Master Thesis

Chromatographic separation of binary and ternary mixtures by Three Columns Intermittent Simulated Moving Bed (3C-ISMB)

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Chromatographic separation of binary and ternary mixtures by Three Columns Intermittent Simulated Moving Bed (3C-ISMB)

A master thesis submitted to the Swiss Federal Institute of Technology (ETH) Zurich for the degree of Master of Science ETH in Process Engineering

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Abstract

Simulated Moving Bed (SMB) is a multi-column chromatographic process, which allows the continuous separation of chemical and biological compounds for the purposes of preparative chromatography. Patented in the early 60s, it finds its first application in the petrochemical industry for the recovery of high pure n-paraffins. However, starting from the 90s, also pharmaceutical companies demonstrated their interest on the SMB technology a cause of the economical importance that gained the separation of racemic mixtures for isolation of highly pure active pharmaceutical ingredients (API). Furthermore, an intense research activity in the chiral resolution, it is demonstrated by the yearly awarded patents and publications at that time. Considering the fact that downstream processing costs can reach 70% to 90% of the total production costs of a drug, may enforce pharmaceutical companies to adapt their plants with chromatographic separation processes, which can deliver superior separation performances compared to the classical batch chromatography, which is the most used separation technique in the industry so far. As a matter of fact, the cost-efficient advantages offered by continuous processes make SMB to find nowadays several areas of application such as fine chemistry, biotechnology and food processing, although limited to binary separations. Recently, a continual development of SMB processes has broken this limitation extending its application on the separation of ternary mixtures, where the major focus relies in the challenging task of recovering the intermediate component. Beside the high purity that SMB processes can achieve by the resolution of species with very low selectivity, the productivity of the process is the most important performance indicator for evident economic reasons. In this regard, a modification of the conventional process, named "Intermittent Simulated Moving Bed" (I-SMB) demonstrates (in 2010) superior productivity when compared to the standard SMB. Furthermore, its "intermittent" mode of operation, i.e. the semi continuous feeding permits to countercurrent undesired dispersion effects which affect the purity levels when the conventional SMB is operated at "optimized" conditions. In the frame of this master thesis, a new SMB process was developed: the Three Columns Intermittent Simulated Moving Bed (3C-ISMB). Especially, when the 3C-ISMB process is compared with its "Four Columns counterpart" (the I-SMB in 1-1-1-1 configuration, which was renamed in this work as "4C-ISMB"), three things could be demonstrated: first, same purity levels by both ISMB processes at the same operating parameters. Second, a superior productivity of 33% at the same operating conditions by simply reducing the number of columns from four to three. More important, these ISMB processes were compared at their optimal operation, i.e. when the maximal allowable pressure drop was fully exploited. In this regard, binary separations at nonlinear chromatographic conditions were performed at different feed concentrations, separating a thermodynamically well known system, the racemic mixture (±)-Tröger’s Base on a laboratory-scale plant. Before implementing the ISMB processes, operating points were designed near to the optimum in order to achieve the highest possible productivity but still having complete separation. In particular, the design work could be accomplished with the Triangle Theory, an extremely useful and
clever short-cut method for SMB process optimization. As far as the productivity is concerned it could be experimentally demonstrated what the Minimum Switch Time Design predicted in advance, namely the superior performances of the 3C-ISMB compared to the 4C-ISMB. In particular, under nonlinear optimized conditions, the 3C-ISMB could deliver steady-state productivities till 149 g/L h, corresponding to a relative improvement of 77% compared to the 4C-ISMB process. As proof of concept, a three fraction separation on the system (±)-γ-Phenyl-γ-butyrolactone and (−)-Tröger’s Base in ethanol was performed under linear chromatographic conditions: the practical realization of the separation could be accomplished with the optimized 3C-ISMB process in a ”two steps cascade” configuration. As far as the purity are concerned, very high levels were achieved by all product streams, i.e. 99.4% for the most retained component, and 99.3% pure streams for the intermediate component and for the less retained component respectively. Moreover, since the racemic system possessed a rather low selectivity ($S = 1.47$), it could be demonstrated that the 3C-ISMB can also deliver high purity levels by more challenging conditions compared to the Tröger’s Base separation ($S = 2.58$). Since SMB optimizations under nonlinear conditions can only be accomplished by fully described system’s thermodynamics, a study on a particular case of Generalized Langmuir isotherm was accomplished. In particular, the adsorption isotherms of the system PNT/TBP in ethanol/water 50%/50% v/v were fully described with an $M_2$-isotherm. Interestingly, an inversion point of the retention order of the binary system at a specific solvent composition could be experimentally documented for the first time (to the best of our knowledge).
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Notation
fitting parameters of a calibration curve

A cross sectional area of a chromatographic column, [cm$^2$]

c$_{i,j}$ concentration of component $i$ in the fluid phase of section $j$, [g L$^{-1}$]

c$_{F}^i$ total feed concentration, [g L$^{-1}$]

c$_F$ concentration of feed, [g L$^{-1}$]

D$_{ax}$ axial dispersion coefficient, [cm$^2$ s$^{-1}$]

D$_{app}$ apparent axial dispersion coefficient, [m]

H$_i$ henry constant of component $i$, [-]

K$_{eq,i}$ equilibrium constant in Langmuir and Anti-Langmuir isotherms

k$_{i,j}$ mass transfer coefficient of solute $i$ in section $j$, [s$^{-1}$]

$a_{i,1}$, $a_{i,2}$, $b_{i,1}$, $b_{i,2}$ parameters in Bi-Langmuir isotherm, [-] resp. [L g$^{-1}$]

h peak height

L column length, [cm]

$m_j$ dimensionless flow rate ratio in section $j$, [-]

$m_j$ dimensionless average flow rate ratio in section $j$, [-]

n$_j$ number of columns per section/module

n$_{i,j}$ concentration of component $i$ in the solid phase of section $j$, [g L$^{-1}$]

n$_{eq,i,j}$ equilibrium solid phase concentration, [g L$^{-1}$]

PR productivity, [g L$^{-1}$ h$^{-1}$]

Q$_j$ volumetric flow rate in section $j$, [mL s$^{-1}$]

S selectivity, [-]

T temperature, [$^\circ$C]

t time, [min]

$t^*$ switch time, [min]

t$_D$ dead time, [min]

t$_{D0}$ hold-up time with dead time, [min]

t$_0$ hold-up time without dead time, [min]

$t_{i,j}$ retention time of component $i$ in section $j$, [min]

$\tau_R$ retention time, [min]

u superficial fluid velocity, [cm min$^{-1}$]

$v_C$ interstitial fluid velocity, [cm min$^{-1}$]

$V$ column volume, [mL]

$V_D$ dead volume, [mL]

$V_j^D$ dead volume per module, [mL]

w peak width at base

$w_{1/2}$ peak width at half height

SC specific solvent consumption, [L g$^{-1}$]

X$_i$ purity of component $i$, [-]

Y$_i$ recovery of component $i$, [-]
Greek letters

\( \alpha, \beta, \gamma \) runs of binary separations at 5g/L, 10g/L and 15g/L respectively
\( \Delta P \) pressure drop, [bar]
\( \epsilon^* \) overall column void fraction, [-]
\( \lambda \) wavelength of signal, [nm]

Subscripts and superscripts

A more retained compound
B less retained comp. in binary separations; intermediate comp. in ternary separations
C less retained compound in ternary separations
D desorbent
E extract
F feed
i index for component
j index for section
R raffinate
I-SMB Intermittent Simulated Moving Bed
SMB Simulated Moving Bed
TMB True Moving Bed
3C-ISMB Three Columns Intermittent Simulated Moving Bed
4C-ISMB Four Columns Intermittent Simulated Moving Bed

Abbreviations

API Active Pharmaceutical Ingredient
EAM Equal Area Method
ETH Swiss Federal Institute of Technology (Eidgenössische Technische Hochschule)
HPLC High Pressure Liquid Chromatography
MSTD Minimum Switch Time Design
UV-VIS Ultraviolet-Visible
Simulated Moving Bed (SMB) is a countercurrent multi-column process for the continuous separation of chemical and biological compounds. In particular, the main area of utilization of this technology resides in the frame of preparative chromatography. Before going into detail in the engineering aspects of the SMB technology, it is worth to elucidate the meaning of terms like "Chromatography", "preparative chromatography", "continuous separation" and "countercurrent" mode of operation with some background information which are presented in this section: the official birth of the concept "Chromatography" in liquid phase separations is related to the first use of the term dated in 1906, when Michael Tswett, a Russian botanist, revealed the basic principles of an adsorption method for the separation and isolation of the green and the yellow chloroplast pigments, which were extracted from green plants [50]. The work of Tswett became a milestone for the development of the basic principles of chromatographic separation [19]. The interpretation of the nomenclature "Chromatography" (from the Greek roots \(\chiρωμα\) and \(\gammaρα\phi\epsilon\nu\)) is referred to the visualization of the multicoloured rings resulting from the separation of the plant pigments investigated at that time [19]. Evolution of chromatographic techniques led to development of batch-techniques as LCC (Liquid Column Chromatography), the GC (Gas Chromatography), and the TLC (Thin-layer Chromatography) as analytical tools, although they were not optimally used prior to the 1967 [36]. In the early 1970s, a powerful LCC, the High Pressure Liquid Chromatography (HPLC) offered better separation performances compared to the classical LCC, permitting the high selective resolution of mixtures by higher efficiency [36]. Nowadays, the importance of chromatographic separation techniques such as HPLC, for qualitative and quantitative studies of chemical and biological substances, is underlined by the worldwide use in laboratories over the past 40-plus years [50]. Furthermore, the advent of the HPLC already brought valuable applications for pharmaceutical studies on drug substances and their metabolites [36]. Regardless the several separation mechanisms of chromatography techniques [18] or their purposes (analytical or preparative), in general, a chromatographic process can be defined
as the resolution of two or multiple substances of a fluid mixture, based on their differential velocity of movement along a stationary phase. In particular, the difference in migration rate is related to the varying degree of adsorption of the dissolved components to the stationary phase when the solvent (mobile phase) is flowing through the stationary phase.

In particular, the reduced consumption of energy compared to other separation technologies like distillation, and the ability of separating thermo-sensitive components make chromatographic process particularly interesting in the pharmaceutical industry, where the quality of the API (Active Pharmaceutical Ingredient) is the most relevant issue for getting the commercialization's approval from authorities like FDA (Food Drug Administration, USA) or Swissmedic (Switzerland). Although traditional batchwise chromatography is widely used in the industry, it suffers of some major drawbacks when applied at the production scale, such as the often inefficient usage of the stationary phase, the large volumes of desorbent needed, the limited purity obtained for components with small adsorptivity differences and the discontinuity of the process. In order to overcome these limitations, some continuous chromatographic methods, like the "cross-current" rotating annular chromatograph and the "counter-current" Simulated Moving Bed were developed. The former can separate multiple component mixtures but its performance is the same as in batch chromatography. On the other hand, although the main application of SMB is restricted to binary separations so far, especially in the field of chiral separations, higher productivity is offered compared to the cross-counter counterpart.

Patented in 1961, the concept of Simulated Moving Bed was originally introduced in the petrochemical industry (by the company UOP) for the recovery of high purity n-paraffins. Beyond the use of SMB in the petrochemical refinery, various applications are proposed in other areas such as fine chemistry, biotechnology and food (sugar) processing. As a matter of fact, the SMB process represented the first of a series of many practical implementations of the True Moving Bed (TMB) for "preparative" purposes. In fact, the separation is not focused to gain qualitative or quantitative "information" on the substances (i.e. the purpose of analytical chromatography) but the intention is to recover the products in the exact condition (i.e without degradation) as before undergoing the separation. The latter is the purpose of preparative chromatography. In the 90s, an increased research activity on SMB, reflected by yearly awarded patents and publications began in the field of chiral separations in order meet the strict regulations in the pharmaceutical industry in the production of enantiopure drugs. It is worth noting that nine of the ten top selling drugs consist of a chiral active pharmaceutical ingredient in 2005. The economic importance of single-enantiomer therapeutics can be highlighted by the sales of US$ 225 billion in 2005, representing 37% of the total pharmaceutical market of $602 billion. Nevertheless, valuable applications beyond the chiral separations, i.e. ternary separations, are already researched and recently proposed in the academic field. Another aspect is worth to be mentioned: considering the fact that, in the pharmaceutical industry, downstream processing costs

...
can reach 70%-90% of the total production costs [45], may enforce pharmaceutical companies to adapt their production pipeline with chromatographic processes with superior separation performances (i.e. more productive, with less solvent consumption and waste). In fact, the performance amelioration offered by the SMB technologies have led to a growing employment of the process over the past decade. Mainly custom manufacturers such as Novasep, Ampac Fine Chemicals and Bayer have been studying the SMB technology, first at small scale, then at pilot-scale and currently several API’s are produced using SMB at hundreds of tons per year [22]. Thus the implementation of SMB processes at production scale of APIs could be a reality for many other industries, especially those involved in the generic’s market, if we consider the fact that when drugs loose their patent protection, the utilization of more economic processes render generics’ manufacturers even more competitive [14]. This scenario can be furthermore supported by the recent behavior of market leading pharmaceutical companies like Roche and Novartis: Roche has recently signed an agreement with ChromaCon AG for the purchase of a pilot plant equipment based on the SMB technology for the purification of monoclonal antibodies (mAb) [1], whereas Novartis has already a functioning SMB plant in the Research & Development (R&D) since 2006 [43]. Some words have to be spent on the work of the Separation Process Laboratory (SPL, ETHZ), which substantially contributed on the development of the SMB technology, since the 90s till today [23]: in 1997, Mazzotti et al developed a powerful short-cut method for the design and optimization of SMB processes, named the ”Triangle Theory”, which is today a standard design tool used both in academia and in the industry [35], leading to more than three hundred citations of the corresponding publication [23]. Later, the main focus of the research by the SPL moved to the control of standard SMB units [22, 30]. Recently, the research interests shifted to study an high productive SMB process, i.e. the Intermittent Simulated Moving Bed (I-SMB) [28, 29, 26] and development of SMB processes allowing three fraction separations [1, 24].

1.1 State of research

The prime focus of this section resides on the state of art of the Simulated Moving Bed technology. The distinctive feature of the SMB technology consists in its ”continuous” mode of operation, namely its ability to process binary systems in a continuous manner [10]. To be more precise, the mixtures to be resolved can be fed continuously in the columns and the products can be collected at the same time in a raffinate stream (containing the less retained component) and in an extract stream (containing the more retained component) [10]. Furthermore, the superior efficiency demonstrated by ”countercurrent” schemes in chemical processes such as distillation, gas absorption, and liquid-liquid extraction [53] has been applied in the SMB technology, as mentioned in the previous section. Since the implementation of a ”True Moving Bed” (TMB) is rather challenging to implement in reality, the SMB columns remain ”fix” as static units (so called ”fixed beds”). On the other hand, the movement of the fluid phase in opposite direction to the stationary can be simulated in a ”discontinuous” manner by synchronously switching
the ports in the direction of the fluid migration \[^{40}\]. Anyway, more accurate aspects on the process schemes of the TMB, SMB and its modifications such as the "Intermittent Simulated Moving Bed" (I-SMB) are presented in the next paragraphs.

### 1.1.1 True Moving Bed (TMB) process

Batch chromatography is one of the most used separation techniques for analytical purposes, mentioning HPLC. Nevertheless, it shows limitations in the field of preparative chromatography such as low productivity and high operating costs \[^{17}\]. This is the reason why chemical and process engineers put a lot of effort in the last 50 years to develop alternatives chromatographic processes. One of these efforts led to the development of the so called True Moving Bed (TMB) process, which represents the first conceptual realization of an ideal continuous counter-current chromatographic process. This process demonstrates several improved features, when compared to the classical batch chromatography. In fact, it demonstrates to have better productivity per unit mass of stationary phase with low solvent consumption due to its continuous mode of operation \[^{17}\]. Furthermore, the counter-current movement of the stationary phase with respect to the mobile phase allows a better mass transfer of the components to be separated, especially useful for challenging separations with low selectivity \[^{17}\]. In Figure 1.1 a scheme of the process is depicted for better understanding its separation mechanism in the case of a binary mixture: the TMB is divided in four sections (labeled as Section I, II, III and IV respectively) by four external streams: two inlet streams (desorbent, feed) and two outlet streams (extract, raffinate). These sections fulfill different functions: in particular, the feed stream enters the unit in a continuous manner between section II and III. This is the zone where the actual separation of two components occurs. In the system, the more retained component, denoted as "A", strongly adsorbs on the solid phase and it is transported downwards, where it is finally collected in the extract outlet stream, which originates from the node connecting section I and II. On the other hand, the weak adsorbing component, denoted as "B", moves upwards in the direction of the mobile phase and it is withdrawn at the "raffinate" outlet between section III and IV. The function of section I is to desorb the strong component A (also called "more retained component") and hence to regenerate the stationary phase before it is moved (free of both A and B) to section IV. On the other hand, the function of section IV is to regenerate the mobile phase (the solvent) by adsorbing the weak component B (also called "the less retained component"). In contrast to batch-wise chromatography, the overlap of both components (A, B) is not a concern for the purity of the outlet stream, as long as the solute concentration’s profiles are resolved enough \[^{17}\]. This feature permits the TMB process to be operated at high solute’s concentrations, which results in a high productivity. Moreover, overloaded conditions decrease the dilution of the solutes, which is favorable for the recovery step in the next phases of the downstream processing \[^{17}\]. The difference between batch chromatography and continuous chromatography can be elucidated with a simple cartoon-analogy \[^{25}\] depicted in Figure 1.2, considering a single chromatographic column and two species, A (yellow) and B (blue), which have a different affinity to the stationary
phase results in a different velocity along the column. The less retained component B will elute before the more retained component A permitting their separated collection if the column is long enough. This situation is similar to the speedy cat and the lazy turtle when running a race, where they both reach the track but at different times. On the other hand, a continuous counter-current device such as TMB (and SMB) can be represented by the situation where the two animals run on a moving belt, whose velocity is intermediate but opposite to theirs: the speedy animal will fall off on the right-hand side, whereas the slower on the left-hand side. Imaging that new animal couples (turtle, cat) are placed continuously on the middle of the moving belt, a ”continuous counter-current turtle-cat separator” can be ideally realized. The same concept can be applied for any continuous counter-current chromatographic process, such as TMB or SMB for a binary separation (i.e. A,B) \cite{25}. Ideally, TMB seems to be a better choice than the classical batch chromatography. However, the TMB is not a practical solution for implementing a continuous counter-current separation due to some drawbacks: mechanical degradation due to considerable shear forces \cite{17} causing attrition, creates the formation of ”fines” . On the other hand, the eddy dispersion of both phases and mass transfer between the two phases lead to uneven packing properties \cite{55}. The attempt to overcome this technical difficulty with moving columns was not successful due to the unreliability of the sealing between the static ports and the moving columns \cite{55}. As solution to this technical issue, the multi-column chromatographic process with conventional packed columns, named ”Simulated Moving Bed” was introduced in 1961 \cite{16}.

