Rita Fonseca e Silva Tavares Ribeiro

Licenciatura Engenharia Química e Bioquímica

8-zone Simulated Moving Bed Chromatography for the separation of ternary mixtures

Dissertação para obtenção do Grau de Mestre em
Engenharia Química e Bioquímica

Orientador: José Paulo Mota, Prof. Catedrático Faculdade de Ciências e Tecnologia da Universidade Nova de Lisboa

Co-orientadores: Andreas Seidel-Morgenstern, Prof. Dr-Ing., Max Planck Institute for Dynamics of Complex Technical Systems

Francisco Vitor Santos da Silva,Dipl.-Ing., Max Planck Institute for Dynamics of Complex Technical Systems

FACULDADE DE CIÊNCIAS E TECNOLOGIA
UNIVERSIDADE NOVA DE LISBOA

Setembro, 2016

Acknowledgements

The work described here was carried out at the Max Planck Institute for Dynamics of Complex Technical Systems.
I would like to express my gratitude to the people that made the realisation of this thesis possible.

First and foremost I would like to thank my supervisors Prof. José Paulo Mota and Prof. Dr.-Ing. Andreas Seidel-Morgenstern for giving me the opportunity to work in the research group for preparative chromatography at Max Planck Institute for Dynamics of Complex Technical Systems. I am grateful for their kindness and support in the development of this project.

Secondly I wish to thank my second supervisor Francisco Vitor Santos da Silva for all his help and encouragement throughout this project. His dedication and willingness to help in difficulty were of great importance for the development of this thesis.
Furthermore I would like to thank Bettina Kattein and Rojiar Pishkari, whose work and cooperation greatly contributed for this project.
I am much obliged to Luise Borchert and Jacqueline Kaufmann for all their helpful instructions on how to best use the HPLC system.

I would also like to thank my friends Tiago Santos and João Chapa for their help and support while living in Magdeburg, and my former colleagues from Faculdade de Ciências e Tecnologias da Universidade de Lisboa: Sara Pereira, Tomás Monteiro and Diogo Mendes that encouraged me to take this opportunity of studying abroad.
Finally I would like to thank my family for believing in me and providing endless support throughout my life and for giving me the opportunity to pursue my goals.

Abstract

Simulated Moving Bed Chromatography is a well-established separation method. Its conventional system has proved its utility for the separation of binary mixtures. Since in industry one of the main challenges is to separate complex mixtures, several studies were conducted and novel structures have been proposed to address this problem, including the 8-zone Simulated Moving Bed.
This works' aim is to experimentally demonstrate the isolation of an intermediary eluting molecule out of a pseudo ternary mixture using 8-zone SMB.
This was performed through adsorption isotherm characterization, using multicomponent single column experiments, and subsequent model and design of the 8-zone SMB process for raffinate recycle configuration. This study was carried out for a mixture of food colorants: Tartrazine, Sunset Yellow, Crystal Ponceau (0,04g/L) and Fast Green (0,012g/L) .The respective solvent had a composition of 20% Ethanol and 80% Acetic Acid 70mmolar.

The analysis of the separation performance is achieved using spectroscopy and Lambert Beer Law to calculate each molecule's concentration at each outlet port.

Resumo

Cromatografia de leito móvel simulado (Simulated Moving Bed Chromatography) é um processo bem estabelecido na indústria química. A sua configuração clássica é bastante útil para a separação de misturas binárias. Sendo que um dos principais desafios na indústria química consiste na separação de misturas complexas, várias técnicas foram desenvolvidas no sentido de resolver este problema, incluindo a 8-zone Simulated Moving Bed.

Este trabalho tem como objetivo a demonstração experimental do isolamento de um composto intermediário a partir de uma mistura pseudo ternária usando 8-zone Simulated Moving Bed.

Isto foi conseguido através da caracterização de isotérmicas, e subsequente modelação matemática do processo para 8-zone SMB com configuração de reciclo do refinado. Este estudo foi realizado para uma mistura de corantes alimentares: Tartrazine, Sunset Yellow, Crystal Ponceau (0,04g/L) e Fast Green (0,012g/L) . O solvente utilizado possuía a seguinte composição: 20% Etanol e 80% Ácido Acético 70mmolar.

A análise da separação foi conseguida utilizando espectroscopia e a Lei de Lambert Beer para o cálculo das concentrações de cada composto em cada corrente de saída.

Nomenclature

Abbreviations

Abbreviations	Description
AR	Alura Red
CP	Crystal Ponceau
Exp	Experimental
FG	Fast Green
PB	Patent Blue
Sim	Simulation
SY	Sunset Yellow
TA	Tartrazine

Acronyms

Acronyms	Description
SMB	Simulated Moving Bed
TMB	True Moving Bed
UV	Ultra Violet

Latin symbols

Notation	Description	Units
A	Absorbance	AU
b	optical path length	cm
B	Calibration curve slope	
C	Concentration in liquid phase	g/L
E	Error	
e	molar absorptivity	$Lmol^{-1}\ cm^{-1}$
H	Henry coefficient	-
I_0	Intensity of incident light	-
I_t	Intensity of light transmitted	-
K	Product between molar	-

	absorptivity and optical path	
m	Flow rate dimensionless ratio	-
N	Total number of mixing cells	-
q	Concentration in the solid phase	g/L
t	Time	min
t_0	Columns dead time	min
t_R	Retention time	min
V_{ads}	Volume of solid phase	m^3
V_c	Column Volume	m^3
V_{int}	Interstitial volume	m^3
V_{pore}	Volume inside of the pores	m^3
$\dot{V}$	Volumetric flow rate	m^3/s

Subscript text

Subscript	Description
F1	Feed 1
R1	Raffinate 1
R2	Raffinate 2
E1	Extract 1
E2	Extract 2
S1	Solvent 1
S2	Solvent 2
ExtR	Extract Recycle
RafR	Raffinate Recycle
n	Number of molecules considered for concentration estimation method
J	Number of wavelengths
i	Molecule
w	Wavelength
J	One mixing cell
k	TMB/SMB zone
s	Solid phase
calc	Calculated

Greek Symbols

Greek letters	
α	Selectivity factor
ε_e	Extragranular pororsity
ε_e	Intragranular porosity
ε_t	Total porosity
σ_t	Second central moment
μ_t	First absolute moment
β	Dimensionless flow rate margin

List of Figures

List of Tables

Contents

1. Introduction

In most chemical processes in industry in order to produce the desired product a lot of side components are also produced. One of the challenges in chemical industry is to separate the desired product while meeting the needs for purity and productivity. Despite being considered an expensive process chromatography has been widely used in pharmaceutical and biotechnological industries to separate complex mixtures. One of the most recent developments in this technique was the Simulated Moving Bed process- a counter current chromatography process in which the liquid and the solid phase move in opposite directions, to maximize mass transfer driving force, and therefore provide a better separation. Its conventional configuration is composed by four zones, each of them with a specific function in the separation process.

1.1. Simulated Moving Bed Chromatography – State of the art

Since its introduction in 1961 the SMB technology has been mostly applied in petrochemical, pharmaceutical and sugar industries.

However the conventional four zones SMB cannot separate a multicomponent mixture in three different fractions which constitutes a major drawback.

In an attempt to solve this problem researchers have proposed several techniques to separate complex mixtures, such as ternary mixtures. One of the possibilities presented in[2] was the use of multiple types of adsorbents with different binding properties for the components to be separated. However in practice it is rather difficult to find suitable adsorbent to perform such a separation. Another possibility would be to use batch and continuous chromatography together [3] .

Some authors described a five zone system with side streams to collect the intermediate component of a mixture [4, 5].

Since a conventional SMB can successfully separate binary mixtures some authors studied the possibility of coupling of two independent four-zone SMB units [6, 7], while other authors suggested the use of cascades systems [8]. A complex installation of a nine zone SMB was suggested in [9].

The analysis of several moving bed configurations performances were characterized for the equivalent True Moving Bed process at the low solvent consumption point in [10]. A similar analysis was presented in [11] for ternary separation under linear conditions.

Recently the concept 8-zone SMB was studied and validated experimentally for a ternary mixture of cycloketones presented in [12] and proved to be capable of isolating intermediately eluting target compounds. Also regarding ternary separations a technique designed for 8-zone TMB with extract and raffinate recycle was presented in [13] for optimizing the operating conditions to maximize the processes' profit. However further investigation is required, namely the extension of the developed models and subsequent validation for more realistic cases of multi-component mixtures, such as pseudo ternary mixtures.

1.2. Objectives and thesis structure

The purpose of this work is to further contribute to the extension of the 8-zone SMB chromatography application for complex mixtures separations. More specifically this works' aim is to experimentally demonstrate the isolation of an intermediary eluting molecule out of a pseudo ternary mixture of food colorants using 8-zone SMB, with one column per zone.

The theoretical background necessary in further chapters is described in Chapter 2. This includes fundamental notions on chromatographic process, as well as detailed description of the True Moving Bed process design for pseudo ternary separations. Chapter 3 provides a description the experimental procedures, including information on chemical substances and equipment used in this work. The experimental results and discussion are included in Chapter 4, while major conclusions and further work are presented in Chapter 5.

2. Theoretical Background

2.1. Chromatographic process

Chromatography from the Greek "writing with colors" is a separation process born in the turn of the 19th century and was first named by M.S. Tswett in his work concerning separation of plant pigments in 1903.[14]

It's a separation process in which a mixture contacts a solid adsorbent and by the difference of affinity with the solid phase one can separate the mixture's components.

In preparative liquid chromatography the mixture (liquid phase) is forced through a column packed with the adsorbent (solid phase). The mixture components elute from the column according to their affinity with the adsorbent, as shown in figure 1 where the component will lower affinity, A will elute first and the component with higher affinity, B, will elute last.
The affinity of each compound with the stationary phase, or in other words the adsorption equilibrium, can described by its respective isotherm, the which relates the concentration of adsorbed compound in the solid phase with its concentration in the liquid phase. Knowing the components isotherms is key to understand and optimize a chromatographic separation process [1]

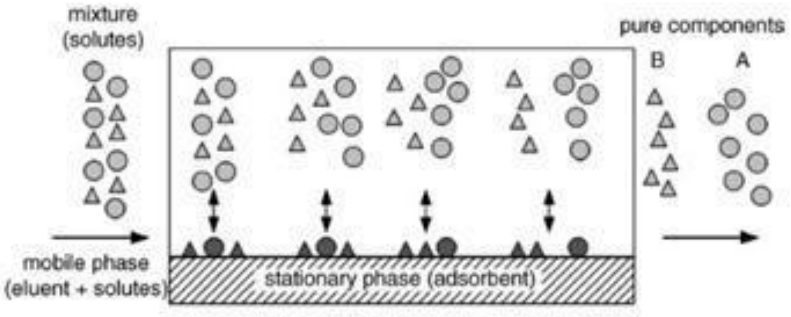


Figure 2. 1 - Principle of adsorption chromatography [1]

Fundamental relations

The retention time of specie corresponds to the time each component takes to elute from the column, and can be determined by analysis of the chromatogram.

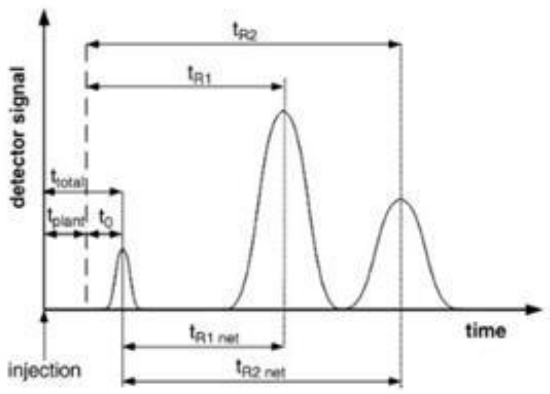


Figure 2. 2 - Chromatogram of two retained components [1]

The retention times for two components are showed in figure 2. To calculate them it is necessary to subtract the total dead volume of the system (capillaries, valves etc. and the columns dead volume). This volume can be determined by measuring how much time it takes for a component to elute from the system without the column t_{plant}.

It is also required to have a reference time, t_0 and this is commonly measured by injecting a molecule that should not interact with the adsorbent, but that has the same size as the molecules of interest, to be sure that they are not excluded from de adsorbent's pores. The relationship between the columns dead time and its total porosity can be described as[1, 15]:

$$\varepsilon_t = \frac{\dot{V} t_0}{V_c} \tag{2. 1}$$

Considering that the adsorbent consists of a series of spherical porous particles, the column will have two different porosities: the extragranular porosity, corresponding to the columns void fraction, and the intragranular porosity, that is the volume inside the spheres pores[1, 15]

- $\varepsilon_e = \frac{V_{int}}{V_c}$
- $\varepsilon_i = \frac{V_{pore}}{V_{ads}}$ (2. 2)

And these can be related with the total porosity as such:

$$\varepsilon_t = \varepsilon_i + (1 - \varepsilon_i)\,\varepsilon_e$$

2.2. Mathematical Modeling

Several models have been developed to describe chromatographic processes. Most of which were developed under the following assumptions:

- The column is radially homogeneous – volume and porosity remain constant
- Isothermal conditions
- Constant fluid velocity
- Absence of chemical reaction

The simplest model is the ideal equilibrium model. Several additional assumptions are made for this model[1, 15]:

- No axial dispersion
- Both phases are in equilibrium
- The column efficiency is infinite

$$\frac{\partial C_i}{\partial t} + \frac{1-\varepsilon}{\varepsilon}\frac{\partial q_i(C_i)}{\partial t} + u\frac{\partial C_i}{\partial z} = 0 \qquad (2.\ 3)$$

This model takes only into account the thermodynamics of the system ignoring axial dispersion influence, mass transfer and kinetic effects.

Mixing cell model

One can also describe a chromatographic column using a mixing cell model. This model, similar to the stirred tank cascades introduced by Martin and Synge (1941)[16], assumes that a column can be divided into several mixing cells, and that for each component in each cell both phases are in equilibrium and the isotherm is constant.

Considering N mixing cells each with a volume of $\frac{V_c}{N}$, and that the concentration inside the pores and at the surface of the adsorbent is the same: [1]:

$$\frac{V_c}{N}\left(\varepsilon_t\frac{\partial C_{i,k}}{\partial t} + (1-\varepsilon_t)\frac{\partial q_{i,k}(C_{i,k})}{\partial t}\right) = \dot{V}(C_{i,(k-1)} - C_{i,k}) \qquad (2.\ 4)$$

In this case, the most relevant parameter for describing transport phenomena is the number of cells, the column efficiency, for it accounts for the effects of mass transfer resistance and axial dispersion, responsible for the band broadening in chromatograms. In short the higher the number of cells the higher the columns efficiency will be. For this case the number of cells can be calculated from a chromatographic peak, using its first absolute moment and second central moment[1]:

$$N = \left(\frac{\mu_t}{\sigma_t}\right)^2 = \left(\frac{t_R}{\sigma_t}\right)^2 \quad (2.\ 5)$$

Since the second central moment (variance) is related to the width of a peak, for highly efficient columns the peak's width will be narrower, which means that the elution profiles will be closer to ideal.

2.3. Linear Chromatography

In the limiting case of nonlinear chromatography, when the concentration of solute in the liquid phase is directly proportional to the amount of solute in the solid phase, the process is considered linear. In this case the isotherm for a given substance is expressed as[1, 15]:

$$q_i = H_i C_i \quad (2.\ 6)$$

In which the equilibrium constant is the Henry coefficient. This parameter can be determined with the following expression:

$$H = \left(\frac{t_R}{t_0} - 1\right)\frac{\varepsilon_t}{1 - \varepsilon_t} \quad (2.\ 7)$$

A substance with a high Henry coefficient, in other words a stronger affinity with the adsorbent, will have a larger retention time. It can also be stated that in order to separate compounds from each other their Henry coefficients need to be different. The separation capability can be described by a selectivity factor α according to:[1, 15]

$$\alpha = \frac{H_B}{H_A} \qquad (2.\ 8)$$

Reversed phase chromatography

As opposed to normal phase chromatography, reversed phase chromatography simply means that the solid phase is hydrophobic, instead of hydrophilic. As the solid phase is hydrophobic it will capture hydrophobic particles and let the polar particles be eluted first. [1]

In this work the mobile phase will be composed by food colorants and a solvent composed of ethanol and a aqueous solution of acetic acid. This mobile phase will enter in contact with the solid phase which is C18 bonded silica (LiChroCART® 100-10 Merck kGaA)

2.4. Counter current chromatography

In 1971 D. Broughton first patented the Simulated Moving Bed process, a counter current chromatography process in which the liquid and the solid phase move in opposite directions, to maximize mass transfer driving force, and therefore provide a better separation.[17]

To best understand this concept it's best to start with its hypothetical equivalent – the True Moving Bed process.

