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

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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.

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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.

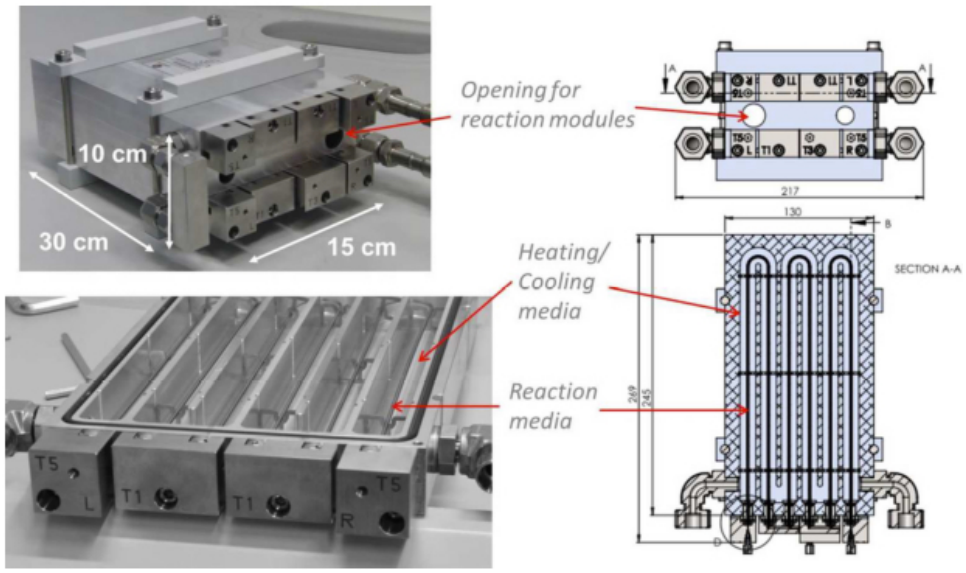

Figure 1: The Plug & Play reactor [8].

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].

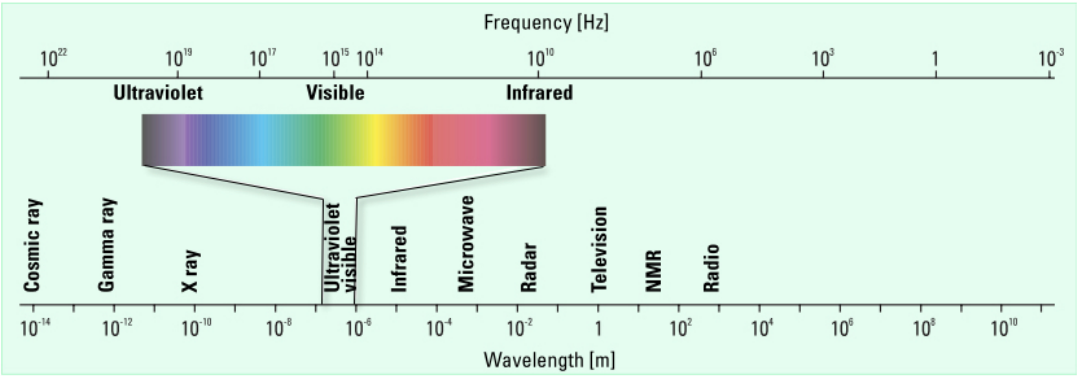

Figure 2: Electromagnetic spectrum [9, p. 2].

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.

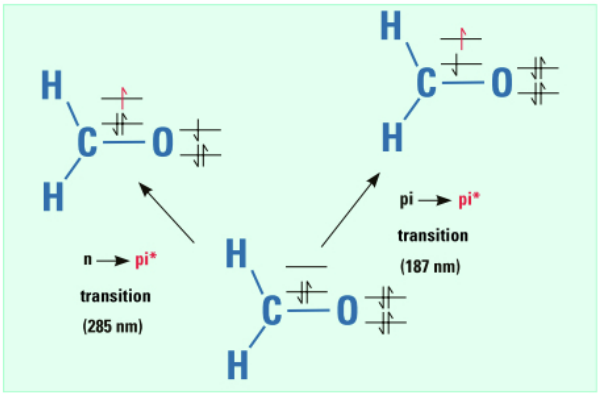

Figure 3: Scheme of the formaldehyde transitions to excited electronic states [9, p. 4].

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].

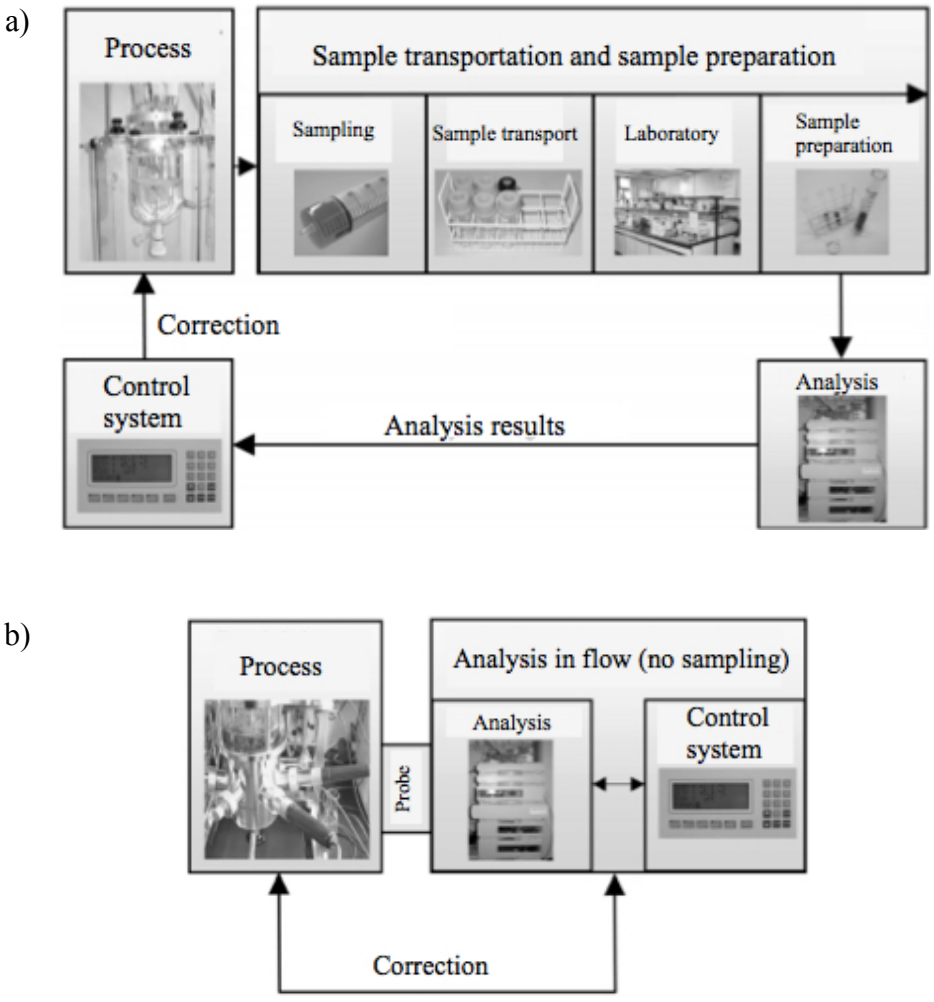

Figure 4: Scheme of offline (a) and inline (b) analytics [9, p. 16, 17].

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.