1.1.2 Simulated Moving Bed (SMB) process

The innovating technical feature of SMB resides in its fixed stationary phase (i.e. a fixed bed). The approximation of the continuous solid flow is ”simulated” by successive synchronized movements of the inlet and outlet ports in the same direction of the fluid phase over a finite length at discrete time steps \cite{25,17}. Although, similar operating principles to the TMB process can be found, i.e. the division of the unit in four sections, which renders it a multi-column process as well. Each of these sections is made of at least one or more fixed-bed columns \cite{17}. Thus, the corresponding solid phase velocity of SMB is defined as the ratio of the column length to the switch time \cite{17}. With reference to figure 1.3, a scheme of a SMB process in (2-2-2-2) configuration is presented: this particular unit consists of eight fixed bed columns, which are connected in series. The four sections fulfill the same functionalities described for the TMB. It is worth mentioning, that the simulation of the counter-current movement is better approximated by increasing the frequency of port switches, which unfortunately requires more columns \cite{40}. Conventionally the SMB process has five degrees of freedom, represented by the four flow rates and the switch time. The number of degree of freedom can be increased by the use of temperature and solvent gradients \cite{40} or by non-constant operating conditions like in the case of the Intermittent Simulated Moving Bed, (I-SMB) \cite{28,29,26}. Since this work focuses on the design and development of an SMB process by isothermal, isochratic conditions, additional details on processes operated at varying temperature and solvent composition,
Figure 1.1: Scheme of a True Moving bed (TMB) process [17].
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Figure 1.2: The "turtle-cat separator" analogy. (a) Analogy for batch-wise chromatography. (b) Analogy for counter-current chromatography. (c) Analogy for counter-current chromatography [40].

are not needed. The I-SMB will be described in detail in the Theory chapter.

1.2 Aim of this thesis

This work reports the design and development of a novel SMB process: the Three columns Intermittent Simulated Moving Bed (3C-ISMB). The scheme presented below is a modification of the I-SMB, which was patented by Nippon Rensui Corporation [47, 48]. In particular, a mathematical design describing the 3C-ISMB process by the use of "average dimensionless flow rate ratios" could be formulated. Furthermore, optimal operating conditions under chromatographic nonlinear conditions have been designed by using a useful short-cut design method, the "Triangle Theory" [35]. This method gives simple constraints in order to determine the optimal operating conditions, namely the operating point where the productivity is maximized and the desorbent requirement minimized, whilst achieving complete separation. Another useful design, the "Minimum Switch Time Design" [28] was applied for SMB optimization in order to account for specific technical constraints of the laboratory plant. In particular, an analytical mathematical expres-
Figure 1.3: Scheme of a Simulated Moving bed (SMB) process. Dashed lines indicate the port positions after the first switch [17].
CHAPTER 1. INTRODUCTION

sion demonstrating the superiority in productivity of the 3C-ISMB over the 4C-ISMB (previously known as "(1-1-1-1) I-SMB") could be derived. Beside designing the new process, the experimental effort of this work consisted in a series of separations using a thermodynamically well described binary racemic mixture, the Tröger’s Base enantiomers. In particular, the binary separations were performed at nonlinear chromatographic conditions with two main objectives: the first objective was to confirm the validity of the model equations by comparing the achieved purities in the frame of Triangle Theory and through detailed simulations. The second objective was to demonstrate the superior productivity of the 3C-ISMB compared to the I-SMB process at comparable purity levels. However, the boundaries of the region of complete separation (the region where 100% pure product streams are achieved) have to be known in advance for SMB (and ISMB) optimization. Therefore, an adequate study of the adsorption isotherms is necessary. Thus the thermodynamics of adsorption for a particular chromatographic nonlinear binary system, that is PNT/TBP in ethanol/water was experimentally studied: in particular, the experimental work was accomplished by means of "Frontal Analysis", where the adsorption isotherm’s parameters could be estimated using the "Equal Area Method" and the "Method of Characteristics". Beside the experimental work, accurate simulations were made to confirm the reliability of the estimated parameters: with this study, the first experimental evidence (to the best of our knowledge) of a system subjected to two particular cases of the Generalized Langmuir Isotherm in dependence of the solvent composition could be done: in particular, the experimental evidence of an inversion between the M1-isotherm and the M2-isotherm in dependence of the solvent composition will be presented considering the system PNT/TBP in ethanol/water. The last step would have been, to implement the 3C-ISMB for the separation of this particular chromatographic nonlinear system. However, major focus was relied on the description of the novel process, and due to limited time at disposal, this task could not be accomplished. In the last part of this work, a three fraction separation was designed and implemented in a "Two steps 3C-ISMB Cascade" configuration.
Chapter 2

Theory

This chapter allows the reader getting familiar with basic definitions used in Batch Chromatography (section 2.1.1). Nevertheless, major focus of the Theory section is relied on presenting the mathematical models supporting the design of the new process, namely the Three Columns Intermittent Simulated Moving Bed, which is called “3C-ISMB”. In addition, analog equations for the 4C-ISMB (known in the literature as ”1-SMB in 1-1-1-1 configuration [28, 29, 26]) will be derived in parallel and presented as well in order to permit a direct performance comparison with this related precursor.

2.1 Batch Chromatography

2.1.1 Definitions

The separation principle of the chromatographic technologies described and used in this work (i.e. the HPLC, 4C-ISMB, 3C-ISMB) are all based on the principle of ”adsorption” chromatography, where the species are separated according to their different retention to the same stationary phase. As mentioned in the introductory section, Chromatography can be distinguished for its analytical or preparative purposes, which were both applied during the thesis: in particular HPLC was used as analytical tool during the study of the isotherms. Furthermore, off-line information about the species’ concentrations and purities during the experimental implementation of the 3C-ISMB process and the 4C-SIMB process could be gained using HPLC as well. On the other hand both ISMB processes were described and implemented according to the purposes of preparative chromatography. The aim of the following section will be to clarify some frequently occurring concepts when dealing with chromatographic studies.

Batch chromatography, when applied in the the elution mode, consists in injecting a sample containing the mixture to be separated (in form of a ”pulse” or ”continuous feed”), into a stream of mobile phase (solvent), which flows at constant velocity through a packed chromatographic column [40, 22]. The solutes’ concentrations in the emerging mobile phase (eluate) is continuously monitored on-line by a detector (i.e. UV-VIS)
plotted at the column outlet. Record of the signal’s intensity against time results in the so called chromatogram, where signal’s peaks, belong to the solutes. With reference to Figure 2.1 an idealized version of such a chromatogram is presented [22].

The most common parameters describing a chromatographic separation are the following: [18] [22]
CHAPTER 2. THEORY

- ""Hold-up time \( t_0 \) Retention time of an unretained compound, i.e. residence time of the eluent.
- Retention time \( t_R \) The time a compound need to travel from the column inlet to the detector.
- Adjusted retention time \( t_R' \) Total retention time less the hold-up time.
- Peak area \( A_{\text{peak}} \) Represents the concentration quantitatively.
- Peak height \( h \) Distance between peak maximum and base.
- Peak width at base \( w \) The segment of the peak base intercepted by the tangents drawn to the inflection points on either side of the peak.

- Peak width at half height \( w_{1/2} \) Length of the line parallel to the peak base at 50% of the peak height that terminates at the intersection with the two limbs of the peak."

Two important limitations of Batch chromatography are related to the adsorption behavior of the system and on the properties of the column. More precisely, low "selectivity" between components and poor "efficiency" of chromatographic columns are of major concern in Batch chromatography and have to be elucidated in the next lines. In particular, Selectivity is a measure for the distance of the peaks in terms of retention times, it is defined \[22\] according to equation (2.1) \[33\] as

\[
S = \frac{t_{R,A} - t_0}{t_{R,B} - t_0} = \frac{t'_{R,A}}{t'_{R,B}}
\] (2.1)

where A represents the more retrained species and B the less retrained species. It is rather obvious that the selectivity is a function of the different adsorbtivities of the species to be resolved \[22\]. In the case of a linear isotherm this functionality is expressed simply as the ratio of the Henry’s constants, i.e.

\[
S = \frac{H_A}{H_B}
\] (2.2)

The term performance on the other hand is related to the peak width \[22\]: The efficiency of a chromatographic column is a measure of the capacity of the column to restrain peak dispersion and thus, provide high resolution. The higher the efficiency, the more the peak dispersion is restrained, and the better the column. For a packed column the efficiency generally decreases with retention \[31\]. Systems showing very narrow peaks are referred to as "high performance systems", whereas "low performance systems" feature broad peaks \[22\]. Quantitatively the performance is expressed through the resolution which is defined according to equation (2.3) \[33\] as

\[
R = \frac{t_{R,A} - t_{R,B}}{1/2 \cdot (w_A + w_B)}
\] (2.3)

Summarizing, both low performance and low selectivity might lead to overlapping of the peaks and therefore to limited purity. This problem is amplified if the separation
is conducted under overloaded conditions, i.e. when the column is operated under high feed concentrations (under nonlinear conditions). However, in order to increase the productivity, it would be desirable if a process could maintain high purity levels even under overloaded conditions [22]. That was the main aim of the design and experimental part of this work, i.e. to provide theoretical and experimental proofs of the improved productivity showed by the 3C-ISMB compared to the 4C-ISMB, especially under chromatographic nonlinear conditions.

2.2 Intermittent Simulated Moving Bed (ISMB)

This section presents the basic principles on which design and development of the 3C-ISMB process was based. In this regard, one of the modified SMB schemes, namely the intermittent SMB (I-SMB) patented by Nippon Rensui Corporation [47, 48], will be presented with the scope to clarify the fundamental features of the new invented process.

2.2.1 Process description

2.2.1.1 Four Columns Simulated Moving Bed (4C-ISMB)

Analog to the standard SMB (figure 1.3), the ISMB unit consists of four sections. Nevertheless, the switch period, the so called switch time, is divided into two substeps (figure 2.2): in substep I the unit is operated as an SMB without flow in section IV. In substep II, all inlets and outlets ports are closed, and the mobile phase is circulated through the column train in order to adjust the relative position of the concentration profiles [23]. In previous publications, it has been demonstrated that the I-ISMB in (1-1-1-1) configuration doubles the productivity of the (1-2-2-1) standard SMB whilst fulfilling high purity specifications [28, 29]. This is a result of the semi-continuous mode of operation, which allows that the leading edge of the more retained component (component A), has a large clearing from the raffinate port at the time of product withdrawal [23]. Similarly, the trailing edge of component B is far from the extract port during the withdrawal phase, hence also a high purity level for the extract can be achieved [23]. On the other hand, by the standard SMB, a drop in purity related to dispersion effects is observed, because the decisive edges of the composition fronts, namely front of A and tail of B, are much closer to the product ports. As mentioned in the introductory section 1.1.2, a higher number of chromatographic columns per section is necessary to better approximate the counter-current movement of the solid phase, and hence to mitigate the dispersion effects, which lowers the purity. Benefits of the intermittent mode of operation by the I-SMB in (1-1-1-1) configuration (4C-ISMB), will be explained in the next sections. ISMB separations are run for a certain number of so called ”cycles”. With reference to figure 2.2 the time sequence of one chromatographic cycle can be explained: in both ISMB processes described in this work (i.e. 4C-ISMB, 3C-ISMB), each cycle consists in a number of switches equal to the number of the columns of the specific ISMB process (i.e. 4 for the 4C-ISMB and 3 for the 3C-ISMB). Analog for both ISMB processes, during each switch
Figure 2.2: Time sequence of one cycle in 3C-ISMB and 4C-ISMB. Every ISMB separation consists of a desired number of cycles (e.g. 30).

time the process is operated continuously during substep I and discontinuously during substep II.

2.2.1.2 Three Columns Simulated Moving Bed (3C-ISMB)

The aim of this section is to describe the new process, i.e. the "Three Columns Intermittent Simulated Moving Bed from a conceptual point of view with the help of an analog scheme, which was used for the description of the 4C-ISMB (section 2.2.1.1). With reference to figure 2.4 we note that the 3C-ISMB process is operated in substep I fully identical to standard four sections I-SMB, whereas substep II consists of only three instead of four sections which results in recycling a stream containing the weak component rather than 

Figure 2.3: Process scheme of closed loop Intermittent Simulated Moving Bed (I-SMB). Feed (F) and fresh solvent (D) supply as well as withdrawal of the product streams raffinate (B) and extract (A) is conducted in substep I, whereas in substep II all inlet and outlet ports are closed. After the end of substep II the ports are switched in direction of the fluid flow and substep I is repeated (according to figure 2.2)
pure solvent. It is worth pointing out that substep I in 3C-ISMB is not only fully identical to I-SMB but also to classical 3-zone open loop SMB and 3-zone SMB with partial recycle (for detailed descriptions on the corresponding process schemes, the reader is referred to the document on the invention disclosure \[23\]). On the other hand, substep II combines the concepts of I-SMB and 3-zone SMB with partial recycle in a novel manner, i.e. all inlet and outlet ports are closed and the fluid is just circulated with identical flow rates in each section along the column train. However, the elimination of the fourth section and thus the recycling of the weak component is similar to the concept of 3-zone SMB with partial recycle \[23\]. With reference to figure 2.4, the process scheme of the 3C-ISMB can be explained: analog to the 4C-ISMB process, a feed stream (containing a mixture of A, B) is injected in the unit with a flow rate \(Q_F\) in substep I. At the same time an extract stream, leaves column I of the unit at flow rate \(Q_E\), containing the more retained component (A), while the less retained component (B) is collected in the raffinate stream, leaving the column III at a flow rate \(Q_R\). In substep II, the mobile phase is circulated through the column train, analogously to the 4C-ISMB (figure 2.3). It is worth to mention the different function of the recycle step (recirculation step): in the case of 4C-ISMB, a regenerated stream consisting of pure solvent (D) leaves the third column of substep II, whereas the mobile phase containing the less retained component B is recycled back to the first column of substep II (D+B).

2.2.2 Mathematical modeling

The intention of this section is to give to the reader an overview of the mathematical models, which were used for the study of the isotherms, the design and the optimization of both ISMB processes: In particular for process design, the "Equilibrium Theory Model of Chromatography" was applied as building block model for single columns. This model, was extremely useful in two cases: first, in the studies of the isotherms where the "Method
of Characteristics” [51] was applied for the Henry’s constants (in the linear and nonlinear cases) and Equilibrium constants estimation. Second, in the SMB modeling, the Equilibrium Theory model was coupled with process specific node mass balance equations in order to be able to connect the single columns in the specific desired configuration, such as the 3C-ISMB or the 4C-ISMB. Detailed simulations of both processes were run in order to validate the concentration and purity profiles by the experimentally implemented separations. Also useful simulations of breakthrough profiles were performed in the frame of the isotherms’ estimation (refer to section 4.1.1.1) in order to check the reliability of the estimated parameters. Therefore also a detailed model of chromatography is briefly presented and explained below. For the sake of transparency, the equations presented in this section are based on the theory published in [35, 40]. In addition, it’s worth to mention, that this description is restricted to the case of a linear isotherm, since detailed equations on the boundaries of the region of complete separation in the nonlinear case can be retrieved in previous publications [35, 34, 40]. Anyway, for all the models described below, the following seven assumptions are made:

1. No radial concentration gradients in the column.
2. Operation under isothermal conditions, namely the temperature is ideally constant.
3. Constant fluid velocity along the column.
4. All columns have identical void fractions.
5. No concentration gradients within the solid particles of the stationary phase.
7. Mass transfer described by the Equilibrium Dispersive Model when necessary (i.e by detailed simulations).

