.

Reaction monitoring experiment #	Integration time [ms]	# calibration	Water bath temperature of the calibration setup [°C]
5	1.05	1.	30
6	1.05	1.	30
7	1.7	2.	84
8	1.7	2.	84
9	1.7	2.	84

After the experiment, the UV/Vis flow cell was flushed by injecting 5 ml of the solvent, followed by a 5 ml Absolute EtOH injection into the cell inlet tube with a 5 ml syringe. Then it was checked for impurities (e.g. dust particles on the inner Quarz surface) and further cleaned on demand as described in chapter 5.3.9. The Plug & Play reactor setup was cleaned with pumping the solvent through the setup for approx. 30 minutes.

5.3.8 Offline HPLC analysis [8], [31]

All the samples used for comparison between UV/Vis inline with the HPLC offline analysis were analysed with offline HPLC Agilent 1100 Series HPLC. This HPLC is equipped with a Poroshell 120 EC-C18 threaded column, a variable wavelength UV/Vis detector, a thermostated column compartment, a vacuum degasser and a pump [31], [32]. Detection of the components was done at 237 nm by UV/Vis spectrometer detector. The UV/Vis retention times at 237 nm of the components analysed are gathered in Table 13. The HPLC settings and mobile phase specifications can be found in Table 14.

The HPLC analytical methods presented in this chapter were developed by Dr. Georg Johannes Lichtenegger in scope of his PhD work within the Plug & Play reactor project and are described in the publications:
[8] G. J. Lichtenegger, V. Tursic, H. Kitzler, K. Obermaier, J. G. Khinast, and H. Gruber-Wölfler, "The Plug & Play Reactor: A Highly Flexible Device for Heterogeneous Reactions in Continuous Flow," Chemie Ing. Tech., vol. 88, no. 10, pp. 1518–1523, 2016.
[31] G. J. Lichtenegger, "Continuous Processes for the Synthesis and Isolation of Functionalized Biphenyls via Suzuki-Miyaura Cross-Coupling Reactions," Doctoral dissertation, Technische Universität Graz, 2016.

Table 13: Retention times detected by UV/Vis detector at 237 nm.

Component	Retention time [min]
SA	3.514
ASA	1.84
Phenylboronic acid	0.893
4-Bromotoluene	4.842
Biphenyl	5.965
4-Phenyltoluene	8.268
4-4'-Dimethylbiphenyl	10.587

Table 14: HPLC analytical method specifications [8], [31].

	ASA production	4-Phenyltoluene production
Injection volume [µL]	1	2
Flow [ml/min]	1	1
Pumping time [min]	6	15
Pressure setting [bar]	199	0-400
Temperature setting [°C]	25	25
Mobile phase, ratio of mobile phase constituents	Ultrapure H_2O, MeOH (>99.9%), ortho-H_3PO_4 (>85%), 300:200:1 (v.v.v.)	Ultrapure H_2O, MeOH (>99.9%), ortho-H_3PO_4 (>85%), 200:300:1 (v.v.v.)

For the reaction monitoring experiments, no internal standard was used. Thus, in case of ASA production, to estimate the concentration of ASA and SA, the mass of the samples had to be determined. Additionally, information about density of eluent and reaction solution was required. Densities of the eluent = 0.946 g/$cm^3$and of the educt solution = 0.908 g/cm^3 (0.108 M SA in EA) were measured at 25°C with density and sound velocity meter (Anton Paar DSA 500 M). Those densities were assumed constant for all offline analyses to minimise number of density measurements. The determined densities and

sample weights were then used to calculate concentration of a component in the sample (i.e. analysed component in the reaction mixture) c_{sample}:

$$c_{sample} = \frac{c_{analysed} \cdot \left(\frac{m_{sample}}{\varphi_{educt\ solution}} + \frac{m_{eluent}}{\varphi_{eluent}}\right)}{\frac{m_{Sample}}{\varphi_{educt\ solution}}} \quad (8)$$

Eq. (8), derived in Appendix H is valid under assumption that density of an educt solution equals that of the sample. Concentration of analysed component after diluting the sample with the eluent ($c_{analysed}$ = k/A) was directly calculated from the areas A measured by HPLC and the calibration factor k obtained from the calibration curves for ASA and SA (Appendix G). Two methods of sample preparation were used:

- *Method 1*: *HPLC vial* was filled with approx. volume x of sampled product solution. Then 1 mL eluent was added.
- *Method 2*: *GC vial* was filled with approx. volume x of sampled product solution. Then 10 mL eluent was added by 10 mL pipette. 1 mL of the obtained solution was then pipetted to a HPLC vial.

The approximate sample volume x and the sample preparation method varied for different reaction monitoring experiments. Two different scales were used. The essay of variations is collected in Table 15.

Table 15: Sample volume, preparation variations, and methods used for the reaction monitoring experiments.