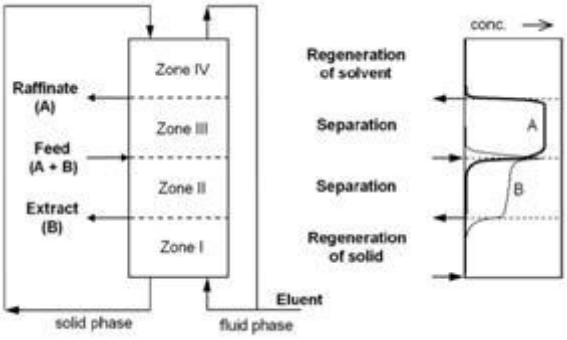


Figure 2. 3 - TMB scheme [4]

A true moving bed, in its simplest structure consists of one chromatographic column that works with the same counter current principle previously stated. This column is divided into four zones, each zone with a specific function. In figure 3 the feed port is between zones II and III, for the case of a binary mixture the

component with lowest affinity with the solid phase, A, will move upwards along with the liquid phase while the other, B, will move downwards along with the solid phase. Component A can therefore be collected in the raffinate port while component B, due to the inlet of fresh solvent in zone I will be collected in the extract port. This separation is ensured by the setting of adequate velocities for the liquid and solid phases.[11]

In practice the movement of the solid phase is very difficult to perform in such a way that it assures reproducibility in experiments. Furthermore pumping adsorbent particles would create solvent back mixing leading to a poor separation. To solve this problem the Simulated Moving Bed (SMB) was created, and instead of pumping the solid phase this one is divided into several columns. The columns movement is simulated by periodic switching the inlet and outlet ports in the direction of the liquid phase. By adjusting the switch time and flow rates it is possible to perform a complete separation of the components.

Both SMB and TMB are divided into four zones, each with a different function in the separation process:

- Zone I: between the solvent and extract ports where desorption of the more retained components takes place. Its flow is adjusted in order to desorb all components, which is why it's also referred to as the solid phase regeneration zone.
- Zone II: between the extract and the feed ports, where desorption of the less retained components takes place.
- Zone III: between the feed and raffinate ports, where adsorption of the more retained components takes place.
- Zone IV: between the raffinate and solvent ports, where adsorption of the less retained components takes place. Its flow is adjusted in order to adsorb all components to be sure that what comes out of this zone, and will enter zone I, is only pure solvent. Hence, this is also referred to as the liquid phase regeneration zone. [12]

Flow rate determination – triangle theory

To ensure that each component migrates in the correct direction one needs to consider the flow rates in each zone and determine which combination will give the expected result.

To determine the flow rates for each zone in the SMB it is more practical to discuss it in terms of flow rate ratios between the solid and liquid phase m [18] for each k zone as follows:

$$m_k = \frac{\dot{V}_k}{\dot{V}_s} \qquad (2.\ 9)$$

Where V_k the volumetric flow is rate in zone k and V_s is the volumetric flow rate in the solid phase.

Considering these ratios one can write the mass balance of the system:

- $m_{F1} = m_{III} - m_{II}$
- $m_E = m_I - m_{II}$
- $m_R = m_{III} - m_{IV}$
- $m_S = m_I - m_{IV}$

(2. 10)

From these equations it can be stated that for a given feed flow rate $m_{III} > m_{II}$ and for a given solvent flow rate $m_I > m_{IV}$.

Knowing the migration directions of each component, one can define borders for these ratios. For a given zone if a component moves along with the liquid phase the ratio for that zone is larger than the component's Henry coefficient. Conversely if a component moves along with the solid phase the ratio for that zone is smaller than the component's Henry coefficient. This can be converted into the following inequalities:

- $m_I > H_B$
- $H_A < m_{II} < H_B$
- $H_A < m_{III} < H_B$
- $m_{IV} < H_A$

(2. 11)

With these border inequalities, according to the triangle theory [18] it's possible to draw a graphic description of the process:

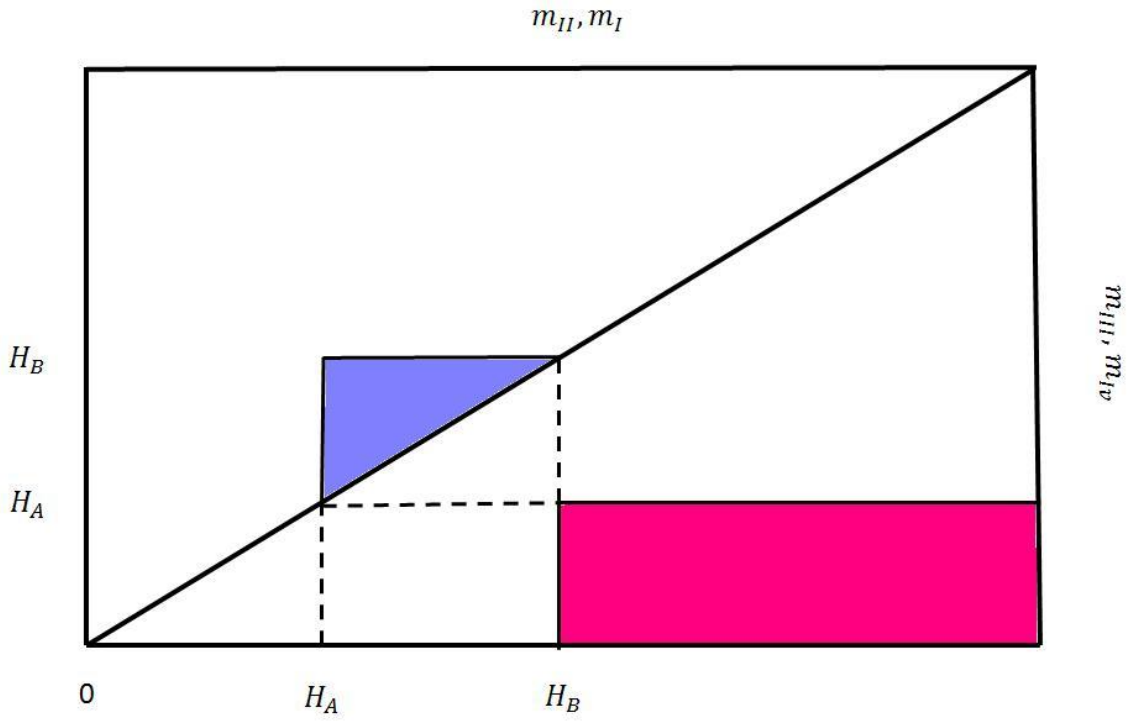


Figure 2. 4 - 4-zone TMB operating regions

As stated before the ratio in zone III needs to be larger that ratio in zone II hence one needs to consider only the area above the diagonal line $m_{III} = m_{II}$. Then considering the border inequalities one can draw the triangle which corresponds to the operating region for zones two and three. If one considers the borders for zones one and four the operating area corresponds to the red rectangle. The triangle theory provides a simple but efficient method to determine TMB operating regions.

2.5. Ternary separations

If one wants to separate a ternary mixture, with the elution order A,B,C where A is the least retained component and C is the most retained component $(H_A < H_B < H_C)$,the most obvious way to do it would be to have two four zone TMBs in cascade, in which the first one separates for example the light fraction A from B and C, and the second one would separate target molecule B from the heavy fraction C, recycling the first unit extract stream(figure 2.5). It could also be performed separating first the heavy fraction C and recycling the raffinate stream into the second unit.

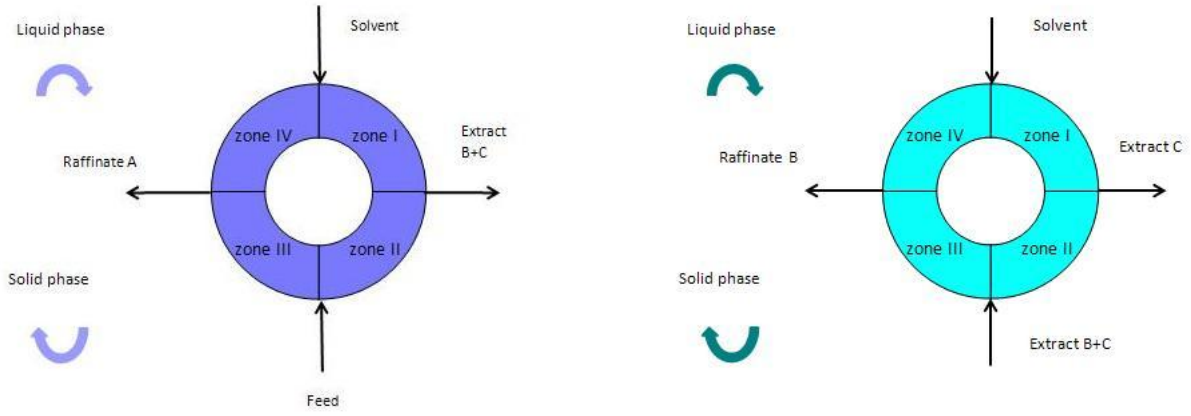


Figure 2. 5 - TMB 4 zone cascade: 1st subunit (left) and 2nd subunit (right)

Several studies [11, 13] state that the best way to operate this system would be to use a buffer tank between the two subunits, which would buffer the different switching times to ensure optimal performance.

To separate ternary mixtures it is also possible to join these two 4-zone TMB into one 8-zone TMB with internal recycle.

Although in this process it's no longer possible to choose independent switch times, it is more economical due to the fact that there's one less pump and since it only uses one rotary valve the dead volume is reduced significantly. [11, 13]

The 8-zone TMB can have two different configurations: extract recycle or raffinate recycle.

In the extract recycle configuration the component with the lowest henry coefficient is separated in the first subunit, while the two remaining components are recycled and separated in the second subunit.

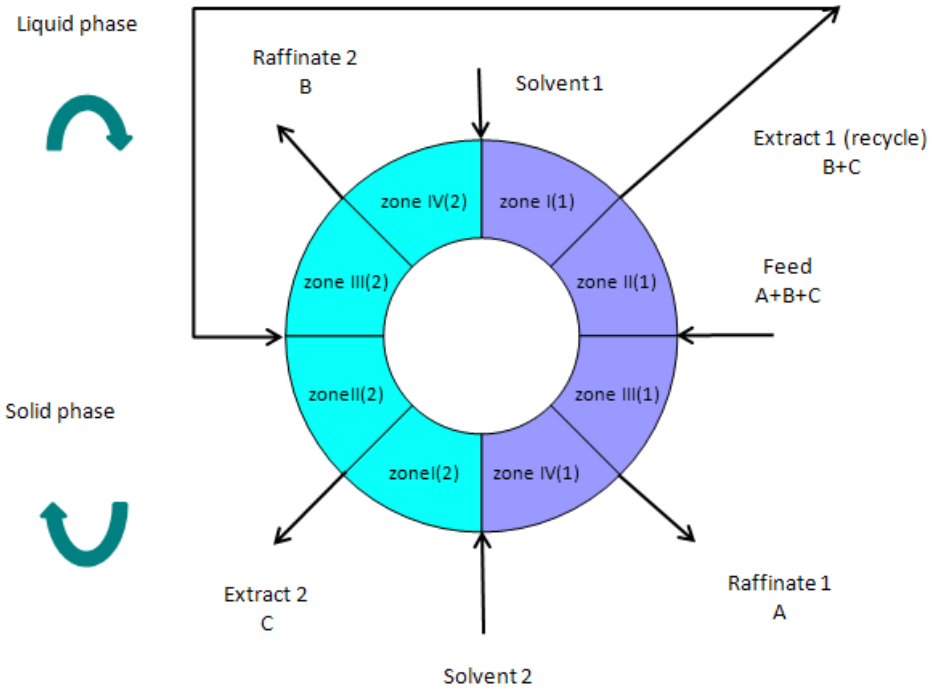


Figure 2. 6 - 8-zone TMB with extract recycle

On the raffinate recycle the opposite happens, that is as the component with the highest affinity with the adsorbent is separated first, and the others are recycled and separated in the second subunit

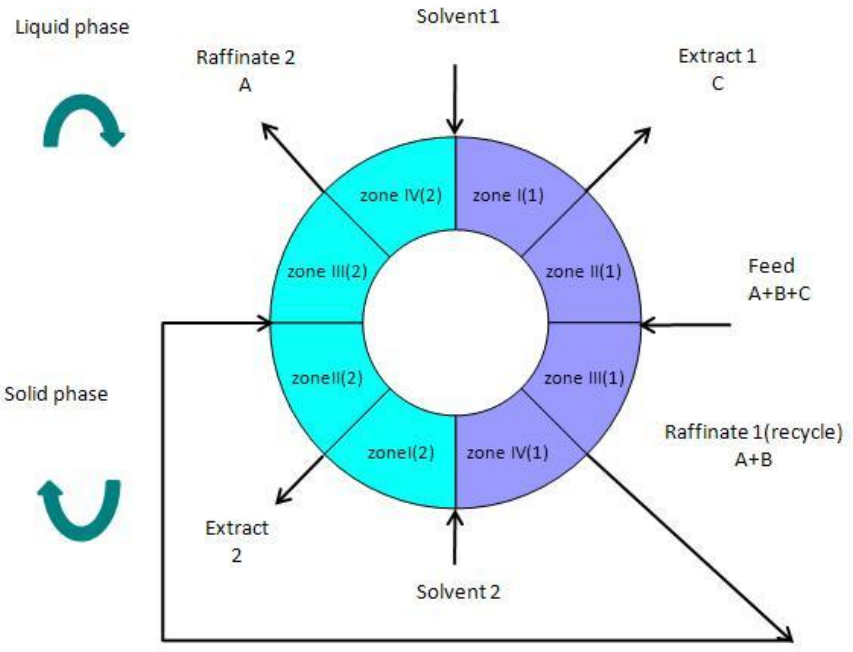


Figure 2. 7 - 8-zone TMB with raffinate recycle

Factors like the selectivity factor between the henry coefficients, the cost of solvent and purity requirements will determine which separation should be done first and therefore which configuration is more suitable.[13]

2.6. Design and modeling TMB

To design an TMB process it's necessary to determine the flow rates, V, in each zone, k, (V_k)

Considering an 8-zone TMB with extract recycle, for a pseudo ternary mixture with five components and elution order A,B,C,D,E where A is the least retained component and E is the most retained component ($H_A < H_B < H_C < H_D < H_E$) the system's mass balance would be [13]:

- $m_{F1} = m_{III(1)} - m_{II(1)}$
- $m_{R1} = m_{III(1)} - m_{IV(1)}$
- $m_{S2} = m_{I(2)} - m_{IV(1)}$
- $m_{E2} = m_{I(2)} - m_{II(2)}$
- $m_{R2} = m_{III(2)} - m_{IV(2)}$
- $m_{S1} = m_{I(1)} - m_{IV(2)}$ (2. 12)
- $m_{ExtR} = m_{I(1)} - m_{II(1)}$
- $m_{ExtR} = m_{III(2)} - m_{II(2)}$
- $m_{I(1)} = m_{II(1)} + m_{III(2)} - m_{II(2)}$

Considering the migration directions of the components:

- $m_{IV(1)} < H_A$
- $H_B < m_{III(1)} < H_C$
- $H_B < m_{II(1)} < H_C$
- $m_{I(1)} > H_E$ (2. 13)
- $m_{IV(2)} < H_C$
- $H_C < m_{III(2)} < H_D$
- $H_C < m_{II(2)} < H_D$

- $m_{I(2)} > H_E$

Using the triangle theory one can draw the respective graph for the system:

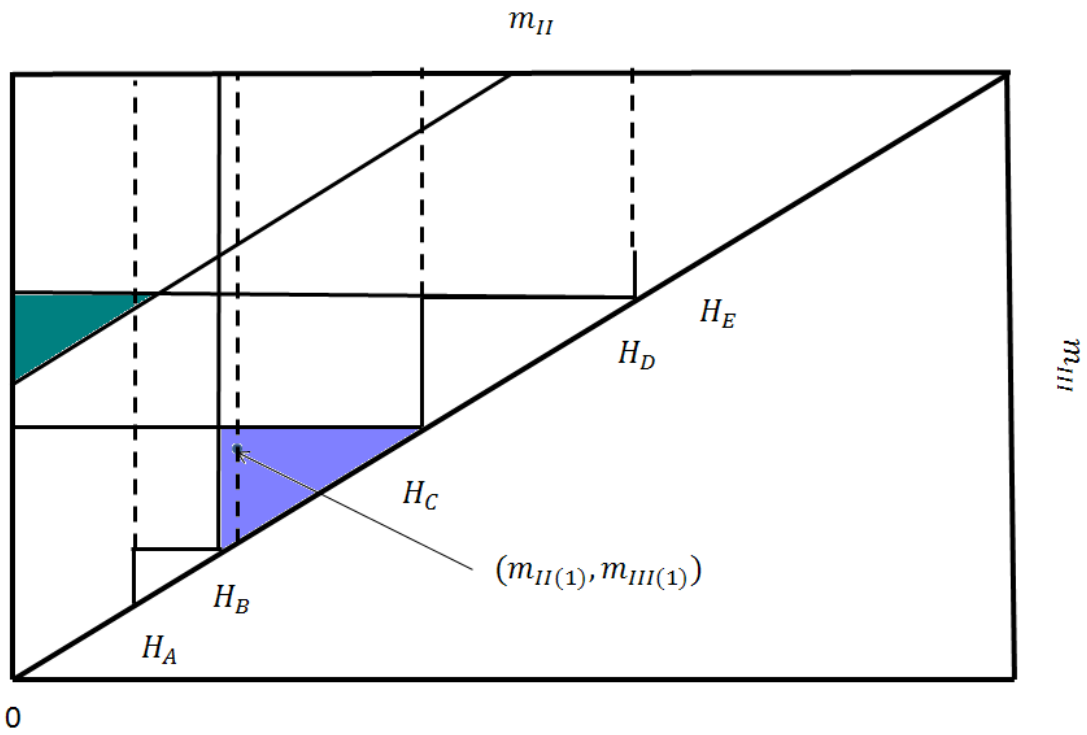


Figure 2. 8 - 8-zone TMB with extract recycle operating regions

As before the purple triangle corresponds to the operating region for zones II and III in this case for the first subunit.