Keeping in mind the seven assumptions listed above, the movement of a solute i along the axial coordinate z of a single chromatographic column in section j of the ISMB unit can be modeled by a Partial Differential Equation, which is based on the material balance of the following form:

\[
v_j \frac{\partial c_{i,j}}{\partial z} + \frac{\partial}{\partial t} \left[ c_{i,j} + \left(1 - \frac{\epsilon^*}{\epsilon^*}\right) n_{i,j} \right] = D_{ax,i,j} \frac{\partial^2 c_{i,j}}{\partial z^2} \tag{2.4}
\]

where \(c_{i,j}\) and \(n_{i,j}\) are the concentrations of the solute in the fluid and solid phase respectively. The void fraction of the column is denoted by \(\epsilon^*\) and \(D_{ax,i,j}\) is the axial dispersion coefficient, which may depend on the mobile phase velocity [40]. On the other hand, The interstitial velocity \(v_j\) in section \(j\), is related to the volumetric flow rate \(Q_j\) through:

\[
v_j = \frac{Q_j}{A\epsilon} \tag{2.5}
\]
where $A$ denotes the cross sectional area of the column. Equation (2.4) is coupled to the mass balance in the solid phase, which takes in consideration mass transfer effects, hence:

$$
\frac{\partial n_{i,j}}{\partial t} = k_{i,j}(n^*_{i,j} - n_{i,j})
$$

(2.6)

where $k_{i,j}$ represents the mass transfer coefficient and $n^*_{i,j}$ stands for the solid phase concentration in equilibrium with the fluid phase. The latter variable can be formulated as a function of the solute’s mobile phase concentration. At constant temperature, the it is described by the adsorption isotherm:

$$
n^*_{i,j} = f_i(c_{A,j}, c_{B,j}) \quad (i = A, B)
$$

(2.7)

Detailed mathematical expressions of the nonlinear adsorption isotherms estimated in this work will be presented in the experimental chapter and results presented as well in the homonymous chapter. When dispersion effects and mass transfer effects can be neglected, the inhomogeneous term of equation 2.4 equals zero. Thus, when a linear adsorption isotherm is assumed, the Equilibrium Theory Model of Chromatography can be formulated:

$$
v_j \frac{\partial c_{i,j}}{\partial z} + (1 + \nu H_i) \frac{\partial c_{i,j}}{\partial t} = 0
$$

(2.8)

Where $H_i$ are the Henry’s constants and $c_i$ the concentration of each component $i$ in the mobile phase. $\nu$ represents the phase ratio. As mentioned before, this model will be solved with the Method of Characteristics delivering expressions, especially useful for the estimation of the isotherm parameters (refer to section 3.3.1).

### 2.2.3 Design with Triangle Theory

The so called Triangle Theory was applied for the design of the 3C-ISMB as well for the 4C-ISMB: this is a very practical short-cut method developed by Mazzotti et al. [35]. Originally developed for the design of conventional SMB processes, the main feature of the method is the introduction of so called dimensionless flow rate ratios $m_j$, defined as:

$$
m_j = \frac{Q_j t^* - V e^*}{V (1 - e^*)} = \frac{\text{net fluid flow rate}}{\text{net solid flow rate}}
$$

(2.9)

where $Q_j$ is the flow rate in section $j$, $t^*$ is the switching time, $V$ the column volume and $e^*$ the overall void fraction. For the sake of simplicity, we can assume two components (A, B respectively) which are both subject to a linear adsorption isotherm:

$$
\begin{cases}
  n^*_{i,j} = H_i c_i \quad (i = A, B) \\
  H_A > H_B
\end{cases}
$$

(2.10)

Like in the case of the conventional SMB, if we assume an infinite column efficiency, the constraints to achieve complete separation in section II and III and complete regeneration
in section I and IV in both ISMB processes (i.e. 3C-ISMB, 4C-ISMB) can be derived by solving the model in equation 2.8 with the Method of Characteristics, leading to:

\[
\begin{align*}
    t_{A,I} & \leq t^* \\
    t_{B,II} & \leq t^* \leq t_{A,II} \\
    t_{B,III} & \leq t^* \leq t_{A,III} \\
    t^* & \leq t_{B,IV}
\end{align*}
\]  

(2.11a)  
(2.11b)  
(2.11c)  
(2.11d)

Considering the fact that each column experiences two different flow rates during each switch period \( t^* \), the retention time of the species \( i \) in the column’s section \( j \) can be formulated as:

\[
t_{i,j} = \frac{V}{Q_j} \left[ \epsilon^* + (1 - \epsilon^*)H_i \right] \quad (i = A, B; j = I, II, III, IV) 
\]

(2.12)

\[
\begin{align*}
    H_A & \leq m_I \\
    H_B & \leq m_{II} \leq H_A \\
    H_B & \leq m_{III} \leq H_A \\
    m_{IV} & \leq H_B
\end{align*}
\]  

(2.13a)  
(2.13b)  
(2.13c)  
(2.13d)

Constraints 2.13b and 2.13c define a triangular region of complete separation in the \((m_{II}, m_{III})\)-plane, whereas constraints 2.13a and 2.13d define a rectangular region of complete regeneration in \((m_I, m_{IV})\)-plane. Under non-linear chromatographic conditions, the triangle gets distorted, but explicit equations for its boundaries are available which makes the theory still easy to use. In fact, the SMB practitioner normally chooses an operating point in the \((m_{II}, m_{III})\)- and in the \((m_I, m_{IV})\)-plane, afterwards the switching time is fixed according to maximum pressure drop considerations (refer to [23] and Minimum Switch Time Design below). When this theory is extended to the ISMB processes, average flow rate ratios has to be defined according to:

\[
\begin{align*}
    \hat{Q}_j &= \alpha Q_j + (1 - \alpha)Q_{IV} \quad (j = I, II, III) \\
    \hat{Q}_{IV} &= (1 - \alpha)Q_{IV}
\end{align*}
\]  

(2.14a)  
(2.14b)

and redefining the \( \hat{m} \)-values as:

\[
\hat{m}_j = \frac{\hat{Q}_j t^* - V\epsilon^*}{V(1 - \epsilon^*)}
\]

(2.15)

Where \( \alpha (0 < \alpha \leq 1) \) indicates the step ratio, which defines the duration of the substep I relative to the whole switch time \( t^* \). Furthermore, based on the process schemes in figures 2.3 and 2.4, the following node mass balances are valid for both ISMB processes:
\[ Q_E = Q_I - Q_{II} \]  
\[ Q_F = Q_{III} - Q_{II} \]  
\[ Q_D = Q_I \]  
\[ Q_R = Q_{III} \] (2.16a, 2.16b, 2.16c, 2.16d)

### 2.2.4 Minimum Switch Time Design (MSTD)

As mentioned previously, the standard SMB process possesses five degree of freedoms. In contrast, six degree of freedom are possessed by both ISMB processes, i.e. the four internal flow rates \( Q_j \) \((j=I, II, III, IV)\), the switch time \( t^* \), and the step ratio \( \alpha \). In order to give numerical values to these variables, leading to a situation where complete separation and complete regeneration is achieved, the constraints expressed by the inequations 2.13 have to be fulfilled. In reality, purities are not the only concern when implementing an ISMB process, especially when dealing with "delicate" stationary phases, which withstand a maximum pressure drop: this is the case when the process is implemented for e.g. on chiral chromatographic columns, which only allow to operate by a maximal pressure drop \( \Delta p_{max} \).

Exceeding this technical constraint would ruin the stationary phase, compromising the separation ability of the chromatographic column. For a single column of length \( L \), the pressure drop can be related to the flow rate \( Q_j \) with Darcy’s Law as follows:

\[ \Delta p_j = \phi \frac{L}{A} Q_j \]  

(2.17)

Where \( A \) is the cross-sectional area of the column, and \( \phi \) is the pressure drop coefficient depending on the property of the packed stationary phase and on the fluid phase. In the case of a multicolumn process, the overall pressure drop consists on the pressure drop over the column’s train (all sections). Considering both substep I and substep II, the constraints for the maximal allowable overall pressure read:

\[
\begin{aligned}
\text{Substep I: } & \Delta p_{max} = \sum_{j=1}^{4} n_j \Delta p_j \quad (j = I, II, III) \\
\text{Substep II: } & \Delta p_{max} = \sum_{j=1}^{3} n_j \Delta p_4 \quad \text{(if } 3C-\text{ISMB : } j = 3; \text{ if } 4C-\text{ISMB : } j = 4) \\
\end{aligned}
\]  

(2.18)

where \( n_j \) is the number of columns per section \( j \). When a set of average dimensionless flow rate ratios \( \hat{m}_j \) is given, the equations listed in 2.18 permit to calculate the minimum switch time and the step ratio \( \alpha \), which therefore gives the minimum duration of substep I [28]. Considering equations 2.15, 2.17 and 2.18 we obtain for the I-SMB (here, called 4C-ISMB) the so called Minimum Switch Time Design [28]:
Figure 2.5: Triangle Theory for SMB and I-SMB design. The complete separation region is represented as right-angled triangle in the (a) \((m_2,m_3)\)-physical plane, whereas the region of complete regeneration is represented in the (b) \((m_1,m_4)\)-physical plane by the boundaries of a rectangle, whose dimensions (length, width) depend on the size of the Henry’s constants \([40, 28]\).
$\alpha_{I-SMB} = \frac{\sum_{j=1}^{III} n_j(\hat{m}_j - \hat{m}_{IV})(1 - \epsilon^*)}{\sum_{j=1}^{IV} n_j(\hat{m}_j(1 - \epsilon^*) + \epsilon^*)}$  \hspace{1cm} (2.19a)

$t^*_I-SMB = \frac{\phi L^2}{\Delta P_{max}} \sum_{j=1}^{IV} n_j (\hat{m}_j(1 - \epsilon^*) + \epsilon^*)$  \hspace{1cm} (2.19b)

Since, the 3C-ISMB can be operated by a minimum of three columns (instead of four columns as in the I-SMB 1-1-1-1), with an analog derivation the Minimum Switch Time Design of the 3C-ISMB reads:

$\alpha_{3C-ISMB} = \frac{\sum_{j=1}^{III} n_j(\hat{m}_j - \hat{m}_{IV})(1 - \epsilon^*)}{\sum_{j=1}^{III} n_j(\hat{m}_j(1 - \epsilon^*) + \epsilon^*)}$  \hspace{1cm} (2.20a)

$t^*_3C-ISMB = \frac{\phi L^2}{\Delta P_{max}} \sum_{j=1}^{III} n_j (\hat{m}_j(1 - \epsilon^*) + \epsilon^*)$  \hspace{1cm} (2.20b)

The reader is referred to Report#10 [7] for a detailed derivation of the minimum switch time and step ratio $\alpha$ in the case of the 3C-ISMB in open loop configuration (according to scheme of figure 2.1.2 in [7]) and in the case of the 4C-SIMB: as a matter of fact, same derivation principles can be applied no matter the three columns I-SMB is operated in open or closed configuration. At this point some considerations about the Minimum Switch Time Design have to be done: it is interesting to note that both expressions for the minimum switch time are mathematically independent to the corresponding step ratio [28]. Furthermore, equations 2.19 can be applied also to the standard SMB process when operated at the same dimensionless flow rates, i.e. $\hat{m}_j = m_j$, where only the first equation of 2.18 is considered [28]. This is an important consideration, that was exploited to demonstrate the superiority in productivity by the I-SMB compared to the conventional SMB process (please refer to the publication of Shige et al [28]).

### 2.2.5 Performance indicators

This section is intended to give to the reader an overview on the "separation performance indicators", which were used to assess the performance behavior of the experimentally implemented 4C-ISMB, 3C-SIMB and "Two steps 3C-ISMB cascade. It has to be mentioned that all indicators are based on an average concentration of the species in the extract and in the raffinate. This reads:

$$c_{p,i} = \int_{Nt^*}^{(N+\alpha)t^*} c_{p,i}^{switch} dt \quad (Binary \ system : i = A, B; \ Ternary \ system : i = A, B, C)$$  \hspace{1cm} (2.21)
binary separation, the recovery reads:

$$X_A = \frac{c_{E,A}}{c_{E,A} + c_{E,B}}, \quad X_B = \frac{c_{R,B}}{c_{R,A} + c_{R,B}}$$  \hspace{1cm} (2.22)$$

For a three fraction separation, considering three components A, B, and C, the purities can be defined as:

$$\begin{align*}
\text{Step I:} & \quad X_A^I = \frac{c_{E,A}}{c_{E,A} + c_{E,B} + c_{E,C}}, \quad X_B^{I+C} = \frac{c_{R,B} + c_{R,C}}{c_{R,A} + c_{R,B} + c_{R,C}} \\
\text{Step II:} & \quad X_A^{II} = \frac{c_{E,A}}{c_{E,A} + c_{E,B} + c_{E,C}}, \quad X_B^{II+C} = \frac{c_{R,B} + c_{R,C}}{c_{R,A} + c_{R,B} + c_{R,C}} \hspace{1cm} (2.23)$$

Important performance indicators for a suitable calculation of the solvent consumption (see definition in next lines), is the recovery of the product. For each component of a binary separation, the recovery reads:

$$Y_A = \frac{Q_E c_{E,A}}{Q_F c_{F,A}}, \quad Y_B = \frac{Q_R c_{R,B}}{Q_F c_{F,B}}$$  \hspace{1cm} (2.24)$$

Whereas for a three fraction separation:

$$\begin{align*}
\text{Step I:} & \quad Y_A^I = \frac{Q_E c_{E,A}}{Q_F c_{F,A}}, \quad Y_B^I = \frac{Q_R c_{R,B}}{Q_F c_{F,B}}, \quad Y_C^I = \frac{Q_R c_{R,C}}{Q_F c_{F,C}} \\
\text{Step II:} & \quad Y_A^{II} = \frac{Q_E c_{E,A}}{Q_F c_{F,A}}, \quad Y_B^{II+C} = \frac{Q_R c_{R,B} + Q_R c_{R,C}}{Q_F c_{F,C}} \hspace{1cm} (2.25)$$

An analog definition can be applied when a three fraction separation is implemented. From an economical point of view the productivity $Pr$ and the solvent consumption $SC$ are of great importance: in particular, the productivity is defined as the total amount of recovered products per unit volume of the stationary phase per unit time:

$$Pr = \frac{\alpha (Q_E c_{E,A} + Q_R c_{R,B})}{\sum_{j=1}^{3} n_j V} \quad (if \ 3C - ISMB : j = 3; \ if \ 4C - ISMB : j = 4)$$  \hspace{1cm} (2.26)$$

Analogously, the productivity during a ”two steps three fraction separation cascade”, reads:

$$\begin{align*}
\text{Step I:} & \quad Pr^I = \frac{\alpha (Q_E c_{E,A} + Q_R c_{R,B} + Q_R c_{R,C})}{\sum_{j=1}^{3} n_j V} \\
\text{Step II:} & \quad Pr^{II} = \frac{\alpha (Q_E c_{E,A} + Q_R c_{R,B} + Q_R c_{R,C})}{\sum_{j=1}^{3} n_j V} \hspace{1cm} (2.27)$$
The second economically relevant performance indicator is the dimensionless specific solvent consumption per unit amount of the recovered products. In the case of a binary separation, it reads:

\[
SC = \frac{(Q_D + Q_F)}{Q_F(Y_{A_F,A} + Y_{B_F,B})}
\] (2.28)

Analogously, the solvent consumption during a "two steps three fraction separation cascade", reads:

\[
\begin{align*}
\text{Step I: } SC^I & = \frac{(Q'_D + Q'_F)}{Q'_F(Y'_{A_F,A} + Y'_{B_F,B} + Y'_{C_F,C})} \\
\text{Step II: } SC^{II} & = \frac{(Q''_D + Q''_F)}{Q''_F(Y''_{B_F,B} + Y''_{C_F,C})}
\end{align*}
\] (2.29)

\[H_A > H_B > H_C\]
Chapter 3
Experimental

The experimental section is intended to give to the reader detailed description of the procedures and methods, which were used in the experimental part of this work. In particular the experimental work relied on both analytical chromatography and preparative chromatography: regarding the chromatographic analytical procedures, HPLC was used for performing so called ”breakthrough experiments” under linear and nonlinear chromatographic conditions for the estimation of the linear and nonlinear isotherms respectively. Furthermore, HPLC was also employed in order to give an off-line feedback about the concentrations and purities achieved during the implemented ISMB separations. On the other hand, in the frame of preparative chromatography, both ISMB processes (3C-ISMB, 4C-ISMB) were implemented on a laboratory plant, based on the process description and design presented in the Theory section 2.2. Process Flow Diagram (PFD) of both processes shall better clarify how the processes were realized.

3.1 Equipment

As mentioned before, the HPLC technology was applied for analytical purposes. On the other hand, ISMB separations were implemented on a laboratory plant based on a modified ÄKTA™ Explorer system.

