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MULTICOMPONENT CHIRAL SEPARATION BY PREPARATIVE AND SIMULATED MOVING BED CHROMATOGRAPHY

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MULTICOMPONENT CHIRAL SEPARATION BY PREPARATIVE AND SIMULATED MOVING BED CHROMATOGRAPHY

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To the magic woman, Baraa

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" علم العلماء هو مايبقي على الأمم "

"The science of scientist is what keeps nations alive"

Jabir ibn Hayyan (721-815)

Abstract

This work deals with the separation of multicomponent chiral mixtures by preparative and simulated moving bed chromatography. The developed study is based on different pseudo-binary and binary separations of nadolol quaternary commercial drug. All preparative separations were performed using an Azura preparative HPLC unit and all the SMB separations were carried out using the LSRE-FlexSMB unit.

Nadolol is a pharmaceutical drug widely used in the treatment of cardiovascular diseases. Nowadays, despite the more and more restricted international legislation towards commercialization of safer pharmaceutical drugs based on single enantiomers, nadolol is still only commercially available as an equal mixture of their four stereoisomers. Although some studies identify the stereoisomer RSR-nadolol as the stereoisomer with the relevant therapeutic action, in this work nadolol it is used as a prototype quaternary mixture to develop different strategies for multicomponent separations.

The main objective of this work is to identify and implement the main experimental strategy for the complete separation of nadolol quaternary mixture into its pure four stereoisomers. Different chiral and achiral adsorbents, with particle size diameters (5, 10 and 20 μm) as well as several solvents and solvent mixtures (aqueous and organic) used.

The selected global strategy for the complete separation of the four nadolol stereoisomers is based on two main steps. The first step is the achiral separation of nadolol racemates (two pairs of enantiomers) by high pH reversed-phase preparative chromatography using C18 adsorbents. Then, the second step is based on two parallel binary chiral separations. It is important to notice that both first and second steps can be accomplished by preparative HPLC or SMB technologies. However, this work presents theoretical and experimental results showing that SMB technology presents higher preparative performance when compared to “equivalent” preparative HPLC operation.

For the achiral nadolol racemates preparative separation the Waters XBridge C18 adsorbent was concluded to be the better solution for the preparative separation of

nadolol racemates, using an ethanol:water: diethylamine (30:70:0.1) solvent composition for both FB and SMB separations. For SMB, the pilot unit's pressure drops limits imposed a maximum internal flow-rate of only 5 mL/min and, for a nadolol feed concentration of 2 g/L, both racemates were recovered 100% pure, with a system productivity of 0.13 g_{feed}/(L_{bed}.hr) and a solvent consumption of 6.19 L_{solvent}/g_{feed}. Additional simulation results showed that a SMB preparative unit can perform the 9 g/L nadolol racemate separation with a system productivity of 3.61 g_{feed}/(L_{bed}.hr) and a solvent consumption of only 1.95 L_{solvent}/g_{feed} using the same average internal flow-rate as in FB operation.

The chiral separation of nadolol racemates into its pure enantiomers by SMB is performed using the Daicel Chiralpak IA adsorbent and a 25:75:0.1 methanol:acetonitrile:diethylamine solvent composition for first racemate and a 5:95:0.1 methanol:acetonitrile:diethylamine solvent composition for second racemate. For SMB, the pilot unit's pressure drops limits imposed a maximum internal flow-rate of only 20 mL/min and, for a nadolol racemate A feed concentration of 2 g/L, both stereoisomers (1 and 4) were recovered 100% pure, with a system productivity of 1.81 g_{feed}/(L_{bed}.hr) and a solvent consumption of 2.47 L_{solvent}/g_{feed}. For a nadolol racemate B feed concentration of 2 g/L, both stereoisomers (2 and 3) were recovered 100% pure, with a system productivity of 0.87 g_{feed}/(L_{bed}.hr) and a solvent consumption of 4.25 L_{solvent}/g_{feed}.

Resumo

Este trabalho foca-se na separação de misturas quirais multicomponente por cromatografia de leito móvel simulado (SMB) e por cromatografia de leito-fixo (FB). O estudo desenvolvido baseia-se em diferentes separações pseudo-binárias e binárias da mistura comercial quaternária do nadolol. Todas as separações preparativas por FB foram realizadas usando uma unidade preparativa Azura de HPLC e todas as separações SMB foram realizadas usando a unidade LSRE-FlexSMB.

O nadolol é um fármaco amplamente utilizado no tratamento de doenças cardiovasculares. Atualmente, apesar da legislação internacional cada vez mais restrita focando a comercialização de fármacos mais seguros baseados em enantiômeros individuais, o nadolol ainda está disponível comercialmente apenas como uma mistura racêmica de quatro estereoisômeros. Embora alguns estudos identifiquem o estereoisômero RSR-nadolol como o estereoisômero com ação terapêutica relevante, neste trabalho o nadolol é utilizado como um protótipo de uma mistura quaternária no desenvolvimento de diferentes estratégias para separações preparativas multicomponente.

O principal objetivo deste trabalho é identificar e implementar a principal estratégia experimental para a separação completa da mistura quaternária de nadolol nos seus quatro estereoisômeros puros. Diferentes adsorventes quirais e aquirais, com diferentes diâmetros de tamanho de partícula (5, 10 e 20 μm), bem como vários solventes e misturas de solventes (aquosos e orgânicos) são utilizados.

A estratégia global selecionada para a separação completa dos quatro estereoisômeros do nadolol é baseada em duas etapas principais. A primeira etapa é a separação aquiral dos racematos de nadolol (dois pares de enantiômeros) por cromatografia preparativa de fase reversa com pH elevado usando adsorventes C18. A segunda etapa é baseada em duas separações quirais binárias paralelas. É importante observar que tanto a primeira como a segunda etapas podem ser realizadas por ambas as tecnologias preparativas de FB ou SMB. Este trabalho apresenta resultados teóricos e experimentais mostrando que a tecnologia SMB

apresenta maior desempenho preparativo quando comparada à operação preparativa “equivalente” de FB.

Para a separação preparativa aquiral dos dois racematos do nadolol, o adsorvente Waters XBridge C18 foi considerado a melhor solução para a separação preparativa, usando uma composição de solvente etanol:água:dietilamina (30:70:0,1) para separações FB e SMB. Na operação por SMB, os limites de queda de pressão da unidade piloto impuseram um caudal interno máximo de apenas 5 mL/min e, para uma concentração de alimentação de nadolol de 2 g/L, ambos os racematos foram recuperados 100% puros, com uma produtividade global de 0.13 g_{alimentação} / (L_{leito}.hr) e um consumo de solvente de 6.19 L_{solvente}/g_{alimentação}. Os resultados adicionais obtidos por simulação mostraram que uma unidade preparativa SMB pode realizar a separação de 9 g/L de racemato de nadolol com uma produtividade global de 3.61 g_{alimentação} / (L_{leito}.hr) e um consumo de solvente de apenas 1.95 L_{solvente}/g_{alimentação} usando o mesmo caudal interno médio equivalente ao utilizado na operação por FB.

A separação quiral dos racematos de nadolol nos seus enantiômeros puros por SMB é realizada usando o adsorvente Daicel Chiralpak IA e uma composição de solvente de metanol:acetonitrilo:dietilamina (25:75:0.1) para o primeiro racemato e uma composição de metanol:acetonitrilo:dietilamina (5:95:0.1) para o segundo racemato. Para a separação por SMB, os limites de queda de pressão da unidade piloto impuseram um caudal interno máximo de 20 mL/min e, para uma concentração de alimentação de racemato A do nadolol de 2 g/L, ambos os estereoisômeros (1 e 4) foram recuperados 100% puros, com uma produtividade global de 1.81 g_{alimentação} / (L_{leito}.hr) e um consumo de solvente de 2.47 L_{solvente}/g_{alimentação}. Para uma concentração de alimentação de racemato B do nadolol de 2 g/L, ambos os estereoisômeros (2 e 3) foram recuperados 100% puros, com uma produtividade global de 0.87 g_{alimentação} / (L_{leito}.h) e um consumo de solvente de 4.25 L_{solvente}/g_{alimentação}.

الخلاصة

يغطي هذا العمل عملية الفصل التحضيرية والكارليالية بطريقة كرماتوغرافيا محاكاة السرير المتحرك, تركز هذه الدراسة على الفصل الثنائي لمركب النادولو الرباعي الطبي التجاري. جميع عمليات الفصل التحضيرية تمت بواسطة جهاز ازورا وجهاز محاكاة السرير المتحرك SMB المتوفر في مختبرات LSRE

يعتبر النادولو مادة صيدلانية شائعة جداً لمعالجة أمراض القلب. في وقتنا الحاضر, بعد التشديدات الكبيرة على سلامة ونقاء الأدوية التجارية والمتمثل في تحضير ادوية بزمرة واحدة نقية فقط, في الواقع يعتبر مازال دواء النادولو يباع في الصيدليات على شكل مركب رباعي. بالرغم من ان بعض الدراسات اشارت إلى أن العنصر الفعال في مركب النادول هو المركب الرابع. في هذا العمل سوف يؤخذ مركب النادول كمثال على فصل المركبات الرباعية لوضع استراتيجية لفصل هذا النوع من المركبات.

الهدف من هذا العمل هو إيجاد وتطبيق استراتيجية لفصل جميع مركبات النادولو الأربعة والحصول على مركبات نقية على شكل اربع نظائر نقية. تم استخدام انواع مختلفة من أعمدة الفصل الكارليالية والغيركارليالية بحجم جزئيات مختلف (5, 10 و 20 μm) بالإضافة إلى استخدام عدة مذيبات مختلفة.

الإستراتيجية الشاملة للفصل الكامل لمركب النادول الرباعي تركز على خطوتين رئيسيتين. الخطوة الأولى تتمثل بفصل غير كاليالي لفصل عناقيد النادول الثنائية باستخدام الطور العكسي عند أس هيدروجيني عالي وبتقنية الكروماتوغرافية التحضيرية باستخدام اعمدة C18. بعد ذلك, الخطوة الثانية تتألف من خطوتين متناظرتين من الفصل الكارليالي. من المهم هنا الإنتباه إلى ان الخطوة الأولى والثانية يمكن تنفيذهما باستخدام تقنية الكروماتوغرافيا التحضيرية بالإضافة إلى تقنية السرير المتحرك. وهذا العمل سوف يعرض الخلفية النظرية لكلا التقنيتين بالإضافة إلى مناقشة النتائج العملية.

لأجل عملية الفصل الغيركاليالي تم استخدام اعمدة Waters XBridge C18 وتم إستنتاج ان هذه الأعمدة تعتبر الأفضل لإجراء عملية الفصل الأولى باستخدام مزيج إيثانول:ماء بنسبة (30:70) لتقنيات الكروماتوغرافي السائلة التحضيرية و تنقية محاكاة السرير المتحرك. كانت هناك معاناة تتعلق بضغط التشغيل لمحاكاة السرير المتحرك وكان الإلتزام بعدم تجاوز تدفق للمذيب بقدر اكبر من 5 mL/min, هذا التدفق كان مصمم لفصل 2 g/L من المحلول المغذي للنادول, كلا العنقودين الناتجين تم إسترجاعها بنقاوة 100% وإنتاجية 0.13 gfeed/(Lbed.hr) وإستهلاكية للمذيب 6.19 Lsolvent/gfeed. تم إجراء محاكاة حسابياً وتبين أن النظام يمكنه إجراء الفصل لـ 9 غرام/بالليتر تركيز من المحلول المغذي لمحلول النادول وإنتاجية تبلغ 3.61 غرام/(ليترسرير/ساعة) وإستهلاكية للمذيب تبلغ فقط 1.95 ليتر لكل غرام من المحلول المغذي.