Sample preparation method #	# Production	Approx. sample volume [µL]	Scale used	Reaction monitoring Experiment #
1	ASA	125 µL	AND GR-120	1
	ASA	75 µL	AND GR-120	2
	ASA	40 µL	AND GR-120	3
	4-Phenyltoluene	125 µL	-	5
	4-Phenyltoluene	125 µL	-	6
	4-Phenyltoluene	125 µL	-	7
	4-Phenyltoluene	125 µL	-	8
	4-Phenyltoluene	125 µL	-	9
2	ASA production	375 µL	Mettler HK 160	4

As can be seen in Table 15, for analysing samples obtained from 4-phenyltoluene production experiments, no scale was used. This is because for this reaction the yield of 4-phenyltoluene was calculated directly from the predicted concentrations ($c_{predicted}$ = k/A) calculated from calibration factor k and the areas A determined by the HPLC:

$$Yield = \frac{c_{4-phenyltoluene}}{c_{4-phenyltoluene} + c_{4-bromotoluene}} \cdot 100\ \% \tag{7}$$

where $c_{4-phenyltoluene}$ is the predicted concentration of 4-phenyltoluene and $c_{4-bromotoluene}$ is the predicted concentration of 4-bromotoluene, both in the analysed product mixture. This simplification assumes there are no by-products in the product mixture. It was only possible to accept this simplification for the calculation because, as shown previously for synthesis of 4-phenyltoluene in the Plug & Play reactor, high selectivity of 99.5% can be reached as well as only a small amount of by-products biphenyl (i.e. < 1% yield) and 4,4'-dimethylbiphenyl (i.e. < 0.2 yield) [8], [31]. Calibration factors k for 4-bromotoluene and 4-phenyltoluene used to calculate concentrations, determined with

a HPLC calibration by Dr. Georg Johannes Lichtenegger in scope of his PhD work within the Plug & Play reactor project, were 0.01641 and 0.00163 respectively [31].

5.3.9 Cleaning the UV/Vis flow cell manually

Impurities (e.g. dust particles) were removed by injecting 1 ml of different acids into the UV/Vis flow cell inlet tube by a 1 ml syringe. Most of the impurities were removed with 4 M hydrochloric acid, prepared by diluting 12 M hydrochloric acid (Roth 37%) with ultrapure water. 1 ml of the 4 M hydrochloric acid was injected. 15 minutes after the injection, the acid was washed out of the UV/Vis flow cell with a careful injection of 3 ml air, followed by 50 ml ultrapure water. For the impurities that could not be removed with this procedure, sulphoric acid (Roth >95%) was used. Again, 1 ml was injected and after 1 hour the acid was washed out in the same way as described for the hydrochloric acid.

5.3.10 Application of the inline system to monitor reactions in flow

Application of the inline system to monitor reactions in flow was used to determine yield of reaction monitoring experiments with CLS method and can be further used for applications of this method to monitor different model reactions. The application is included on the CD attached to this thesis. In scope of this thesis this application was used to monitor 4-phenyltoluene production yield. The application included the Excel file (i.e. In-line.xlsm) which was coupled with AVASOFT (version 7.7.2, available at the IPPE). AVASOFT was used to continuously measure absorbance spectra while the Excel file was used to perform automated solver and 4-phenyltoluene yield calculation.

Prior to using this application to monitor the reaction, the following procedure was performed:

1) The heat exchanger water bath thermostat (Chapter 5.2.2) was preheated to the desired temperature (84°C) and the Avantes light source was turned on. Both preheating the water bath and the light source was done for min. 2 hours prior to the experiment.

2) Integration time (1.7 ms) and the number of averaged spectra (2000) were selected (Appendix J) in the main window of AVASOFT (version 7.7.2, available at the IPPE).
3) Light source was turned off. The black background was measured and saved by clicking: 'File', 'Save Dark'.
4) Light source was turned on. 30 ml of EtOH:H_2O 6:4 (v.v.) were injected with a syringe into the UV/Vis flow inlet tubing. During injection of the last ml of this solvent, the white background was measured and saved by clicking: 'File', 'Save Reference'.
5) The concentration of 4-bromotoluene in the reaction mixture was entered to the Excel file. This was later needed to perform calculation of 4-phenyltoluene yield from the concentration of 4-phenyltoluene, obtained with inline UV/Vis measurement, and concentration of 4-bromotoluene in the reaction mixture.
6) The Excel automation was adjusted (Appendix J) to continuously overwrite data to a fixed position in an existing workbook (In-line.xlsm).
7) The history function entry was adjusted for the History channel F1 (Appendix J). The adjustment of the history function entry included selection of function type, measurement mode, function definition, adjustment of function display settings, all as specified in Appendix J.
8) The Excel output was enabled by clicking: 'Application', 'Excel output' and 'Enable'.
9) Automated measurements were started in AVASOFT by clicking: 'Application', 'History', 'Start measuring'. This executed continuous overwriting data to a fixed position in the workbook (In-line.xlsm).

The first calculation of yield in the workbook was executed when the inline experiment started by pressing on the 'Show yield' button in the workbook. This was done in order to provide the same time scale for pressure and flow monitoring obtained with a Labview and yield obtained with the inline UV/Vis monitoring. Further calculations of yield were again executed by pressing the 'Show yield' right after the AVANTES spectral data overwriting took place.