Because there are now two TMB units there's a recycle stream that needs to be considered. Depending on the value chosen for $m_{II(1)}$ (dot on the graph) there will be a different setting. Rewriting the recycle equation with the lower boundary for $m_{I(1)}$ $m_{I(1)} = H_E$ it becomes [13]:

$$m_{III(2)} = m_{II(2)} + H_E - m_{II(1)} \quad (2.\ 14)$$

This corresponds to the green diagonal line on the graph. In order to respect the restriction that $m_I > H_E$ values for $m_{II(2)}$ and $m_{III(2)}$ need to be chosen above this line. As it's also need to respect that $H_B < m_{III} < H_C$ the operating region for zones II and III of the second subunit will correspond to the green trapeze area represented on the graph.

For the raffinate recycle there will be a similar process. For this case the mass balance becomes[13]:

- $m_{F1} = m_{III(1)} - m_{II(1)}$
- $m_{E1} = m_{I(1)} - m_{II(1)}$
- $m_{S1} = m_{I(1)} - m_{IV(2)}$
- $m_{R2} = m_{III(2)} - m_{IV(2)}$
- $m_{E2} = m_{I(2)} - m_{II(2)}$
- $m_{S2} = m_{I(2)} - m_{IV(1)}$
- $m_{RafR} = m_{III(1)} - m_{IV(1)}$
- $m_{RafR} = m_{III(2)} - m_{II(2)}$
- $m_{IV(1)} = m_{II(2)} + m_{III(1)} - m_{III(2)}$

(2. 15)

The ratio borders will then be:

- $m_{IV(1)} < H_A$
- $H_C < m_{III(1)} < H_D$
- $H_C < m_{II(1)} < H_D$
- $m_{I(1)} > H_E$
- $m_{IV(2)} < H_A$
- $H_B < m_{III(2)} < H_C$
- $H_B < m_{II(2)} < H_C$
- $m_{I(2)} > H_c$

(2. 16)

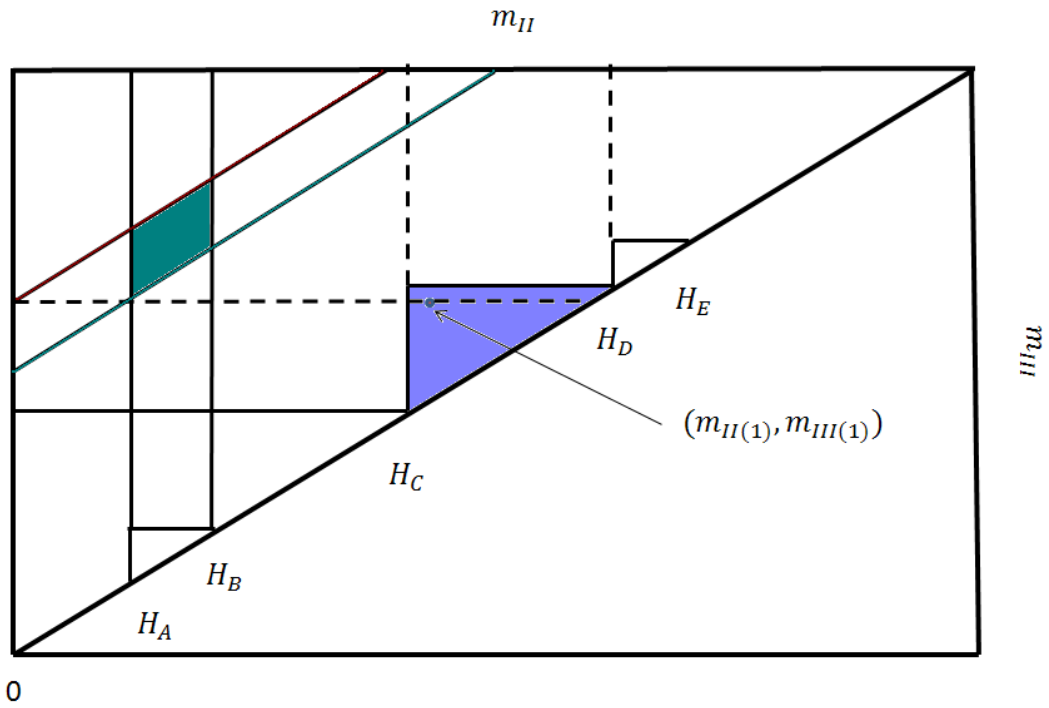


Figure 2. 9- 8-zone TMB with raffinate recycle operating regions

As in the extract recycle the recycle diagonal line depends on the value chosen for $m_{II(1)}$ (dot on the graph). If for the recycle equation one sets $m_{IV(1)} = H_A$ [13]:

$$m_{III(2)} = m_{II(2)} + m_{III(1)} - H_A \tag{2. 17}$$

For this configuration an extra condition is necessary because $m_{IV(1)}$ can't be smaller than zero, hence he following equation needs to be added to the chart [13]:

$$m_{III(2)} = m_{II(2)} + m_{III(1)} \tag{2. 18}$$

Equations 18 and 19 correspond respectively to the green and red lines on the graph. As $m_{IV} < H_A$ values for zones II and III need to be chosen between these two lines, within the green area on the graph.

Mathematical modeling - Mixing cell equilibrium model

The 8-zone SMB can be described by a stage model, or as series of mixing cells. The system can be divided into a series of mixing cells, in each cell the liquid and solid phase are in equilibrium. If one considers a column packed with adsorbent porous particles, in which the liquid and solid phases are in equilibrium the mass balance for one cell J is [19]:

$$C_{J-1}(t) = C_J(t) + \frac{t_0}{N}\frac{dC_J(t)}{dt} + \frac{(1-\varepsilon_e)}{\varepsilon_e}\frac{t_0}{N}\frac{dq_J(t)}{dt} \quad (2.\ 19)$$

Where N is the total number of cells, and q_J represents the concentration in the solid phase both outside and inside of the pores. As before, a high number of mixing cells will assure the lessening of broadening effects.

2.7. Food colorants

The molecules chosen for this study of pseudo-ternary mixtures separation are food colorants. These are organic molecules soluble in water and some are negatively charged. [20, 21] In practical terms when compared to other molecules used in similar works[22], food colorants present several advantages for this study because they're not toxic, not volatile which would cause problems in the collection of samples and yield calculation, and absorb both in UV and visible light which makes their identification and detection easier. Furthermore they are colorful allowing a closer follow up of the process, since it's possible to see the molecules through the systems plastic capillaries and the outlets of the system.

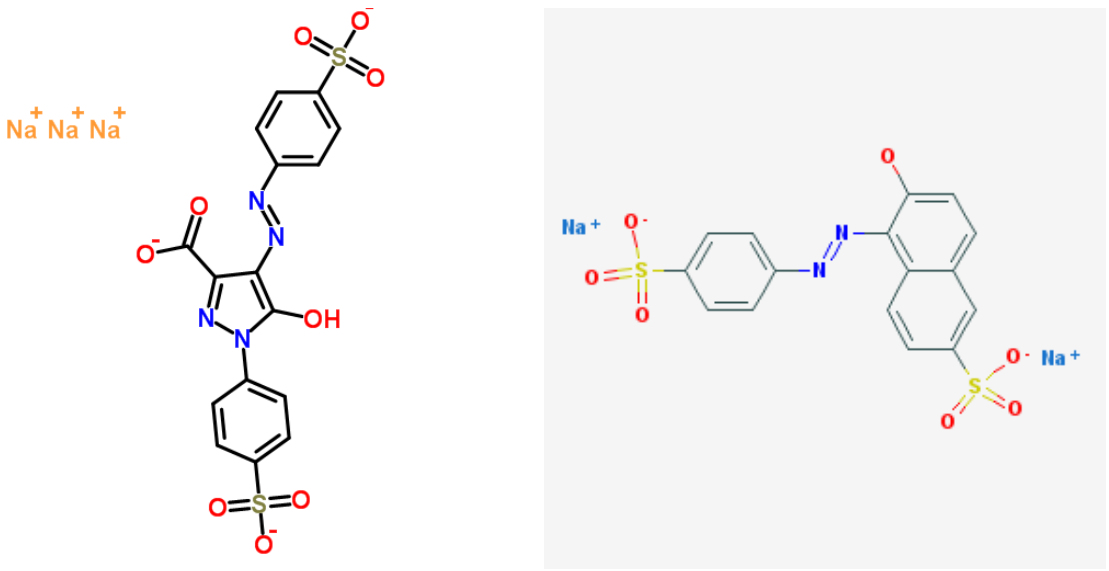


Figure 2. 10 - Food colorants Tartrazine (on the left) and Sunset Yellow (on the right)

2.8. Spectroscopy

In chromatography the study of the molecules absorption spectra is important as it provides information on which range of wavelengths (UV or visible light) one should measure to obtain a clearer chromatogram.

The absorption spectra analysis is also useful in the calculation of the process outlet stream concentrations, using the Lambert Beer Law that states that the relationship between the intensity of incident light I_0 and the intensity of transmitted light I_t is as follows[23]:

$$\log\left(\frac{I_0}{I_t}\right) = A = ebC \qquad (2.\ 20)$$

Where A is the absorbance, which is directly proportional to: the substance concentration, C, the length of the measuring cell, b, and the molar absorption coefficient or extinction coefficient e.

This law is only valid if the following conditions are met[24]:

- The concentrations must be low
- The light used must be monochromatic
- The solution must be homogeneous and non-fluorescent
- There can be no photochemical reactions
- The solute cannot form variable associations with the solvent

3. Experimental Procedure

3.1. Chemicals

This work's goal is to study the separation of a pseudo ternary mixture of food colorants. All the substances used in this work are listed in table 2.1.

Table 3. 1 - Chemicals and respective manufactures

Designation	Brand/Manufacture
Tartrazine	Alfa Aesar
Sunset Yellow FCF (90% dye content)	Sigma Aldrich
Alura Red AC (80% dye content)	Sigma Aldrich
Crystal Ponceau 6R	Sigma Aldrich
Fast Green FCF, Electrophoresis Reagent	Alfa Aesar
Patent Blue VF	Abcam Biochemicals®
Ethanol Absolute (>99,7%)	VWR Chemicals
Acetic Acid 100%	Carl Roth
Blue Dextran	Sigma Aldrich

3.2. Outlet concentration measurement – Spectroscopy

Spectroscopy was used to estimate the SMB outlet concentrations by measure the samples' absorbance.

The spectra were measured using a spectrophotometer Genesys and precision cells Hellma Quartz Suprasil 100-QS 10mm. The baseline was set using the solvent.

The samples spectra were measured within the visible light range (400-800nm). The spectra were measured in 5nm steps.

3.2.1. Concentration Estimation Method

To make this calculation first a calibration curve is required. To do this five concentration values were chosen for each molecule so that the maximum absorbance wouldn't be higher than 3AU. The absorbances were measured for these concentrations and calibration curves were built using the Lambert Beer's Law [23, 24]

For each wavelength there were five calibration curves, one for each molecule, and each of them can be written as:

$$A_i(w_j) = C_i K_i(w_j) \tag{3. 1}$$

$w_1 = 400$

$w_j = 800$

$\Delta j = 5$

Where A is the absorbance for a wavelength w, .C is the concentration of molecule i, and K is the product of the optical path length and molar absorptivity. The index j represents the wavelength in question. The range of wavelengths used for this method was the visible light range (400-800 nm) with 5nm interval between each measurement.

Since all calibration curves are linear and independent of each other they can be added. Rewriting the equation it becomes:

$$A_{total}(w_j) = \sum_{i=1}^{n=5} C_i K_i(w_j) \tag{3. 2}$$

Written in vector form the measured absorption spectra can be written as:

$$A = \begin{bmatrix} A_{total}(w_1) \\ \dots \\ A_{total}(w_j) \end{bmatrix} \tag{3. 3}$$

And the respective wavelengths:

$$W = \begin{bmatrix} w_1 \\ \dots \\ w_j \end{bmatrix} \tag{3. 4}$$

For each food colorant i the slopes of the respective calibration curves

$$B_i = \begin{bmatrix} K_{i,1} \\ \dots \\ K_{i,j} \end{bmatrix} \tag{3. 5}$$

Joining all slopes:

$$B = \begin{bmatrix} K_{1,1} \dots & K_{i,1} \\ \dots & \\ K_{1,j} \dots & K_{i,j} \end{bmatrix} \quad (3.\ 6)$$

The concentration can then be calculated solving the following equation in order to C:

$$A = B * C$$

This calculation was performed recurring to a Matlab function - lsqnonneg.[25].This function calculates the concentration by minimizing the associated error using the least squares method. Through this method the error E is defined as the squared difference between the measured or real absorbance A and the calculated absorbance $(B * C_j)$:

$$E = \sum_{n=1}^{N} \left(A - (B * C_i)\right)^2 \quad (3.\ 7)$$

The program then returns the concentrations vector:

$$C = \begin{bmatrix} 1 \\ \dots \\ i \end{bmatrix} \quad (3.\ 8)$$

3.3. Isotherms determination –Equipment

The isotherms were determined using an HPLC system (Agilent Technologies 1260). This system comprises a binary pump, a degasser, an autosampler, a column compartment with temperature control, and a UV detector (fig 2.1). For all experiments the flow rate was set to 2mL/min. Due to the columns dimensions the temperature control was not possible.

3.4. Isotherms determination –Method

A sample for a given molecule is prepared for a certain concentration. This sample is then filtered, using a cellulose nitrate filter with pore size 0,2 μm (Sartorius Biolab Products)and a vacuum pump. Both sample and solvent are ultrasonically degassed (Elmasonic 550R) to make sure that no air enters the column.

The isotherms were determined using the dynamic method of frontal analysis. This method based on the determination of breakthrough curve times for different initial concentrations[26].
Writing the mass balance for a column the following expression is obtained:

$$\dot{V}C_2t_b - \dot{V}C_1t_b = V_LC_2 + V_Sq(C_2)$$

$$q_2 = \left(\frac{t_b}{t_0} - 1\right)\left(\frac{(C_2 - C_1)}{F}\right) + q_1 \quad (3.\ 9)$$

Knowing the breakthrough times one can calculate the concentration in the solid phase, and plot it along the liquid concentration

To get a full isotherm one needs different breakthrough times for different solute concentrations. Using the HPLC gradient elution system a method was developed so that one could have several steps in only one experiment.

Table 3. 2 - Isotherms step method

Time	%Solvent	%Sample
0	100	0
20	87,5	12,5
40	75	25
60	62,5	37,5
80	50	50
100	37,5	62,5
120	25	75
140	12,5	87,5
160	0	100

This way the experiment starts with 100% solvent and 0% of sample and progressively increases the amount of sample, every twenty minutes, creating eight steps, and therefore eight points in the isotherm.

For each sample a different wavelength was set according to their adsorption spectra maximum. The reference wavelength was set to 800nm for at this wavelength none of the molecules absorbs.

3.5. SMB

3.5.1. System Equipment

The SMB unit is composed of 16 column positions and nine 16 position switching valves (Valco Intruments Co,Inc) to control the flow rates direction. Each of these valves is associated with an inlet or outlet stream (feed, solvent, raffinate and extract ports) and will select one of 16 dead-end streams (SD), directing the flow through the valve to the correct column. The valves maximum operating pressure is 3800 PSI (aprox, 260 bar). The valves position is set by the computer program.

The system has 6 HPLC isocratic pumps from Agilent Technologies, were some are from 1200 series (1,2 and 3 in fig 2.2) and others from 1100 series (4,5,6, and 7 in fig 2.2). The feed is impelled by a quaternary pump equipped with a degasser from Agilent Technologies 1200 series. All pumps can provide flow rates between 0,01mL/min and 5mL/min and maximal pressure should not exceed 200 bar. The system is also equipped with a two way valve (Valco Intruments Co,Inc) which allows two configurations: open or closed loop, the first one lets the outlet stream of IV(1) go to waste and the latter recycles it into zone I(2).

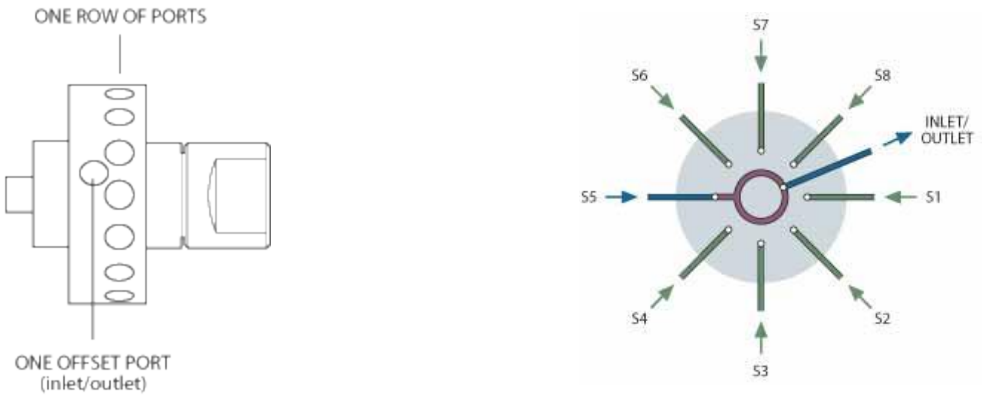


Figure 3. 1 - Valco position switching valves

3.5.2. Methods: Dead volumes

In order to model the 8 zone SMB unit it is necessary to calculate the dead volume of the system, or the volume without columns.

The system is set so that it can operate with 16 columns. Since in this case only 8 will be used there is a larger part of dead volume. More specifically to make a correct model one needs to know how much dead volume exists per column, and volumes of the recycle streams, for the extract and the raffinate. For all experiments the configuration of the SMB unit was set as presented in figure 3.2.