الفصل الكارليالي لعناقيد النادول تم بواسطة السرير المتحرك من خلال استخدام اعمدة مقدمة من شركة Daicel Chiralpak IA وفصل العنقود الأول تم باستخدام مذيب 25:75:0.1 ميثانول:اسيتونيتريول:داي إيثيل امين, اما العنقود الثاني تم باستخدام مذيب 5:95:0.1 ميثانول:اسيتونيتريول:داي إيثيل امين. كان ضغط التشغيل للسرير المتحرك يمنحنا مجال للتدفق الداخلي إلى حدود 20 mL/min وتركيز المحلول المغذي للنادول 2 غرام/بالليتر, تم إسترجاع نظائر (1 و 4) بنقاوة 100% وإنتاجية 1.81 gfeed/(Lbed.hr) وإستهلاكية للمذيب 2.47 Lsolvent/gfeed. تم استخدام تركيز

2 غرام/بالليتر للعنقود الثاني (2 و 3), تم إسترجاع نظائر بنقاوة 100% وإنتاجية 0.87 g_{feed}/(L_{bed}.hr) وإستهلاكية للمذيب 4.24 L_{solvent}/g_{feed}.

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List of symbols

C_i^F	Concentration of component i in the feed solution, g/L
V_c	Column volume, cm ³
V^d	Eluted volume, cm ³
b_i	Model parameter for species i of the equilibrium adsorption isotherm (L/g)
c_i^d	Concentration of component i in the eluted volume, g/L
q_i^*	Concentration of component i in the particle, g/L
c	Fluid phase, g/L
d_p	Particle diameter, μm
k	Mass transfer coefficient, s ⁻¹
L_c	Length of column, mm
N_c	Number of columns per section in the SMB system
Pe	Peclet number
PR	System productivity, grams/L _{bed} .h
PUR	Raffinate Purity (%)
PUX	Extract Purity (%)
SC	Solvent consumption, L/g
t	Time variable, s
t^*	Switch time interval, s
u_0	Linear velocity, m s ⁻¹
x	Dimensionless axial coordinate
z	Axial coordinate, cm
M	Total number of experimental points for the equilibrium adsorption isotherm
N	Total number parameters to be estimated for the equilibrium adsorption isotherm model
Q	Capacity factor of the equilibrium adsorption isotherm of the Langmuir term (g/L)
St	Stanton number
m	Numerical parameter of the equilibrium adsorption isotherm

Greek Symbols

α	Selectivity factor
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α	Number of mass transfer units
β	Safety margin (SMB operation)
γ	Ratio between fluid and solid interstitial velocities in the TMB operation
γ^*	Ratio between fluid and solid interstitial velocities in the SMB operation
ε	Bed porosity
θ	Dimensionless time variable
μ_1	First moment of the peak
μ_k	Moment of order k
σ^2	Peak variance
τ	Tortuosity factor

Subscripts and Superscripts

A	Less retained component (binary mixture)
B	More retained component (binary mixture)
E	Eluent
F	Feed
<i>i</i>	Component index (<i>i</i> = 1, 2, 3, 4)
R	Raffinate
REC	Recycle
X	Extract

List of Abbreviations

ACN	Acetonitrile
C7	Heptane
CSP	Chiral Stationary Phase
DEA, D	Diethylamine, strong basic modifier
E	Ethanol
HETP	Height Equivalent to a Theoretical Plate
Hex	Hexane
HPLC	High-Performance Liquid Chromatography
IPA	Iso-propanol alcohol
LDF	Linear driving force
LG3	Langmuir isotherm model
LLG4	Linear+Langmuir equilibrium adsorption isotherm model
MeOH, M	Methanol
RSD	Relative Standard Deviation
SMB	Simulated Moving Bed
TMB	True Moving Bed
UV	Ultraviolet

Chapter 1 Introduction

This chapter provides a general survey of the preparative separation for chiral drugs, with the main technologies being used for the complete separation of the multicomponent nadolol mixture into its four pure stereoisomers. The objectives for the Ph.D work are established.

1.1 Motivation and Outline

The continuous interest for the commercialization of safer and more efficient drugs together with the more and more restrictive international legislation is pushing the pharmaceutical industry in the direction of the development and production of chiral optically pure form pharmaceutical drugs [1, 2]. Enantioselective synthesis, also called asymmetric synthesis, followed by a crystallization process, is the traditionally method to obtain single pure form enantiomers [4, 5]. Often, the synthesis involves several consecutive and specific reactions directed to the production of one target single molecule, imposing a time-consuming process. As, in industrial operations, time-consuming processes are associated with high costs, alternative approaches are welcome. The two main alternatives are the indirect chromatographic resolution of a racemic mixture using chiral derivatization reagents to obtain one pure form enantiomer and the direct chromatographic resolution using a chiral adsorbent that allows the simultaneous production of the two enantiomers [3, 4].

High performance liquid chromatography using chiral stationary phases is a long-time established technique to perform the analytical separation and quantification of chiral compounds. Simulated moving bed (SMB) technology is an approach already accepted and adopted in the pharmaceutical industry for the large-scale production of chiral drugs. SMB technology allows, in a shorter period, the availability of both the

single enantiomers presented in a binary mixture, essential in the initial stages for the development of a pharmaceutical chiral drug. This can be confirmed by the increasing number of SMB preparative and industrial units already installed in the pharmaceutical industry worldwide. Some other important advantages also reinforce the success of the SMB technology, as the fact of being a continuous process simulating the counter-current movement of liquid and solid phases and, thus, maximizing the mass transfer driving force and succeeding in more difficult separations. Additionally, as the solvent is recycled and the adsorbent is re-used continuously, the SMB operation introduces a significant improvement in productivity and decrease in solvent consumption, when compared with the traditional fixed-bed chromatographic operation [5].

This thesis succeeded to set and implement a global strategy for complete separation of the chiral quaternary mixture by combining preparative fixed-bed and simulated moving bed chromatography at normal-phase and high pH reversed-phase. By the end of this study, a justification of the selection of the most efficient strategy for complete separation of nadolol; the justification will be supported by extensive experimental and simulation results of fixed-bed and simulated moving bed chromatography.

In *Chapter 2* presents the experimental procedures used for packing the preparative columns, and for the pulses experiments using IA stationary phase with different mobile-phase compositions. In this chapter are also presented the experimental procedures, modelling and simulation tools used to describe the fixed-bed adsorption behavior. Finally, are presented the tools used to estimate the initial SMB operation conditions as well the experimental operation of the FlexSMB unit and fixed chromatography.

Chapter 3 will present the chemical reagents, adsorbents, analytical and preparative columns, together with different chiral and achiral bulk materials. A description of the SMB pilot unit with the commercial preparative Azura HPLC system used in this work.

Chapter 4 introduce the separation of the more retained enantiomer from the quaternary nadolol stereoisomers mixture. Experimental results are presented and discussed such solvent screening, adsorption equilibrium isotherms measurements and breakthrough measurements with the SMB separation.

In *Chapter 5* strategies for the complete separation of the multicomponent nadolol mixture into its four pure stereoisomers are defined, a screening for mobile phase composition using different columns was important to evaluate the one step separation of all nadolol stereoisomers, it is possible to separate the more retained stereoisomer from the quaternary nadolol mixture using pure alcohol or alcohol-acetonitrile solvents, but no effective separation between the three first stereoisomers. There is a need for a different separation strategy that combines achiral and chiral separation steps.

Chapter 6 presents the first step achiral separation of nadolol quaternary mixtures into pure racemates, Three Waters C18 adsorbents (XBridge, Shield and XSelect) are compared for the separation of nadolol racemates using ethanol:water:diethylamine solvent mixtures. Experimental and simulation results are presented to compare the separation performances at preparative scale using both the fixed-bed and the simulated moving bed operations.

In *Chapter 7* the complete separation of all the stereoisomers of nadolol is presented by experimental preparative FB and SMB chiral chromatographic separations of the two SMB products streams obtained from previous achiral step. One commercial fixed-bed preparative HPLC Azura system equipped with one single

column of preparative dimensions (30 mm ID x 250 mm L) and one lab-scale SMB apparatus (the FlexSMB-LSRE® pilot unit) equipped with six semi-preparative columns (19 mm ID x 100 mm L) were used.

The main conclusions of the thesis are presented in *Chapter 8* and future work is addressed.

1.2 Chiral drugs and nadolol stereoisomers

The story of chiral chemicals started in 1848 when Louis Pasteur separated by hand sodium ammonium tartrate [6]; it took a complete century after that to realize how chirality is an important topic to study, especially knowing that it is involved in our living system in all aspects, such proteins, enzymes, amino acids, carbohydrates, nucleosides and several alkaloids and hormones are chiral compounds [7].

Chirality can be described by its original Greek name, "cheir" which means 'handedness.' It is like left and right hands; the two non-superimposable mirror-image forms of chiral molecules are called enantiomers. The structure of chiral molecules is usually a tetrahedral geometric configuration with carbon as the central atom attached to four different groups [8]. The physical and chemical properties are identical for optically active enantiomers. Oppositely, the non-optically active known as achiral molecules, each enantiomer of the chiral molecule has an equal position with opposite optical rotation. Enantiomers can rotate in a clockwise direction –right handed- it is referred to as (+) dextrorotary. In contrast, left-handed (-) levorotary, can be rotated in a counter-clockwise direction [9]. To better understand enantiomers, we can take the stereochemical structure of butan-2-ol (Figure 1.1). The structure of S-butan-2-ol is similar to R-butan-2-ol, but they are not superimposed images.

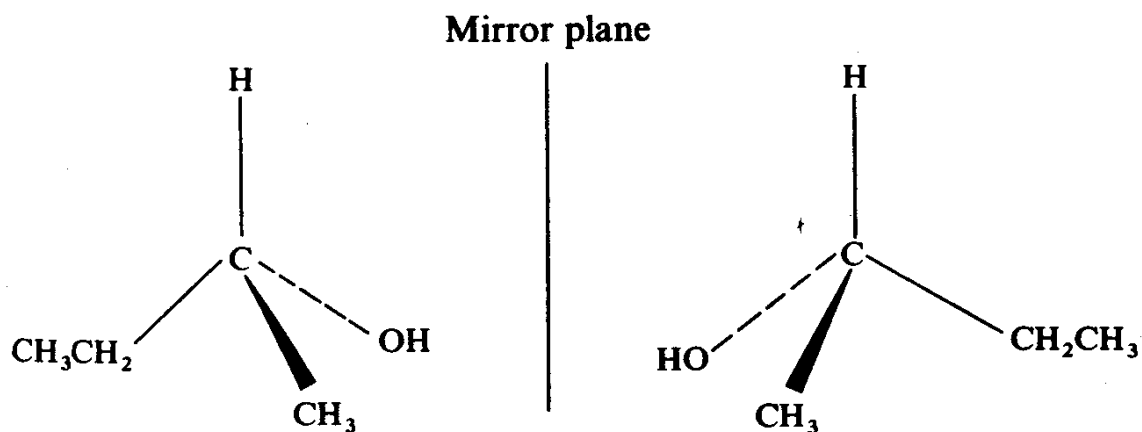


Fig 1.1. Stereo-chemical structure of butan-2-ol. [10].