6. References

[1] K. Plumb, “Changing the Mind Set,” *IChemE*, vol. 83 (A6), no. June, pp. 730–738, 2005.

[2] A. E. Cervera-padrell, T. Skovby, S. Kiil, R. Gani, and K. V Gernaey, “Active pharmaceutical ingredient (API) production involving continuous processes – A process systems engineering (PSE) - assisted design framework,” *Eur. J. Pharm. Biopharm.*, vol. 82, no. 2, pp. 437–456, 2012.

[3] FDA, “Guidance for Industry PAT — A Framework for Innovative Pharmaceutical Development, Manufacturing, and Quality Assurance,” p. 16, 2004.

[4] R. W. Kessler, *Prozessanalytik: Strategien und Fallbeispiele aus der industriellen Praxis*. Weinheim: WILEY-VCH Verlag GmbH, 2006.

[5] ICH Expert Working Group, “Pharmaceutical Development Q8 (R2),” *ICH Harmon. Tripart. Guidel.*, no. August, pp. 1–24, 2009.

[6] K. Sutcliffe, “In-line analysis (PAT) in drug substance development,” 2011. [Online]. Available: www.rsc.org/events/download/Document/566c6bd5-ba0f-47db-8fb0-599c8777c038. [Accessed: 23-Mar-2017].

[7] T. Kourti and B. Davis, “The Business Benefits of Quality by Design (QbD),” *Pharm. Eng.*, vol. 32, no. 4, pp. 1–10, 2012.

[8] G. J. Lichtenegger, V. Tursic, H. Kitzler, K. Obermaier, J. G. Khinast, and H. Gruber-Wölfler, “The Plug & Play Reactor: A Highly Flexible Device for Heterogeneous Reactions in Continuous Flow,” *Chemie Ing. Tech.*, vol. 88, no. 10, pp. 1518–1523, 2016.

[9] T. Owen, *Fundamentals of UV-visible spectroscopy*. Germany: Agilent Technologies, 2000.

[10] J. Metternich, S. Ritzenhoff, E. Busker, M. Dopheide, and G. Scholz, *Betriebsanalytik*, 2nd ed. VERLAG EUROPA-LEHRMITTEL, 2013.

[11] G. Schwedt, T. Schmidt C., and O. J. Schmitz, *Analytische Chemie*. Wiley-VCH, Weinheim, 2016.

[12] K. A. Bakeev, *Process Analytical Technology*. Blackwell Publishing, 2005.

[13] R. Li-Hua, "Examining the appropriateness and effectiveness of technology transfer in China," *J. Technol. Manag. China*, vol. 1, no. 2, pp. 208–223, 2006.

[14] J. Chaudhary, J. Akash, and V. Saini, "Simultaneous estimation of multicomponent formulations by UV-Visible spectroscopy: An overview," *Int. Res. J. Pharm.*, vol. 2, no. 12, pp. 81–83, 2011.

[15] E. Dinç and D. Baleanu, "Two new spectrophotometric approaches to the multicomponent analysis of the acetaminophen and caffeine in tablets by classical least-squares and principal component regression techniques," *Farmaco*, vol. 57, no. 1, pp. 33–37, 2002.

[16] D. M. Bourg, *Excel Scientific and Engineering Cookbook*. O'Reilly Media, Inc., 2006.

[17] J. B. Ashie, "Study on Methods of Simultaneous Multi-Component Analysis.," East Tennessee State University, 2008.

[18] P. C. Schmidt and B. W. Glombitza, "Quantitative multicomponent analysis of

aspirin and salicylic acid in tablets without separation of excipients by means of principal component regression and a classical least squares algorithm," *Trends Anal. Chem.*, vol. 14, no. 2, pp. 45–49, 1995.

[19] A. W. Owen, E. A. J. McAulay, A. Nordon, D. Littlejohn, T. P. Lynch, J. S. Lancaster, and R. G. Wright, "Monitoring of an esterification reaction by on-line direct liquid sampling mass spectrometry and in-line mid infrared spectrometry with an attenuated total reflectance probe," *Anal. Chim. Acta*, vol. 849, pp. 12–18, 2014.

[20] P. Edinger, J. Schneebeli, R. P. W. J. Struis, S. M. A. Biollaz, and C. Ludwig, "On-line liquid quench sampling and UV-Vis spectroscopy for tar measurements in wood gasification process gases," *Fuel*, vol. 184, pp. 59–68, 2016.

[21] K. Palur, B. Koganti, S. C. Archakam, S. Chenchugari, B. Nagireddy, M. B. Devabhaktuni, and M. Sankranthi, "Simultaneous Estimation of Atorvastatin and Aspirin in Bulk and Capsule Dosage Form by Chemometric Assisted Spectrophotometric Methods," *J. Young Pharm.*, vol. 8, no. 4, pp. 424–429, 2016.