3.1.1 Set-up for analytical chromatography

In the frame of analytical studies, adsorption isotherms and feedback information on the implemented separations of the ISMB processes were performed using the standard analytical tool ”High Pressure Liquid Chromatography” (HPLC): in particular, adsorption isotherm parameters could be estimated by performing experiments on an HPLC unit based on the system Agilent Technologies 1200 Series. On the other hand, a HPLC unit based on the system DIONEX Ultimate 3000 Column was employed for the analysis of extract and raffinate product streams during the implementation of the 4C-ISMB, 3C-ISMB and Two steps 3C-ISMB cascade. For analysis of the samples, only a single chromato-
graphic column was necessary. Furthermore, reliable analysis of the samples with high selectivity and with high efficiency of the column are of major concern, therefore suitable stationary phases for the system to be analyzed have to be properly selected. In this regard, details on the column’s properties, i.e. on the stationary phase properties and on the column dimension will be given below. Since the functioning principles of HPLC is based on Batch chromatography, the reader is referred to the Theory section 2.1.1 for clarifications on the fundamentals of the technique. Solute samples were weighted with an high precision \( (d=0.01mg) \) balance purchased from Mettler Toledo (Mettler Toledo AX205 Delta \( \text{Range}^\text{R} \)) and solvent’s masses were weighted with the former balance and with METTLER AE200 \( (d=0.01g) \). Furthermore, homogenization of the prepared solution could be accomplished using a standard mixer (e.g. IKA\( ^\text{R} \)RCT basic). With reference to figure 3.4, the set-up for the study of the linear isotherms is conceptually explained: the small bottle in red represents the HPLC vial containing the sample to be analyzed. On the other hand, the big bottle in blue represents the solvent reservoir which is continuously fed. During, the experiment, a continuous stream of solvent (blue) is fed by means of a pump through the chromatographic column, which was previously ”equilibrated” at the thermal set point \( (T=23^\circ\text{C}) \). The actual breakthrough experiment starts as soon as a small pulse (20\( \mu \text{L} \)) of sample containing the desired dissolved species is injected in the multiposition valve. The solute travels with the fed solvent along the chromatographic column, where it is adsorbed. After a time period of length \( t_R \), the species breakthroughs the column’s end, where it is detected by the UV-VIS detector at a desired wavelength. As result, the chromatogram contains a concentration peak of gaussian form (red concentration profile). With reference to figure 3.5, the set-up for study of the nonlinear isotherms is briefly explained: analog to the set-up described in figure 3.4, a solvent stream (blue) is continuously fed through the chromatographic column. This time, the sample was previously loaded on a loop (green) of 5mL capacity and injected in the mobile phase’s stream for a specific time of desired duration \( (t_F=4\text{min}) \). Measurement of the time is started as soon as the sample is injected by the manual change of the multiposition-valve’s position, permitting the solvent to flow through the loop. The shape of the eluted species (green concentration profile) depends on the specific nonlinear adsorption behavior of the injected species.

### 3.1.2 Set-up for preparative chromatography

ISMB separations were all implemented on a plant of laboratory scale, which is based on an adapted version of the purchased \( \overset{\text{T}}{\tilde{\text{A}}}KTA^\text{T}\text{M Explorer System} \) (GE Healthcare), whose modifications fulfilled the requirements of this work (Figure 3.1). With reference to figure 3.2 and figure 3.3, the Process Flow Diagrams describing the set-up of the 4C-ISMB and 3C-ISMB respectively are shown. With reference to figure 3.2, the set-up of the 4C-ISMB can be explained: The equipment is provided with four columns which are connected with seven multiposition valves (indicated with ”\( \text{V} \)” and manifolds (indicated as white circles with black border, where the filled black circles on the border indicates the inlet and the outlet ports of the manifold). Four reservoirs are indicated
with four Schott flasks, i.e. the feed (green), the desorbent (black), the extract (blue) and the raffinate (red). A buffer tank "BT" is placed between the recycle outlet and the sucking side of the recycle pump. In particular, the buffer tank permits the recycle line to release possible air bubbles. Four lines per manifold, as well four lines per multiposition valve (V-1, V-2, V-3, V-6, V-7, V-8) are necessary to fulfill the change of configuration during one cycle in the 4C-ISMB. Worth to mention is that the color notation (feed in green, desorbent in black, extract in blue and raffinate in red) is maintained as in the process scheme of figure 2.3. With reference to figure 3.3 the set-up of the 3C-ISMB can be briefly described: The 3C-ISMB construction has a similar configuration as by the 4C-ISMB. Same notation for multiposition valves, reservoirs, manifolds, pumps, lines and BT are used. In this process only three columns instead of four are needed, but the same number of pumps is employed. Furthermore, only three lines per manifold, as well three lines per multiposition valve (V-1, V-2, V-3, V-6, V-7, V-8) are necessary to fulfill the change of configuration during one cycle in the 3C-ISMB. Worth noting to mention is that the color notation (feed in green, desorbent in black, extract in blue and raffinate in red) is maintained as in the process scheme of figure 2.4. The similarity in both ISMB processes permitted to perform separations with simple modifications of the set-up and in a so called UNICORN code. In fact, by the implementation of the ISMB processes, all the parts of the plant, i.e. the gradient pumps (P-900), the detectors (UV-900) and the multiposition valves were controlled by the software UNICORN™ (UNICORN 5.20 Workstation, General Electric Company) [1, 27]. Balances were used for monitoring the injected mass of feed and desorbent respectively. Two gradient pumps are used for injecting and pushing the mobile phase through the column train. With reference to the figure 3.1 pumps are contained in two boxes ("Pump 2", "Pump 1") on the bottom of the photograph. Additionally, on the top right side of the photograph, two pill glasses are used to collect the extract and raffinate streams respectively: At the end of every cycle (refer to figure 2.2), the netto weights of the products (extract, raffinate) has been estimated for gaining information about the real flow rates and for checking the mass balances (refer to Report#12 [10] for detailed equations). Afterwards, the same samples were analyzed with HPLC to check the evolution of the concentrations and purities during the separation. In the center of the picture, the degasser is shown (white box). Operation of the plant was realized writing a proper code (an example can be seen in Appendix B of Report#13 [9]) using the UNICORN software, according to the temporal sequence of figure 2.2, the desired operating parameters of the specific experiment, and the configuration (4C-ISMB, 3C-ISMB). The reader is referred to Report#11 [6] for a detailed explanation on the implementation of a chromatographic cycle with the help of PFDs. Especially problematic for a correct process design is the so called "dead volume", which consists in the parts (capillary-tubes) connecting the columns. This volume was accurately estimated using HPLC (please refer to Report#13 [10] for the detailed experimental procedures): the connecting part between the inlet manifold and the column is \( V_{D1} = 0.018mL \), the connecting part between the outlet manifold and the column is \( V_{D2} = 0.017mL \). Finally, the dead volume of the connecting line between two
consecutive columns is $V_{D3} = 0.060mL$. For the implemented design by both processes, an overall dead volume of $V_D = 0.095mL$ per section was assumed.
Figure 3.1: Photograph of laboratory plant from side (left) and front (right) view. With reference to the photo on the left: three columns (vertical) are placed on the ÄKTA system for the implementation of the 3C-ISMB process. On the same unit a fourth column can be added to implement the 4C-ISMB process. In red are the inlet and outlet capillary tubes connecting the columns with the corresponding manifolds. With reference on the photo on the right: on the top left side, the feed reservoir (schott flask) and the desorbent reservoir (ethanol white bottle) are placed on the corresponding balances (left-balance (feed): Mettler Toledo PG1003-S, d=0.001g; right-balance (desorbent): Mettler Toledo PG4002-S, d=0.01g).
Figure 3.2: Process Flow Diagram of 4C-ISMB.
Figure 3.3: Process Flow Diagram of 3C-ISMB.
3.2 Materials

Since this work has dealt with several different systems, whose adsorption thermodynamics was studied with a suitable specific column, it is worth to make some clarifications on all substances and chromatographic columns which were used in this work. The implementation of the 3C-ISMB and 4C-ISMB was realized by using a racemic mixture containing the Tröger’s Base, (±)-2,8-dimethyl-6H,12H-5,11-methanodibenzo[b,f][1,5]diazocine (purchased from Sigma-Aldrich, 98% pure, Lot#S20991V). This racemate was used for the implemented separations after complete dissolution in ethanol (Scharlau, ethanol absolute, analytical grade, ACS, Reag. Ph Eur). Its solubility at room temperature was assumed in this work to be around 18g/L according to previous studies [29]. 1,3,5-Tris-tert-butylbenzene (Fluka, 97% pure, was dissolved in ethanol as well and injected in the HPLC for the estimation of the hold-up time \( t_0 \) in order to be able to estimate the overall void fraction \( \epsilon^* \) of the used column according to:

\[
\epsilon^* = \frac{(t_{D0} - t_D)Q}{V} = \frac{t_0Q}{V} \tag{3.1}
\]

Where \( t_{D0} \) is the measured hold-up time by HPLC, which has to be adjusted considering the dead time \( t_D \) in order to get the real hold-up time \( t_0 \). In the case of the Chiral Pak AD columns used in this study (Chiral Pak AD, 0.46 x 15 cm, Chiral Technologies Europe: Column#1: #AD10235-04; Column#2: #AD10235-05; Column#3: #AD10235-06; Column#4: #AD10235-08) an average overall void fraction of \( \epsilon^*=0.68 \) was estimated; on the other hand, the 5cm long Zorbax column (ZORBAX 5µm, 300A Stable Bond-C18 with dimensions 0.46 x 5 cm, S.N. USIW001273) had \( \epsilon^*=0.60 \), whereas the 25cm long column Zorbax (ZORBAX 5µm, 300A Stable Bond-C18 with dimensions 0.46 x 25 cm, S.N. USHH005155, purchased from Agilent) had \( \epsilon^*=0.58 \). Furthermore, the semi preparative Jupiter column (Jupiter 15µm 300A Stable Bond-C18 with dimensions 1 x 10 cm, S.N. 613685, purchased from Phenomenex) had \( \epsilon^*=0.73 \). The maximal allowable pressure drop by Chiral Pak AD column was \( \Delta p_{max} = 40 \text{bar} \) and by Jupiter column \( \Delta p_{max} = 240 \text{bar} \). No pressure drop restriction is set by the Zorbax columns.

Off-line measurements about concentration and purity levels achieved during the ISMB separations of the Tröger’s Base enantiomers were accomplished with the HPLC (DIONEX) on another chiral selective column, namely the analytical column Chiral Pak \( R \) IA (20µm, 0.46 x 25 cm, S.N. IA08064-04, Chiral Technologies Europe). Furthermore, the racemate \( \gamma \)-Phenyl-\( \gamma \)-butyrolactone (Sigma-Aldrich, 99%pure, Lot#BCBB6395V) was employed in the two steps three fraction 3C-ISMB cascade together with (−)-Tröger’s Base, which was obtained previously by separating a mixture of (±)-Tröger’s Base (Sigma-Aldrich) using a conventional SMB process according to a procedure reported elsewhere [30]. Regarding the study of the nonlinear isotherms of the system PNT/TBP in ethanol/water: the organic compound phentole (Sigma-Aldrich, 99% pure, Batch#59397MJ), abbreviated PNT in this work, and 4-tert-butylphenol (Sigma-Aldrich, 99% pure, Lot#STBB3768), abbreviated TBP in this work, were used in the isotherms’ studies in dissolved form, i.e. in a mixture consisting of ethanol and deionized water (microfilter, Millipore, Advantage
before their injection in the HPLC unit (Agilent). Worth to be mentioned is that PNT was previously distilled three times to get rid of some impurities (for explanations on the reason, please refer to figure 2.2.4 of Report#5). TBP is completely soluble (at least 90g/L) at room temperature for the ethanol/water compositions considered in this work. The miscibility of PNT was estimated experimentally at 23°C using the device EasyMax 102 (Mettler Toledo): it has been observed, that the miscibility strongly decreases with higher water content (miscibility of at least 77g/L in ethanol/water 65%/35% v/v; miscibility of 15g/L ethanol/water 50%/50% v/v; miscibility of 7g/L ethanol/water 45%/55% v/v). Uracil (Sigma-Aldrich, 99%pure,Lot#027K0719) was used as non retained component in the estimation of the hold-up time $t_0$ in the two analytical ”columns Zorbax” and in the semi-preparative ”column Jupiter”. In particular, the three latter columns were all used for the investigation of the $M_1$ and $M_2$ adsorption isotherms considering the system PNT/TBP in ethanol/water.

### 3.3 Adsorption isotherms

This section shall give to the reader an overview about the experimental procedures, which aimed at describing the thermodynamic adsorption behavior of the substances studied in this work. Linear and nonlinear isotherms could be estimated by so called ”breakthrough experiments”. All measurements were carried out using a HPLC unit at isothermal conditions, i.e. at T=23°C ±1°C, although, a slight different set-up was employed by the breakthrough experiments for the measurement of the chromatograms under nonlinear conditions. In section 3.3.1 and 3.3.2, the different set-up will be explained. Worth to be mentioned is that all the chromatograms presented in this work, are shown using ”real concentrations”, i.e. ”mass of solute” per ”unit of volume of the solution”. Especially during the study of the nonlinear isotherms, very high signal intensities were achieved due to the high concentrations. Therefore, saturation of the signal’s intensity by high concentrations led to a nonlinear relation between signal intensity and concentration, thus a proper calibration curve was needed. In particular, the conversion of the absorbance intensity, expressed as ”arbitrary unit” (mAU) was realized with a wavelength ($\lambda$) specific calibration curve, whose mathematical expression has the following form:

\[
\text{Abs} (\lambda, c_i) = \frac{A c_i}{1 + B c_i}
\]

(3.2)

Where $\text{Abs}$ denotes the wavelength and concentration dependent absorbance. $A \left[ \frac{\text{mAU} \cdot L}{g} \right]$ and $B \left[ \frac{L}{g} \right]$ are the fitting parameters. The concentration of the solute $c_i$ will be expressed in this work with units ”gram of solute” per ”liter of solution”, where the density of the solution was fairly approximated with the solvent’s density. For additional details on the calibration curves, the reader is referred to figures 2.1.2a and 2.1.2b of Report#6.


3.3.1 Linear adsorption isotherms

With reference to figure 3.4, a breakthrough experiment consists in the injection of a sample containing the desired dissolved solute at highly diluted concentrations (around 0.05g/L). As a matter of fact, when the injected species travels through the column, its time of retention $t_R$ will only be affected by the size of its Henry’s constant. Therefore, at diluted conditions, the adsorption isotherm can be approximated from equation 2.7 to:

$$n_i^* \approx H_i c_i$$  \hspace{2cm} (3.3)

Where the adsorption of species $i$ on the stationary phase of the chromatographic column is expressed as a linear function of the solute concentration in the mobile phase $c_i$, where the Henry’s constant $H_i$ is the linear factor. A proof of the reasonableness of this approximation can be found in the peak’s shape of the resulting chromatogram after the breakthrough experiment: when the peak possesses a gaussian concentration’s profile it indicates the absence of nonlinear effects, namely the concentration does not influence the retention time. Now, assuming a linear isotherm, the Equilibrium Theory Model presented in equation 2.8 can be solved by means of the Method of Characteristics, leading to:

$$H_i = \frac{(t_{R,i} - t_D) v_C}{L} - 1 \frac{1}{\nu}$$  \hspace{2cm} (3.4)

Where $\nu$ denotes the phase ratio between the solid and liquid phase, i.e. $\nu = \frac{1 - \epsilon^*}{\epsilon^*}$. During a breakthrough experiment, the flow rate is set to a constant value and its relation to the interstitial velocity is:

$$v_C = \frac{u}{\epsilon^*} = \frac{Q}{A\epsilon^*}$$  \hspace{2cm} (3.5)

Where $A$ denotes the cross-sectional area of the chromatographic column. When the column properties are known, i.e. the column’s length $L$ and the overall void fraction $\epsilon^*$ (estimated with equation 3.1), the Henry’s constant can be estimated as a function of the retention time of the solute $t_{R,i}$.