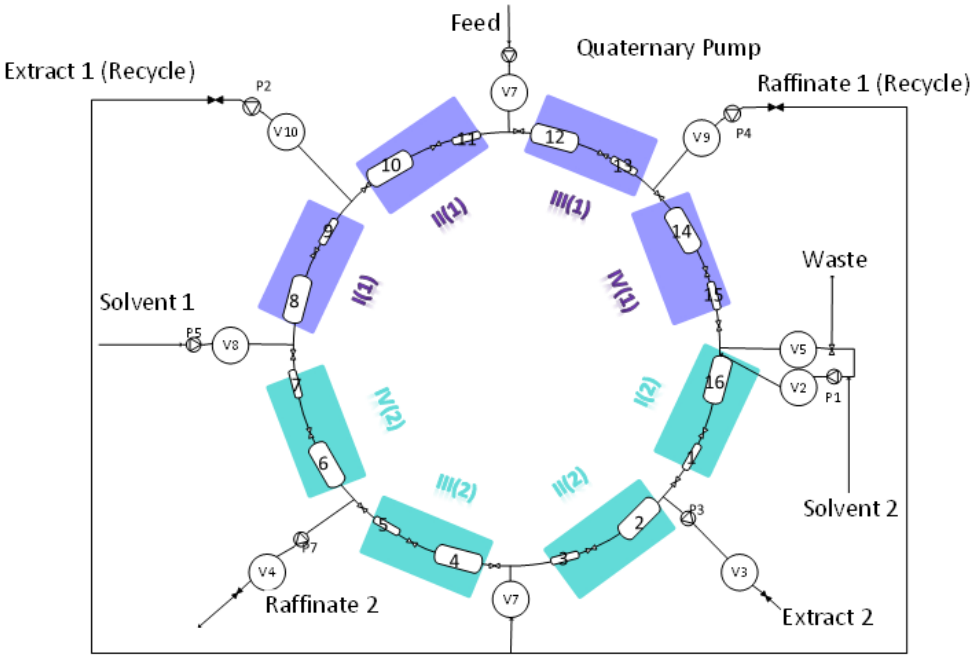
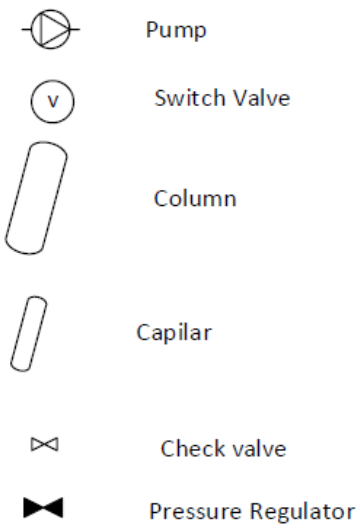


Figure 3. 2 - SMB scheme



For these experiments two food colorants solutions were used: Fast Green (0,01g/L), and Alura Red (0,2g/L). These concentrations were chosen so that one could see the solution passing through the plastic capillary. The solvent used was Ethanol 99, 7%.

To calculate the dead volumes several measurements were made, using Fast Green solution in the feed stream and Alura Red on the Solvent 1 stream, for each measurement was chosen a flow rate for the respective pump while all others were set to 0mL/min.

With this method these set of volumes was measured:

1. just before column 12 to waste /zones III(1) and IV(1)
2. just before column 8 to waste / first subunit
3. just after column 9 to just after column 4 / Extract recycle
4. just after column 13 to just after column 4 / Raffinate recycle
5. just after column 10 to just after column 6 / zones I(1) and IV(2)
6. just after column 14 to just after column 1 / zones IV(1) and I(2)

Measurements 1, 2 and 5 were done to calculate the dead volume per column, 3 and 4 for the recycle streams, and 6 for the volume between valves 5 and 2.

For the first two, after setting the pump flow rate, the solvent that was still in the system is collected until the food colorant starts to come out in the waste, and this way one knows this volume.
For the remaining measurements the collection starts when the food colorant solution reaches the respective exit position, and its volume is measured. Also a capillary was used to connect the food colorant solution to each entry port.
Because when making these measurements there is still solvent in the system the color will appear more diluted and progressively more concentrated which makes it difficult to be sure of how long it takes for the colorful solution to fill said volume. As such an air bubble can be forced into the capillary, to separate the food colorant from the solvent.

For each experiment the real flow rate was measured to make sure that the pumps were working correctly.

4. Results and Discussion

4.1. Spectroscopy

4.1.1. Wavelengths Choice

In order to get a clear chromatogram it is necessary to choose the right wavelength and for that the spectra of all molecules at 0,01g/L concentration was measured from 190 to 800 nm with 1nm interval between each measurement. (Figure 4.1)

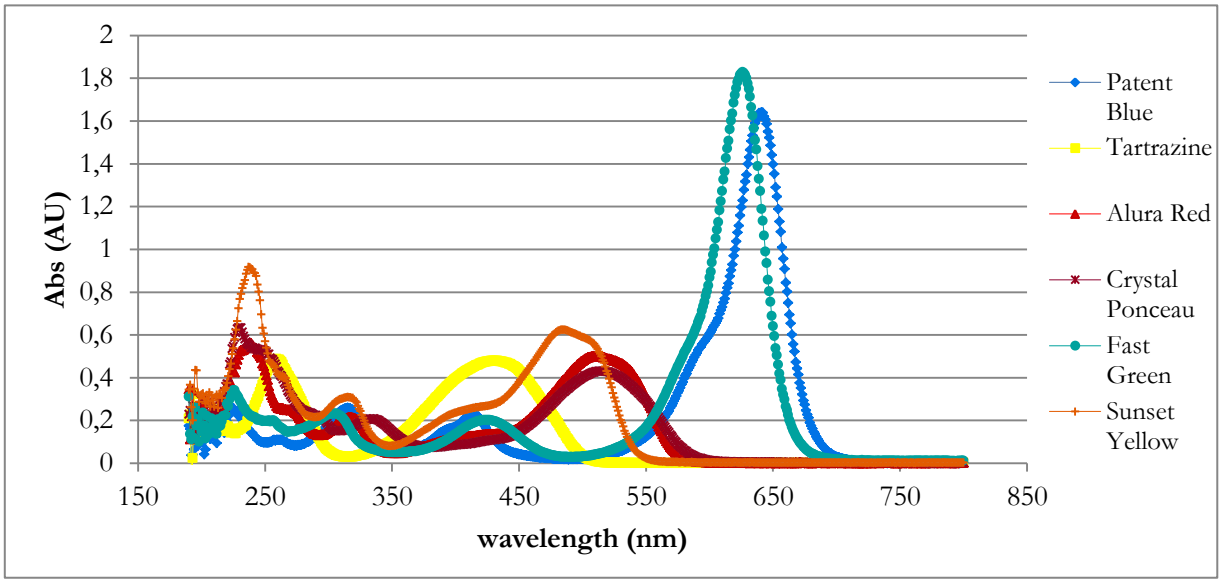


Figure 4. 1 - Spectra Overlap

This experiment had two goals:

To know for which wavelength range is it possible to analyze a mixture of food colorants while being able to tell them apart; And to be able to choose the best wavelengths for the isotherm measurements. The spectra overlap considerably, particularly in the UV region (190-400) where often the molecules have more than one absorption peak. This makes molecule identification impossible based on the UV spectra and therefore in the experiments to estimate isotherms and to measure outlet concentrations through spectroscopy, the absorbance measurements were made only within the visible light range (400-800).

This way, provided that one choses the proper wavelength, it is possible to perform isotherm estimation experiments with mixtures instead of single component experiments which are time consuming and don't show possible interactions between the molecules' adsorption.

4.1.2. Calibration Surfaces

To measure outlet concentrations of the SMB system the linear regression method was used. This was done only for five of the molecules, since the goal is to separate a pseudo ternary mixture. These

molecules were chosen based on the chromatographic experiments of mixtures described further. For the calibration method the following concentrations were chosen:

Table 4. 1 - Concentrations chosen for calibration

Molecule	Concentration g/L
Tartrazine	0,008
	0,016
	0,024
	0,032
	0,04
Sunset Yellow	0,008
	0,016
	0,024
	0,032
	0,04
Crystal Ponceau	0,008
	0,016
	0,024
	0,032
	0,04
Fast Green	0,0024
	0,0048
	0,0072
	0,0096
	0,012
Patent Blue	0,003
	0,006
	0,009
	0,012
	0,015

And the following calibration surfaces were obtained:

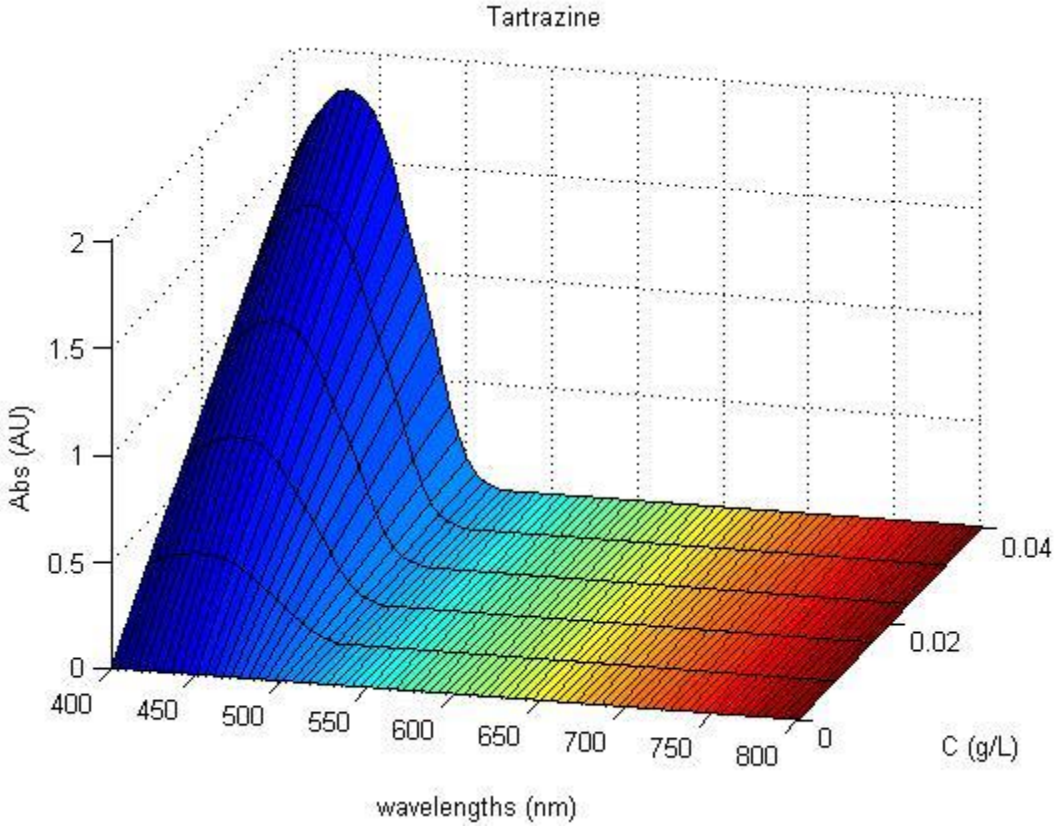


Figure 4. 2 - Tartrazine Calibration Surface

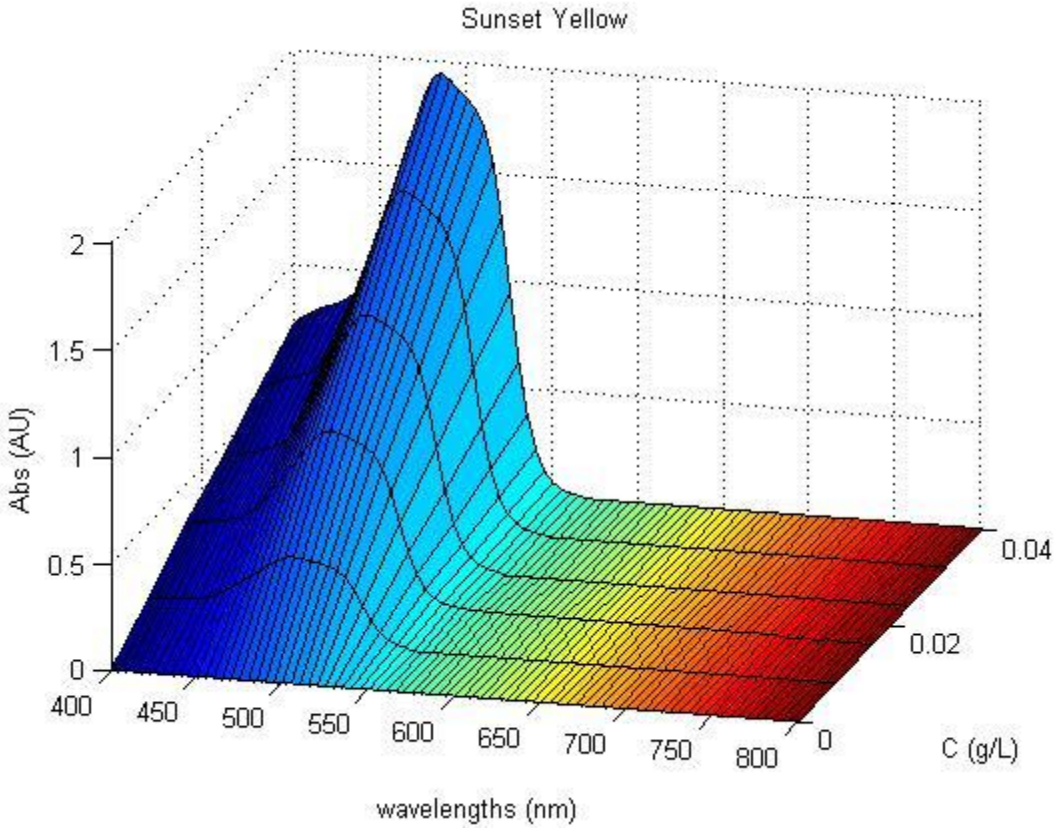


Figure 4. 3 - Sunset Yellow Calibration Surface

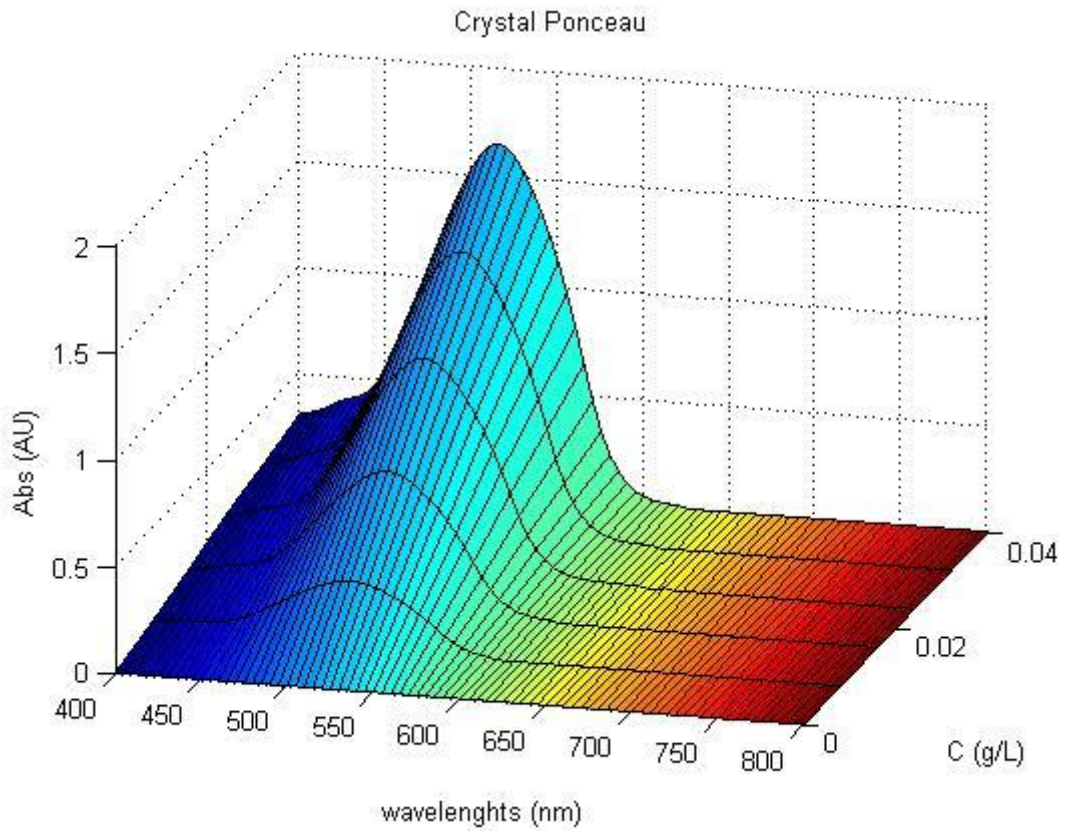


Figure 4. 5 - Crystal Ponceau Calibration Surface

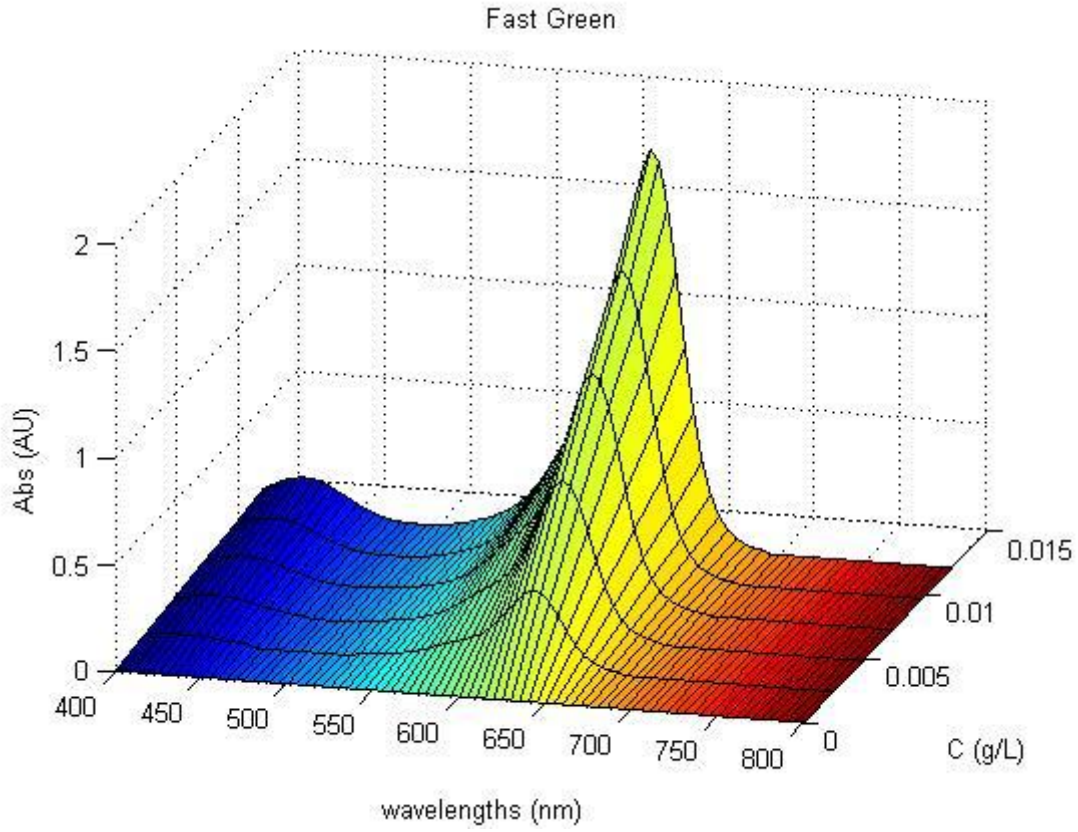


Figure 4. 4 - Fast Green Calibration Surface

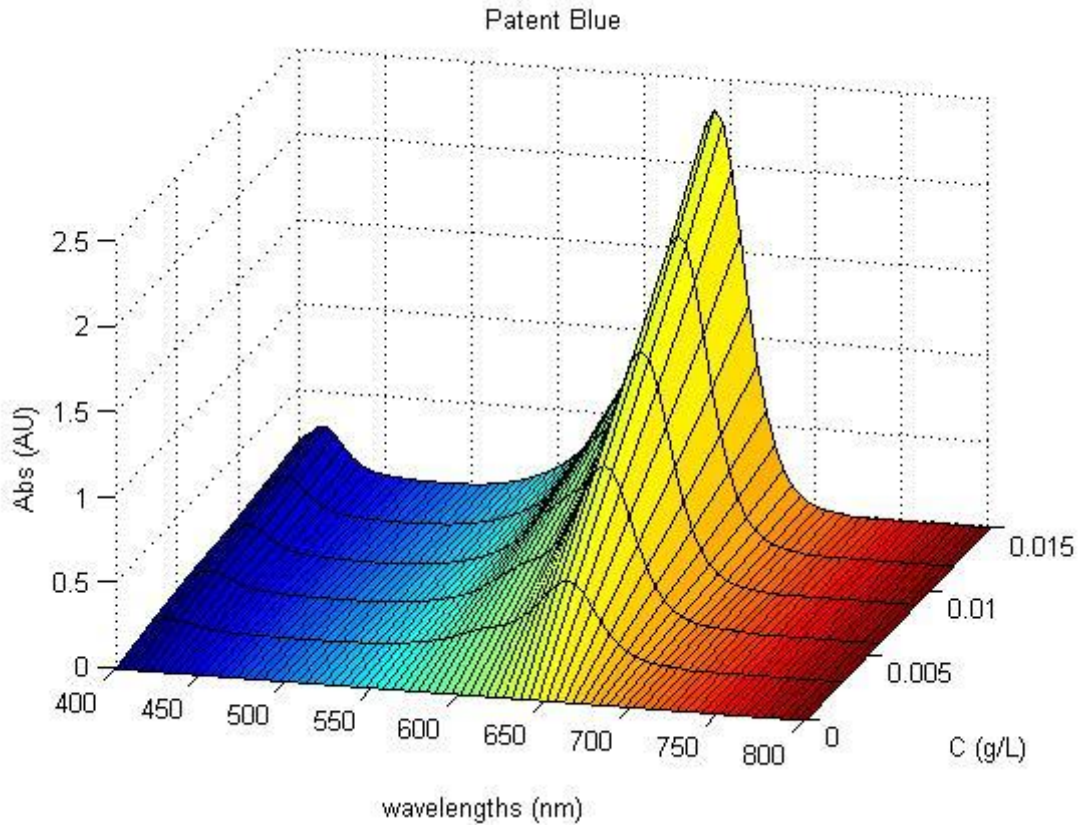


Figure 4. 6 - Patent Blue Calibration Surface

In all of them, the Lambert Beer Law applies, as the absorbance relationship with the concentration is linear. At first the concentrations for the calibration were chosen so that the maximum absorbance wouldn't be higher than 3AU since this is the maximum absorbance possible. To make sure that the resulting calibration curves, as well as the isotherms, were linear the calibration was remade for low concentrations, with maximum being 1mg/L. However it was discovered that for such low concentrations the absorbance values were too close to the detectors' noise making it impossible to calculate the concentrations with certainty. So ultimately the calibration curves were made for a slightly lower concentration than the original setting, with their maximum absorbance set for 2AU instead of 3AU.

4.1.3. Concentration Estimation Method Test

To test the concentration calculation method described previously a series of experiments were made. In this experiments a mixture of food colorants was prepared, each of them with the maximum concentration chosen for the respective calibration curves. The solvent composition was 20%Ethanol and 80% Acetic Acid 70mmolar. From this mother mixture the remaining solutions were prepared in which one of the food colorants was considerably more concentrated than the others: for solutions 1 to 4 one of the molecules is twice more concentrated than the others and for solutions 5 to 8 one of the molecules is five times more concentrated than the others. This was meant to simulate possible results from the SMB experiments, and confirm that the method can calculate concentrations accurately.

The other experiments in which the original mixture was diluted five and ten times were made to test the method for lower concentrations.

The results are summarized in table 4.2

Table 4. 2 – Concentration Estimation Method Test Results

Mixtures	Molecule	Real Concentration (g/L)	Calculated Concentration (g/L)	Error
Mixture	Tartrazine	0,04	0,0408	2%
	Sunset Yellow	0,04	0,0402	0%
	Crystal Ponceau	0,04	0,0413	3%
	Fast Green	0,012	0,0125	4%
5 times diluted	Tartrazine	0,008	0,0085	6%
	Sunset Yellow	0,008	0,0086	8%
	Crystal Ponceau	0,008	0,0083	4%
	Fast Green	0,0024	0,0026	8%
10 times diluted	Tartrazine	0,004	0,0042	5%
	Sunset Yellow	0,004	0,0042	5%
	Crystal Ponceau	0,004	0,0042	5%
	Fast Green	0,0012	0,0013	8%
Solution 1	Tartrazine	0,04	0,0404	1%
	Sunset Yellow	0,02	0,0211	6%
	Crystal Ponceau	0,02	0,0205	3%
	Fast Green	0,006	0,0063	5%
Solution 2	Tartrazine	0,02	0,0208	4%
	Sunset Yellow	0,04	0,0442	11%
	Crystal Ponceau	0,02	0,0204	2%
	Fast Green	0,006	0,0063	5%
Solution 3	Tartrazine	0,02	0,0208	4%
	Sunset Yellow	0,02	0,0211	6%
	Crystal Ponceau	0,04	0,0429	7%
	Fast Green	0,006	0,0063	5%
Solution 4	Tartrazine	0,02	0,0208	4%
	Sunset Yellow	0,02	0,0212	6%
	Crystal Ponceau	0,02	0,0205	3%
	Fast Green	0,012	0,0128	7%

Solution 5	Tartrazine	0,04	0,0419	5%
	Sunset Yellow	0,008	0,0081	1%
	Crystal Ponceau	0,008	0,0081	1%
	Fast Green	0,0024	0,0025	4%
Solution 6	Tartrazine	0,008	0,008	0%
	Sunset Yellow	0,04	0,0413	3%
	Crystal Ponceau	0,008	0,0077	4%
	Fast Green	0,0024	0,0024	0%
Solution 7	Tartrazine	0,008	0,008	0%
	Sunset Yellow	0,008	0,0084	5%
	Crystal Ponceau	0,04	0,0415	4%
	Fast Green	0,0024	0,0025	4%
Solution 8	Tartrazine	0,008	0,0085	6%
	Sunset Yellow	0,008	0,0086	8%
	Crystal Ponceau	0,008	0,0083	4%
	Fast Green	0,012	0,0125	4%

In table 4.2 the calculated values for the concentration are showed along with the respective error calculated as such:

$$\sqrt{\left(\frac{C_{calc} - C_{real}}{C_{real}}\right)^2} * 100 \qquad (4.\ 1)$$

These calculations have a small associated error, the highest being 11%. However this method has its limitations as it is only valid for a small range of concentrations – for lower concentrations absorbance values are too close to the detectors noise and the calculation no longer work, and for higher concentrations the light gets blocked causing the relationship between concentration and absorption to be nonlinear. Still it proved to be a practical and effective method. Furthermore it was not as time consuming as other methods such the ones that rely on the HPLC system.

To get further confirmation on the methods reliability, an inverse calculation was performed using the calculated concentrations to estimate the absorbance, which would then be compared with the measured absorbance. For a reference pair of values for absorption and concentration used in the calibration curve for a molecule i, the Lambert Beer Law can be written as:

$$A_{iref}(w_j) = C_{iref} K_i(w_j) \quad (4.\ 2)$$

For the calculated values it becomes:

$$A_{icalc}(w_j) = C_{icalc} K_i(w_j) \quad (4.\ 3)$$

Since the slope K is the same for both equations it is possible to write:

$$A_{icalc}(w_j) = \frac{A_{iref}(w_j)}{C_{iref}} * C_{icalc} \quad (4.\ 4)$$

Using equation 3.11 the absorbance was calculated and compared to measured one in figure 4.7.

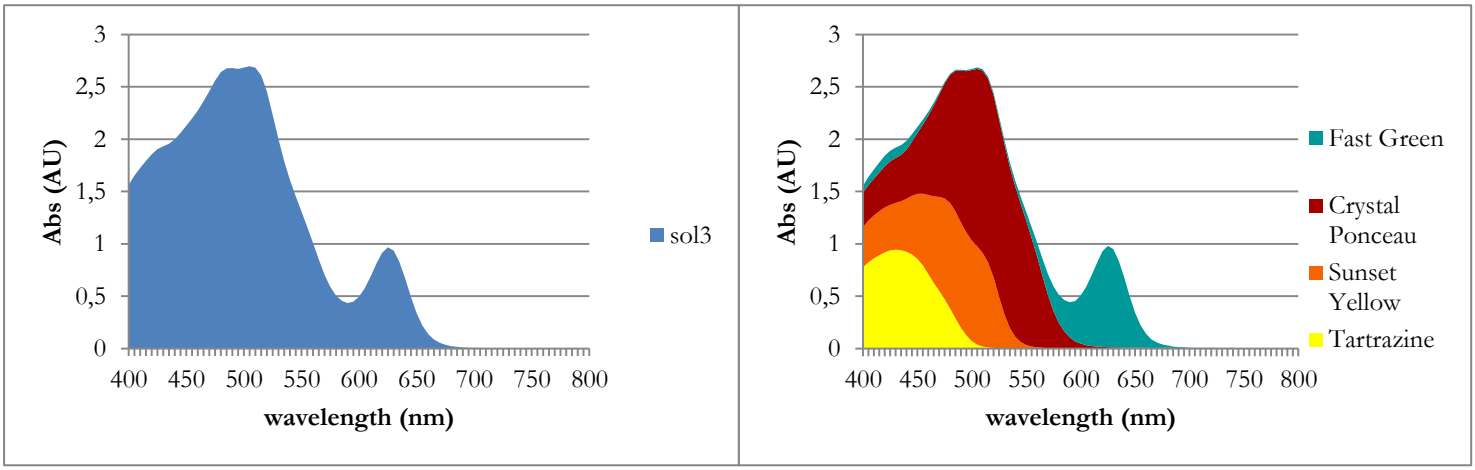


Figure 4. 7 - Measured spectrum of solution 3 (left) and deconvoluted spectrum calculated using the method described (right)

In this figure Lambert Beer's Law additive property can be confirmed.

4.2. Isotherm Estimation

4.2.1. Dead times and porosity

In order to estimate isotherms, dead times of the system and column need to be determined. Since some of the molecules are sterically excluded from the pores of the particles it was decided to consider the external porosity, using Blue Dextran as a reference molecule to measure t_0 . Since for the design of SMB experiments the porosity is one auxiliary term used for the calculation of the retention times, it is only important to consider the same porosity for all further calculations.

The porosity was measured for only four of the columns to be used in the SMB experiments due to time containments. The results below correspond to the average measurements for these parameters which were then used for further calculations.

Table 4. 3 - Dead times and porosity

t_{BD} (min)	2,351
t_{plant} (min)	1,112
t_0 (min)	1,239
ε_e	0,316
Column Volume (mL)	7,853

4.2.2. Retention Times

For the isotherm estimation multicomponent experiments were conducted. For each food colorant a different wavelength was chosen according to their absorption maxima. In this experiment it was used a mixture of Tartrazine, Sunset Yellow, Crystal Ponceau and Fast Green, in which the first three components had a concentration of 0,04g/L and the latter had a concentration of 0,012g/L. Based on figure 4.1 four wavelengths were chosen such that the identification of each molecule was easier. The chromatograms are presented in figure 4.8

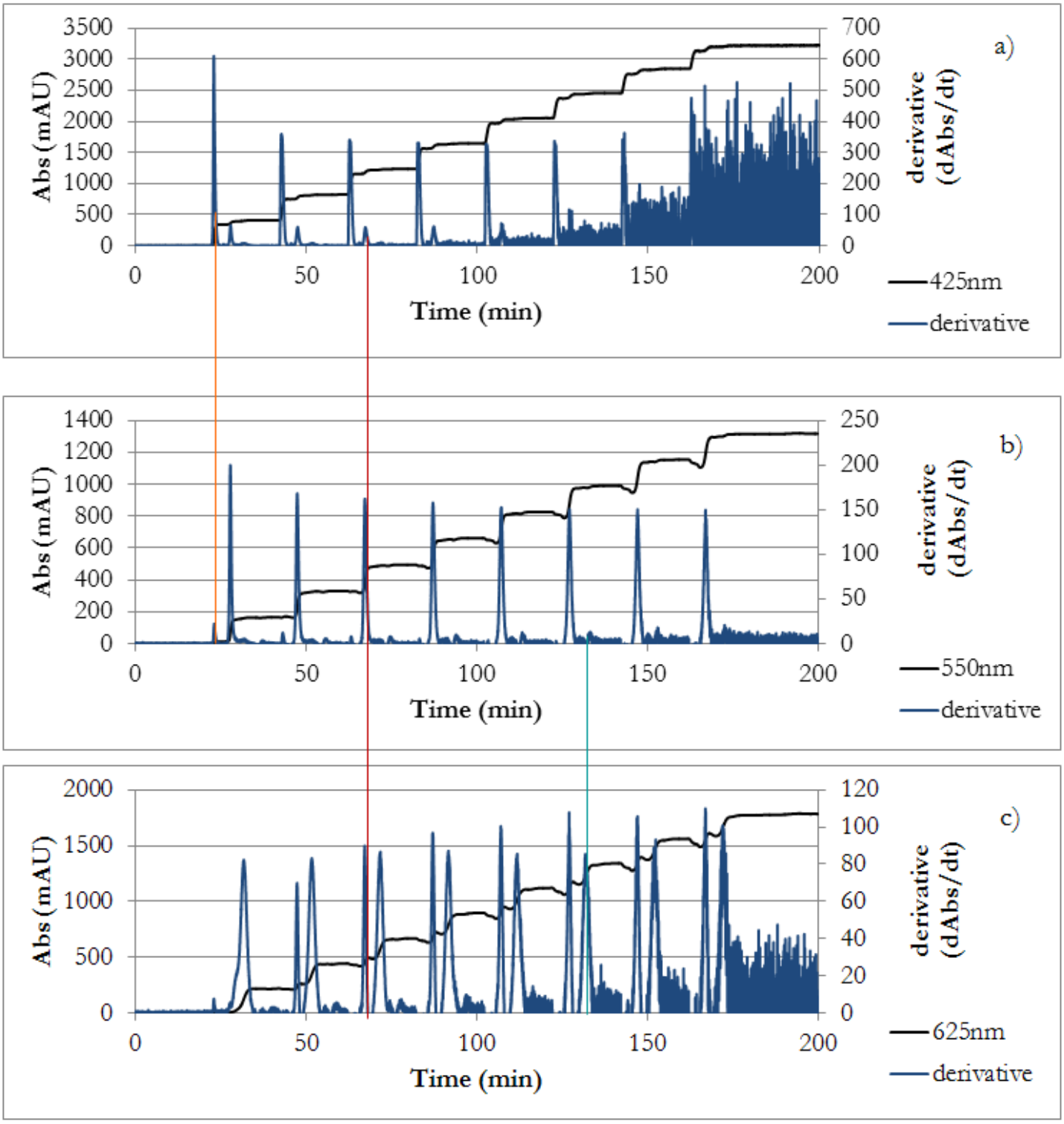


Figure 4. 8 - Breakthrough curves and retention times

In picture 4.8 three chromatograms are presented, in which the black line corresponds to the breakthrough curve and the blue line corresponds to its derivative on time. In a typical breakthrough graph for a single molecule there are several steps equally spaced, and the retention times would be obtained through the curves' inflection points. In this experiment with four components, because all molecules absorb in the visible light range all molecules breakthrough curves are visible in all wavelengths. So for each molecule a wavelength was chosen at which it absorbs more than the others, so that they can be told apart in the chromatogram.