Nowadays, more than 40% of marketed drugs have chiral active ingredients, and almost half of these drugs are marked as racemic mixtures [11]. According to market research future magazine [12], the world contribution of the chiral chemicals market was valued in 2021 at 5.8 billion USD; this value was expected to be expanded with a Compound Annual Growth Rate (CAGR) of 11.25 % to reach 14.8 billion USD by 2030. However, due to the COVID-19 outbreak, it is expected to contract a CAGR of 3% by the end of 2030.

Nadolol is one representative beta-blocker pharmaceutical drug prescribed worldwide to relieve several diseases mainly related to the cardiovascular system. However, like other pharmaceutical drugs, it is also associated with severe risks, such as depression, insomnia, and cardiovascular failure. Some authors refer that these side effects could be related to the fact that the nadolol drug is still marketed as a mixture of equal amounts of its four stereoisomers. Additionally, studies are referring that some therapeutic effects of this drug are related to only one of the four stereoisomers. Despite the international regulation agencies' growing pressure for pharmaceutical drug safety, pure single nadolol stereoisomers are still not commercially available.

The chemical structure of nadolol has three stereo-genic centers, which allows for eight possible stereoisomers. However, the two hydroxyl substituents on the cyclohexane ring are fixed in the cis-configuration, which precludes four stereoisomers [13]. Regardless of considerable evidence that it is essential to characterize the stereochemical components when describing the pharmacodynamics and pharmacokinetics of a racemic drug, the narrow international legislation concerning chiral drugs safety still allows nadolol commercialization in the form of a racemic mixture of four stereoisomers [14] (Figure 1.2).

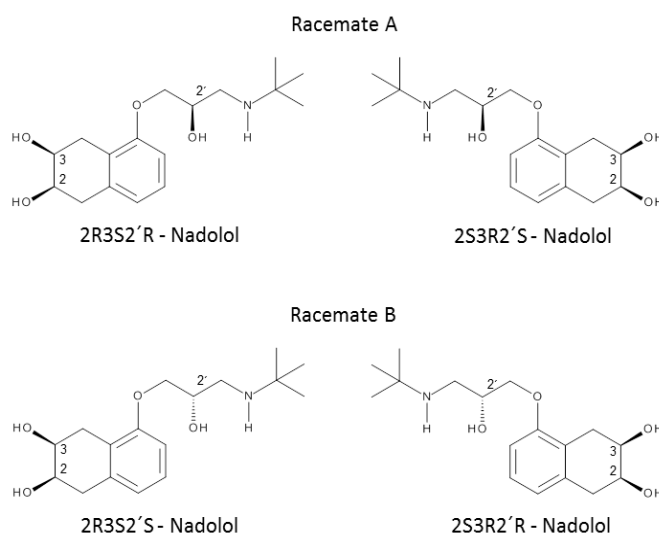


Fig 1.2. Molecular structures of the four nadolol stereoisomers. [15]

1.3 Fixed-bed and simulated moving bed preparative chromatography

The design for a successful simulation moving bed separation asks for precise fixed-bed measurements; several fixed-bed experiments were performed, starting with proper packing of the preparative columns; it is an essential step since good separation performance also depends on the adequate hydrodynamic behavior of the chromatographic columns. Solubility limitations are considered when selecting the most promising solvents after a wide range of screening mobile phase compositions. Equilibrium adsorption isotherm measurements were performed to build SMB separation region based on the equilibrium theory, kinetic data were estimated by

validating the equilibrium adsorption isotherms via breakthrough measurements. Figure 1.3 presents a flowchart for the main fixed-bed measurements needed for a pilot simulated moving bed separation.

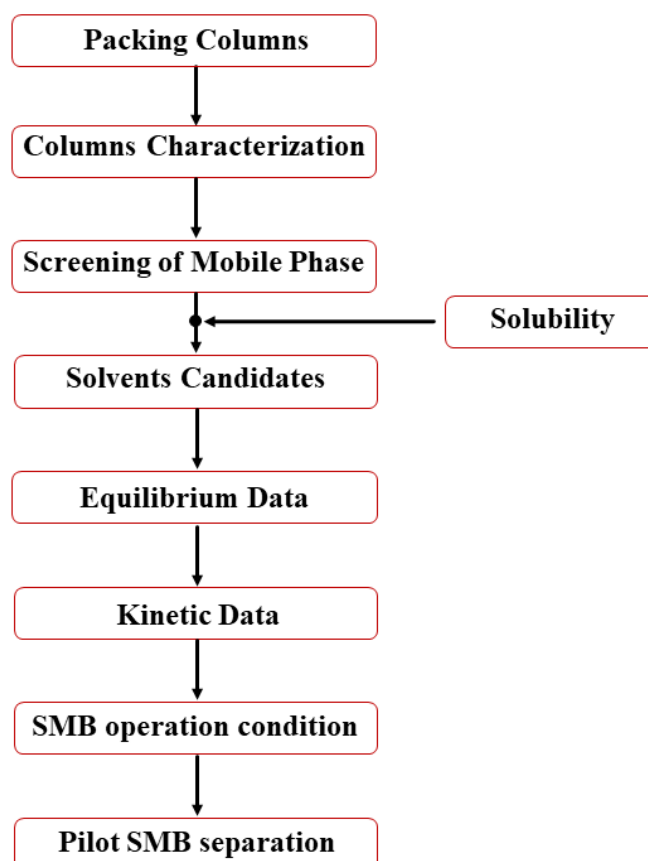


Fig 1.3. Flowchart for the fixed-bed adsorption process

Simulated Moving Bed technology is one of the most powerful techniques used for preparative chromatography. One of the essential advantages of this technology is that it allows the continuous injection and separation of binary mixtures, and the mass-transfer driving force is maximized by the counter-current contact between the solid and liquid phases. The counter-current contact in SMB was initially achieved using a rotary valve that periodically shifted the feed, eluent, raffinate, and extracts streams at selected time intervals in the direction of the fluid flow phase [8].

The first appearance of the SMB technology in the chemical engineering field was in 1961 when the first patent UOP (Universal Oil Products, Des Plaines, Illinois, USA) appeared. Then this technology was applied to petroleum refining and petrochemicals and became known as the Sorbex process, which was designed for *p*-xylene recovery from mixed xylenes.

The SMB is a much more helpful tool in the pharmaceutical industry, particularly for small-scale tests, where only a few grams of chiral drugs are needed. However, the use of SMB technology in the pharmaceutical industry is not limited to laboratory tests. Large-scale chromatographic separations were limited mainly due to the high cost of the adsorbent, the high dilution of products, and the large amounts of mobile phase needed. With the introduction of SMB technology, large-scale separation can now be carried out under cost-effective conditions. A diagram of a Simulated Moving Bed is presented in Figure 1.4.

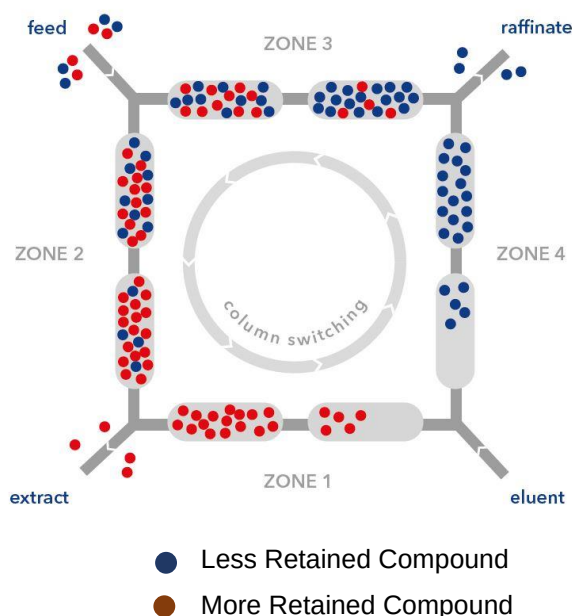


Fig 1.4. Schematic diagram of a Simulated Moving Bed.

There are few published works reporting the separation of nadolol, table 1.1 presents the main separation of nadolol at analytical scale, while table 1.2 shows the recent binary simulated moving bed separations of nadolol.

Table 1.1 main separation of nadolol at analytical scale.

Author	Year	Column	Mobile Phase Composition	UV(nm)
McCarthy [14]	1994	Analytical: Chiralpak® AD, 10 µm	EtOH/Hex/IPA (25/75/0) to (75/0/75) EtOH/Hex/DEA (20/80) Hex/EtOH/IPA (77/15/8) all with 0.3%DEA H ₂ O/EtOH (75/25) with 0.5%DEA	270
Aboul-Enein and Aboul-Basha [16]	1996	Analytical: Chiralcel OD	EtOH/Hex/DEA (x/y/0.4) different x/y	254
Wang and Ching [17]	2002	Analytical: β-cyclodextrin, 15 µm	H ₂ O/MeOH/TEAA (80/20/1) pH=5.5	280
Wang and Ching [18]	2003	Analytical: β-cyclodextrin, 15 µm	H ₂ O/MeOH/TEAA (80/20/1) pH=5.5	280
Wang and Ching [19]	2004	SMB (2-2-2-2): β-cyclodextrin, 15-25 µm Analytical: β-cyclodextrin, 5 µm	H ₂ O/MeOH/TEAA (80/20/1)	280
Wang and Ching [20]	2005	SMB (2-2-2-2): β-cyclodextrin, 15-25 µm Analytical: β-cyclodextrin, 5 µm	H ₂ O/MeOH/TEAA (80/20/1)	280
Singh <i>et al</i> [21]	2006	Analytical: Chiralpak® OD, 10 µm	EtOH/Hex/DEA/HAc (15/87/0.2/0.2)	276
Wang and Ching [22]	2007	Analytical: Chiralpak® AD-H, 5 µm	EtOH/Hex/DEA (20/80/0.3)	280
Van Arnum [23]	2008	Analytical: Chiralpak® AD-H	0.1%ESA in Hex/EtOH	---
Sung <i>et al</i> [24]	2010	Preparative HPLC: JAIGEL- ODS-BP-L Analytical: Chiralpak® AD-H	MeOH/H ₂ O	230

The chiral separation of all four stereoisomers at the analytical scale was first reported by McCarthy in 1994 [14]. In that work, the analytical chiral separation was performed with normal and reverse-phase methods, using a tris 3,5-dimethylphenyl carbamate (Chiralpak® AD, 10 mm) chiral stationary phase. Several mobile-phase compositions were tested using hydrocarbons, alcohols, and acetonitrile. All four stereoisomers' complete analytical separation was reported under the normal-phase mode, using a 20:80:0.3 %ethanol:%hexane:%diethylamine mobile-phase composition. The last retained compound, referred to as the most active stereoisomer (RSR-nadolol), eluted the chiral chromatographic column after 32 min using a flow rate of 1.2 mL/min.