3.3.2 Nonlinear adsorption isotherms

With reference to figure 3.5, the breakthrough experiment under nonlinear chromatographic conditions is shown. an overloaded, i.e. an highly concentrated sample is continuously fed for a specific time ($t_F = 4\text{min}$). The peak’s shape reveals the nature of the nonlinearity, whereas the position of the shock is influenced by the feed time and the specific retention time of the solute. Worth to be mentioned is that, in the case of nonlinear isotherms, a single breakthrough experiment is sufficient for estimating the unique isotherm parameter, i.e. the Henry’s constant. On the other hand, in the case of nonlinear estimations, multiple injections by several solute’s concentrations are necessary in order to get reliable fittings of the isotherm’s parameters. For the estimation of the adsorption
isotherm’s parameters, two methods were applied independently to each other: the Equal Area Method and the Method of Characteristics. The former relies on pure mass balances calculations, whereas in the latter, the nature of the adsorption isotherm has to be assumed in advance. With reference to figure 3.6, the Frontal Analysis with both methods can be explained. The key principle of the Equal Area Method relies on estimating the amount of solute adsorbed on the stationary phase by different feed concentrations, and making a suitable fitting of these data against the corresponding feed concentration (refer to equation 2.7). Considering a single breakthrough experiment by a specific concentration (e.g. 14.6g/L, figure 3.6), an arbitrary concentration-to-time” rectangular region is chosen for the mass balance: based on this region, first the area ”concentration per time” is converted in ”concentration per volume” considering the applied flow rate. Now, the rectangular ”control area” is a ”control volume” and can be used for further mass balance calculations: in particular, the amount of solute which adsorbed on the stationary phase is estimated subtracting the solute amount in the dead volume and in the mobile phase inside the column from the total amount of solute of the control volume. With reference to figure 3.6, the control volume is based on the area represented by the green rectangle. Anyway, the reader is referred to Report#7 [12] for detailed derivations of the equations used in the Equal Area Method. Regarding the Method of Characteristics, the nature of the adsorption isotherm has to be known in advance to permit a relation between the amount of adsorbed solute on the stationary phase and that in the mobile phase. Since PNT, showed a typical Anti-Langmuir behavior, where the simple wave is expressed on the front of the elution profile (refer e.g. to chromatogram in figure 3.6), the following relations are valid, at equilibrium, for an Anti-Langmuir adsorption isotherm:

\[
\begin{align*}
  n_i^* &= n_i^* (t_S, c_F) = \left[ (t_S - t_D - t_F) \frac{c_F}{L} - 1 \right] \frac{c_F}{\nu} \\
  n_i^* &= \frac{H_i c_i}{1 - K_{eq,i}}
\end{align*}
\]

(3.6)

Where \(t_S, t_D, t_F\) refer to the shock-front’s time-position, the dead time, and the feed time respectively. Furthermore, \(c_F\) denotes the feed concentration (also called mobile
Figure 3.5: Scheme of set-up for breakthrough experiments by nonlinear isotherms.

phase concentration at plateaux), \( v_C \) is the interstitial velocity inside the column (refer to equation 3.5) during the breakthrough experiment, whereas \( L \) is the column’s length and \( \nu \) represents the phase ratio. When a Langmuir adsorption isotherm is considered the relation modifies to:

\[
\begin{align*}
\frac{n^*_i}{n^*_i} &= \frac{n^*_i(t_S, c_F)}{n^*_i(t_D, c_F)} = \left[ \left( \frac{t_S}{t_D} - 1 \right) \frac{v_C}{\nu} \right] \frac{c_F}{n^*_i} \\
H_i &= \frac{H_i}{1 + K_{eq,i}}
\end{align*}
\]

(3.7)

Where the unique difference to equation 3.6 consists in the absence of the feed time variable \( t_F \), since the shock’s front of a solute subject to a Langmuir isotherm breakthrough on the front side of the elution profile (please refer to the Results section). Furthermore, the Langmuir isotherm differs to the Anti-Langmuir isotherm from the opposite sign of the equilibrium constant. In addition to TBP, fitting with a Langmuir isotherm was also performed on the single components TBP, (-)-Tröger’s Base, and (+)-Tröger’s Base. Although, a more accurate description of the Tröger’s Base system could be described by Shige et al [29] by binary breakthrough injections and fitting a competitive Bi-Langmuir adsorption isotherm of the form:

\[
\begin{align*}
\frac{n^*_i}{n^*_i} &= \frac{a_{i,1}c_i}{1 + b_{A,1}c_A + b_{B,1}c_B} + \frac{a_{i,2}c_i}{1 + b_{A,2}c_A + b_{B,2}c_B} \\
H_i &= a_{A,1} + a_{A,2} > a_{B,1} + a_{B,2} = H_B
\end{align*}
\]

(3.8)
Figure 3.6: Frontal Analysis. Elution profile during a breakthrough experiment after injection of PNT with concentration 14.6 g/L in ethanol. More precisely, the sample was fed for 4 minutes, causing the elution profile to reach a concentration plateaux value equal to the feed concentration of the injected solute. Areas with different meanings are marked with different colors: the dead area is in turquoise, the area covered by the solute in the mobile phase in grey, whereas that in the stationary’s phase in orange, and the area corresponding to the eluted solute is colored in yellow. The vertical dotted red line indicates the starting point for the elution’s profile integration. Relevant for the application of the Method of Characteristics, the position of the shock’s front is marked with a vertical dark blue line: in particular, its position is defined by the tiny equivalent (green) areas at the tail’s edges (in the case of a Langmuir isotherm, front edges) of the elution profile, which are determined applying the Equal Area Method as well.
CHAPTER 3. EXPERIMENTAL

3.4 Implementation of Design

This section is intended to explain how the design was experimentally implemented considering the specific properties of the plant and of the system to be separated. In the frame of Triangle Theory, the dimensionless average flow rate ratios (equation 2.15) will be adjusted considering the module’s dead volume (for the corresponding value please refer to section 3.1.2). On the other hand, in the frame of the Minimum Switch Time Design, equations for the minimum switch time, and the step ratio, both accounting for the module’s dead volume and the plant’s specific pressure drop coefficients, will be presented. Worth to be mentioned is that, the dead volume is a property depending on the plant’s geometry, whereas the size of the pressure drop coefficients depend on the plant’s geometry (i.e. tubes, valves, manifolds), on the particle size of the column’s packing and it is also a strong function of the mobile phase viscosity (refer to figure 2.3.1 of Report#5 [5] and figure 4.7 of [43]).

3.4.1 Triangle Theory

As mentioned in section 3.1.2, the extra-column dead volume has to be considered, especially when implementing a process on a plant of lab-scale where the volume of the tubes and of the connecting components can not be neglected when compared with the column volume [29]. With reference to equation 2.15, the dimensionless flow rate ratios, when considering the plant specific module’s dead volume $V^*_D$ read:

$$\hat{m}_j = \frac{\hat{Q}_j t^* - V e^* - V^*_D}{V (1 - e^*)} \quad (j = I, II, III, IV) \quad (3.9)$$

Worth to be mentioned, that this expression was formulated analogously to the dimensionless flow rate ratios of the conventional SMB [37], according to previous publications [27, 29]. This expression will be exploited for the design of both ISMB processes, i.e. the 4C-ISMB and 3C-ISMB. Worth to be mentioned is that, the same numerical value for the module’s dead volume $V^*_D$ can be applied for every average flow rate ratio $\hat{m}_j$, since every module was previously built with tubes of same diameter and length, therefore $V^*_D=V_D = 0.095mL$. The final version of the dimensionless flow rate ratios reads:

$$\hat{m}_j = \frac{\hat{Q}_j t^* - V e^* - V_D}{V (1 - e^*)} \quad (j = I, II, III, IV) \quad (3.10)$$

3.4.2 Experimental Minimum Switch Time Design

As mentioned before, a Minimum Switch Time Design considering the plant geometry, and the pressure drop produced through the plant, has to be formulated. With reference to figure 3.7, the pressure drop coefficient of the connecting tube $\phi_t$, between consecutive columns could be estimated through:
Figure 3.7: Pressure drop coefficients in PFD’s zoom in of (a) 4C-ISMB process and (b) 3C-ISMB process respectively.

Table 3.1: Experimentally estimated pressure drop coefficients. Estimated values and the corresponding goodness of fit, according to figure 3.7 and refined values according to equations 3.12.

<table>
<thead>
<tr>
<th>Pressure Drop coefficient</th>
<th>Estimated $[\text{bar min/mL}]$</th>
<th>Goodness of fit</th>
<th>Refined $[\text{bar min/mL}]$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\phi_m$</td>
<td>18.68</td>
<td>0.999</td>
<td>19.14</td>
</tr>
<tr>
<td>$\phi_c$</td>
<td>5.53</td>
<td>0.938</td>
<td>3.49</td>
</tr>
<tr>
<td>$\phi_t$</td>
<td>13.15</td>
<td></td>
<td>15.65</td>
</tr>
</tbody>
</table>

$$\phi_t = \phi_m - \phi_c$$ (3.11)

where $\phi_m$, $\phi_c$ denote the pressure drop coefficient of one module and through a column respectively. Numerical values of the coefficients $\phi_c$, $\phi_m$ could be experimentally estimated by injecting pure ethanol at different flow rates through the column an through one module respectively. Then, the measured pressure drop by different flow rates allowed to fit a linear regression according to the function described in equation 2.17 and numerical values to the slopes $\phi_c$ and $\phi_m$ could be assigned. With reference to equation 3.11 the unknown $\phi_t$ could be estimated indirectly. Table 3.1 summarizes the “estimated” pressure drop coefficients and the “refined” values after implementation of an ISMB process considering a maximal overall pressure drop of $\Delta p_{\text{max}}$. The “refined” pressure drop coefficients were then used for the experimental design of all ISMB separations presented below.

In order to develop analytical expressions for the minimum switch time and step
ratio specific for the laboratory plant described in section 3.1.2, equations 2.18 can be reformulated considering the experimental pressure drop coefficients:

\[
\begin{align*}
\text{Substep I} : \quad & \Delta p_{\text{max}} = \phi_c Q_1 + (\phi_c + 2\phi_t) Q_2 + \phi_c Q_3 \\
\text{Substep II} : \quad & \Delta p_{\text{max}} = (f_1\phi_c + f_2\phi_t) Q_4
\end{align*}
\] (3.12)

Where \( f_1 \) and \( f_2 \) are process specific constant factors: if the 4C-ISMB process is considered \( f_1=4 \) and \( f_2=3 \); on the other hand if the 3C-ISMB process is considered \( f_1=3 \) and \( f_2=2 \).
Finally, the minimum switch time and step ratio for the 4C-ISMB process and for the 3C-ISMB process read as follows:

\[\alpha_{4C-ISMB} = \frac{V (1 - \epsilon^*) [\phi_c \dot{m}_I + (\phi_c + 2\alpha) \dot{m}_I I + \phi_c \dot{m}_II + (3\phi_c + 2\alpha) \dot{m}_IV] + V_D (3\phi_c + 2\alpha)}{V (1 - \epsilon^*) [\phi_c \dot{m}_I + (\phi_c + 2\alpha) \dot{m}_I I + \phi_c \dot{m}_II + (3\phi_c + 2\alpha) \dot{m}_IV] + V (1 - \epsilon^*) (\phi_c + \phi_I) \dot{m}_IV + V_D (7\phi_c + 5\alpha)}\]

\[t_{4C-ISMB} = \frac{\{V [\dot{m}_IV (1 - \epsilon^*) + \epsilon^*] + V_D\} (4\phi_c + 3\alpha) \{V [1 - \epsilon^*] [\phi_c \dot{m}_I + (\phi_c + 2\alpha) \dot{m}_I I + \phi_c \dot{m}_II + (3\phi_c + 2\alpha) \dot{m}_IV] + V (1 - \epsilon^*) (\phi_c + \phi_I) \dot{m}_IV + V_D (7\phi_c + 5\alpha)\}}{\Delta \rho_{\text{max}} (V \epsilon^* (4\phi_c + 3\alpha) (3\phi_c + 2\alpha) \dot{m}_IV + V (1 - \epsilon^*) (\phi_c + \phi_I) \dot{m}_IV + V_D (4\phi_c + 3\alpha))}\]

\[\alpha_{3C-ISMB} = \frac{V (1 - \epsilon^*) [\phi_c \dot{m}_I + (\phi_c + 2\alpha) \dot{m}_I I + \phi_c \dot{m}_II + (3\phi_c + 2\alpha) \dot{m}_IV] + V_D (3\phi_c + 2\alpha)}{V (1 - \epsilon^*) [\phi_c \dot{m}_I + (\phi_c + 2\alpha) \dot{m}_I I + \phi_c \dot{m}_II + (3\phi_c + 2\alpha) \dot{m}_IV] + V (1 - \epsilon^*) (\phi_c + \phi_I) \dot{m}_IV + V_D (3\phi_c + 2\alpha)}\]

\[t_{3C-ISMB} = \frac{\{V [\dot{m}_IV (1 - \epsilon^*) + \epsilon^*] + V_D\} (3\phi_c + 2\alpha) \{V [1 - \epsilon^*] [\phi_c \dot{m}_I + (\phi_c + 2\alpha) \dot{m}_I I + \phi_c \dot{m}_II + (3\phi_c + 2\alpha) \dot{m}_IV] + V (1 - \epsilon^*) (\phi_c + \phi_I) \dot{m}_IV + 2V_D (3\phi_c + 2\alpha)\}}{\Delta \rho_{\text{max}} (V \epsilon^* (4\phi_c + 3\alpha) (3\phi_c + 2\alpha) \dot{m}_IV + V (1 - \epsilon^*) (\phi_c + \phi_I) \dot{m}_IV + V_D (3\phi_c + 2\alpha))}\]

For additional information about the derivation of the analytical expressions for the minimum switch time and the step ratio of both processes (4C-ISMB, 3C-ISMB), the reader is referred to Report#15&16 [13].
3.4.2.1 Experimental Design of Binary separations

With reference to figure 3.8 according to the dimensionless flow rate ratios (section 3.4) and the experimental Minimum Switch Time Design (section 3.4.2), operating points for three binary separations (so called run α, run β, and run γ) were designed. Each run corresponds to a binary separation of the Troeger’s Base enantiomers by different concentrations, namely 5g/L (run α), 10g/L (run β) and 15g/L (run γ) respectively, with the aim to create chromatographic nonlinear conditions, as indicated by the distorted triangles of figure 3.8. Each experiment was realized in three steps: (a) start-up in 4C-ISMB mode with optimized conditions (according to the designed average flow rate ratios of table 3.2 and according to the experimental Minimum Switch Time Design of equations 3.13), (b) switch to 3C-ISMB without changing the operating conditions (3C-ISMB non optimized) and (c) 3C-ISMB with optimized operating conditions (according to equations 3.14 and table 3.2). The operating points (depicted as ”stars” in figure 3.8) were chosen near to the triangle’s tip in order to achieve the highest possible productivity whilst being in the complete separation region (100% purity region for raffinate and extract). However, for robustness reasons, a safety margin of 5% from the boundaries of the complete separation region was applied, in order to account for the slight deviations of the experimental points from the designed operating conditions (compare experimentally designed operating parameters of table 3.2 with real experimental values of table 4.3). Table 3.3 reports the system characteristics. In particular, the equilibrium constants of the Bi-Langmuir isotherm were kept the same as in Shige et al [26], whereas the parameters $a_1^i$ and $a_2^i$ were adjusted with constant specific factors based on estimated Henry’s constants under linear conditions, (please refer to ”adjusted values” in table 4.2).
Figure 3.8: Experimental design with Triangle Theory for Binary separations. The three colored stars (green, blue, red) in the \((\hat{m}_{II}, \hat{m}_{III})\)-plane represent designed operating points for complete separation of the binary mixture \((\pm)\)-Tröger’s Base in ethanol for an overall feed concentration of 5g/L (green), 10g/L (blue) and 15g/L (red) respectively. The boundaries of the corresponding complete separation region by the feed concentrations mentioned above, were calculated with the Triangle Theory considering a Bi-Langmuir adsorption isotherm. Three black stars in the \((\hat{m}_{I}, \hat{m}_{IV})\)-plane overlap in the region of complete regeneration.
Table 3.2: Designed operating conditions and predicted separation performances (productivity $Pr$, specific solvent consumption $SC$) from the experimental MSTD for the 4C-ISMB (known as I-SMB in the literature [28, 29, 26]) and for the 3C-ISMB (both under optimized conditions, "opt"). Purities are predicted according to simulations with an Equilibrium Dispersive Model considering $D_{app} = 10^{-3}$ and $k_i = 1s^{-1}$. The position of the operating points in the operating parameter plane are shown in figure 3.8.

<table>
<thead>
<tr>
<th>Run</th>
<th>$c_T$ [g/l]</th>
<th>Operating mode</th>
<th>Average Flow rate ratio</th>
<th>$t^*$ [min]</th>
<th>$\alpha$ [-]</th>
<th>Flow rate [mL/min]</th>
<th>Purity [%]</th>
<th>$Pr$ [g/L h]</th>
<th>$SC$ [L/g]</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 $\alpha$</td>
<td>5 I-SMB opt</td>
<td>5.715</td>
<td>1.857</td>
<td>4.086</td>
<td>1.090</td>
<td>5.210</td>
<td>0.223</td>
<td>3.18</td>
<td>0.53</td>
</tr>
<tr>
<td>5 $\beta$</td>
<td>10 I-SMB opt</td>
<td>5.715</td>
<td>1.755</td>
<td>3.786</td>
<td>1.090</td>
<td>5.118</td>
<td>0.209</td>
<td>3.45</td>
<td>0.50</td>
</tr>
<tr>
<td>5 $\gamma$</td>
<td>15 I-SMB opt</td>
<td>5.715</td>
<td>1.703</td>
<td>3.570</td>
<td>1.090</td>
<td>5.067</td>
<td>0.201</td>
<td>3.63</td>
<td>0.48</td>
</tr>
<tr>
<td>5 $\delta$</td>
<td>10 3C-ISMB opt</td>
<td>5.715</td>
<td>1.755</td>
<td>3.786</td>
<td>1.090</td>
<td>3.846</td>
<td>0.278</td>
<td>3.45</td>
<td>0.50</td>
</tr>
<tr>
<td>10 $\alpha$</td>
<td>15 3C-ISMB opt</td>
<td>5.715</td>
<td>1.703</td>
<td>3.570</td>
<td>1.090</td>
<td>3.795</td>
<td>0.268</td>
<td>3.63</td>
<td>0.48</td>
</tr>
</tbody>
</table>
Table 3.3: Characteristics of the binary model system.