In figure 4.8 a) the wavelength chosen 425nm, which according to figure 3.1 Tartrazine is the molecule that absorbs the most. When plotting the time derivative each inflection point is shown by peak. Since Tartrazine absorbs more in this wavelength the highest step corresponds to Tartrazine's retention time (orange line). Comparing chromatogram a) with b) one can still see a peak in the same region as the one of the previous graph. This is because Tartrazine also absorbs light at 550nm but absorbs much less than Crystal Ponceau, the molecule that absorbs the most at this wavelength. In graph b) the largest step corresponds to the red line which corresponds to a smaller step in graph c). It is also possible to see Fast Greens' retention time in graphs b) and c) because this molecule also absorbs in these wavelengths, but considerably more at 625nm.
In short based on these graphs it can be said that the molecules absorb independently of each other and that their isotherms are linear. It is important to state that these results were obtain through a single experiment, which is an advantage as it allows for several solvent compositions tests in a shorter amount of time when compared to single component experiments.

The resulting isotherms, calculated according to equation 3.8 (chapter 3.4) are shown in figure 4.9.

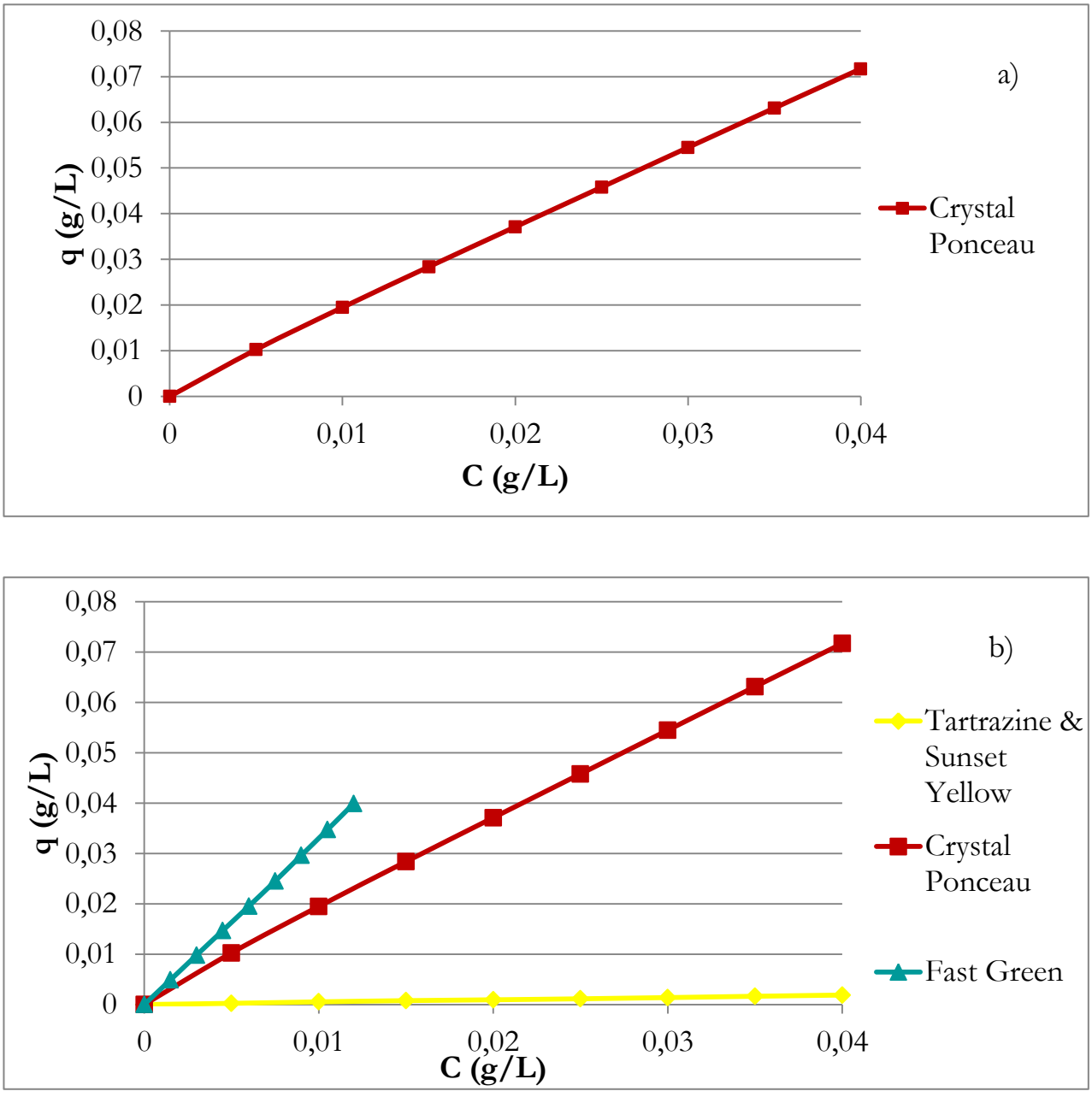


Figure 4. 9 – a) Crystal Ponceau Isotherm for 20% Ethanol b) Isotherms for Tartrazine, Sunset Yellow, Crystal Ponceau and Fast Green for 20% Ethanol

Table 4. 4 - Henry coefficients for figure 4.9 isotherms

Molecule	H_i	R
Tartrazine	0,047	0,997
Sunset Yellow	0,047	0,997
Crystal Ponceau	1,818	0,999
Fast Green	3,299	0,999

Table 4. 5 - Selectivity Factors for Table 4.4 Henry coefficients

$\alpha_{j,i}$	
$\alpha_{SY,TA}$	1
$\alpha_{CP,SY}$	38,617
$\alpha_{FG,CP}$	1,815

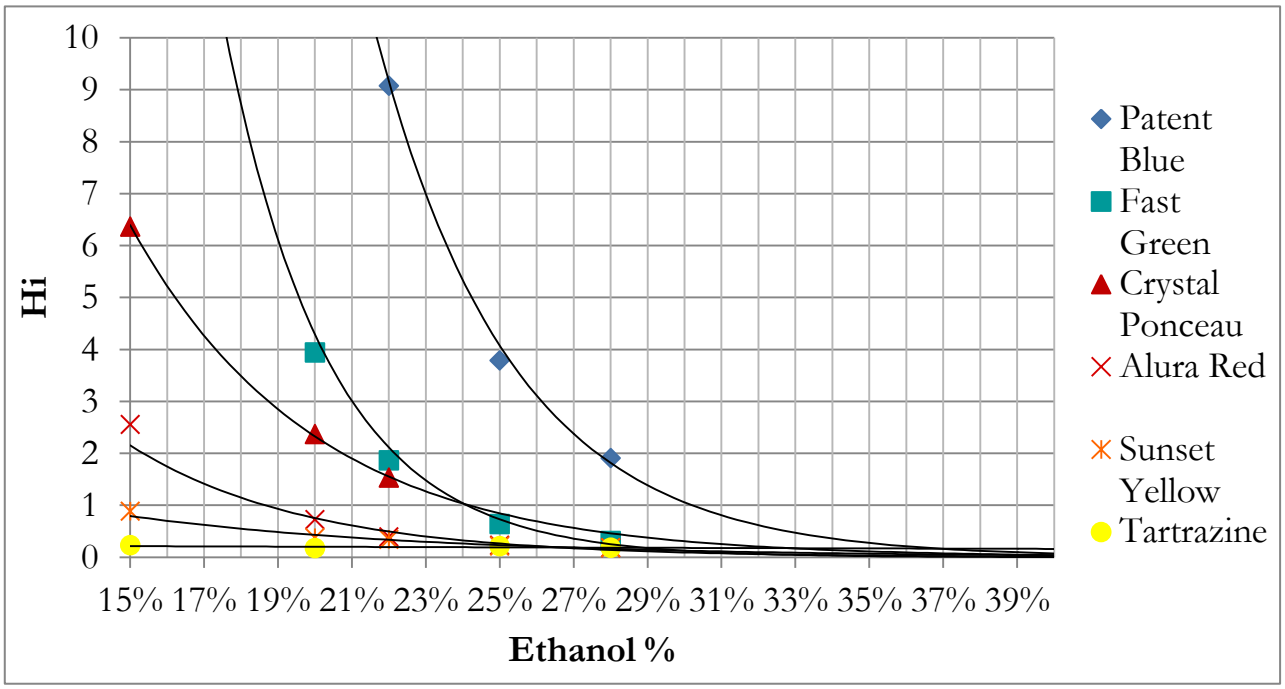


Figure 4. 10 - Henry coefficients vs Ethanol percentage

Through the isotherm calculation it can be confirmed the previous conclusion that they are linear. Also the Henry coefficients are fairly separated from each other which makes the separation possible. There can be a mixture separated in three fractions with Crystal Ponceau being the target molecule, Tartrazine and Sunset Yellow as the weakly adsorbing fraction and Fast Green the strong absorbing fraction.

In order to choose the amount of ethanol that allows successful SMB separation, different solvent compositions were chosen according to figure 4.10.

Analyzing figure 4.10 it is clear that there are no five molecules that would be separated in to three fractions as intended. For high ethanol concentrations the Henry coefficients are too close to each other, making the separation impossible. For lower percentages Henry coefficients of Patent Blue and Fast Green start to become too high for SMB experiments. The higher the Henry coefficients are the longer the molecule will take to elute. This means that to elute the molecule either a very high flow rate has to be set, meaning higher pressure drop, or higher switching times, which would cause the experiment to last too long. Furthermore, Tartrazine, Sunset Yellow and Alura Red have very close Henry coefficients, which implies that it is not be possible to separate them.
Even though there can be no five molecule mixture with the desired characteristics it is still possible to separate a four molecule mixture in three fractions. Because all isotherms are linear and the molecules do not interfere with each other's' adsorption a separate experiment with Patent Blue alone can be done with a solvent composition of 25% Ethanol. If Patent Blue would elute in the correct port it would prove that a pseudo ternary separation is possible to perform using 8 zones SMB.
Based on this chart the following settings were chosen for the SMB experiments:

- Solvent: 20% Ethanol and 80% Acetic Acid 70 mM
- Feed: a mixture of Tartrazine, Sunset Yellow, Crystal Ponceau (0,04g/L) and Fast Green (0,012g/L)

4.3. SMB

Before conducting SMB experiments it is necessary to measure the systems' dead volume.

4.3.1. Dead Volumes Measurement

The volumes measured are presented in the following table:

Table 4. 6 - SMB Measured Volumes

Experiments	$V_{C12-waste}$	$V_{C8-waste}$	$V_{Extract\ Recycle}$	$V_{Raffinate\ Recycle}$	V_{C10-C6}	V_{C14-C1}
Volume (mL)	3,5	5	3,75	3,75	4,5	8
Time (min)	5,97	4,55	1,67	1,73	2,08	4,38
Flow rate set (mL/min)	0,5	1	2	2	2	2
Measured Flow rate (mL/min)	0,58	1,09	2,25	2,16	2,16	1,83

With these measurements it was possible to calculate the volume per column, which was needed to perform the simulation. The dead volume per column corresponds to the dead volume per zone, so to calculate it the volumes of the first two measurements are subtracted and divided it by two (figure 3.2)

Table 4. 7 - Volume per Column Calculation

	Zone I (1)	Zone II (1)	Zone III (1)	Zone IV (1)	Zone I (2)	Zone II (2)	Zone III (2)	Zone IV (2)
$V_{C12-waste}$								
$V_{C8-waste}$								
V_{C8-C12}								
$V_{per\ column}$								

Knowing the dead volume per column the volume between valves 2 and 5 can be calculated:

$$V_{val5-val2} = V_6 - (2 * V_{per\ column}) \quad (4.5)$$

The results are summarized in table 4.8

Table 4. 8 - SMB Dead Volumes

Volumes (mL)	$V_{per\ column}$	$V_{val5-val2}$	Extract Recycle	Raffinate Recycle	Total
	0,75	6,5	3,75	3,75	20

These results are important because they provide a better understanding of the SMB system. However the most relevant one is the dead volume per column that will be included in further calculations.

4.3.2. Flow Rate Choice

The next step is to calculate the necessary flow rates for the separation to occur.
Because the SMB system is built for 16 column positions and only eight will be used this dead volume needs to be taken into account. The triangle theory that enables the calculation of the flow rates is made for TMB in which there are no extra dead volumes because of the hypothetical continuous movement of the solid phase. So before the flow rate calculation the Henry coefficients have to be corrected to include the delay caused by the dead volumes. Considering the difference between the corrected Henry coefficient $H'$and the uncorrected one H , rewetting equation 2.7 yields:

$$H' - H = \frac{\varepsilon}{1-\varepsilon}\left(\frac{t_R' - t_R}{t_0}\right) \qquad (4.\ 6)$$

Considering that $t_R' - t_R = \frac{0,75}{2}$ the corrected Henry coefficients for all molecules are:

Table 4. 9 - Corrected Henry coefficients

Molecules	H_i
Tartrazine	0,187
Sunset Yellow	0,187
Crystal Ponceau	1,957
Fast Green	3,334
Patent Blue	3,439

Using these Henry coefficients in table 4.9 and the triangle theory mentioned chapter 2.6 the flow rates were calculated for Raffinate recycle configuration

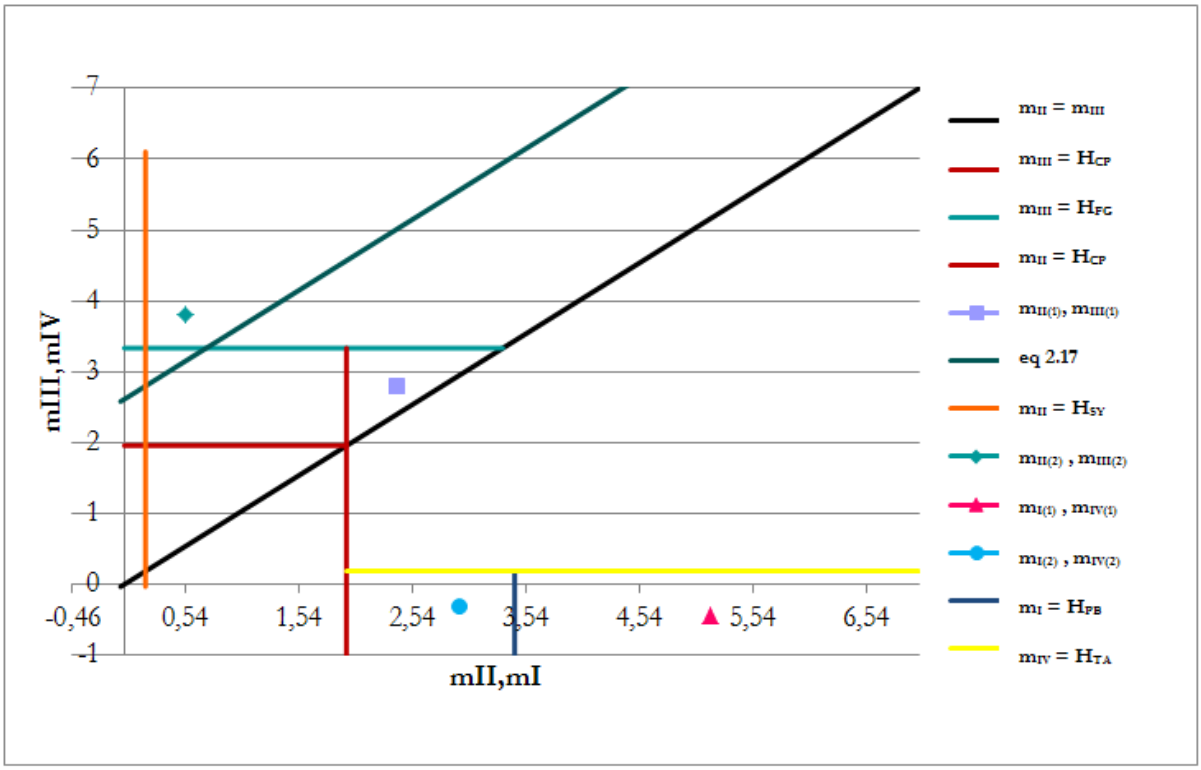


Figure 4. 11 - Triangle method for SMB experiment with Raffinate Recycle

Using the method described in chapter 2.6 the dimensionless ratios $m_{II(1)}, m_{III(1)}, m_{II(2)},$ and $m_{III(2)}$ were chosen from the graph and using equation 2.15 the ratio $m_{IV(1)}$ was determined. However the extra condition that states that $m_{IV(1)}$ cannot be smaller than zero was not considered. The respective flow rate for this zone was deliberately set to zero, and therefore its ratio has the minimum possible value, to prevent the fastest molecules that should come out in the raffinate 1 port to be recycled from going into the second subunit. This minimum value can be determined using the following equation

$$m_{minimum} = -\frac{\varepsilon_e}{1-\varepsilon_e} \qquad (4.\ 7)$$

For this case $m_{minimum} = -0{,}46$

To determine the remaining ratios it's necessary to attribute margins, β to the inequalities that define the system [27] :

- $m_{I(1)} = \beta H_{TA}$
- $m_{I(2)} = \beta H_{CP}$
- $m_{IV(2)} = \frac{H_{TA}}{\beta}$

(4. 8)

Usually the value chosen for β is the same for every ratio. However for this experiment in order for the ratios to be within the desired values they had to be chosen separately.