Wang and Ching reported in 2004, for the first time, the separation of nadolol stereoisomers at preparative scale. However, the adsorbent used was based on perphenyl carbamoylated β -cyclodextrin (15–25 μm) that normally presents low saturation capacity (0.1–5 mg solute/g CSP) and, consequently, low preparative performance when compared with the polysaccharide-derivatives-based CSP (5–150 mg solute/g CSP) [19-20].

In 2007, Wang and Ching published another study reporting the analytical separation of nadolol stereoisomers using Chiralpak[®] AD column (5 μm), but no further work was reported considering the experimental preparative chiral separation using this chiral stationary phase [22].

Table 1.2. Recent separations of nadolol using SMB chromatography.

Author	Year	Column	Mobile Phase Composition	UV(nm)
Ribeiro <i>et al</i> [15]	2013	SMB (1-2-2-1): Chiralpak [®] AD	80E/20C7/0.3DEA	230/270
Jermann <i>et al</i> [25]	2015	3C-ISMB: Chiralpak [®] AD	70E/30C7/0.3DEA	240
Arafah <i>et al</i> [26]	2016	SMB (1-2-2-1): Chiralpak [®] IA	100M/0.1DEA	270
Arafah <i>et al</i> [27]	2019	SMB (1-2-2-1) Chiralpak [®] IA	25M/75ACN/0.1DEA	270
Arafah <i>et al</i> [28]	2020	SMB (1-2-2-1) XBridge C18 Shield XSelect	Simulation 30E/70W/0.1DEA	---
Arafah <i>et al</i> [29]	2022	SMB (1-2-2-1) Fixed-bed XBridge C18	30E/70W/0.1DEA	270
Arafah <i>et al.</i> <i>To be</i> <i>Submitted</i> [30]	2023	SMB (1-2-2-1) Fixed-bed Chiralpak [®] IA	5M/95ACN/0.075DEA 25M/75ACN/0.1DEA	270

Ribeiro *et al.* published a work in 2013 reporting the preparative pseudo-binary separation by chiral liquid chromatography of the four nadolol stereoisomers using the

Chiralpak® AD adsorbent. That work shows how retention and resolution depend on the polarity of the solvent composition (ethanol-hexane and ethanol-heptane mixtures). The selected mobile phase composition for the preparative separation of the more retained stereoisomer (RSR-nadolol) was an 80:20:0.3 %ethanol:%heptane:%diethylamine mixture. The SMB separation was carried out using a 2 g/L total feed concentration. Both recovery and purity were 100%, with system productivity of PR=0.65 g/(L.h) and a solvent consumption of SC = 9.6 L/g [15].

Jermann et al., in 2015, studied and applied the three columns intermittent simulated moving bed technology (3C-ISMB) to separate nadolol stereoisomers. The separation was carried out using the Chiralpak® AD 20 mm stationary phase using a 30:70:0.3 %heptane:%ethanol:%diethylamine solvent composition and cascade productivity of 2.10 g/(L h) and solvent consumption of 12 L/g of the product was obtained by using total feed concentrations of 5 g/L, 10 g/L, 15 g/L, and 20 g/L. The individual elution profiles of each component were afterward derived from the offline analysis of the collected fractions [25].

In 2016, Arafah et al. presented the pseudo-binary separation of the RSR-stereoisomer of nadolol, using a more efficient immobilized Chiralpak® IA CSP and pure methanol with 0.1% of diethylamine modifier as solvent composition. This work proved that the immobilized Chiralpak® IA is a better alternative to the former coated Chiralpak® AD CSP since it extends the range of compatibility between adsorbent and solvent compositions, promoting new possibilities for preparative separation of nadolol stereoisomers [26].

Arafah et al. 2019 published a new work on improving the chiral separation of nadolol stereoisomers using Chiralpak® IA with a more efficient mobile phase composition of 25%methanol:75% acetonitrile:0.1% diethylamine. A 2 g/L total feed

concentration was used to perform pseudo-binary separation of nadolol stereoisomers via SMB operation. The more retained component was recovered 100% pure, with system productivity of 0.77 g/(L.h) and a solvent consumption of 9.62 L/g. Comparing these results with the ones reported by Arafah et al. (2016), this work shows that the 25:75:0.1 methanol acetonitrile-diethylamine solvent composition is a better alternative to 100:0.1 methanol-diethylamine for the preparative separation of nadolol stereoisomers by SMB chromatography [27].

Arafah et al. 2020 optimized the first separation of nadolol racemates under pH reversed phase under preparative conditions. The optimization for SMB operation was concluded after comparing three waters C18 adsorbents, namely, Xbridge, Shield and XSelect. Using ethanol:water solvent mixture with diethylamine as a basic modifier. The Waters XBridge C18 adsorbent was concluded to be the better solution for the preparative separation of nadolol racemates, using ethanol:water: diethylamine (30:70:0.005) solvent composition (pH=11) and under preparative and simulated moving bed (SMB) chromatography [28].

Arafah et al., 2022 performed the real separation of nadolol racemates under high pH reversed phase using both fixed-bed and simulated moving bed; the separation two pairs of enantiomers was carried out using one commercial fixed-bed Azura HPLC system, equipped with sole Water Xbridge C18 of preparative dimensions (30 mm ID x 250 mm L). The same separation was excited using lab-scale SMB apparatus (the FlexSMB-LSRE pilot unit) equipped with six semi-preparative columns (19 mm ID x 100 mm L) (Chapter 4). A feed concentration of 9 g/L used for FB, the purities for both outlet streams were 99.9%, with a global system productivity of 3.06 $\text{g}_{\text{feed}}/(\text{L}_{\text{bed}}.\text{hr})$ and a solvent consumption of 4.21 $\text{L}_{\text{solvent}}/\text{g}_{\text{feed}}$. For SMB, the pilot unit's pressure drops limits imposed a maximum internal flow rate of only 5 mL/min and, for

a nadolol feed concentration of 2 g/L, both racemates were recovered 100% pure, with a system productivity of $0.13 \text{ g}_{\text{feed}}/(\text{L}_{\text{bed}}.\text{hr})$ and a solvent consumption of $6.19 \text{ L}_{\text{solvent}}/\text{g}_{\text{feed}}$ [29].

Recently, Arafah et al., 2022 submitted the final stage of complete separation for nadolol stereoisomers; the chiral separation of (2/3) and (1/4) was done using fixed-bed and simulated moving bed chromatography, and both systems were attached with Chiralpak®IA adsorbent with exact dimensions used in previous work. The separation of 2/3 was carried out using 5M/95ACN solvent composition, while 1/4 separation was executed using 25M/75ACN solvent composition. Both separations achieved 100% purities for all outlet streams [30].

Chapter 2 Modelling and simulation tools

This chapter focuses on the main experimental and simulation methods for predicting the adsorption equilibrium isotherms using the adsorption-desorption method. All equations to calculate the performance parameters for fixed bed and simulated moving bed chromatography are presented.

2.1 Adsorption equilibrium isotherms

An accurate method is advised to experimentally measure the equilibrium adsorption isotherm since equilibrium data dramatically impacts the quality of the preparative separation process performance. In this work, we focus on separating nadolol stereoisomers at the preparative scale. Due to that, it is advisable to obtain the competitive isotherm experimentally instead of predicting those from single component isotherms.

The experimental determination of the competitive adsorption isotherms was carried out using the adsorption-desorption method. According to this method, the preparative column is saturated with a large amount of the feed solution with known racemic nadolol concentration (2, 4, 6, 8, and 10 g/L) until equilibrium is achieved. The column is then completely regenerated with pure eluent. The eluted volume resulting from this desorption step is collected and analysed using the analytical Knauer HPLC system in order to measure each enantiomer concentration (Figure 2.1).

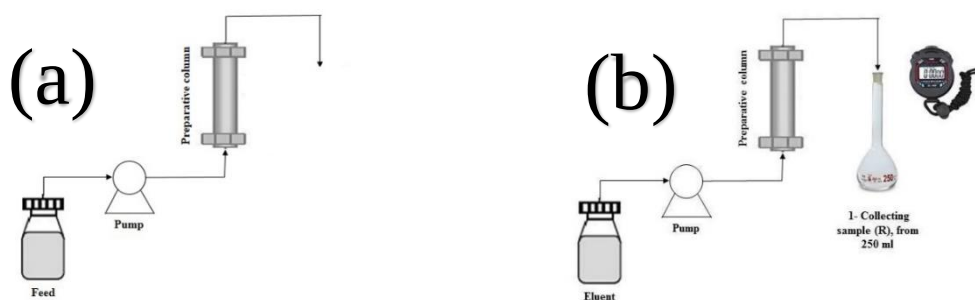


Fig 2.1. The adsorption–desorption method; (a) saturation step, (b) regeneration step.

Under this saturation scenario, the concentration of component i in the solid phase, q_i^* , in equilibrium with the concentration of component i in the liquid phase (that is equal to feed concentration, C_i^F), can be calculated using the following mass balance:

$$V^d c_i^d = \varepsilon V_c C_i^F + (1 - \varepsilon) V_c q_i^* \quad (1)$$

where:

V^d : Eluted volume [cm³];

c_i^d : Concentration of each component in the eluted volume [g/L];

ε : The bed porosity, equal to 0.4;

V_c : The column volume [cm³];

C_i^F : The concentration of component i in the feed solution [g/L];

q_i^* : Concentration of each component retained in the particle [g/L]

In fact, q_i^* is considered as the total concentration retained in the adsorbent, considering the mass of each species retained in the adsorbent and the mass of each species retained inside the particle porous. This procedure is consistent with the simulation of the chromatographic process, based in a model that considers the particles as homogeneous.

The previous procedure produces one single point of the adsorption isotherm for each component and a given feed nadolol solution concentration. Due to that, it is necessary to apply the mass balance equation for different feed solution concentrations to obtain the complete adsorption isotherm.