[22] United Nations Office On Drugs and Crime (UNODC), *Guidance for the Validation of Analytical Methodology and Calibration of Equipment used for Testing of Illicit Drugs in Seized Materials and Biological Specimens*. New York, 2009.

[23] "General criteria of competence for calibration and testing laboratories, UKAS, Teddington, UK."

[24] H. J. Kisner, C. W. Brown, and G. J. Kavarnos, "Multiple analytical frequencies and standards for the least-squares spectrometric analysis of serum lipids.," *Anal. Chem.*, vol. 55, no. 11, pp. 1703–7, 1983.

[25] D. Lewis, *Aspirin*, 2nd ed. London: Royal Society of Chemistry, 2003.

[26] A. Suzuki, “Recent advances in the cross-coupling reactions of organoboron derivatives with organic electrophiles, 1995–1998,” *J. Organomet. Chem.*, vol. 576, no. 1–2, pp. 147–168, 1999.

[27] N. Miyaura and A. Suzuki, “Palladium-Catalyzed Cross-Coupling Reactions of Organoboron Compounds,” *Chem. Rev.*, vol. 95, no. 7, pp. 2457–2483, 1995.

[28] I. Maluenda and O. Navarro, “Recent developments in the Suzuki-Miyaura reaction: 2010-2014,” *Molecules*, vol. 20, no. 5, pp. 7528–7557, 2015.

[29] A. Suzuki, “Cross-coupling reactions via organoboranes,” *J. Organomet. Chem.*, vol. 653, no. 1–2, pp. 83–90, 2002.

[30] G. J. Lichtenegger and H. Gruber-Woelfler, “Strategies to develop leaching-free heterogeneous catalysts.,” *Chim. Oggi*, vol. 33, no. 4, Suppl., pp. 12–18, 2015.

[31] G. J. Lichtenegger, “Continuous Processes for the Synthesis and Isolation of Functionalized Biphenyls via Suzuki-Miyaura Cross-Coupling Reactions,” Doctoral dissertation, Technische Universität Graz, 2016.

[32] A. Technologies, *Agilent 1100 Series HPLC Value System User’s Guide*, vol. 11. Waldbronn, 1999.

7. Appendix

Appendix A Stock solutions and single component mixtures used for sizing of the inline sensor for reaction monitoring

Table 16: The essay of stock solutions and single component mixtures used for sizing of the inline sensor for reaction monitoring.

#	Component #	Mass of the component weighted [g]	The component concentration in the mixture [mM]
stock solution 1	SA	1.1050	400.02
stock solution 2	ASA	1.4412	400.00
stock solution 3	SA	0.0441	15.97
stock solution 4	ASA	0.0578	16.04
SCM 1	SA	✗	0.80
SCM 2	ASA	✗	0.80
stock solution 5	Phenylboronic acid	0.1325	54.36
stock solution 6	4-Bromotoluene	0.1231	35.977
stock solution 7	4-Phenyltoluene	0.1184	35.27
SCM 3	Phenylboronic acid	✗	0.11
SCM 4	4-Bromotoluene	✗	0.07
SCM 5	4-Phenyltoluene	✗	0.07

Appendix B Single component and binary mixtures used for calibration in flow for acetylsalicylic acid production

Table 17: SA and ASA single component mixtures.

#	Component	Concentration [M]
stock solution	SA	0.9993
SCM 1	SA	0.4997
SCM 2	SA	0.3997
SCM 3	SA	0.2498
SCM 4	SA	0.1249
SCM 5	SA	0.0625
SCM 6	SA	0.0312
SCM 7	SA	0.0156
SCM 8	SA	0.0078
stock solution	SA	1.3028
SCM 9	SA	0.7817
SCM 10	SA	0.6514
SCM 11	SA	0.5211
SCM 12	SA	0.3257
SCM 13	SA	0.1629
SCM 14	SA	0.0814
SCM 15	SA	0.0407
SCM 16	SA	0.0204
stock solution	ASA	0.3993
SCM 17	ASA	0.2795
SCM 18	ASA	0.1997
SCM 19	ASA	0.1597
SCM 20	ASA	0.0998
SCM 21	ASA	0.0499
SCM 22	ASA	0.0250
SCM 23	ASA	0.0125
SCM 24	ASA	0.0062

Table 18: Preparation specifications and concentrations of binary mixtures for validation of calibration in flow for acetylsalicylic acid production.

#	Single component mixtures used for preparation	Concentration of ASA obtained [M]	Concentration of SA obtained [M]
BM 1	SCM 16, SCM 19	0.0799	0.0102
BM 2	SCM 14, SCM 20	0.0499	0.0407
BM 3	SCM 13, SCM 21	0.0250	0.0814
BM 4	SCM 15, SCM 22	0.0125	0.0204
BM 5	SCM 2, SCM 23	0.0062	0.1999
BM 6	SCM 4, SCM 24	0.0031	0.0625

Appendix C Single component mixtures used for calibration in flow for 4-phenyltoluene production

Table 19: Single component mixtures used for the first calibration in flow for 4-phenyltoluene production.