Table 4. 10 - Margins for dimensionless ratios

Dimensionless Ratios	β
$\boldsymbol{m_{I(1)}}$	1,499
$\boldsymbol{m_{I(2)}}$	1,500
$\boldsymbol{m_{IV(2)}}$	-0,599

Since all these calculations are made for a TMB process when calculating the flow rates for SMB a correction needs to be made. From equation 1.9 the flow rate for a zone k in TMB would be:

$$\dot{V}_k^{TMB} = m_k * \dot{V}_s^{TMB} \quad (4.\ 9)$$

Correcting it for the SMB it becomes:

$$\dot{V}_k^{SMB} = m_k * \dot{V}_s^{TMB} + \frac{\varepsilon V_c}{t^*} \quad (4.\ 10)$$

Where t^* corresponds to the switch time. For this experiment, t^* was set to 6,7min so that steady state was reached within a reasonable amount of time. The calculated flow rates are presented in table 4.11.

Table 4. 11 – Estimated Flow Rates for SMB experiment with Raffinate Recycle

Dimensionless ratios		Flow rates (mL/min)	
$m_{I(1)}$	5,158	$\dot{V}_{I(1)}$	4,508
$m_{II(1)}$	2,399	$\dot{V}_{II(1)}$	2,295
$m_{III(1)}$	2,799	$\dot{V}_{III(1)}$	2,616
$m_{IV(1)}$	-0,461	$\dot{V}_{IV(1)}$	0
$m_{I(2)}$	2,937	$\dot{V}_{I(2)}$	2,726
$m_{II(2)}$	0,538	$\dot{V}_{II(2)}$	0,802
$m_{III(2)}$	3,799	$\dot{V}_{III(2)}$	3,418
$m_{IV(2)}$	-0,311	$\dot{V}_{IV(2)}$	0,12
m_{D1}	5,469	$\dot{V}_{D1}$	3,847
m_{E1}	2,759	$\dot{V}_{E1}$	1,497
m_{F1}	0,400	$\dot{V}_{F1}$	0,347
$\dot{V}_{R1}$	3,261	$\dot{V}_{R1}$	2,601
m_{D2}	3,398	$\dot{V}_{D2}$	2,606
m_{E2}	2,398	$\dot{V}_{E2}$	1,513
m_{F2}	3,261	$\dot{V}_{F2}$	2,601
m_{R2}	4,111	$\dot{V}_{R2}$	3,564

4.3.3. Simulation

To test these settings a simulation was prepared using Chromworks™2016. The model chosen was the mixing cell model for linear isotherms with the henry coefficients from table 4.4., considering a dead volume per column of 0,75mL, external porosity of 0,316 and 200 plates per column [22]

The following results were obtained:

Table 4. 12 – Simulation Results for Raffinate 2

Molecule	M (mg)	Purity (%)	Recovery (%)	Productivity(mg/mL/h)	C (g/L)
Tartrazine	0,688	49,997	99,907	0,012	0,004
Sunset Yellow	0,688	49,997	99,907	0,012	0,004
Crystal Ponceau	0	0,005	0,009	0	0
Fast Green	0	0	0,002	0	0
Patent Blue	0	0,001	0,007	0	0
Total	1,375	100	54,374	0,024	0,008

Table 4. 13 - Simulation Results for Extract 2

Molecule	M (mg)	Purity (%)	Recovery (%)	Productivity(mg/mL/h)	C (g/L)
Tartrazine	0	0	0	0	0
Sunset Yellow	0	0	0	0	0
Crystal Ponceau	0,686	99,986	99,726	0,012	0,007
Fast Green	0	0,003	0,009	0	0
Patent Blue	0	0,011	0,029	0	0
Total	0,686	100	27,140	0,012	0,007

Table 4. 14 - Simulation Results for Extract 1

Molecule	M (mg)	Purity (%)	Recovery (%)	Productivity(mg/mL/h)	C (g/L)
Tartrazine	0	0	0	0	0
Sunset Yellow	0	0	0	0	0
Crystal Ponceau	0	0,007	0,005	0	0
Fast Green	0,207	44,434	100,380	0,004	0,002
Patent Blue	0,259	55,558	100,410	0,005	0,002
Total	0,466	100	18,442	0,008	0,004

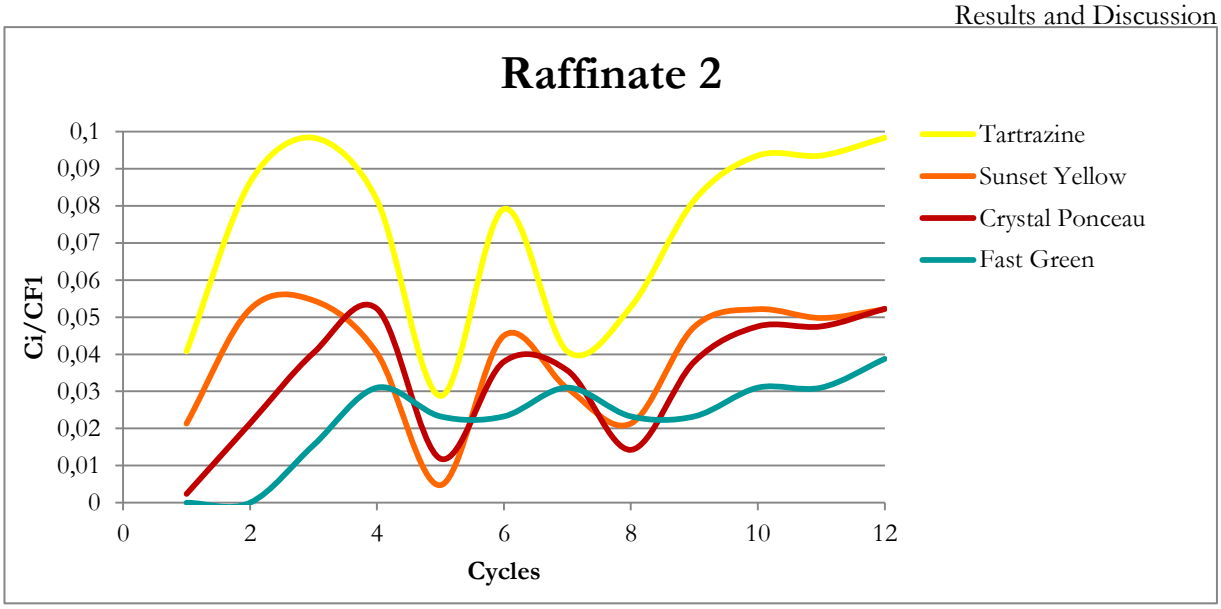
According to these results the separation should occur with almost 100% recovery for all molecules and 99% purity for the target molecule Crystal Ponceau. Based on these results an SMB experiment was conducted for a mixture with 0,04g/L of Tartrazine, Sunset Yellow and Crystal Ponceau and 0,012mg/L of Fast Green, with a solvent composition of 20%Ethanol and 80%Acetic Acid 70mmolar.

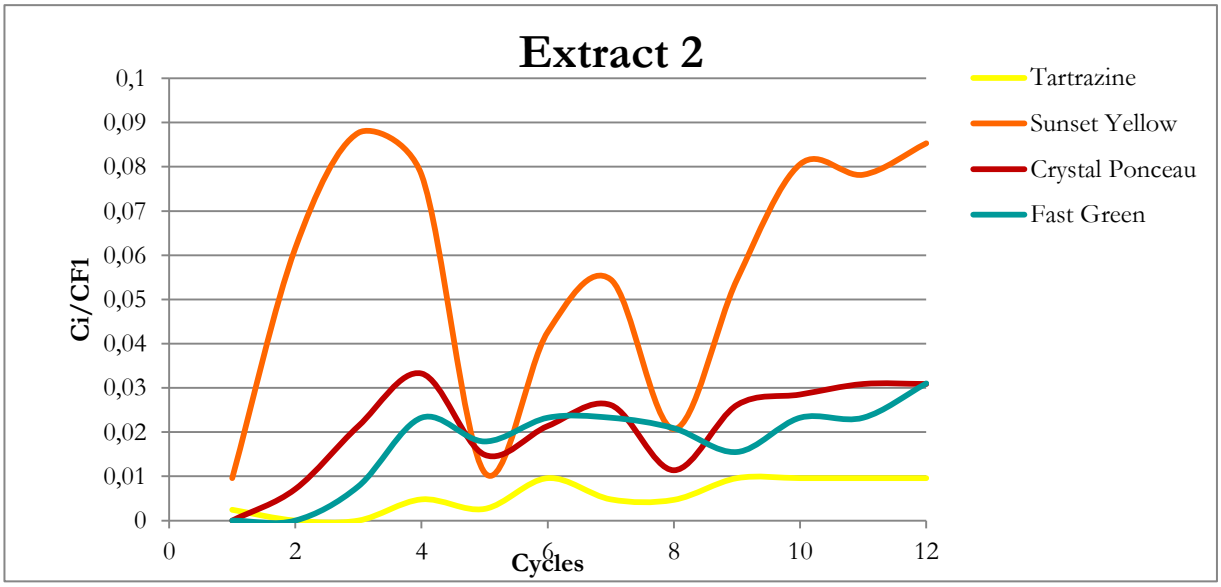
4.3.4. SMB experimental results

During the experiment the flow rates in the outlet ports were measured, to confirm the previous settings. The associated error was calculated according to equation 4.1. The temperature was also measured throughout the experiment and had an average value of 23 °C. The measurements are presented in table 4.15 and the outlet concentrations in figure 4.12

Table 4. 15 - Measured Flow Rates and associated error

	Raffinate 2		**Extract 2**		**Extract 1**	
Cycle	Measured Flow Rates (mL/min)	Error	Measured Flow Rates (mL/min)	Error	Measured Flow Rates (mL/min)	Error
1	3,078	7%	1,978	3%	2,201	1%
2	3,134	5%	2,034	6%	2,239	1%
3	3,116	6%	2,052	7%	2,201	1%
4	2,500	24%	1,978	3%	2,220	0%
5	2,425	26%	1,978	3%	2,201	1%
6	3,097	6%	2,052	7%	2,220	0%
7	2,910	12%	2,015	5%	2,220	0%
8	2,948	11%	2,034	6%	2,220	0%
9	3,097	6%	2,090	9%	2,220	0%
10	3,060	7%	2,090	9%	2,220	0%
11	3,097	6%	2,090	9%	2,239	1%
12	3,060	7%	2,090	9%	2,220	0%
Temperature	23°C					





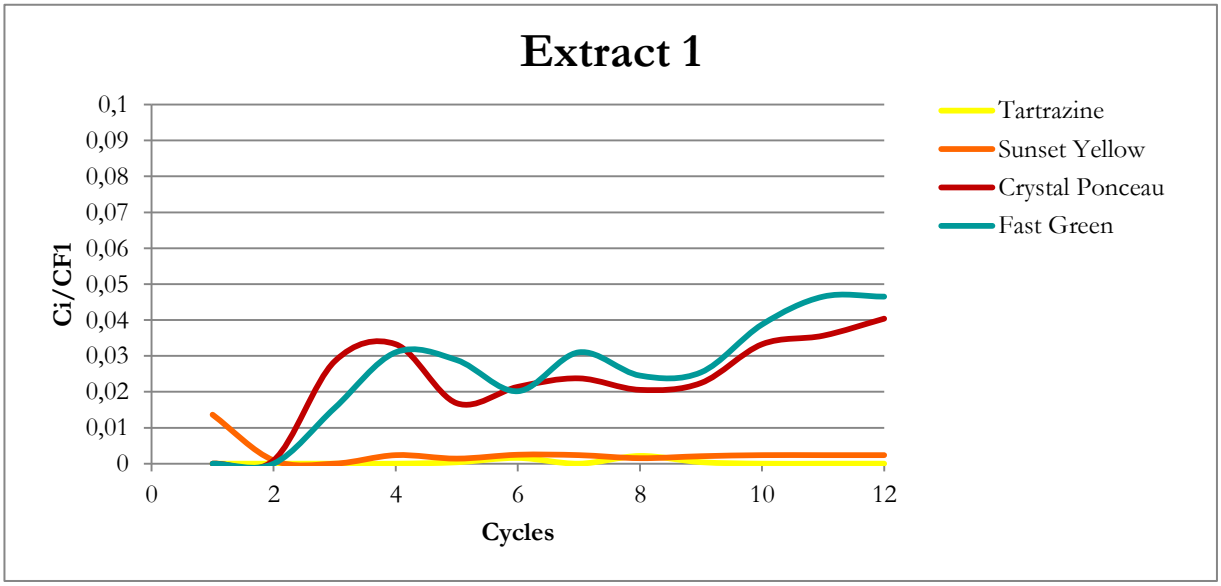


Figure 4. 12 - Outlet Concentrations of SMB Experiment with Raffinate Recycle

Table 4. 16 - Mass Balance for SMB experiment with Raffinate Recycle

	In (mg)	Raffinate 2 (mg)	Extract 1 (mg)	Extract 2 (mg)	Total out (mg)	In-Out (mg)	(In-Out)/In
Tartrazine	8,257	6,452	0,000	0,248	6,700	1,559	0,189
Sunset Yellow	8,257	3,518	0,059	2,712	6,289	1,969	0,238
Crystal Ponceau	8,257	2,987	0,973	0,980	4,940	3,319	0,402
Fast Green	2,477	0,619	0,320	0,227	1,166	1,312	0,529

During this experiment the feed inlet was not uniform due to what appeared to be a decalibration of one of the switch position valves causing the pressure to suddenly rise and switch off the pump, which explains the drops in concentration in the cycles 5 and 8. It also explains the increase of the associated error in flow rate measurement for the same cycles.

Apart from those two cycles the measured flow rates are very close to the selected ones, with a small associated error.

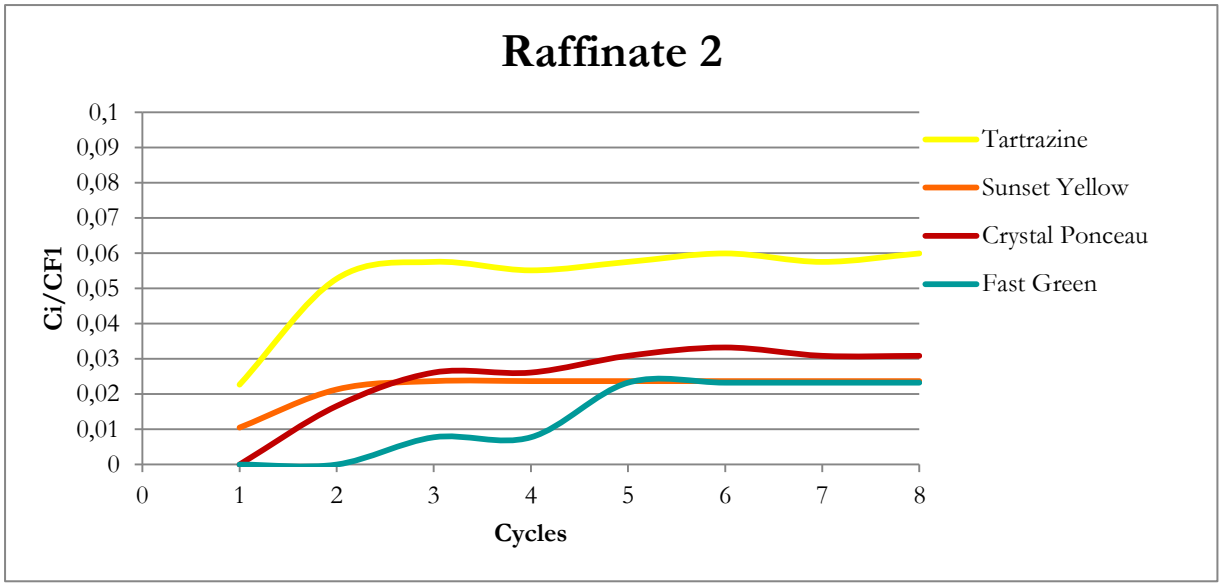
Still it's possible to see that the separation didn't work as expected. Fast Green was supposed to come out in extract 1 and it appears that it stays inside the columns for longer than predicted, causing it to elute in every port.