For chiral mixtures, the Langmuir model is the most used one. However, this model typically fails to predict the preparative separation process. It is well known that, when leading with chiral mixtures under competitive conditions (high concentrations), the selectivity factor decrease with the increase of species concentration. The Langmuir model thus not considers this behavior since it predicts a constant value over

the entire range of concentrations. A practical way to overcome this limitation is to introduce a linear term into the Langmuir model. This “linear+Langmuir” model (LLG4) can be expressed for a quaternary mixture as:

$$q_i^* = \frac{Qb_iC_i}{1+\sum_{j=1}^2 b_jC_j} \quad (\text{Langmuir}) \quad (2)$$

$$q_i^* = mC_i + \frac{Qb_iC_i}{1+\sum_{j=1}^2 b_jC_j} \quad (\text{Linear + Langmuir}) \quad (3)$$

where:

$i = 1, 2$ (number of species): 1 is the less returned component(s)

: 2 is the more returned component(s)

m : Linear term of the equilibrium adsorption isotherm

C_i : Concentration of species i in the liquid phase (g/L)

Q : Capacity factor of the equilibrium adsorption isotherm of the Langmuir term (g/L)

b_i : Model parameter for species i of the equilibrium adsorption isotherm (L/g)

The adsorption isotherm parameters were estimated using the Levenberg-Marquardt algorithm written on FORTRAN language [31-32]. For these simulations the objective function was defined to minimize the SD parameter, defined as,

$$SD = \sqrt{\frac{SQ}{M - N}} \quad (4)$$

with,

M : Total number of experimental points for the equilibrium adsorption isotherm

N : Total number parameters to be estimated for the equilibrium adsorption isotherm model

$$SQ = \sum_{j=1}^{M/2} \left[(q_{1j}^{*T} - q_{1j}^{*E})^2 + (q_{2j}^{*T} - q_{2j}^{*E})^2 \right] \quad (5)$$

were,

q_{ij}^{*T} and q_{ij}^{*E} are the equilibrium solid concentration of species i predicted from the model and experimentally obtained, respectively.

2.2 Fixed-bed chromatography

Generally, in preparative competitive liquid chromatography, the modelling of fixed-bed saturation and regeneration steps can be determined using the Linear Driving Force (LDF) model. This model assumes that the adsorbent particles are homogeneous, the flow is composed by the sum of a convective term and a dispersive term, and the mass transfer resistance is described by a linear driving force model. This methodology has been used in the majority of the published work in the field [33-34]. The model is based on the mass balance equations:

$$\frac{\partial C_i}{\partial \theta} = \frac{1}{Pe} \frac{\partial^2 C_i}{\partial x^2} - \frac{\partial C_i}{\partial x} - \frac{1 - \varepsilon}{\varepsilon} St(q_i^* - q_i) \quad (6)$$

And

$$\frac{\partial C_i}{\partial \theta} = St(q_i^* - q_i) \quad (7)$$

$i = 1, 2$ (number of species): 1 is the less returned component(s)

: 2 is the more returned component(s)

$$q_i^* = f_i(C_1, C_2) \quad (8)$$

The initial conditions are:

(i) For saturation:

$$\theta = 0, \forall x, C_i = 0; q_i = 0 \quad (9)$$

(ii) For regeneration:

$$\begin{aligned} \theta = 0, \forall x, C_i &= C_i^F \\ q_i &= q_i^{*F} = f_i(C_1^F, C_2^F) \end{aligned} \quad (10)$$

The boundary conditions are:

(i) For saturation:

$$x = 0, C_i - \frac{1}{Pe} \frac{\partial C_i}{\partial x} = C_i^F \quad (11)$$

$$x = 1, \frac{dC_i}{dx} = 0 \quad (12)$$

(ii) For regeneration:

$$x = 0, C_i - \frac{1}{Pe} \frac{dC_i}{dx} = 0 \quad (13)$$

$$x = 1, \frac{dC_i}{dx} = 0 \quad (14)$$

In these equations, C_i is the concentration of species i in the liquid; q_i is the average adsorbed concentration of species i in equilibrium with dimensionless axial distance:

$$x = \frac{z}{Lc} \quad (15)$$

And the dimensionless time variable:

$$\theta = \frac{t}{\tau} \quad (16)$$

With space time:

$$\tau = \frac{Lc}{u_i} \quad (17)$$

ε is the bed porosity; Peclet number:

$$Pe = \frac{u_i Lc}{D_{ax}} \quad (18)$$

k is the mass transfer coefficient, and the mass Stanton number:

$$St = k \tau \quad (19)$$

The validation of the selected adsorption isotherm mode was carried out through frontal chromatography experiments as it is presented in figure 2.2.

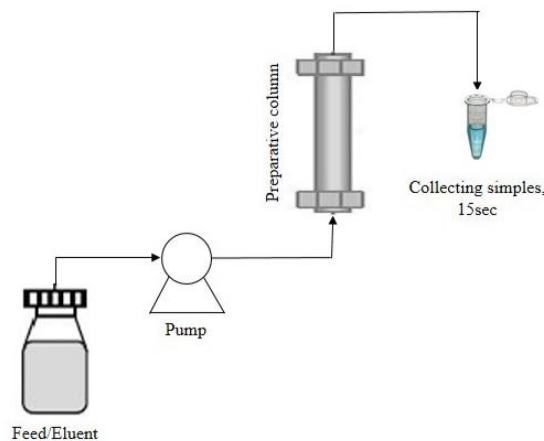


Fig 2.2. Experimental scheme for breakthroughs measurements.

A preparative Knauer HPLC system equipped with a preparative column dimensions, a 10 g/L of nadolol feed concentration was used to perform the saturation-

regeneration steps. During these steps, several different samples were collected immediately at the end of the chromatographic column each 15 seconds, all collected samples on the saturation and regeneration steps, were then analysed on the analytical Knauer HPLC system.

The modelling and simulation were performed using a PDECOL subroutine written in FORTRAN, the used code was developed by former students.

2.3 Simulated moving bed chromatography

The transient SMB model was used to perform the simulation for the prediction of the SMB operation conditions. This model also accounts for the mass transfer resistances and the particular FlexSMB unit dead volumes and switching time delay. The simulations were done using the gPROMS software package (v.3.0.4) from Process System Enterprise (UK), being the flow rates for sections II and III chosen inside the separation region defined by the Triangle Theory [34].

The modelling and simulation of SMB processes usually uses two different approaches. The first is based on the equivalence with the true moving bed (TMB), considering that liquid and solid move in opposite directions. The second is based on the simulation of the real system, by considering its intermittent behavior, that is, the shift of all ports in the direction of the fluid flow. Both mathematical models are based on the following assumptions:

The movement of the fluid phase is described by an axial dispersion flow; the adsorbent particles are assumed as homogeneous material, and the equilibrium between liquid and solid is described by the linear + Langmuir.

The equivalence between the two approaches can be established in terms of interstitial velocities.

$$v_j^* = v_j + v_s \quad (20)$$

The SMB liquid velocity is equal to the sum of the liquid and solid velocities in the TMB approach. The solid velocity in the TMB model can be evaluated using the switch time interval, t^* of the SMB model, by:

$$v_s = \frac{Lc}{t^*} \quad (21)$$

considering LC as the length of one SMB column. The equivalence can also be expressed in terms of the liquid flow-rate by:

$$Q_j^* = Q_j + \frac{\varepsilon Q_s}{(1 - \varepsilon)} \quad (22)$$

Where:

$$Q_s = (1 - \varepsilon) \frac{V_c}{t^*} \quad (23)$$

Q_s is the TMB solid flow-rate, V_c being the volume of one SMB column. Despite the different evolutions in the transient state of the SMB and TMB models, both approaches have similar steady states performances, provided that all sections have a plural number of chromatographic columns, that is, a total minimum of eight columns. In these situations, the TMB model gives satisfactory results and also avoids a considerable computing time. However, when a smaller number of columns are used, the real and more accurate SMB model should be used. In the present work, an SMB unit with six columns was used and, therefore, the real transient SMB model was applied, being the multicomponent adsorption equilibrium described by the linear + Langmuir isotherm model (LLG4).

Table 2.1 Transient SMB model equations

Mass balance over a volume element of the bed, k :

$$\frac{\partial C_{ik}}{\partial \theta} = \gamma_k^* \left\{ \frac{1}{Pe_k} \frac{\partial^2 C_{ik}}{\partial X^2} - \frac{\partial C_{ik}}{\partial X} \right\} - \frac{1-\varepsilon}{\varepsilon} \alpha_k (q_{ik}^* - q_{ik}) \quad \begin{array}{l} i = 1, 2 \text{ (component)} \\ k = 1, \dots, N_C = 6 \text{ (column)} \end{array} \quad (27)$$

Mass balance in the particle:

$$\frac{\partial q_{ik}}{\partial \theta} = \alpha_k (q_{ik}^* - q_{ik}) \quad (28)$$

Multicomponent adsorption equilibrium isotherms:

$$q_{1k}^* = f_1(C_{1k}, C_{2k}) \text{ and } q_{2k}^* = f_2(C_{1k}, C_{2k}) \quad (29)$$

Initial conditions:

$$\theta = 0 : C_{ik} = q_{ik} = 0 \quad (30)$$

Boundary conditions for column k :

$$x = 0 : C_{ik} - \frac{1}{Pe_k} \frac{dC_{ik}}{dx} = C_{ik,0} \quad (31a)$$

Where $C_{ik,0}$ is the inlet concentration of species i in column k .

$$x = 1 : \frac{dC_{ik}}{dx} = 0 \quad (31b)$$

Node balances:

For a column inside a section and for extract and raffinate nodes:

$$C_{ik} = C_{ik+1,0} \quad (32)$$

For the eluent node:

$$C_{ik} = \frac{V_I^*}{V_{IV}^*} C_{ik+1,0} \quad (33)$$

For the feed node:

$$C_{ik} = \frac{V_{III}^*}{V_{II}^*} C_{ik+1,0} - \frac{V_F}{V_{II}^*} C_i^F \quad (34)$$

Global balances:

Eluent node:

$$V_I^* = V_{IV}^* + V_E \quad (35)$$

Extract node:

$$V_{II}^* = V_I^* - V_X \quad (36)$$

Feed node:

$$V_{III}^* = V_{II}^* + V_F \quad (37)$$

Raffinate node:

$$V_{IV}^* = V_{III}^* - V_R \quad (38)$$

Parameters of the SMB model:

Ratio between solid and fluid phases:

$$(1-\varepsilon)/\varepsilon \quad (39)$$

Ratio between fluid and solid interstitial velocities:

$$\gamma_k^* = \frac{v_k^*}{L_C/t^*} \quad (40)$$

Peclet number:

$$Pe = \frac{v_k^* L_k}{D_{ax_k}} \quad (41)$$

The number of mass transfer units:

$$\alpha_k = k t^* \quad (42)$$

where t^* is switch time for the SMB operation, D_{ax_k} is the axial dispersion coefficient and k is the intraparticle mass transfer coefficient.