#	Component	Concentration [mM]
stock solution	Phenylboronic acid	70.17
SCM 1	Phenylboronic acid	56.14
SCM 2	Phenylboronic acid	35.09
SCM 3	Phenylboronic acid	22.46
SCM 4	Phenylboronic acid	17.54
SCM 5	Phenylboronic acid	8.77
SCM 6	Phenylboronic acid	11.23
stock solution	4-4'-Dimethylbiphenyl	1.42
SCM 7	4-4'-Dimethylbiphenyl	1.28
SCM 8	4-4'-Dimethylbiphenyl	1.14
SCM 9	4-4'-Dimethylbiphenyl	0.71
SCM 10	4-4'-Dimethylbiphenyl	0.64
SCM 11	4-4'-Dimethylbiphenyl	0.57
SCM 12	4-4'-Dimethylbiphenyl	0.28
stock solution	Biphenyl	29.14
SCM 13	Biphenyl	14.58
SCM 14	Biphenyl	7.29
SCM 15	Biphenyl	3.64
SCM 16	Biphenyl	1.82
SCM 17	Biphenyl	0.91
SCM 18	Biphenyl	0.46
stock solution	4-Bromotoluene	36.14
SCM 19	4-Bromotoluene	25.30
SCM 20	4-Bromotoluene	18.07
SCM 21	4-Bromotoluene	12.65
SCM 22	4-Bromotoluene	9.04
SCM 23	4-Bromotoluene	4.52
SCM 24	4-Bromotoluene	6.32
stock solution	4-Phenyltoluene	35.13
SCM 25	4-Phenyltoluene	17.57
SCM 26	4-Phenyltoluene	8.78
SCM 27	4-Phenyltoluene	4.39
SCM 28	4-Phenyltoluene	2.20
SCM 29	4-Phenyltoluene	1.10
SCM 30	4-Phenyltoluene	0.55

Table 20: Single component mixtures used for the second calibration in flow for 4-phenyltoluene production.

#	Component	Concentration [mM]
stock solution	Phenylboronic acid	67.32
SCM 1	Phenylboronic acid	53.85
SCM 2	Phenylboronic acid	33.66
SCM 3	Phenylboronic acid	21.54
SCM 4	Phenylboronic acid	16.83
SCM 5	Phenylboronic acid	8.42
SCM 6	Phenylboronic acid	10.77
stock solution	Biphenyl	23.55
SCM 7	Biphenyl	11.78
SCM 8	Biphenyl	5.89
SCM 9	Biphenyl	2.94
SCM 10	Biphenyl	1.47
SCM 11	Biphenyl	0.74
SCM 12	Biphenyl	0.37
stock solution	4-Bromotoluene	51.99
SCM 13	4-Bromotoluene	41.59
SCM 14	4-Bromotoluene	26.00
SCM 15	4-Bromotoluene	20.80
SCM 16	4-Bromotoluene	13.00
SCM 17	4-Bromotoluene	6.50
SCM 18	4-Bromotoluene	10.40
stock solution	4-Phenyltoluene	35.39
SCM 19	4-Phenyltoluene	17.70
SCM 20	4-Phenyltoluene	8.85
SCM 21	4-Phenyltoluene	4.42
SCM 22	4-Phenyltoluene	2.21
SCM 23	4-Phenyltoluene	1.11
SCM 24	4-Phenyltoluene	0.55

Appendix D Specifications of calibration setups

Table 21: Specifications of calibration setups.

Calibration setup	ASA production	4-Phenyltoluene production	
Water bath temperature [°C]	25	30	84
Thermostat stirring setting [rpm]	690	690	690
HPLC pump flow rate [ml/min]	1	0.5	0.5
HPLC pump (Knauer, Azura P4.1 S)	✓	✓	✓
2x PEEK union (Vici Jour union JR-1061)	✓	✓	✓
Starna flow cell (584.4-Q-0.01), Avantes cuvette sample holder connected with 2 fibre-optic cables to the spectrometer (AvaSpec-ULS2048-USB2-UA-50) and the light source (AvaLight-D-S-DUV).	✓	✓	✓
Heating thermostat	✓	✓	✓
Heating thermostat with isolated sample holder	✗	✗	✓
Averaged spectra	2000	2000	2000
Integration time [ms]	1	1	1.7

Appendix E Setup specifications of reaction monitoring experiments

Table 22: Setup specifications for reaction monitoring experiments.