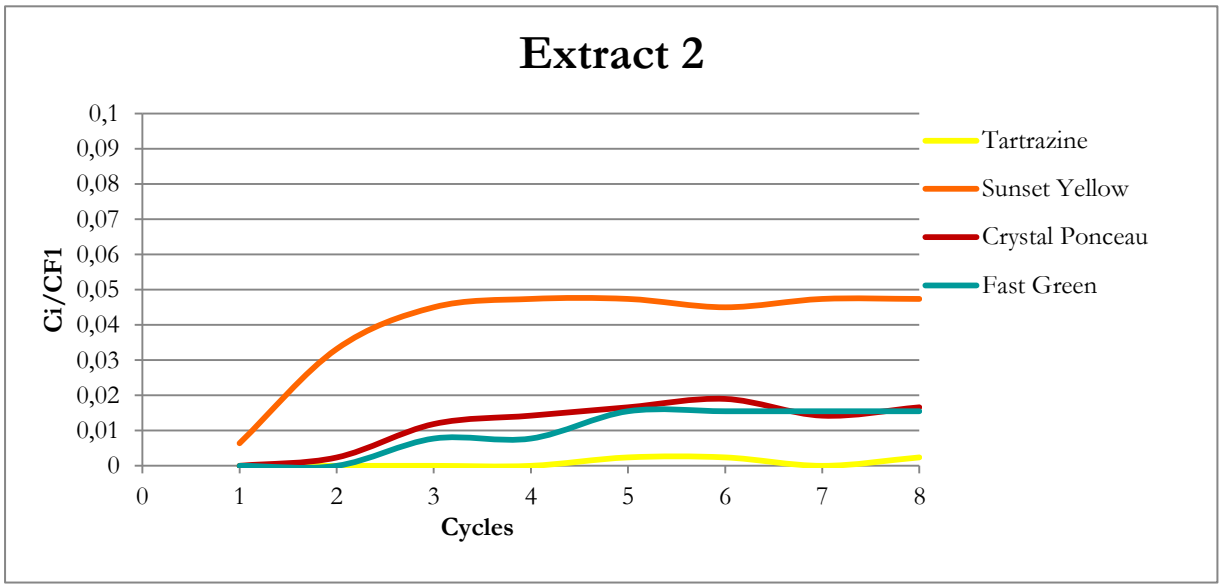
The target molecule Crystal Ponceau also goes out in every port. The only molecule which behavior was as predicted was Tartrazine that elutes in raffinate 2 and is effectively separated from the other molecules. In light of these results a second experiment was performed with some changes:

- $m_{I(1)}$ was increased in an attempt to force Fast Green to come out in extract 1
- $m_{III(1)}$ was considerably increased to make sure that Crystal Ponceau doesn't go to extract 1. Even though $m_{III(1)}$ shouldn't be larger than Fast Greens' Henry coefficient, from this experiment it's is clear that it's retention time is much larger than what was initially considered.
- $m_{IV(2)}$ was set to minimum because it was not to be small enough as Crystal Ponceau still comes out in extract 1.
- $m_{II(2)}$ was increased so that Sunset Yellow leaves the system in raffinate 2 instead of exract 2.

Also the switch time was increased to 8min, so that the dimensionless flow rates could be increased without increasing the flow rates.

The results of the new experiment are presented in figure 4. 13





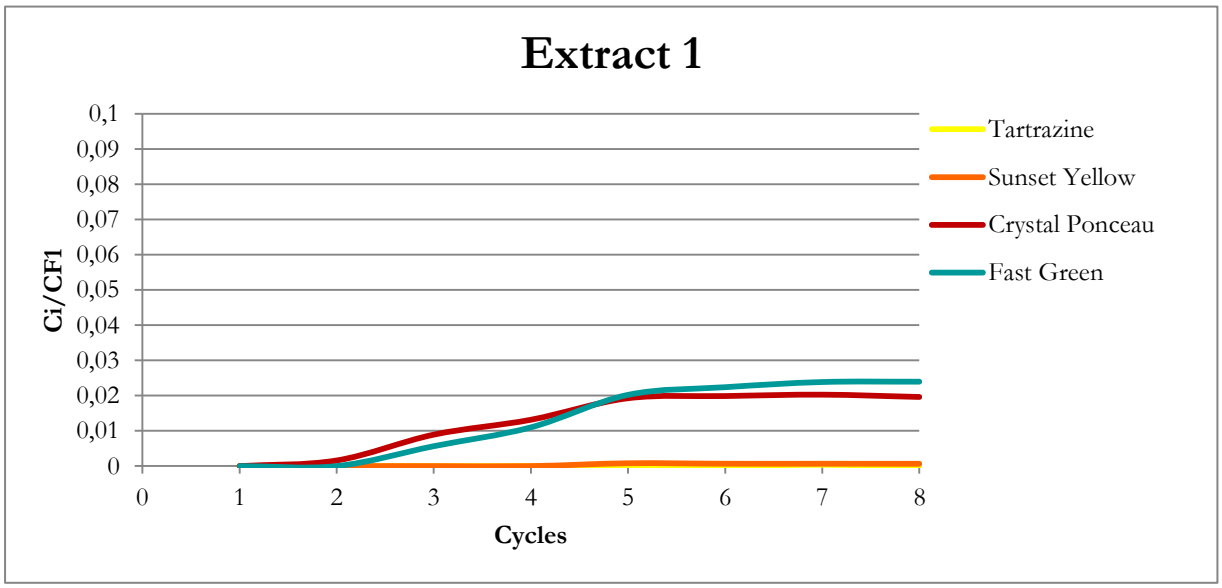


Figure 4. 13 - Outlet concentrations for the second SMB experiment with Raffinate Recycle

Table 4. 17 - Mass Balance for SMB experiment with Raffinate Recycle

	In (mg)	Raffinate 2 (mg)	Extract 1 (mg)	Extract 2 (mg)	Total out (mg)	In-Out (mg)	(In-Out)/In
Tartrazine	4,11648	4,068	0,003	0,052	4,123	0,168	0,039
Sunset Yellow	4,11648	1,693	0,018	2,352	4,063	0,280	0,064
Crystal Ponceau	4,11648	1,890	0,670	0,698	3,258	1,074	0,248
Fast Green	1,234944	0,323	0,214	0,175	0,711	0,616	0,464

This second experiment despite all changes had similar results, which means that some wrong assumption was made. Clearly Fast Greens' retention time is much higher than anticipated based on the isotherm estimation experiments, meaning that its Henry coefficient must be much higher than estimated. The same appears to be the case for Crystal Ponceaus' Henry coefficient to cause it to come out on every port. Also Sunset Yellow is coming out on the extract 2 instead of raffinate 2. This means that Sunset Yellows' Henry coefficient must be larger than $m_{II(2)}$, but it still should be lower than $m_{II(2)}$ because it does not come out in extract 1, which means that the first subunit is working well for this molecule.

To test this hypothesis a simulation was run in Chromworks™2016 with new Henry coefficients:

Table 4. 18 - New Henry coefficients for hypothesis test

Molecule	New H_i
Sunset Yellow	2
Crystal Ponceau	50
Fast Green	50

The results obtained are summarized in tables 4.19, 4.20 and 4.21

Table 4. 19 - Simulation results for hypothesis test comparison with experimental results (Raffinate 2)

Raffinate 2										
Molecules	Mass (mg)		Purity (%)		Recovery (%)		Productivity (mg/mL/h)		C (g/L)	
	Exp	Sim	Exp	Sim	Exp	Sim	Exp	Sim	Exp	Sim
Tartrazine	0,509	0,516	51,016	58,905	98,823	99,811	0,002	0,008	0,002	0,002
Sunset Yellow	0,212	0,157	21,230	17,972	41,123	30,452	0,001	0,002	0,001	0,001
Crystal Ponceau	0,236	0,156	23,706	17,787	45,921	30,138	0,001	0,002	0,001	0,001
Fast Green	0,040	0,047	4,047	5,336	26,134	30,138	0,000	0,001	0	0
Patent Blue	0	0	0	0	0	0	0	0	0	0
Total	0,997	0,876	100	100	212	46,107	0,004	0,013	0,004	0,004

Table 4. 20 - Simulation results for hypothesis test comparison with experimental results (Extract 2)

Extract 2										
Molecules	Mass (mg)		Purity (%)		Recovery (%)		Productivity (mg/mL/h)		C (g/L)	
	Exp	Sim	Exp	Sim	Exp	Sim	Exp	Sim	Exp	Sim
Tartrazine	0,007	0	1,598	0	1,272	0	0	0	0	0
Sunset Yellow	0,294	0,361	71,765	46,366	57,133	69,778	0,002	0,005	0,002	0,002
Crystal Ponceau	0,087	0,245	21,310	31,435	16,965	47,308	0,000	0,004	0,001	0,001
Fast Green	0,022	0,073	5,327	9,431	14,138	47,308	0,000	0,001	0	0
Patent Blue	0	0,099	0	12,768	0	51,238	0	0,001	0	0,001
Total	0,410	0,778	100	100	89,508	40,950	0,002	0,012	0,002	0,004

Table 4. 21 - Simulation results for hypothesis test comparison with experimental results (Extract 2)

Extract 1										
Molecules	Mass (mg)		Purity (%)		Recovery (%)		Productivity (mg/mL/h)		C (g/L)	
	Exp	Sim	Exp	Sim	Exp	Sim	Exp	Sim	Exp	Sim
Tartrazine	0	0	0,301	0	0,066	0	0	0	0	0
Sunset Yellow	0,002	0	2,022	0	0,444	0	0	0	0	0
Crystal Ponceau	0,084	0,076	74,030	39,257	16,265	14,752	0,001	0,001	0,001	0
Fast Green	0,027	0,023	23,647	11,777	17,318	14,752	0	0	0	0
Patent Blue	0	0,095	0	48,965	0	49,965	0	0,001	0	0,001
Total	0,113	0,194	100	100	34,094	10,225	0,001	0,003	0,001	0,001

Analyzing these results the hypothesis seems to be correct, as the concentration, purity and recovery are very similar to the ones obtained in the experiment.

The fact that Fast Greens' estimated Henry coefficient is much higher than estimated indicates that a possible source of error could be related to the isotherm estimation experiments. These could be for example related to experimental error in the preparation of the solvent, or in the preparation of the samples for the isotherm estimation experiments.

Another possible explanation could be that some of the assumptions made for the system were not true. This could be the case for the consideration that all columns are identical, and that therefore the molecules should behave in the same way for all of them. Since the isotherms were only measured for half of the columns that were used in the experiments this could be a reasonable cause for the error. If the molecules don't exhibit the same adsorption behavior for the remaining columns the system was poorly design as it didn't take this fact into account.

Other factors may also interfere to a lesser degree as the lack of temperature control during the experiments.

Further experiments need to be conducted to clarify these reasons. These should include the estimation of the isotherms of all molecules for all columns and the repetition of these experiments.

To further extend this study the extract recycle configuration could be studied for this mixture and compared to the raffinate recycle results.

5. Conclusions

The SMB process has been widely used for binary mixture separation. Though there are still several limitations its study for complex mixture separations has been investigated for several configurations.
The goal of this work was to broader the existing knowledge on the 8-zones SMB process for pseudo ternary separations, since these provide a more realistic multicomponent mixture.

In this work several techniques were used in order to design the SMB experiments and analyze its results. The study of spectroscopy was essential to confirm that the molecules chosen for this work adsorb independently from each other and can be identifiable in mixture experiments. Also spectroscopy proved , despite its limitations to be an efficient way to measure the experiments outlet concentrations for this system, as opposed to the usual HPLC process which complex and time consuming.
Isotherm estimation was crucial for the subsequent design of the SMB process, modeling of the SMB system for raffinate recycle configuration and experimental validation was performed. The estimated isotherms for the tested range of concentrations were found to be linear which was of great advantage for this study as the molecules do not compete with each other for the solid phase, meaning that each molecules' retention time is independent of the presence of the other molecules in the mixture. This allowed the performance of multicomponent experiments, in which it was possible to get every molecule's retention times in one experiment.
The SMB systems' dead volume characterization was also an important result which allowed for a better design, in which the real dead volume per column was considered.

Using the isotherm estimation and dead volume measurement results the SMB experiments were conducted based on the design and model for the raffinate recycle configuration using triangle theory. Though the separation didn't occur as desired, its results provided information on which experimental errors could have occurred and which assumptions should be confirmed for future experiments.

Future work should include:

- Isotherm estimation experiments for all columns to be used in SMB experiments;
- Additional SMB experiments with this configuration;
- SMB design and experiments for extract recycle configuration.

References

1. Schmidt-Traub, H., *Preparative Chromatography of Fine Chemicals and Pharmaceutical Agents*. 2005.
2. Hashimoto, K.S., Y.; Adachi, S., *<A Simulated Moving Bed Adsorber for the Separation of Tricomponents>.* Journal of Chemical Engineering of Japan, 1993. **26**(1): p. 52-56.
3. Shan, Y.S.-M., A., *<Isolation of a Target Compound from a Multi-component Mixture by coupling Batch and Continuous Countercurrent Chromatography >.* Chemical Engineering & Technology, 2005.
4. Abel, S., et al., *Two-fraction and three-fraction continuous simulated moving bed separation of nucleosides.* Journal of Chromatography A, 2004. **1043**(2): p. 201-210.
5. Beste, Y.A.W., A., *<Side-Stream SImulated Moving Bed Chromatography for Multicomponent Separation>.* Chemical Engineering & Technology, 2002. **25**: p. 956-962.
6. Chiang, A.S.T., *<Continuous Chromatographic Process Based on SMB Technology>.* AIChE 1998. **44**(8): p. 1930-1932.
7. Hritzko, B.J.X., Y.; Wooley, R.J.;Wang, N-H. L, *<Standing-Wave Design of Tandem SMB for Linear Multicomponent Systems>.* AIChE, 2002. **42**(12): p. 2769-2787.
8. Wankat, P.C., *<Simulated Moving Bed Cascades for ternary Separations>.* Industrial & Engineering Chemistry Research, 2001. **40**(26): p. 6185-6193.
9. Wooley, R., *<A Nine-Zone Simulating Moving Bed for the Recovery of Glucose and Xylose from Biomass Hydrolyzate>.* Industrial & Engineering Chemistry Research, 1998. **37**: p. 3699-3709.
10. Nicolaos, A.M., L.; Gottelandb,P.; Nicoud,R.M; Bailly,M., *<Application of equilibrium theory to ternary moving bed configurations (four plus four, five plus four, eight and nine zones) I. Linear case.pdf>.* Journal of Chromatography A, 2001. **908**: p. 71-86.
11. Kessler, L.C. and A. Seidel-Morgenstern, *Theoretical study of multicomponent continuous countercurrent chromatography based on connected 4-zone units.* J Chromatogr A, 2006. **1126**(1-2): p. 323-37.
12. Nowak, J., D. Antos, and A. Seidel-Morgenstern, *Theoretical study of using simulated moving bed chromatography to separate intermediately eluting target compounds.* J Chromatogr A, 2012. **1253**: p. 58-70.
13. Santos da Silva, F.V. and A. Seidel-Morgenstern, *Evaluation of center-cut separations applying simulated moving bed chromatography with 8 zones.* J Chromatogr A, 2016. **1456**: p. 123-36.
14. Tswett, M.S., *<About a new category of adsorption phenomena and their application for biochemical analysis>.* Chemical Reviews, 1989. **89**(2): p. 281-285.
15. Guiochon, G.F., A.;Shirazi,D.G.;Katti,A.M., *< Fundamentals of Preparative and Nonlinear Chromatography>*. second ed. 2006.
16. Martin, A.J.P.S., R.L.M., *<A New Form of Chromatogram Employing Two Liquid Phases>.* Biochemical Journal. **35**(12): p. 1358-1368.
17. Brougton, D.B., *<Continuous Sorption Process Employing Fixed Bed of Sorbent and Moving inlets and outlets>*.
18. Storti, G.M., M.; Morbidelli,M.; Card, S., *<Robust Design of Binary Countercurrent Adsorption Separation Processes.>.* AIChE, 1993. **39**(3): p. 471-492.
19. Nicoud, R.-M., *Chromatographic Processes, Modeling, Simulation and Design*. 2015: Cambridge University Press.
20. Fu, R., *<The Separation of Seven Synthetic/Artificial Food Colors on Agilent HC(2) TC(2) Reversed Phase Columns>*. 2008, Agilent Technologies:.
21. Fu, R., *<Fast Separation of Syntetic/Artificial Food Colors on Agilent Poroshell 120 EC-C18>*. 2012, Agilent Technologies.

22. Kattein, B., *Experimentelle Studie zur Anwendbarkeit des SMB-Prozesses zur Trennung von pseudo-ternaren Gemischen*. 2016, Technische Universitat Braunschweig.
23. Hammes, G.G., *Spectroscopy for the biological sciences*. 2005.
24. A, R.F.R., *Chemical Analysis, Modern Instrumentation Methods and Techniques*. 2000.
25. [cited 2016; Available from: http://de.mathworks.com/help/matlab/ref/lsqnonneg.html.
26. Seidel-Morgenstern, A., *Experimental determination of single solute and competitive adsorption isotherms.* Journal of Chromatography A, 2004. **1037**(1-2): p. 255-272.
27. Ruthven, D.M.C., C.B., *<Counter-Current and Simulated Counter-Current Adsorption Separation Processes>.* Chemical Engineering Science. **44**(5): p. 1011-1038.
28. Agrawal, G. and Y. Kawajiri, *Comparison of various ternary simulated moving bed separation schemes by multi-objective optimization.* J Chromatogr A, 2012. **1238**: p. 105-13.
29. Beltscheva, D., *Linear two-step gradient counter-current chromatography Analysis based on a recursive solution of an equilibrium stage model.* Journal of Chromatography A, 2003. **989**(1): p. 31-45.
30. Faria, R.P. and A.E. Rodrigues, *Instrumental aspects of Simulated Moving Bed chromatography.* J Chromatogr A, 2015. **1421**: p. 82-102.
31. Seidel-Morgenstern, A., L.C. Keßler, and M. Kaspereit, *New Developments in Simulated Moving Bed Chromatography.* Chemical Engineering & Technology, 2008. **31**(6): p. 826-837.
32. Agrawal, G., B. Sreedhar, and Y. Kawajiri, *Systematic optimization and experimental validation of ternary simulated moving bed chromatography systems.* J Chromatogr A, 2014. **1356**: p. 82-95.
33. Ching, C.B.R., D.M; Hidajat,K., *<Experimental Study of a Simulated Counter-Current Adsorption System III. Sorbex Operation.pdf>.* Chemical Engineering Science, 1985. **40**(8): p. 1411-1417.