The adsorption equilibrium parameters (depending on the isotherm model used):

$$q_{1k}^* = f_1(C_{1k}, C_{2k}) \text{ and } q_{2k}^* = f_2(C_{1k}, C_{2k})$$

The SMB operating conditions are usually obtained from the constraints for the net fluxes of each component (in this case “enantiomer”) in each of the four sections of the equivalent TMB approach. In section I, both enantiomers must be completely desorbed to clean the solid phase at the beginning of this zone. In sections II and III, the two enantiomers must move in opposite directions, which means, the more retained enantiomer to be adsorbed and carried by the solid phase, while the less retained enantiomer must be desorbed and carried by the liquid phase. Finally, in section IV, both enantiomers must be adsorbed in order to regenerate completely the liquid that will be recycled to the beginning of the first section. The constraints will define the regeneration (sections I and IV) and the separation (sections II and III) regions:

$$\begin{aligned} \frac{Q_I C_{2,I}}{Q_s q_{2,1}} > 1; \frac{Q_{II} C_{1,II}}{Q_s q_{1,II}} > 1 \text{ and } \frac{Q_{II} C_{2,II}}{Q_s q_{2,II}} < 1; \\ \frac{Q_{III} C_{1,III}}{Q_s q_{1,III}} > 1 \text{ and } \frac{Q_{III} C_{2,III}}{Q_s q_{2,III}} < 1; \frac{Q_{IV} C_{1,IV}}{Q_s q_{1,IV}} < 1 \end{aligned} \quad (43)$$

where Q_j is the volumetric liquid flow-rate in section j , Q_s is the solid flow-rate, $Q_{1,j}$ and $Q_{2,j}$ are the liquid phase concentration, and $q_{1,j}$ and $q_{2,j}$ are the adsorbed concentrations of species 1 and 2 in section j of the equivalent TMB operation. These constraints can also be expressed in terms of liquid and solid interstitial velocities using the dimensionless ratio between liquid and solid volumes, $\varepsilon/(1 - \varepsilon)$, and the dimensionless ratio between liquid and solid interstitial velocities in each section,

$\gamma_j = v_j/u_s$. In this way, the previous constraints become:

$$\begin{aligned} \gamma_I > \frac{(1 - \varepsilon) q_{2,I}}{\varepsilon C_{2,I}}; \frac{(1 - \varepsilon) q_{1,II}}{\varepsilon C_{1,II}} < \gamma_{II} < \frac{(1 - \varepsilon) q_{2,II}}{\varepsilon C_{2,II}} \\ \frac{(1 - \varepsilon) q_{1,III}}{\varepsilon C_{1,III}} < \gamma_{III} < \frac{(1 - \varepsilon) q_{2,III}}{\varepsilon C_{2,III}}; \gamma_{IV} < \frac{(1 - \varepsilon) q_{1,IV}}{\varepsilon C_{1,IV}} \end{aligned} \quad (44)$$

The definition of the above constraints had led to several design methodologies that allows, in several cases, a good approach to the separation region. The simplest method is developed over a complete design methodology called the “Triangle Theory” [35-36]. This method is based on the assumptions that mass transfer resistances and axial dispersion are negligible, and the adsorption equilibrium can be described by a Langmuir isotherm model (LG3) or by a linear + Langmuir isotherm model (LLG4). However, when mass transfer resistances and axial dispersion cannot be neglected, the critical values are more restrictive and should be evaluated through simulation using more precise models.

In the present study, the design of the SMB operation is obtained by simulation, taking into account mass transfer resistances and axial dispersion and by evaluating the separation regions where both outlet streams (extract and raffinate) are, at least, 99.0% pure. The maximum pressure allowed in the FlexSMB-LSRE[®] unit is near 50 bar due to a trade-off between maximum adsorbent pressure drop and hardware limitations. In view of these limitations, it was decided to work with a maximum system pressure around 35 bar, measured at the beginning of section I. This pressure limit implies fixing the internal flow-rate in section I Q_I^* . The critical values for γ_I and γ_{IV} were also fixed by using the following equations:

$$\gamma_I = \frac{(1 - \varepsilon)}{\varepsilon} (m_2 + Qb_2)\beta \quad (45)$$

$$\gamma_{IV} = \frac{(1 - \varepsilon)}{\varepsilon} (m_1 + Qb_1)\beta \quad (46)$$

where m_1 ; m_2 , Q , b_1 , and b_2 are the parameters of the LLG4 adsorption isotherm model, and β is the safety margin, considering here as 25%, that is, $\beta = 1.25$. Afterwards, the switching time was fixed using:

$$t^* = \frac{\varepsilon V_c}{\varepsilon} (\gamma_I + 1) \quad (47)$$

After fixing Q_I^* , γ_I , γ_{IV} , and t^* for each mobile phase composition, the SMB operating conditions were obtained by evaluating, through simulation, the $(\gamma_{II} X \gamma_{III})$ separation regions. The experimental operation of the SMB unit is then carried out by choosing a pair of $(\gamma_{II} X \gamma_{III})$ values inside the separation region evaluated before. The corresponding SMB γ_j^* values can be determined by using:

$$\gamma_j^* = \gamma_j + 1 \quad (48)$$

and the internal SMB flow-rates in sections II, III, and IV, by using

$$Q_j^* = \frac{\varepsilon V c}{\varepsilon} \gamma_j^* \quad (j = II, III, IV) \quad (49)$$

(note that Q_I^* is already fixed by the pressure limit described before). The inlet, outlet and recycle SMB flow-rates are then evaluated by

$$Q_E = Q_I^* - Q_{IV}^* \quad (50)$$

$$Q_X = Q_I^* - Q_{II}^* \quad (51)$$

$$Q_F = Q_{III}^* - Q_{II}^* \quad (52)$$

$$Q_R = Q_{III}^* - Q_{IV}^* \quad (53)$$

$$Q_{REC} = Q_{IV}^* \quad (54)$$

2.4 Performance parameters

2.4.1 SMB performance parameters

The SMB operation can be characterized in terms of performance parameters, namely, purity, recovery, productivity and solvent consumption. For the pseudo-binary separation of nadolol compounds it is expected that component 1, the more retained component, to be completely recovered and collected in the extract, while the less retained component 2 to be completely recovered and collected in the raffinate stream.

The extract purity (PU_X , %) is defined as the ratio between the mean concentration of the more retained component 1 and the sum of the mean concentrations of the two components in the extract stream. Similarly, the raffinate purity (PU_R , %) is defined as the ratio between the mean concentration of the less retained component 2 and the

sum of the mean concentrations of the two components in the raffinate stream. The recovery in the extract stream (RC_X , %) is defined as the ratio between the amount of the more retained component 1 collected in the extract and the total amount of the same component 1 fed into the system. The recovery in the raffinate stream (RC_R , %) is defined as the ratio between the amount of the less retained component 2 obtained in the raffinate and the total amount of the same component 2 fed into the system. Inside a SMB complete separation region both purities and both recoveries are 100%, as $\bar{C}_2^X = 0$ and $\bar{C}_1^R = 0$, as well as $\bar{C}_1^X = Q_F C_1^F / Q_X$ and $\bar{C}_2^R = Q_F C_2^F / Q_R$.

The system productivity of component 1 (PR_1 , g/L_{bed}.hr) is defined as the amount of the more retained component 1 recovered in the extract stream per total volume of bed and per time. Similarly, the system productivity of component 2 (PR_2 , g/L_{bed}.hr) is defined as the amount of the less retained component 2 recovered in the raffinate stream per total volume of bed and per time. A global system productivity can also be defined as the sum of these two amounts. Inside a SMB complete separation region, as purities and recoveries are 100%, this global system productivity is also equal to the amount of nadolol components fed into the system per total volume of bed and per time, $PR = Q_F(C_1^F + C_2^F)/V_T$.

The solvent consumption (SC, L_{solvent}/g) is defined as the ratio between the total amount of solvent fed into the system (in eluent and feed) and the total amount of nadolol components recovered in the extract (component 1) and in the raffinate (component 2) streams. As well, inside a SMB complete separation region, as purities and recoveries are 100%, solvent consumption is also equal to the ratio between the total amount of solvent fed into the system (in eluent and feed) and the amount of nadolol components fed into the system, $SC = (Q_E + Q_F)/[Q_F(C_1^F + C_2^F)]$ or $SC = (Q_E + Q_F)/(PR \times V_T)$. The SMB performance parameters are summarized in Table 2.1.

Table 2.2 SMB performance parameters. 1 represents the more retained racemate to be recovered in the extract and 2 represents the less retained racemate to be recovered in the raffinate stream.

Extract Purity PU_X (%)	$PU_X = \frac{\bar{C}_1^X}{\bar{C}_1^X + \bar{C}_2^X}$	(55)
Raffinate Purity PU_R (%)	$PU_R = \frac{\bar{C}_2^R}{\bar{C}_1^R + \bar{C}_2^R}$	(56)
Extract Recovery RC_X (%)	$RC_X = \frac{Q_X \bar{C}_1^X}{Q_F \bar{C}_1^F}$	(57)
Raffinate Recovery RC_R (%)	$RC_R = \frac{Q_R \bar{C}_2^R}{Q_F \bar{C}_2^F}$	(58)
Global Recovery RC (%)	$RC = \frac{Q_X \bar{C}_1^X + Q_R \bar{C}_2^R}{Q_F (\bar{C}_1^F + \bar{C}_2^F)} = \frac{RC_X + RC_R}{2}$ if $\bar{C}_1^F = \bar{C}_2^F$	(59)
Productivity of racemate 1 PR_1 (g/L _{bed} .hr)	$PR_A = \frac{Q_X \bar{C}_1^X}{V_T}$	(60)
Productivity of racemate 2 PR_2 (g/L _{bed} .hr)	$PR_2 = \frac{Q_R \bar{C}_2^R}{V_T}$	(61)
Global Productivity PR (g/L _{bed} .hr)	$PR = \frac{Q_X \bar{C}_1^X + Q_R \bar{C}_2^R}{V_T} = PR_1 + PR_2$	(62)
Solvent Consumption SC (L _{solvent} /g)	$SC = \frac{Q_E + Q_F}{Q_X \bar{C}_1^X + Q_R \bar{C}_2^R}$	(63)

2.4.2 FB performance parameters

The FB performance is determined based in a equivalence with the SMB operation taking into consideration the fractions of pure compounds recovered and the waste(s) fraction(s) in each run. For each injection, the less retained component 2 is recovered in the first product fraction (fraction 2), while the more retained component 1 is recovered in the second product fraction (fraction 1), ensuring the minimum purity standards. Between the first and the second product fractions there is a waste fraction containing both components 1 and 2.

The purity of each FB product fraction, PU_1 and PU_2 (%), is calculated as the ratio between the concentration of the desired component and the total concentration of both components in that fraction. The recovery of each component, RC_i (%), is defined as the ratio between the amount of that component collected in its fraction and the total amount of that component fed into the system. As in FB operation the product fractions are not collected continuously, as in SMB operation, a global recovery, RC (%), can be defined as the ratio between the total amount of components 1 and 2 collected in the desired product fractions (fraction 1 and 2, respectively) and the total amount of components 1 and 2 fed into the system. Contrary to SMB operating inside its complete separation region, FB does not usually operate with 100% recovery since that would impose a baseline separation of the two components. In that way, FB operation usually has a waste fraction between the two pure product fractions. If both product fractions are pure and considering that components 1 and 2 are detected the same way by the UV equipment, the global recovery can also be experimentally calculated using the chromatogram areas of each fraction solution, that is, $RC = \frac{A_2 + A_1}{A_2 + A_w + A_1}$, where A_2 , A_w and A_1 are the chromatogram areas of the less retained component 2 fraction, of the waste W fraction and of the more retained component 1 fraction, respectively.