Reaction monitoring experiment #	1	2	3	4	5	6	7	8	9
HPLC pump (Knauer, Azura P4.1 S)	✓	✓	✓	✓	✓	✓	✓	✓	✓
2x PEEK union (Vici Jour union JR-1061)	✓	✓	✓	✓	✓	✓	✓	✓	✓
Kern balance (EWJ 600 2M) to measure mass flow connected via a RS-232 to a PC	✓	✓	✓	✓	✓	✓	✓	✓	✓
Labview pressure and mass flow monitoring	✓	✓	✓	✓	✓	✓	✓	✓	✓
Temperature monitoring with K-type thermocouple attached to reactor	✓	✓	✓	✓	✓	✓	✓	✓	✓
Reactor heating with Lauda P18 thermostat	✓	✓	✓	✓	✓	✓	✓	✓	✓
Starna flow cell (584.4-Q-0.01), Avantes cuvette sample holder connected with 2 fibre-optic cables to the spectrometer (AvaSpec-ULS2048-USB2-UA-50) and the light source (AvaLight-D-S-DUV).	✓	✓	✓	✓	✓	✓	✓	✓	✓
Whatman TM 52 filter papers with pore size 7 µm used for the inline filtration	✗	✗	✗	✗	✓	✓	✓	✓	✓
Heating thermostat including: (i) helical tube heat heat exchanger, and (ii) magnetic stirrer (IKA C MAG HS7 digital) equipped with heating plate.	✓	✓	✓	✓	✓	✓	✓	✓	✓
Isolated sample holder	✗	✗	✗	✗	✗	✗	✓	✓	✓

Appendix F Technical data of the UV/Vis equipment

Avantes light source (AvaLight-D-S-DUV):

- Wavelength range: 190-400 nm
- Light type: Deuterium
- 50 µm SMA-905 connector
- Warm up time: 30 min
- Max. drift: ± 0.5 %/h
- Lamp lifetime: 2000 h

Avantes Fibre-optic Spectrometer (AvaSpec-ULS2048-USB2-UA-50):

- Wavelength range: 200-1100 nm
- 50 µm SMA-905 connector

Starna flow cell (584.4-Q-0.01)

- Optical path length: 0.01 mm
- Absorption measurements in range from 170 to 2700 nm
- M6 threaded connectors sealing interior and connecting it to the PTFE tube with OD=1.6 mm and ID=0.8 mm
- Tested up to pressure of 5.1 bar
- Chemical resistance: good resistance to strong acids (e.g. concentrated H2SO4) and poor resistance to strong bases (e.g. 0.1 M NaOH solutions may damage the internal Quartz surface)

Avantes Fibre-optic cable (FC-UV400)

- Fibre core diameter: 400 µm
- Thermal resistance up to 100 °C except of the PVC jacketing (up to 65 °C)

- Optimised for absorption measurements in the UV/Vis wavelength range
- Low tensile strength. Thus the cable should be bended with care

Appendix G HPLC calibration curves

Table 23: Concentrations and areas for HPLC calibration of SA and ASA.

Concentration ASA [mM]	Area ASA [mVs]	Concentration SA [mM]	Area SA [mVs]
9.89	3432.7	1.01	459.4
9.89	3429.9	1.01	460.1
9.89	3464.4	1.01	459.6
7.41	2637.6	10.14	4661.8
7.41	2609.3	10.14	4663.6
7.41	2660.0	10.14	4668.7
4.94	1740.5	2.54	1174.3
4.94	1733.3	2.54	1175.6
4.94	1739.8	2.54	1163.3
2.47	872.8	5.07	2334.9
2.47	871.4	5.07	2302.9
2.47	883.2	5.07	2315.9
0.99	371.7	7.61	3502.8
0.99	358.2	7.61	3500.9
0.99	343.7	7.61	3511.6

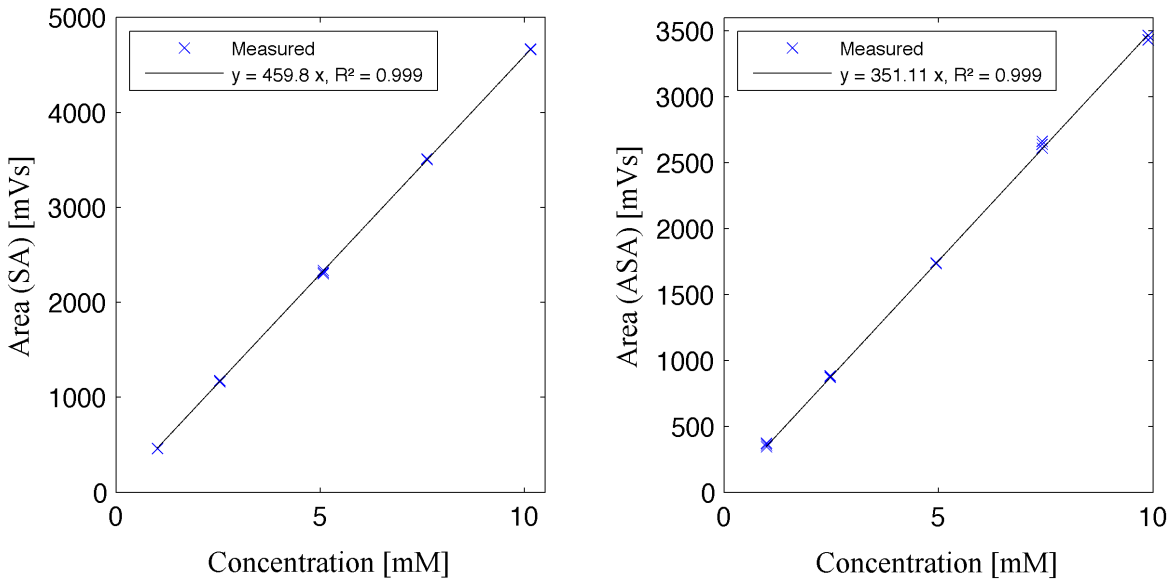

Figure 33: Calibration curve for SA (left) and ASA (right).