<table>
<thead>
<tr>
<th>Column</th>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A [cm$^2$]</td>
<td>0.166</td>
<td></td>
</tr>
<tr>
<td>L [cm]</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>$\epsilon^*$ [-]</td>
<td>0.68</td>
<td></td>
</tr>
<tr>
<td>$\Delta P_{\text{max}}$ [bar]</td>
<td>40</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bi-Langmuir Isotherm</th>
<th>Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+)-Tröger’s Base</td>
<td>(-)-Tröger’s Base</td>
</tr>
<tr>
<td>$a_{i,1}$ [-]</td>
<td>1.64</td>
</tr>
<tr>
<td>$b_{i,1}$ [L/g]</td>
<td>0.0132</td>
</tr>
<tr>
<td>$a_{i,2}$ [-]</td>
<td>0.32</td>
</tr>
<tr>
<td>$b_{i,2}$ [L/g]</td>
<td>0.136</td>
</tr>
</tbody>
</table>

3.4.2.2 Experimental Design of a Ternary separation

In this study, a ternary separation of the system (-)-Troeger’s Base, (most retained component A), and (±)-γ-Phenyl-γ-butyrolactone enantiomers (intermediate retained component B and less retained component C) dissolved in ethanol was implemented in a so called "Two-steps 3C-ISMB cascade". Starting feed concentrations were 0.5g/L for each component (A, B, C). Set-up of both steps (also called "stages") of cascade was realized with the 3C-ISMB process, according to the process scheme of figure 2.4 and PFD of figure 3.2. The prepacked Chiral Pak stainless steel AD columns (Column#1, Column#2, Column#3, referring to section 3.2) were used for the implementation of the cascade whereas the fourth column (Column#4) was used for the analysis of the product streams at each cycle end with an HPLC unit (DIONEX). The system characteristics are briefly summarized in table 3.4. Worth to be mentioned is the smaller values in all three Henry’s constants than the one estimated in [21] since another batch of chiral stationary phase was used in this study. Linear adsorption isotherms were estimated at T=23°C ±1°C according to equation 3.4. Since a Chiral Pak AD column with another serial number was used for the estimation of the Henry’s constants, a slight different value for the (-)-Tröger’s Base ($H_A = 5.00$) was estimated (table 3.4), when compared with the value ($H_A = 5.06$) in table 3.3.
Figure 3.9: Experimental design with Triangle Theory for Ternary separation. The Ternary separation, under linear conditions, was implemented in a cascade in two steps ("stages"), therefore two separated designs are needed: the complete separation region (right-angled triangles) and the complete regeneration region (dashed rectangles) and corresponding operating points (stars) are drawn for both steps of the cascade (step1 in black and step2 in violet).
Table 3.4: Characteristics of the ternary model system.

<table>
<thead>
<tr>
<th>Column</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A$ [cm$^2$]</td>
<td>0.166</td>
</tr>
<tr>
<td>$L$ [cm]</td>
<td>15</td>
</tr>
<tr>
<td>$\epsilon^*$ [-]</td>
<td>0.68</td>
</tr>
<tr>
<td>$\Delta P_{\text{max}}$ [bar]</td>
<td>40</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Component</th>
<th>Linear isotherm</th>
<th>$H_i$ [-]</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\pm$-\text{-Phenyl-}$\gamma$-butyrolactone</td>
<td>1.48</td>
<td>2.33</td>
</tr>
<tr>
<td>$(-)$-Tröger's Base</td>
<td></td>
<td>5.00</td>
</tr>
</tbody>
</table>
Table 3.5: Designed operating conditions and predicted separation performances (productivity Pr, specific solvent consumption SC) from the experimental MSTD for the Two steps 3C-ISMB cascade (both under optimized conditions, "opt"). Purities are predicted according to Triangle Theory assuming complete separation region. The position of the operating points in the operating parameter plane are shown in figure 3.9.

<table>
<thead>
<tr>
<th>Run</th>
<th>$e_X$ [g/L]</th>
<th>Operating mode</th>
<th>Average Flow rate ratio</th>
<th>$t^*$ [min]</th>
<th>$\alpha$ [-]</th>
<th>Flow rate [mL/min]</th>
<th>Purity [%]</th>
<th>Pr [g/L h]</th>
<th>SC [L/g]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st stage</td>
<td>1.50</td>
<td>3C-ISMB opt</td>
<td>$\dot{m}_I$ 5.715, $\dot{m}_II$ 2.597, $\dot{m}_III$ 4.736, $\dot{m}_IV$ 0.799, $\dot{m}_V$ 4.497</td>
<td>0.437</td>
<td>$Q_I$ 2.00, $Q_{II}$ 0.73, $Q_{III}$ 1.60, $Q_{IV}$ 0.96</td>
<td>100</td>
<td>100</td>
<td>14.3</td>
<td>2.20</td>
</tr>
<tr>
<td>2nd stage</td>
<td>2.80</td>
<td>3C-ISMB opt</td>
<td>$\dot{m}_I$ 2.800, $\dot{m}_II$ 1.600, $\dot{m}_III$ 2.150, $\dot{m}_IV$ 0.560, $\dot{m}_V$ 3.561</td>
<td>0.335</td>
<td>$Q_I$ 1.47, $Q_{II}$ 0.80, $Q_{III}$ 1.04, $Q_{IV}$ 0.96</td>
<td>100</td>
<td>100</td>
<td>1.2</td>
<td>7.29</td>
</tr>
</tbody>
</table>
Chapter 4

Results and Discussion

This chapter is substantially dedicated to the presentation and discussion of three main studies. First, estimation of the isotherm parameters are presented for the binary system (±)-Tröger’s Base in ethanol, and for the system PNT/TBP in ethanol/water. Both systems were determined by performing Frontal Analysis of the single components. However, the parameters of the latter system were not employed in the implementation of the binary separations, since they didn’t fit a Bi-Langmuir isotherm, although the Langmuir did. In the following sections the experimental results will be presented and commented with the help of detailed simulations on the breakthrough profiles. Second, resulting concentration profiles, purity profiles, productivity profiles of the three performed runs (α, β, γ) with the binary system Tröger’s Base in ethanol will be shown and discussed. As proof of validity of the experimental Minimum Switch Time design (Section 3.4.2) a pressure drop profile will be presented as well. Finally, as third study, the results of the ternary separation will be presented in form of concentration profiles, purity profiles and in form of a productivity profile as well.

4.1 Parameter estimation of adsorption isotherms

This section is intended to present the adsorption isotherm parameters, which were estimated in this work. Major focus will be placed on the study of the $M_1$- and $M_2$-isotherms, since the original plan of the thesis was to study the PNT/TBP system in ethanol/water in great detail for the implementation of an SMB process (or related) under nonlinear chromatographic conditions. Furthermore, single isotherms for the system (±)-Tröger’s Base in ethanol will be presented.

4.1.1 Binary systems

In the following sections the adsorption isotherms for two binary systems, i.e. (±)-Tröger’s Base in ethanol and PNT/TBP system in ethanol/water respectively, will be presented.
CHAPTER 4. RESULTS AND DISCUSSION

4.1.1.1 $M_1$- and $M_2$- isotherms

With reference to figure 4.1, the Henry’s constants of the single components PNT and TBP respectively are represented as a function of the volume percent of water in ethanol. In particular, the Henry’s constants could be experimentally estimated by means of pulse injection experiments under linear conditions by several ethanol/water compositions (blue and red crosses) on the Zorbax column (ZORBAX 5µm, 300Å Stable Bond-C18 with dimensions 0.46 x 25 cm, S.N. USHH005155, Agilent), then the experimental points were fitted with a suitable fitting’s curve, of the form according to Abel et al. [2]:

$$H_i(x) = \frac{H_0^i x}{(1-k_i x)^{n_i}} \quad (i = PNT, TBP)$$  \hspace{1cm} (4.1)

Where $H_0^i$, $k_i$ and $n_i$ are the fitting parameters which permit the correlation of the Henry’s constant (at T=23°C) for a specific component $i$ as a function of the solvent content. In this case, the Henry’s constants of PNT and TBP respectively were described as a function of the percent water content in ethanol. Worth to be mentioned that also in the case of the 5cm-long Zobax, comparable values could be achieved, (e.g. compare the values in Figure 4.1 with: $H_{PNT}(x=50%; L=5cm) = 1.94$, $H_{TBP}(x=50%; L=5cm) = 2.21$.

As a general trend we note an increase of the Henry’s constants by both components when the water content is increased. This observation can be reasonably explained by the stronger interaction of the hydrophobic part (e.g. the aromatic group) of both components with the hydrophobic chains of the stationary phase ($C_{18}$).

Regarding the initial purpose of the thesis, the attempt was to separate with a Simulated Moving Bed process (or related SMB processes) a system subject to an $M_2$ adsorption isotherm. This particularly isotherm conceptually consists on a binary system where an Anti-Langmuir component elutes faster than a Langmuir component. Considering the PNT/TBP system in ethanol/water, a suitable $M_2$ adsorption isotherm for the purpose of an implementation on a SMB process (or related), could be found by Selectivity $S = 1.2$ at 50% water content (figure 4.1), where PNT represents the less retained component and TBP the more retained component. At these conditions, Frontal Analysis using the Equal Area Method and the Method of Characteristics were applied to estimate the amount of adsorbed solute at equilibrium with the mobile’s phase corresponding concentrations and experimental points were fitted with a suitable fitting’s curve. With reference to figure 4.2 TBP follows a Langmuir behavior a cause of two reasons: first, the shift of the shock’s fronts (indicated by the sharp fronts) by smaller retention times when increasing the feed concentration. Second, the monotonically decreasing concentration at the tail, indicates the presence of a simple wave. Therefore, fitting of the adsorbed solute’s concentrations in equilibrium with the corresponding mobile’s phase concentration was performed with a Langmuir function. On the other hand, with reference to figure 4.3 the retarded shock’s tails by increasing feed concentration and the monotonically increasing concentrations at the front of the elution profiles by PNT, suggest an Anti-Langmuir adsorption isotherm. Fitting of both single components was performed with the following functions:
Figure 4.1: Henry’s constants of PNT and TBP respectively as a function of solvent content of water in ethanol. An inversion of the retention order occurs around 41% water content, where the selectivity between PNT and TBP is one.
Table 4.1: Adsorption isotherm’s parameters of PNT and TBP. The adsorption isotherm’s parameters, i.e. the Henry’s constant and the Equilibrium constant were estimated with three methods: the Equal Area Method (EAM), the Method of Characteristics (MOC) and the One-Parameter fitting combining the knowledge of the Henry’s constants at linear conditions with the experimental points given by both previous methods (i.e. EAM, MOC).

<table>
<thead>
<tr>
<th></th>
<th>$H_{TBP}$ $[-]$</th>
<th>$K_{eq}^{TBP} [L/g]$</th>
<th>$R_{TBP}^{2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>EAM</td>
<td>2.20</td>
<td>0.0396</td>
<td>0.9996</td>
</tr>
<tr>
<td>MOC</td>
<td>2.23</td>
<td>0.0428</td>
<td>0.9997</td>
</tr>
<tr>
<td>One-Par</td>
<td>2.21</td>
<td>0.0410</td>
<td>0.9980</td>
</tr>
<tr>
<td></td>
<td>$H_{PNT}$ $[-]$</td>
<td>$K_{eq}^{PNT} [L/g]$</td>
<td>$R_{PNT}^{2}$</td>
</tr>
<tr>
<td>EAM</td>
<td>1.93</td>
<td>0.0316</td>
<td>0.9997</td>
</tr>
<tr>
<td>MOC</td>
<td>1.84</td>
<td>0.0322</td>
<td>0.9998</td>
</tr>
<tr>
<td>One-Par</td>
<td>1.94</td>
<td>0.0306</td>
<td>0.9985</td>
</tr>
</tbody>
</table>

\[
\begin{align*}
H_{i} (i) &= \frac{H_{ci}^{eq}}{1+pK_{eq,i}c_{i}} \quad (\text{if } i = TBP : p = +1; \text{ if } i = PNT : p = -1) \\
H_{PNT} &< H_{TBP}
\end{align*}
\] (4.2)

With reference to figure 4.2 (b), the fitting of the experimental data was performed with "One-Paramter"-fitting curves, i.e. only the Equilibrium constants $K_{eq,i}$ were estimated, since the Henry’s constants of both components were previously estimated by pulse injection experiments. Good fitting is demonstrated by the high R-squared values ($R_{TBP}^{2} = 0.9980; R_{PNT}^{2} = 0.9985$) and the very good overlapping of the simulated elution profiles on the experimental breakthrough profiles by both components (refer to figure 4.2 and figure 4.3 respectively). Worth to be mentioned, is that the numerical simulations were carried out applying fifty discretization cells (i.e. fifty Ordinary Differential Equations) on the Equilibrium Dispersive Model (Partial Differential Equation), and coupled with the proper adsorption isotherm (Langmuir for TBP, Anti-Langmuir for PNT). In particular, the apparent dispersion coefficient was set to a low value, i.e. $D_{app} = 10^{-8} m$.

On an analog way, an $M_{1}$-isotherm could be observed: pulse injection experiments, indicate an inverted elution order of the two components compared to the $M_{2}$-isotherm (i.e. PNT elutes later than TBP), when the solvent composition resides below 41% of the water content, although the non-linear nature was maintained (i.e. Langmuir for TBP and Anti-Langmuir for PNT). The reader is referred to figure 2.3.1 and 2.3.2 of Report#2 [11] for a detailed analysis.

4.1.1.2 Bi-Langmuir and Langmuir isotherms

The system (±)-Tröger’s Base in ethanol was chosen as model compound because, its characteristics, i.e its adsorption isotherms are well known from previous publications [54].
Figure 4.2: Experimental vs Simulation by Frontal Analysis of TBP. (a) Simulated (blue lines) and experimental (dashed red lines) breakthrough profiles at different concentrations (b) Experimentally estimated adsorbed TBP against mobile phase concentration (red crosses) and fitted Langmuir adsorption isotherm (blue line).
Figure 4.3: Experimental vs Simulation by Frontal Analysis of PNT. (a) Simulated (blue lines) and experimental (dashed red lines) breakthrough profiles at different concentrations. (b) Experimentally estimated adsorbed PNT against mobile phase concentration (red crosses) and fitted Anti-Langmuir adsorption isotherm (blue line).
Table 4.2: Adsorption isotherm’s parameters of Tröger’s Base enantiomers assuming Bi-Langmuir, Linear and Langmuir isotherms respectively.

<table>
<thead>
<tr>
<th>Bi-Langmuir (Shige et al) [26]</th>
<th>(a_1[-])</th>
<th>(b_1[L/g])</th>
<th>(a_2[-])</th>
<th>(b_2[L/g])</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-)-Tröger’s Base (A)</td>
<td>3.99</td>
<td>0.0107</td>
<td>0.986</td>
<td>0.601</td>
</tr>
<tr>
<td>(+)-Tröger’s Base (B)</td>
<td>1.56</td>
<td>0.0132</td>
<td>0.304</td>
<td>0.136</td>
</tr>
<tr>
<td>Bi-Langmuir (adjusted values)</td>
<td>(a_1[-])</td>
<td>(b_1[L/g])</td>
<td>(a_2[-])</td>
<td>(b_2[L/g])</td>
</tr>
<tr>
<td>(-)-Tröger’s Base (A)</td>
<td>4.06</td>
<td>0.0107</td>
<td>1.00</td>
<td>0.601</td>
</tr>
<tr>
<td>(+)-Tröger’s Base (B)</td>
<td>1.64</td>
<td>0.0132</td>
<td>0.32</td>
<td>0.136</td>
</tr>
<tr>
<td>Linear isotherms (pulse injections)</td>
<td>(H_i[-])</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(-)-Tröger’s Base (A)</td>
<td>5.06</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(+)-Tröger’s Base (B)</td>
<td>1.96</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Langmuir (One-Par Frontal Analysis)</td>
<td>(H_i[-])</td>
<td>(K_{eq,i}[L/g])</td>
<td>(R_i^2)</td>
<td></td>
</tr>
<tr>
<td>(-)-Tröger’s Base (A)</td>
<td>4.82</td>
<td>0.0366</td>
<td>0.9947</td>
<td></td>
</tr>
<tr>
<td>(+)-Tröger’s Base (B)</td>
<td>1.88</td>
<td>0.0233</td>
<td>0.9947</td>
<td></td>
</tr>
</tbody>
</table>

Nevertheless, the adsorption properties of one column differ with the time, and columns with same stationary phase and packing properties can also slightly differ from batch to batch. For these reasons, pulse injection experiments under linear conditions, were performed. With reference to table 4.2 relevant differences can be observed when compared with the values proposed by Shige et al \[26\]. Since, an accurate determination of the isotherms requires a lot of experimental work, the Bi-Langmuir parameters \(a_{1,i}\) and \(a_{2,i}\) of equation 3.8 were adjusted from the values of (Shige et al) \[26\] according to the following factors:

\[
\begin{align*}
F_A &= \frac{H_A}{a_{A,1} + a_{A,2}} \quad A = (-) - Tröger’s Base \\
F_B &= \frac{H_B}{a_{B,1} + a_{B,2}} \quad B = (+) - Tröger’s Base \\
H_A &= a_{A,1} + a_{A,2} > a_{B,1} + a_{B,2} = H_B
\end{align*}
\]  (4.3)

Where the values for \(a_{A,1}\), \(a_{A,2}\), \(a_{B,1}\), \(a_{B,2}\) can be read in table 4.2. For the sake of simplicity, the equilibrium constants of the Bi-Langmuir isotherms were kept the same as by Shige et al (refer to table 4.2 as well). As additional "short-cut method", the adsorption isotherms were estimated by Frontal Analysis with overloaded pulses of the single components. According to the experimental data, good fits (see R-squared values in table 4.2) could be achieved with Langmuir isotherms. Nevertheless, the implemented design of the binary separations with the racemic mixture \((\pm)-Tröger’s Base\) in ethanol relied on the Bi-Langmuir parameters, which were estimated applying the "adjustment" with the constant factors \(F_A\), \(F_B\), because better predictions through Triangle Theory of the complete separation regions by the performed binary separations (run \(\alpha\), run \(\beta\), run \(\gamma\)) could be reached.
CHAPTER 4. RESULTS AND DISCUSSION

4.1.2 Ternary systems

4.1.2.1 linear isotherms

Since the Henry’s constants were estimated for the unique purpose of implementing the Two steps 3C-ISMB cascade, the reader is referred to table 3.4 of section 3.4.2.2 for the corresponding values of the Henry’s constants by A, B and C respectively. These values were considered in the experimental design of the cascade.