The system productivity of each component (PR_i , g/L_{bed}.hr) is defined as the amount of that component recovered in its product fraction per total volume of bed and per time. A global system productivity can also be defined as the sum of these two amounts. The solvent consumption (SC, L_{solvent}/g) is defined as the ratio between the total amount of solvent fed into the system and the total amount of components recovered in its product fractions. The FB performance parameters are summarized in Table 2.2

It can also be defined the equivalent inlet and outlet flow-rates for the FB operation: the feed flow-rate as $Q_F = V_{inj}/\Delta t_{inj}$, the eluent flow-rate as $Q_E = Q - Q_F$, the raffinate flow-rate as $Q_R = (\Delta t_{f_2}/\Delta t_{inj})Q$, the extract flow-rate as $Q_X = (\Delta t_{f_1}/\Delta t_{inj})Q$, and a waste flow-rate as $Q_W = (\Delta t_{f_W}/\Delta t_{inj})Q$, where Δt_{f_j} is the collecting time period of the fraction j , $\Delta t_{f_2} + \Delta t_{f_W} + \Delta t_{f_1} = \Delta t_{inj}$ and $Q = Q_E + Q_F = Q_R + Q_W + Q_X$. Using these equivalent flow-rates, the FB performance equations presented in Table 2.2 can be rewritten the same way as the performance equations for SMB operation presented in Table 2.1.

Table 2.3. FB performance parameters. 1 and 2 represent the more and the less retained component, respectively.

$$\begin{array}{ll} \text{Purity of fraction 1} & \\ PU_1 (\%) & PU_1 = \frac{\bar{C}_1^{f_1}}{\bar{C}_1^{f_1} + \bar{C}_2^{f_1}} \end{array} \quad (64)$$

$$\begin{array}{ll} \text{Purity of fraction 2} & \\ PU_2 (\%) & PU_2 = \frac{\bar{C}_2^{f_2}}{\bar{C}_1^{f_2} + \bar{C}_2^{f_2}} \end{array} \quad (65)$$

$$\begin{array}{ll} \text{Recovery in fraction 1} & \\ RC_1 (\%) & RC_1 = \frac{V_{f_1} \bar{C}_1^{f_1}}{V_{inj} C_1^F}, \text{ with } V_{f_1} = Q \Delta t_1 \end{array} \quad (66)$$

$$\begin{array}{ll} \text{Recovery in fraction 2} & \\ RC_2 (\%) & RC_2 = \frac{V_{f_2} \bar{C}_2^{f_2}}{V_{inj} C_2^F}, \text{ with } V_{f_2} = Q \Delta t_{f_2} \end{array} \quad (67)$$

$$\begin{array}{ll} \text{Global Recovery} & \\ RC (\%) & RC = \frac{V_{f_1} \bar{C}_1^{f_1} + V_{f_2} \bar{C}_2^{f_2}}{V_{inj} (C_1^F + C_2^F)} = \frac{RC_1 + RC_2}{2} \text{ if } C_1^F = C_2^F \end{array} \quad (68)$$

$$\begin{array}{ll} \text{Productivity of component 1} & \\ PR_1 (\text{g/L}_{\text{bed}} \cdot \text{hr}) & PR_1 = \frac{V_{f_1} \bar{C}_1^{f_1}}{V_T \Delta t_{inj}} = RC_1 \frac{V_{inj} C_1^F}{V_T \Delta t_{inj}} \end{array} \quad (69)$$

$$\begin{array}{ll} \text{Productivity of component 2} & \\ PR_2 (\text{g/L}_{\text{bed}} \cdot \text{hr}) & PR_2 = \frac{V_{f_2} \bar{C}_2^{f_2}}{V_T \Delta t_{inj}} = RC_2 \frac{V_{inj} C_2^F}{V_T \Delta t_{inj}} \end{array} \quad (70)$$

$$\begin{array}{ll} \text{Global Productivity} & \\ PR (\text{g/L}_{\text{bed}} \cdot \text{hr}) & PR = PR_1 + PR_2 = \frac{V_{f_1} \bar{C}_1^{f_1} + V_{f_2} \bar{C}_2^{f_2}}{V_T \Delta t_{inj}} \\ & = RC \frac{V_{inj} (C_1^F + C_2^F)}{V_T \Delta t_{inj}} \end{array} \quad (71)$$

$$\begin{array}{ll} \text{Solvent Consumption} & \\ SC (\text{L}_{\text{solvent}}/\text{g}) & SC = \frac{Q \Delta t_{inj}}{V_{f_1} \bar{C}_1^{f_1} + V_{f_2} \bar{C}_2^{f_2}} = \frac{1}{PR} \frac{Q}{V_T} \end{array} \quad (72)$$

C_i^F is the feed concentration of racemate i , $\bar{C}_i^{fj}$ is the average concentration of racemate i in product fraction j , V_{inj} is the feed injection volume, Δt_{inj} is the injection time interval (time between two successive feed injections), V_{fj} is the volume of product fraction j , Δt_{fi} is the collecting time period of product fraction j , V_T is the volume of the FB column, and Q is the FB flow-rate.

Chapter 3 Experimental materials and equipment

This chapter will present the chemical reagents, adsorbents, analytical and preparative columns, together with different chiral and achiral bulk materials. A description of the SMB pilot unit with the commercial preparative Azura HPLC system will be presented.

3.1 Reagents and adsorbents

The mixture of the four nadolol stereoisomers of nadolol and 1,2,3-tri-*tert*-butylbenzene used as the non-retained compound were obtained from Sigma-Aldrich (Schnelldorf, Germany). The basic modifier (DEA, diethylamine), methanol (M), acetonitrile (ACN) and ethanol (E), all HPLC grade solvents and were obtained from Fluka (Buchs, Switzerland). All reagents and solvents were used without further purification. Type I ultrapure water was obtained with a Merck Millipore Direct Q3 UV lab equipment.

3.2 Experimental materials and equipment

3.2.1 Equipment for Analytical and Preparative Chromatography

Two Knauer HPLC systems (Germany) were used to perform elution chromatography, experimental measurements of the adsorption equilibrium isotherms, and breakthroughs experiments.

The analytical experiments were obtained in a Knauer HPLC system equipped with one Smartline 1050 pump with a 10 mL pump head, a manual 6-port/3-channel injection valve with 20 and 100 μ L loops, and two detectors in series: a Smartline UV detector 2520 set at 230 or 270 nm wavelengths and a polarimeter detector (Chiralser IBZ, Messtechnik, Germany). Chiralpak IA (Daicel, Japan) was used for analytical chiral separations, and three C18 analytical columns (XBridge C18, XBridge Shield RP18 and XSelect CSH C18) were obtained from Waters (USA) manufacturer. All the

four analytical columns have the same dimensions (4.6 mm ID x 250 mm L) and are packed with 5 µm particle size materials. A typical flow-rate of 1 mL/min was used with the analytical HPLC Knauer system.

The preparative experiments were carried out in a Knauer HPLC system equipped with a Smartline UV detector 2520 set at 270 nm wavelength, two Smartline 1050 pumps with 50 mL pump heads, and a manual 6-port/3-channel injection valve with a 1000 µL loop. The preparative reversed-phase separations used a SiliaChrom XT C18 column (20 mm ID x 150 mm L) obtained from Silicycle manufacturer (Canada), three different C18 columns obtained from Waters manufacturer (19 mm ID x 100 mm L). The Waters preparative columns are packed with the same three materials previously referred (XBridge, Shield and XSelect), but with a particle size diameter of 10 µm. preparative (SMB) columns (100mm L x 21.2mm ID) were home-packed with Chiralpak® IA. A typical flow-rate of 5 mL/min was used with the preparative HPLC Knauer system, all IA columns were home-packed using a Knauer Wellchrom Pneumatic pump K-1900 equipped with a 250 mL/min pump head (Figure 3.2). The packing solvent was pure iso-propanol.

The Azura® preparative HPLC system is equipped with one Waters XBridge Prep C18 column of preparative dimensions (30 mm ID x 250 mm L) packed with the same adsorbent as the SMB columns (10 µm particle size diameter). The system was used at normal phase using Chiralpak® IA with preparative dimensions (30 mm ID x 250 mm L). This system is equipped with two preparative HPLC pumps P2.1L model with two 250 mL/min pump heads and one UV/VIS detector UVD2.1L set at 270 nm wavelength. One loop of 10 mL was used for all fixed-bed preparative separations.

All equipment used at analytical and preparative scale are presented in figures 3.1, 3.2 and 3.3

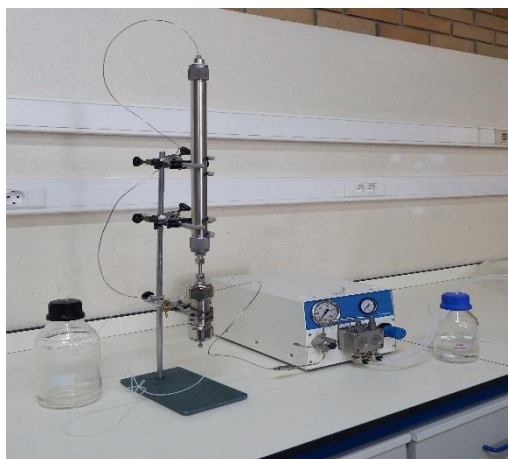


Fig 3.1. Preparative column packing system.

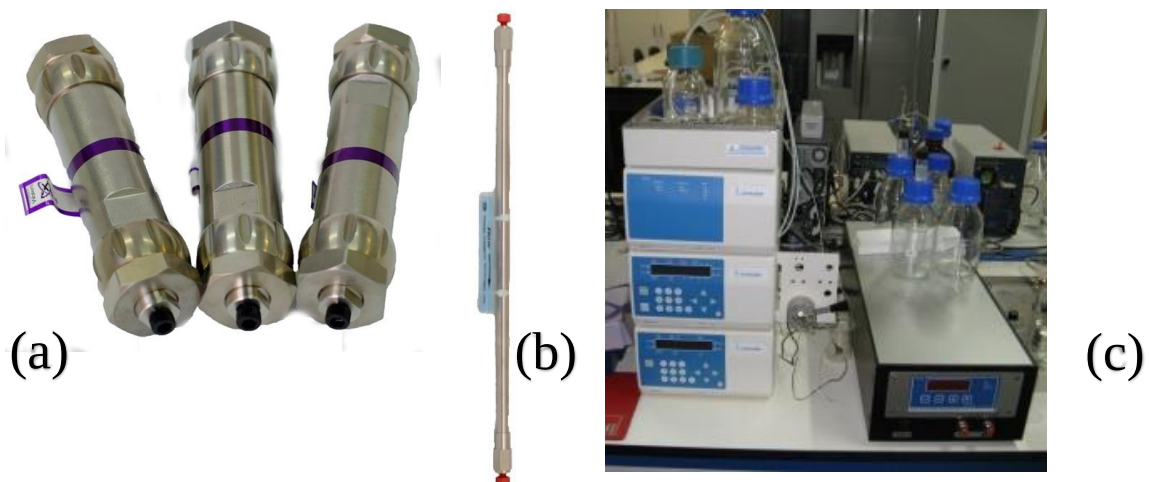


Fig 3.2. (a)Preparative columns, (b) Analytical column, (c) Knauer HPLC system

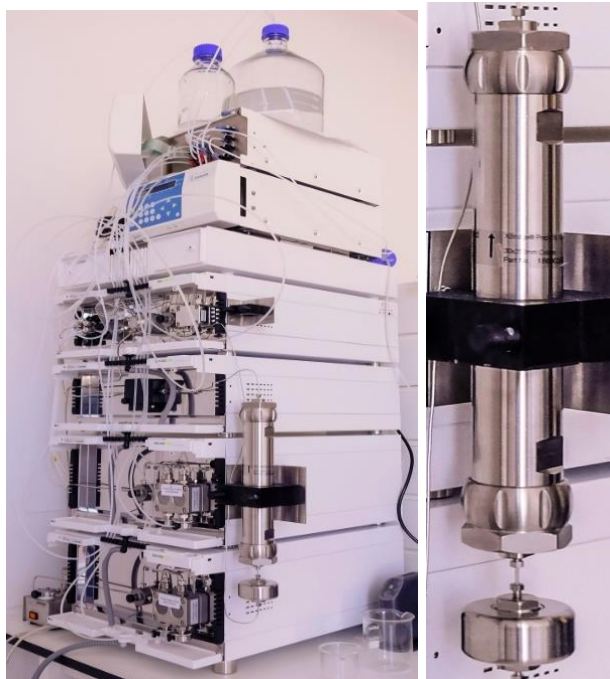


Fig 3.3. commercial fixed-bed preparative HPLC Azura system equipped with one sole column of preparative dimensions (30 mm ID x 250 mm L)