Appendix H Derivation of concentration of reaction mixture

Dilution factor can be expressed as $r = V_{sample+eluent}/V_{sample}$ where V_{sample} is volume of a sample and $V_{sample+eluent}$ is volume of a solution of the sample after dilution with an eluent. Concentration of the analysed component in the sample (c_{sample}) can be written as $c_{sample} = c_{analysed} \cdot r \; = c_{anaylsed} \cdot V_{sample+eluent}/V_{eluent}$ where $c_{analysed}$ is the concentration of the analysed component after diluting sample with the eluent. Assuming an ideal mixture, rewriting $c_{sample} = c_{analysed} \cdot (V_{sample} + V_{eluent})/V_{sample}$ while substituting volume with density $\varphi = m/V$ and simplifying $\varphi_{educt\ solution} = \varphi_{sample}$ finally yields:

$$c_{sample} = \frac{c_{analysed} \cdot \left(\frac{m_{sample}}{\varphi_{educt\ solution}} + \frac{m_{eluent}}{\varphi_{eluent}}\right)}{\frac{m_{Sample}}{\varphi_{educt\ solution}}} \tag{8}$$

Appendix I Acetylsalicylic acid reaction monitoring experiment 2 results

Reaction monitoring experiment 2 results (Figure 34):

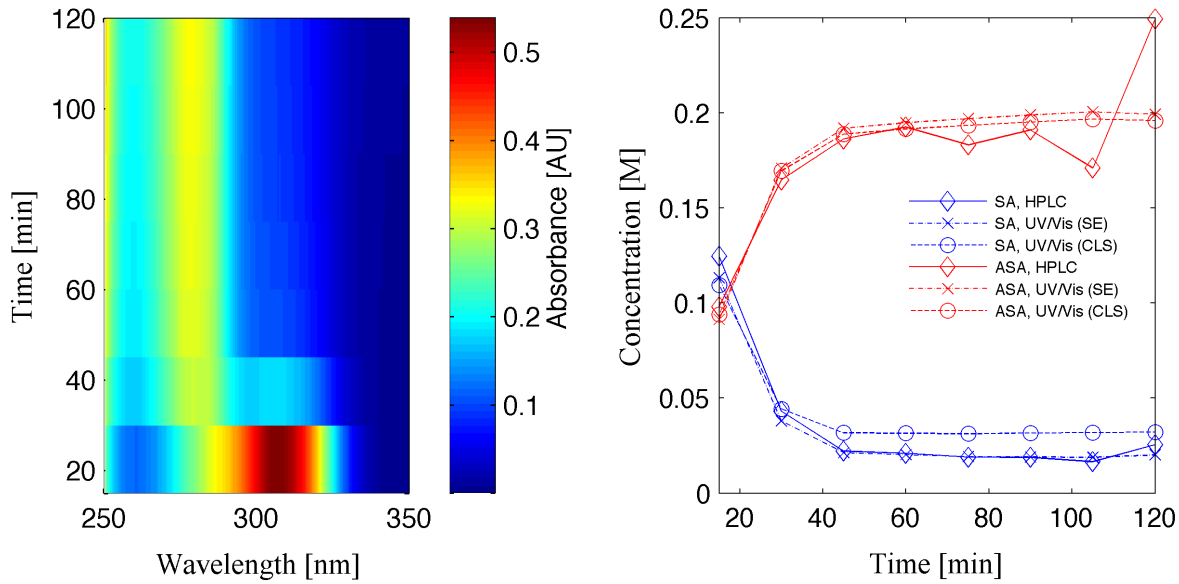

Figure 34: Absorbance after single point (354.35 nm) correction (left). Comparison of the concentrations obtained by the inline UV/Vis CLS (269.034 - 295.95 nm) and SE with offline HPLC (right). Reaction was carried out with 0.200 M SA, 0.317 M AH, both in EA, 1.106 g Amberlyst 15, at 60°C and with a flow rate of 1 ml/min.

Appendix J Specifications for the application of the inline system to monitor 4-phenyltoluene production in flow

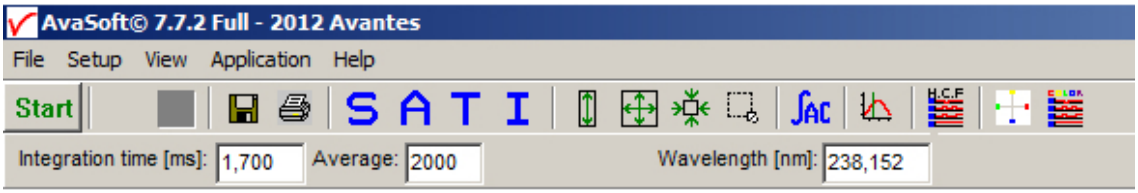

Figure 35: Choosing the integration time and the number of the averaged spectra.