4.2 Implementation of ISMB processes

This section is intended to present the results regarding the experimentally implemented ISMB processes. In particular, the binary separation of the system (±)-Tröger’s Base in ethanol was performed under three different modes of operation, according to the Triangle Theory design of figure 3.8: I-SMB (called here ”4C-ISMB”) under optimized conditions (according to the experimental MSTD of equations 3.13), then 3C-ISMB (not optimized), finally 3C-ISMB (according to the experimental MSTD of equations 3.14). this mode of operation was repeated under different nonlinear conditions, i.e. by total overall feed concentrations of \( c_F^T = 5\text{g/L} \) (run \( \alpha \)), \( c_F^T = 10\text{g/L} \) (run \( \beta \)), and \( c_F^T = 15\text{g/L} \) (run \( \gamma \)) respectively, with the key intention to demonstrate the superior productivity performed by the novel process, i.e. by the 3C-ISMB process compared to the I-SMB process by comparable purity levels. Major interest will be relied on the discussion about the productivity by comparable purity levels under optimized conditions, which permit a direct performance comparison of the 3C-ISMB opt. with the I-SMB opt. Finally, a three fraction separation could be realized by using the 3C-ISMB in a cascade of two steps (also called ”stages”). Major interest of discussion will be relied on the achieved purity levels.

4.2.1 Binary nonlinear separations

With reference to figure 4.4, the experimental operating points of the three runs (run \( \alpha \) in green, run \( \beta \) in blue, run \( \gamma \) in red) by the three mode of operations (4C-ISMB opt., 3C-ISMB, 3C-ISMB opt.) are presented on the (a) four-dimensional physical plane and on a zoom in of the \((m_{II}, m_{III})\)-plane. Every operating mode is marked with a point of different shape (squares for 4C-ISMB opt., circles for 3C-ISMB, diamonds for 3C-ISMB opt.). In this regard, it is important to make the reader aware that an experimental realization of the three modes of operation by exactly the same specific \( \dot{m}_j \) set (j=I, II, III, IV) is rather difficult, leading to a split of the design point (marked with a ”star” in figure 3.8) in three experimental points of slightly different \( \dot{m}_j \) values. In particular, these deviations from the design values (listed in table 3.2) are mainly caused by slight varying flow rates from the set point during the separation. However, all three operating modes, as depicted in figure 4.4a and figure 4.4b, and as demonstrated by the \( \dot{m}_j \) values presented in table 4.3 are very close to each other and close to the designed values (table 3.2). Therefore, pure product streams are expected for all three runs.
Since the separations were carried out using pressure sensitive chromatographic columns, which withstand a pressure drop of a specific value (i.e. 40 bar in the case of the used Chiral Pak AD columns), some considerations have to be made about the importance of respecting the maximal overall pressure drop in relation to the Minimum Switch Time Design: In this regard, a typical pressure drop (during substep II) versus cycle number (please refer to the corresponding process schemes of 4C-ISMB, in figure 2.3 and of 3C-ISMB, in figure 2.4) is shown in figure 4.4. As previously mentioned, each run started in I-SMB under optimized conditions (during cycle 1 to 12), then the same parameters’ set \( t^*, \alpha, \) internal flow rates \( Q_j \) was maintained (see design parameters in table 3.2 and experimental parameters in table 4.3) but the separation continued in 3C-ISMB configuration (during cycle 13 to 20). However, at this point of the separation’s run, the step ratio \( \alpha \) and the minimum switch time \( t^* \) are no longer optimal according to the Minimum Switch Time Design (presented in equation 3.14a and equation 3.14b), since the pressure drop in substep II is smaller during the 3C-ISMB configuration (approx. 28 bar), when compared to the maximal allowable pressure drop \( \Delta p_{\text{max}} = 40 \text{ bar} \), with reference to table 3.3 which was enforced in the design and confirmed during the separation (cycle 1-12 and cycle 21-28). This means, that during the second mode of the run, the process is operated under not optimal conditions (named “3C-ISMB” in table 3.2 and in table 4.3). Considering the smaller pressure drop in substep II, the flow rate \( Q_{IV} \) is allowed to be increased, permitting to increase the step ratio one one hand and to decrease the value of the minimum switch time on the other hand. In this regard, operation of the 3C-ISMB under optimal conditions (named ”3C-ISMB opt.”) was designed according equation 3.14a and equation 3.14b, where the optimal operation is confirmed by the fact that after cycle 20, the pressure drop is adjusted back to the maximal allowable pressure drop for the remaining eight cycles of the separation’s run (cycle 21 to 28, figure 4.5).
Figure 4.4: Operating points in the \((m_{II}, m_{III})\)-plane (top and bottom pictures) and \((m_{I}, m_{IV})\)-plane (picture on top). Solid lines indicates the regions of complete separation and regeneration for the three runs \((\alpha, \beta, \gamma)\), i.e. by total feed concentrations of 5g/L (green), 10g/L (blue), and 15g/L (red) respectively. Within a single run, the three modes of operation are marked with markers of different shape, i.e. squares for 4C-ISMB opt., circles for 3C-ISMB, and diamonds for 3C-ISMB opt. For the sake of comparison, the region of complete separation under linear conditions, i.e. by highly diluted feed concentrations is showed in black dashed lines. Worth to be mentioned, the figure on the bottom, represents a squared zoom in of the zone around the optimal operating point from the figure on the top.
Figure 4.5: Pressure drop vs cycle number (during substep II of run $\gamma$). The vertical black lines divide the pressure drop profile in three regions corresponding to mode of operation 4C-ISMB opt (cycles 1-12), 3C-ISMB (cycles 13-20), and 3C-ISMB opt. (cycles 21-28) respectively.
Table 4.3: Operating conditions and separation performances of the experimental ISMB runs. The position of the experimentally determined operating points in the operating parameter plane are shown in figure 4.4.

<table>
<thead>
<tr>
<th>Run</th>
<th>$c_f^R$ [g/l]</th>
<th>Operating mode</th>
<th>Average Flow rate ratio $\bar{m}$</th>
<th>$t^*$ [min]</th>
<th>$\alpha$</th>
<th>Flow rate $Q$ [mL/min]</th>
<th>Purity [%]</th>
<th>Pr [g/L]</th>
<th>SC [L/g]</th>
</tr>
</thead>
<tbody>
<tr>
<td>α</td>
<td>5.02</td>
<td>I-SMB opt</td>
<td>$\bar{m}_I = 5.70$ $\bar{m}_II = 1.86$ $\bar{m}_III = 4.07$ $\bar{m}_IV = 1.10$</td>
<td>5.21</td>
<td>0.22</td>
<td>3.16 0.52 2.04 0.66</td>
<td>100</td>
<td>98.9</td>
<td>30.1</td>
</tr>
<tr>
<td></td>
<td>5.02</td>
<td>3C-ISMB</td>
<td>$\bar{m}_I = 5.70$ $\bar{m}_II = 1.86$ $\bar{m}_III = 4.07$ $\bar{m}_IV = 1.11$</td>
<td>3.94</td>
<td>0.29</td>
<td>3.16 0.52 2.04 0.96</td>
<td>100</td>
<td>99.0</td>
<td>41.3</td>
</tr>
<tr>
<td></td>
<td>9.87</td>
<td>I-SMB opt</td>
<td>$\bar{m}_I = 5.54$ $\bar{m}_II = 1.60$ $\bar{m}_III = 3.63$ $\bar{m}_IV = 0.99$</td>
<td>5.12</td>
<td>0.21</td>
<td>3.39 0.46 1.97 0.64</td>
<td>100</td>
<td>98.5</td>
<td>57.5</td>
</tr>
<tr>
<td></td>
<td>9.87</td>
<td>3C-ISMB</td>
<td>$\bar{m}_I = 5.54$ $\bar{m}_II = 1.62$ $\bar{m}_III = 3.63$ $\bar{m}_IV = 0.99$</td>
<td>5.12</td>
<td>0.21</td>
<td>3.40 0.47 1.97 0.64</td>
<td>100</td>
<td>97.9</td>
<td>60.6</td>
</tr>
<tr>
<td>β</td>
<td>9.87</td>
<td>I-SMB opt</td>
<td>$\bar{m}_I = 5.54$ $\bar{m}_II = 1.60$ $\bar{m}_III = 3.63$ $\bar{m}_IV = 0.99$</td>
<td>3.85</td>
<td>0.28</td>
<td>3.41 0.48 1.98 0.93</td>
<td>100</td>
<td>98.1</td>
<td>82.0</td>
</tr>
<tr>
<td></td>
<td>9.87</td>
<td>3C-ISMB</td>
<td>$\bar{m}_I = 5.57$ $\bar{m}_II = 1.63$ $\bar{m}_III = 3.65$ $\bar{m}_IV = 0.99$</td>
<td>3.85</td>
<td>0.28</td>
<td>3.41 0.48 1.98 0.93</td>
<td>100</td>
<td>97.5</td>
<td>82.0</td>
</tr>
<tr>
<td>γ</td>
<td>15.00</td>
<td>I-SMB opt</td>
<td>$\bar{m}_I = 5.86$ $\bar{m}_II = 1.81$ $\bar{m}_III = 3.69$ $\bar{m}_IV = 1.18$</td>
<td>5.07</td>
<td>0.20</td>
<td>3.66 0.49 1.96 0.67</td>
<td>100</td>
<td>99.3</td>
<td>79.0</td>
</tr>
<tr>
<td></td>
<td>15.00</td>
<td>3C-ISMB</td>
<td>$\bar{m}_I = 5.80$ $\bar{m}_II = 1.80$ $\bar{m}_III = 3.65$ $\bar{m}_IV = 1.18$</td>
<td>5.07</td>
<td>0.20</td>
<td>3.62 0.49 1.93 0.67</td>
<td>100</td>
<td>99.4</td>
<td>107.1</td>
</tr>
<tr>
<td></td>
<td>15.00</td>
<td>3C-ISMB opt</td>
<td>$\bar{m}_I = 5.82$ $\bar{m}_II = 1.81$ $\bar{m}_III = 3.66$ $\bar{m}_IV = 1.17$</td>
<td>3.79</td>
<td>0.27</td>
<td>3.64 0.50 1.95 0.98</td>
<td>100</td>
<td>99.6</td>
<td>149.4</td>
</tr>
</tbody>
</table>
With reference to figure 4.6, the concentration profiles of the product streams (i.e. raffinate and extract) against the cycle number are presented for all three runs (α, β, γ). Worth to be mentioned, is that the conversion from the arbitrary Absorbance unit "mAU" to the real concentration "mass of solute per solution's volume" (g/L) was made applying a linear calibration since the wavelength of choice by the analysis of the product streams still respected the Lambert-Beer’s law (especially true when the signal resided under 1000mAU). Although the three runs were performed at different concentrations (i.e. 5g/L, 10g/L, 15g/L), a general trend by both product streams can be observed. In fact, after start-up of the plant, the concentrations monotonically increase till reaching a plateau value, which corresponds to the steady state situation. In particular, a cyclic steady state can be recognized after approximately 6 cycles. Some peculiarities originated by the different configuration of the 4C-ISMB process compared to the 3C-ISMB have to be mentioned: the extract concentrations are not influenced by the switch from 4C-ISMB opt., to 3C-ISMB, whereas the raffinate concentration suddenly drops after the switch at cycle’s end 12. Then, after few cycles, the profile recovers the same steady state concentration value as during the 4C-ISMB. The reason relies in the different functionality of the processes during substep II: since the recycle stream contains pure desorbent during the 4C-ISMB, the less retained component (B) will be diluted after the switch to the 3C-ISMB process, thus the concentration of B falls consequently. After some cycles, the concentration of B recovers the same plateau value which had before the switch from 4C-ISMB to 3C-ISMB, because the diluted stream residing in the previous cycles could be entirely flushed out. During the 3C-ISMB opt, the concentration’s profile of both products streams continue at the same level because the same internal flow rates during substep I are kept approximately invariant as in the previous operating modes (refer to table 4.3).

Before going into detailed considerations about the productivity and solvent consumption of both processes, it is reasonable to convert the concentration profiles in purity values of the product streams (raffinate and extract) according to equations 2.22. With reference to figure 4.7, the purity levels achieved by the product streams (raffinate, extract) during each run can be analyzed: we note that the raffinate stream was 100% pure during the whole separation by each run, with the small exception of run γ, where at cycle 13 the purity in raffinate slightly falls, although the 100% purity is recovered right after the next cycle. Worth to be mentioned is that 100% purity doesn’t necessary mean a "perfect" purity but it means that the concentration of component A was under the detection limit of the HPLC’s measurements. Nevertheless, it is reasonable to make this approximation a cause of the high measured absorbance of both enantiomers at wavelength $\lambda = 285nm$. In this regard a purity equal greater to 99.5% can be considered as criteria for "complete separation". On the other hand, the extract purity had a fluctuating trend at steady state in each run: in particular, with reference to table 4.3, steady state purity levels at each operating mode for each run were estimated considering average values. It is worth to mention that the experimental operating points of run β reside outside the boundaries delimiting the region of complete separation where usually Triangle Theory predicts a
Figure 4.6: Concentration profiles during (top) run α ($c_T^F=5g/L$), (middle) run β ($c_T^F=10g/L$), (bottom) run γ ($c_T^F=15g/L$): for each run both (a) the raffinate stream and (b) the extract stream are shown. Vertical black lines at the cycle’s end 12 and 20 indicate the switches in operating mode from 4C-ISMB opt. over 3C-ISMB to 3C-ISMB opt.
pollution of the extract stream (figure 2.5) and therefore the design predicted very well the effect of the deviating experimental points from the designed operating point (figure 3.8). Only run $\gamma$ barely satisfied the purity specification, whereas it is interesting to note that the overlapping operating points of run $\alpha$ had comparable $\hat{m}_2$ values to run $\gamma$ but performed worse purities. This can be explained considering the Triangle Theory as well: in fact, the shift of the distorted region of the complete separation region (from the linear case) permits to operate with complete separation by lower $\hat{m}_2$ values, i.e. the operating point by run $\gamma$ has a greater safety margin against potential dispersion effects, because positioned more far away from the “extract boundary” compared to the operating points of run $\alpha$.

4.2.1.1 Separation performance of Binary separations

This section is intended to present the separation performance of the implemented 4C-ISMB process and 3C-ISMB process respectively in term of productivity, which represents the most interesting performance indicator from an economic point of view. Since the superior productivity of I-SMB compared to the conventional SMB was demonstrated in previous studies [23, 28, 29, 26], a comparison of the novel process with the conventional SMB is not interesting as far as the productivity is concerned. Therefore, the key intention of this section will be to show the performance superiority of the novel invented 3C-ISMB process when compared to I-ISMB (named 4C-ISMB in this work).