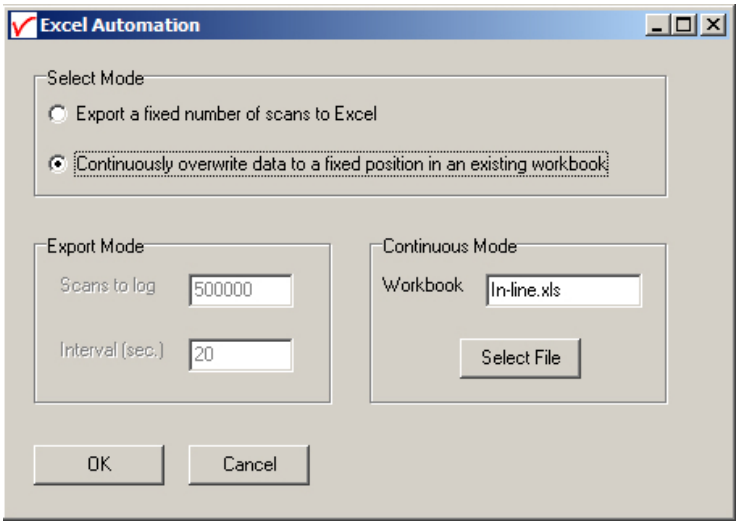

Figure 36: The Microsoft Excel automation adjustment to continuously overwrite data to the fixed position in an existing workbook (In-line.xlsm).

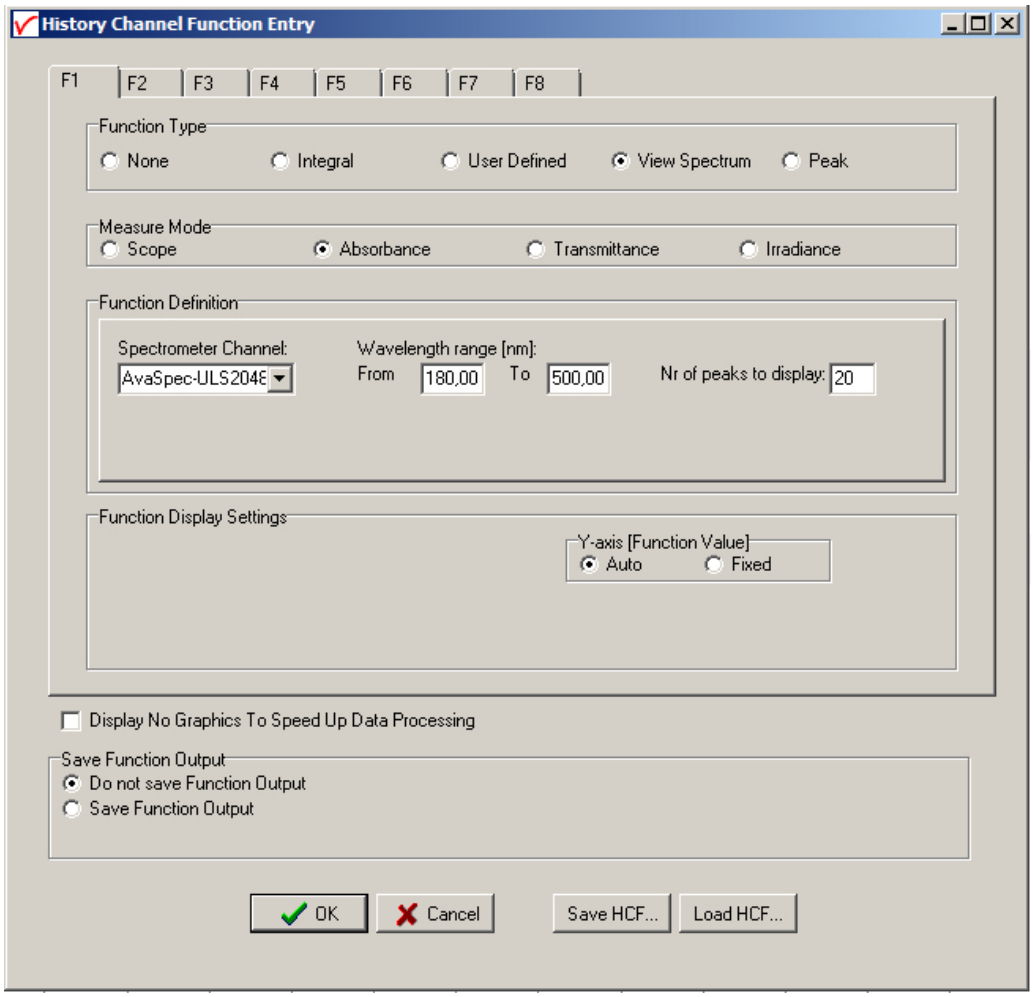

Figure 37: Adjustment of function type, measurement mode, function display settings and function definition of the history channel F1. The specified selection was used to monitor 4-phenyltoluene production yield.