With reference to figure 4.8, the productivity profiles during the three performed runs can be analyzed: each run possesses an analog trend, although increasing the total feed concentration (from 5g/L over 10g/L to 15/L) make the productivity at steady state increase even further (refer to figure 4.8b). Considering the productivity profile against the cycle number of a single run, it is worth to observe that the steady state productivity is already enhanced by switching from 4C-ISMB opt. to 3C-ISMB although the operating parameters were not changed (refer to designed operating parameter’s values in Table 3.2 and to experimental values of table 4.3). As a matter of fact, the productivity was normalized with the number of columns, since the “capital costs” represent an economically relevant issue. Therefore, a relative improvement of 33% of throughput is uniquely caused by the decreased number of columns, i.e. from 4 columns of the 4C-ISMB to only 3 columns of the 3C-ISMB. Worth to be mentioned is that the experimental improvement in each run was estimated around 35-37% due to the slight deviations of the experimental parameters (table 4.3) from the designed parameters (table 3.2). Furthermore, when comparing both processes under optimized conditions (4C-ISMB opt, 3C-ISMB opt.), namely when the maximal allowable pressure drop is fully exploited (compare pressure drop profile during cycle 1-12 with the pressure drop profile during cycle 20-28), the productivity relative improvement increase to 76-78%. Considering the derived step ratio and minimum switch time under optimal conditions, namely according to the Minimum Switch Time Design derived previously (ideal MSTD: equation 2.19 for 4C-ISMB, equation 2.20 for 3C-ISMB; experimental MSTD: equation 3.13 for 4C-ISMB, equation 3.14 for 3C-ISMB), the experimental productivity values are fully predicted: the reduction of
Figure 4.7: Purity vs cycle number by the binary separations. Purity profiles of the product streams during each run: (a) purity vs cycle number by run $\alpha$ ($c_T=5\text{g/L}$), (b) purity vs cycle number by run $\beta$ ($c_T=10\text{g/L}$), and (c) purity vs cycle number by run $\gamma$ ($c_T=15\text{g/L}$). Vertical black lines at the cycle’s end 12 and 20 respectively, indicate the switches in operating mode from 4C-ISMB opt. over 3C-ISMB to 3C-ISMB opt.
one column from 4C-ISMB to 3C-ISMB, and optimization with the MSTD make the step ratio increase one one hand, and make the minimum switch time decrease on the other hand, which allows for increasing the internal flow rate \(Q_{IV}\). Therefore, both effects increase the productivity (please refer to equation 2.26 for the formulation of the productivity). Worth to be noted is that the shift of the optimal operating point ”left-down” when the total feed concentration is increased (refer to figure 3.8 figure 4.4) causes a slight growth of the relative productivity improvement from 76% to 78% (refer to table 3.2 and table 4.3). On the other hand, with reference to figure 4.8, although the extract purity specification was not fulfilled in run \(\alpha\) and \(\beta\), a very slight deviation of the productivity can be observed by comparing the experimental productivity values at steady state (black points) with the productivity values predicted by the Minimum Switch Time Design (blue points) assuming complete separation according to the Triangle design. One more consideration about the productivity: since the solubility limit of the (±)-Troeger’s Base resides at approximately 18g/L at 23°C [26], a further increase in productivity at higher total feed concentrations can not be accomplished with this system, therefore the showed productivity in figure 4.8 is a good reference for the maximal achievable productivity on the described plant at the conditions presented previously (refer to pressure drop coefficients in table 3.1 and to system characteristics in table 3.3). Nevertheless, a modification of the plant reducing the highly relevant pressure drop inside the connecting tubes would allow to increase the internal flow rates an hence the productivity of the separation.

Regarding the other economic relevant performance indicator a final consideration has to be made about the implemented binary separations: the specific solvent consumption per unit amount of recovered product (refer to equation 2.28) could be predicted according to the designed parameters (table 3.2) and estimated from the experimental values (table 4.3): within a run the processes are operated at the same specific solvent consumption, which makes sense, since the desorbent flow rate \(Q_D\) and the feed flow rate \(Q_F\) during all the three operating modes remain exactly the same, and the recovery as well. Due to an increase in the total feed concentration, the specific solvent consumption decreases, although the processed solvent amount per unit of time is larger.

### 4.2.2 Ternary linear separation

This section aims at doing a proof of concept regarding the realization of a three fraction separation implementing a two steps separation cascade using the 3C-ISMB under optimized conditions. With reference to the scheme of figure 4.9, the conceptual realization can be explained: the cascade process consists in two 3C-ISMB units, which divide the process in two steps. In particular, in the first unit a ternary mixture (containing the components A, B, C where \(H_A > H_B > H_C\)) is fed \((F1)\), then the raffinate reservoir \((R1)\) of the first unit is used to feed the second unit. In particular, The raffinate product \((R1)\) is chosen as feed for further processing, because major interest is focused on the recovery of the component with intermediate retaining behavior (component B). On the other hand, collection of the most retained component A is collected at the extract
CHAPTER 4. RESULTS AND DISCUSSION

Figure 4.8: Productivity levels by the Binary separations. (a) Productivity vs cycle number during runs $\alpha (c_T^{\alpha}=5\,\text{g/L})$, $\beta (c_T^{\beta}=10\,\text{g/L})$, and $\gamma (c_T^{\gamma}=15\,\text{g/L})$. (b) Productivity vs total feed concentration for each run at steady state in comparison with predicted productivity by the Minimum Switch Time Design (MSTD) assuming complete separation according to the design based on Triangle Theory (refer to Figure 3.8). Note the point at approx. 17g/L corresponds to an additional binary separation, where only the 3C-ISMB opt. was implemented.

Stream in the first step of the cascade ($E_1$), whereas the less retained component C is collected in the raffinate stream of the second step ($R_2$). Ideally the cascade could be realized in a synchronous manner, namely A, B, C could be collected by operating both steps (semi)continuously during one stage only. Nevertheless, only one AKTA unit was at disposition during this work, therefore a first separation was performed in the first step of the cascade (called ”1st stage”), and afterwards the raffinate stream of the separation, containing a mixture of B and C, was further processed in a second separation (called ”2nd stage”) in 3C-ISMB opt. as well. The operating parameters were designed according to the Triangle Theory considering a system under linear conditions (figure 3.9) for the purpose of an easy proof of concept, since on one hand the linear isotherms of the three components could be quickly estimated by pulse injection experiments and on the other hand the nonlinear isotherms of ($\pm$)-γ-Phenyl-γ-butyrolactone were not known. The characteristics of the system are summarized in table 3.4. However, working under linear conditions, enforces to feed with diluted concentrations. In fact, in this study a ternary mixture with total feed concentration of $c_T^{T}=1.5\,\text{g/L}$ (with component’s concentrations $c_A^{T}=0.5\,\text{g/L}$, $c_B^{T}=0.5\,\text{g/L}$ and $c_C^{T}=0.5\,\text{g/L}$ respectively) was initially fed in the first stage of the cascade. Since the raffinate stream of stage 1 is used to feed the second unit, a further dilution of the products streams by B and C has to be taken in account (figure 4.13b and figure 4.13c), which negatively affects the productivity (table 4.4, figure 4.14). Experimental operating parameters by the implemented cascade are briefly summarized.
in table 4.4. Nevertheless, a fairly good prediction of productivity levels by the MSTD is again demonstrated (compare experimental productivity values from table 4.4 to designed values of table 3.5) as in the case of the previously presented binary separations.

With reference to figure 4.11 and table 4.4, the purity of the three components (A, B, C) at cyclic steady state, can be discussed. Very high purities were achieved by all three final products streams, i.e. 99.4% for the most retained component A in the extract 1 (denoted "E1" in figure 4.9), 99.3% for component B in the extract 2 (E2), and 99.3% as well by component C in the raffinate 2 (R2). Worth to be mentioned is that, the high purity achieved by the intermediate component B is a very good result, since it is usually the product of interest in a three fractions separation. Furthermore, this result is additionally appreciated by the fact that the separated system B+C during stage 2, has a very low selectivity, i.e. of $S = 1.47$ under linear conditions. Therefore, the application of the 3C-ISMB in a cascade configuration showed its powerful application also in case of even more challenging separations, if we compare the selectivity of the system ($\pm$)-\(\text{γ-Phenyl-γ-butyrolactone}\) in ethanol to the system ($\pm$)-Tröger’s Base (in ethanol), whose selectivity was $S = 2.58$ (under linear conditions). Although in this study, poor productivity values were achieved, it could be considerably increased by implementing the 3C-ISMB cascade for separations under nonlinear conditions, ones the adsorption
Figure 4.10: Experimental operating points of the Two steps 3C-ISMB cascade in the four dimensional physical plane. In black, the region of complete separation for the first stage is delimited by the Henry’s constants of component A and B. In violet, the region of complete separation for the second stage is indicated, where the Henry’s constants of components B and C respectively, create the purity boundaries. The region of complete regeneration for stage 1 and 2 are indicated with dashed black lines and violet dashed lines respectively. These are created by the Henry’s constants of A and C in stage 1, whereas A and B in stage 2.
isotherms of the system (±)-γ-Phenyl-γ-butyrolactone in ethanol are mathematically described. Concluding, some considerations about the technical realization of a synchronous operation of the two cascade’s stages have to be made: since a continuous operation of the cascade, where the feed flow rate of the first unit equals that of the second, is not feasible \[23\] when all six columns have the same size, a buffer tank connecting the two units is needed. As shown in table 3.5 and table 4.4 the feed flow rate in the second stage is smaller compared to the first stage. Therefore, the separation with the bigger complete separation region (figure 4.10) was carried out in the first step in order to mitigate the dilution to a certain extend, which would anyway occur. However, a continuous synchronous operation of the cascade could be realized without a buffer tank, e.g. by increasing the column’s volume in the 3C-ISMB unit placed downstream in the cascade, in order to match the feed flow rate of the second unit with the flow rate of the raffinate by the first unit (R1).
Figure 4.11: Chromatograms of the ternary feed mixture and of the product streams at steady state conditions during both stages. With reference to the rectangle-box on the bottom: the zone around the baseline is zoomed in order to highlight the extremely high purities of the product streams. Letters mean: "A" for (-)-Tröger’s Base, "B" and "C" for (±)-γ-Phenyl-γ-butyrolactone enantiomers. "P" stands for injection peak.
Figure 4.12: Purity profiles during the Two Steps 3C-ISMB cascade of components A, B, C respectively. The vertical black line indicates the switch from stage 1 to stage 2.
Table 4.4: Experimental operating conditions and separation performances of the Two steps 3C-ISMB cascade. The position of the operating points in the four dimensional physical plane is shown in figure 4.10.

<table>
<thead>
<tr>
<th>Run</th>
<th>$c_F^*$ [g/l]</th>
<th>Operating mode</th>
<th>Average Flow rate ratio $\alpha$ [-]</th>
<th>Flow rate $Q$ [mL/min]</th>
<th>Purity Pr [%]</th>
<th>SC [g/L h]</th>
<th>Raffinate</th>
<th>Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>1.50</td>
<td>3C-ISMB opt</td>
<td>5.78 2.56 4.74 0.71</td>
<td>4.50</td>
<td>99.6</td>
<td>15.3</td>
<td>99.4</td>
<td>2.20</td>
</tr>
<tr>
<td>2nd</td>
<td>0.58</td>
<td>3C-ISMB opt</td>
<td>2.84 1.82 2.13 0.59</td>
<td>3.56</td>
<td>99.3</td>
<td>1.3</td>
<td>99.3</td>
<td>7.35</td>
</tr>
</tbody>
</table>
Figure 4.13: Concentration vs cycle number by the ”Two Steps 3C-ISMB cascade”. (a) Concentration vs cycle number of A, B, C respectively in extract 1 (E1). (b) Concentration vs cycle number of A, B, C respectively in raffinate 1 (R1) during cycles 1-15, and raffinate (R2), during cycles 16-47. (c) Concentration vs cycle number of A, B, C respectively in raffinate 1 (R1) during cycles 1-15, and extract 2 (E2), during cycles 16-47. Vertical black lines at end of cycle 15 indicate the switch from stage 1 to stage 2 in the cascade.
Figure 4.14: Productivity during the Two steps 3C-ISMB cascade. The vertical black line at end of cycle 15 indicates the switch from stage 1 to stage 2.
This thesis focuses on two types of research, namely the development of a novel process for binary and ternary separations and the fully description of a particular nonlinear adsorption isotherm. Although thermodynamic studies on the isotherms are especially relevant for SMB optimization under nonlinear conditions, the major focus of the thesis relied on proof of concept experiments on the novel process, namely the Three Columns Intermittent Simulated Moving Bed (3C-ISMB). In particular the first aim was to demonstrate, that the same purity levels compared to I-SMB (in this work, called 4C-ISMB) could be achieved when the same operating parameters, i.e. the same internal flow rate set $Q_j$, switch time and step ratio were implemented. This was the case in all performed runs, where the separation of the $(\pm)$-Tröger’s Base in ethanol was realized by three different feed concentrations (5g/L, 10g/L, 15/L). In the design part, the Triangle Theory was exploited for defining the operating points under nonlinear chromatographic conditions by the highest possible productivity whilst ensuring complete separation. Regarding the performed experiments, both processes delivered comparable purity levels, where in one case the purity specification of 99.5% was respected. Due to a shift of the experimental operating parameters, the purity specification was evidently not fulfilled by the extract stream (approx. 97%) of run $\beta$, although the raffinate stream was 100% pure. Although residing in the region of complete separation, the extract purity (approx. 98%) of run $\alpha$ didn’t fulfilled the purity specification. Nevertheless, both processes delivered comparable purities, which was a proof of validity of the recycle concept by the 3C-ISMB. Since the productivity is the most important performance indicator from an economic point of view, particular attention was relied on its study. The key idea was to compare the separation performances of the 3C-ISMB with the 4C-ISMB according to a process specific Minimum Switch Time Design. In this regard, explicit analytical equations for the minimum switch time and for the step ratio could be derived by both ISMB processes, allowing a productivity comparison at fully optimized operating conditions, i.e when the pressure drop of the system is maximized to the maximal allowable pressure drop according to the column specific technical constraints. Furthermore, the validity of the derived equations for the Minimum Switch Time Design could be sustained by performing the binary separations mentioned below, which were carried out on a laboratory plant. Con-
sidering the operation of one run: start-up of the binary separation was implemented in the 4C-ISMB configuration under optimized conditions (according to the MSTD) for 12 cycles. In a ”steady-state” situation the process was switched in 3C-ISMB configuration but keeping the same operating parameters and operated for 8 cycles. Afterwards, the separation was continued in the 3C-ISMB configuration but the recycle flow rate $Q_{IV}$ and the step ratio could be increased whereas the switch time additionally ”minimized” to exploit the full potential of the 3C-ISMB under optimized conditions. Although the same operating parameters were applied after the switch from the optimized 4C-ISMB process to the not optimized 3C-ISMB process, a relative productivity improvement of 33% could accomplished because the 3C-ISMB only needs three columns instead of four. Moreover, when comparing both processes at their optimal operation, i.e. according to the MSTD, the productivity relative improvement increases to 76-78%. As far as the productivity levels are concerned, ”Pareto optimal studies” could be performed in future works, e.g. in order to be able to describe operating points by optimal productivity and solvent consumption. On the other hand, at a given total feed concentration, further economic validity of the 3C-ISMB is supported by the same specific solvent consumption performed as by the 4C-ISMB process. Worth to be mentioned is that the operation of both units under nonlinear conditions, i.e. by high total feed concentrations decreased the specific solvent consumption by a factor of 2.65 when the feed concentration is risen from 5g/L to 15g/L.

In the last part of the thesis, a ternary separation applying the 3C-ISMB in a ”Two steps cascade” was realized achieving very high purity levels: in particular, at steady state, 99.4% pure streams for the most retained component, and 99.3% pure streams by the intermediate component and less retained component, respectively could be achieved. Although the separation of the system $(\pm)$-γ-Phenyl-γ-butyrolactone and $(-)$-Tröger’s Base in ethanol was not run at high concentrations, the successful proof of concept under linear conditions, could allow an implementation of the ”Two steps 3C-ISMB cascade” with same design principles separations under nonlinear conditions, as soon as the adsorption isotherms are mathematically described.

Although the major focus of this thesis relied in the study of the 3C-ISMB, this work also aimed at the description of a particular case of Generalized Adsorption Isotherm since the initial purpose of the thesis, aimed at separate a system subject to an $M_2$-isotherm with a Simulated Moving Bed process. In this regard, the system PNT/TBP in ethanol/water was fully described by a so called $M_2$ adsorption isotherms by estimating the adsorption isotherm’s parameters of the single components by 50% water content. This specific water content was chosen by the estimation of the equilibrium constants a cause of several reasons: reasonable selectivity ($S = 1.14$), reasonable low Henry’s constants ($H_{PNT} = 1.94; H_{TBP} = 2.21$) and reasonable miscibility levels of PNT (15g/L) could allow an implementation of the binary separation using e.g. the 3C-ISMB process on semi-preparative columns with a reversed phase stationary phase such as the alkyl chain C-18. Interestingly, an inversion point of the elution order at 41% is observed when studying the effect of the Henry’s constants by different water contents in ethanol on the analytical 25-cm
long column Zorbax. This behavior would lead to a switch of the adsorption behavior of
the PNT/TBP system from an $M_2$-isotherm to an $M_1$-isotherm by simply adjusting the
water content below 41%, since the Langmuir character of TBP and the Anti-Langmuir
character of PNT was presumed to be maintained. Anyway, further studies through over-
loaded pulses should be performed in order to ensure the validity of this evidence, since
the experiments performed at 25% water content (which aimed at highlighting the Lang-
muir and Anti-Langmuir character by TBP and PNT respectively) didn't show a clear
nonlinear behavior, although an evident shift of the shock’s fronts could be observed (re-
fer to Report#2 [11]). As future work regarding the $M_2$-isotherm system (PNT/TBP in
ethanol/water 50%/50% v/v) the implementation on the 3C-ISMB has to be performed
by using C-18 chromatographic columns, which possess suitable packing properties to
avoid a too high overall pressure drop in the plant: a suitable particles size shall pos-
sesses at least 15µm in size, which was observed in this thesis to be a suitable size by the
semi-prep. column Jupiter. Nevertheless, the different loading capacity of the stationary
phase of Jupiter, compared to that of the analytical column Zorbax (particle size 5µm)
caused undesired high Henry’s constants, which made the implementation on a SMB-
based process not feasible so far (a cause of limited time at disposal). Anyway, future
work will aim at showing the experimental implementation of this particular nonlinear
separation with proper preparative chromatographic columns.
Appendix A

Appendix
Bibliography


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