3.2.2 SMB Pilot Unit

The FlexSMB-LSRE1 unit is composed of one two-way valve per column (Select Dead-end flow path, Vici-Valco, Switzerland), two SD valves per stream for the extract (SD2 and SD5), and raffinate stream (SD4 and SD6) and one SD valve per stream for the feed (SD1) and eluent stream (SD3). There is only one pump within the main system line (the “recycle pump”), connected to the raffinate line at the beginning of section IV (see Fig. 3.4). The SMB unit use 4 HPLC pumps (VWR P130, VWR International, USA), 3 pumps with 50mL/min heads (Eluent, Extract and Raffinate), and 1 pump with 10mL/min head (Feed), aided by two Coriolis flow meters M53-ABD-22-0-B (Bronkhorst B.V., Netherlands) in order to reduce fluctuations inside the all unit. There are also 4 security valves (Purge valves, Swagelok, USA) after each pump head and one extra valve after SD4 valve on the raffinate stream used as a safety valve and to adjust the internal pressure manually. The outlet stream of each column is targeted to the extract or to the raffinate line through a two-way valve (acting as “on-off” valve) between each column. All the SMB experiments presented in this work were performed using six columns and a (1-2-2-1) configuration. The system (valves, pumps, and flow meters) is automatically controlled by means of 5 USB data acquisition boards, connected to a personal computer.

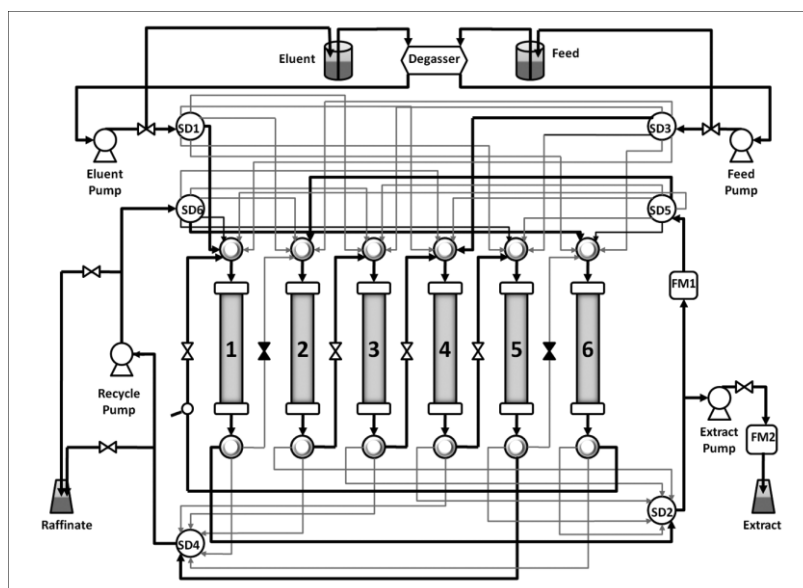


Fig 3.4 Scheme of the FlexSMB-LSRE1 unit, during the first step, using a six-column (1-2-2-1) configuration [34]

3.3 Packing of preparative chromatographic columns

Six preparative columns (i.e., preparative dimensions) were packed with Chiralpak® IA, a proper packing of the preparative column is one important step since good separation performances also depend on the adequate hydrodynamic behavior of the chromatographic column. A methodology was developed for packing the preparative columns and is based on the following steps:

- a) 21.5 g of the CSP bulk material is weighted and pure iso-propanol (used as the packing solvent) is added, obtaining a 90 mL of slurry mixture;
- b) This mixture is let for 30 min and two different phases are obtained. After this time, around 25 mL of liquid phase and 65 mL of slurry are obtained. Then with a syringe the upper liquid was removed and the slurry was energetically mixed;
- c) The column was completely filled with pure and cold iso-propanol ($T=4^{\circ}\text{C}$).
- d) All amount of slurry was inserted in the upper packing reservoir. The reservoir was closed and connected to the pneumatic pump;
- e) Then the packing system was operated using cold iso-propanol to compact the adsorbent bed, using the five stages of the procedure described in Table 3.1;
- f) After the final packing 45-min stage, the pump was turned off, and the upper reservoir was opened and the remaining solvent removed. The reservoir was disconnected from the column and using a blade the extra slurry on the top of the column cut. Finally, the column was closed;
- g) The packed new column was inverted and cold iso-propanol was circulated for more 5 minutes using a liquid pressure of 50 bar

Table 3.1. Packing procedure for preparative chromatographic columns.

Stage #	Liquid pressure (bar)	Air pressure (bar)	Time (min)
1	50	0.7	0-5
2	100	1	5-10
3	150	1.7	10-15
4	200	2	15-25
5	250	2.6	25-45

After packing the six preparative columns using Chiralpak® IA the quality of all the columns packing was evaluated using elution chromatography. This evaluation

was done by analysing the dispersion and measuring variation of the retention time of both the non-retained compound (tracer) and nadolol racemic mixture. The efficiency of all SMB columns was also tested by measuring the influence of the flow-rate upon the height equivalent to a theoretical plate.

To have good separation in simulation moving bed, it's very important to pack all columns with exact the same procedure to have the same adsorption behavior for all preparative columns. Figure 3.5 presents the elution chromatographic pulses of 2 g/L nadolol and tracer (1,3,5-tri-*tert*.-butylbenzene).

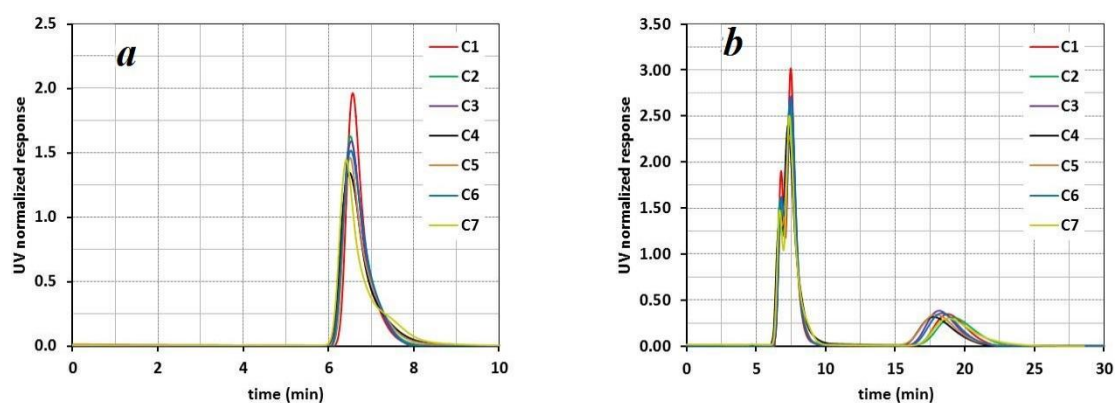


Fig 3.5. UV normalized response to pulses experiments of tracer (a) and nadolol (b) on seven SMB columns packed with Chiralpak® IA, using 100%methanol:0.1%diethylamine solvent ($C_F^T = 2.0$ g/L; $Q = 5.0$ mL/min; Injection volume = 100 μ L).

Chapter 4 Separation of the more retained enantiomer from the quaternary nadolol stereoisomers mixture

The pseudo-binary preparative separation of nadolol stereoisomers is performed by simulated moving bed chromatography (SMB). Using the Chiralpak® IA adsorbent, a methanol-acetonitrile-diethylamine 25:75:0.1 (v/v/v) solvent composition was selected to perform the experimental SMB separation. Using a 2 gL⁻¹ total feed concentration, an equimolar mixture of the four stereoisomers of nadolol, the more retained component was recovered 100% pure, with a system productivity of 0.77 gL⁻¹h⁻¹ and a solvent consumption of 9.62 gL⁻¹. Comparing these results with the ones recently reported by Arafah et al. (2016), this work shows that the solvent composition of methanol-acetonitrile-diethylamine 25:75:0.1 is a better alternative compared with pure methanol for the preparative separation of nadolol stereoisomers by SMB chromatography. The present experimental and simulation results also stresses that the performance of the preparative separation was improved by a careful selection of the solvent composition.

This chapter is based on the following publication:

Arafah, R.S., Ribeiro, A.E., Rodrigues, A.E., and Pais, L.S., Improving the performance of nadolol stereoisomers' preparative separation using Chiralpak IA by SMB chromatography, Chirality 31 62-71 (2019) DOI: 10.1002/chir.23034.

4.1 Introduction

The direct chromatographic resolution of enantiomers using chiral stationary phases (CSPs) is actually a very well established separation technique. Several reasons were responsible for its growing success. The continuous technical development of new CSPs combined with their commercial availability has been, certainly, one of the most important reasons.

A very important step forward was made in the 80s by the Okamoto group when the idea of polysaccharides derivatives, namely cellulose and amylose (Chiralcel and Chiralpak, respectively), was explored in order to enhance the ability of chiral recognition contained into their natural structures.[37-38] Among the wide range of currently available CSPs, the polysaccharide-based are recognized as the most powerful packing material for chiral separation at both analytical and preparative scales, due to their versatility, durability and upper loading capacity.[39-41] These cellulose or amylose-based CSPs are produced by physical coating of the chiral polymer on a matrix. However, due to their coated nature, they can only be used with a limited range of polar (alcohols) or non-polar (alkanes) solvents in combination with some polar additives as modifiers. To overcome this constraint several research groups have develop efforts to immobilize several polysaccharide derivatives onto chromatographic matrices. [42-47] Another important step forward was achieved by the Daicel Corporation in 2004 with the introduction in the commercial market of CSPs manufactured through the immobilization of the chiral selector (polysaccharide-derivative) on the silica support. In a first stage, they introduce the Chiralpak® IA, IB and IC stationary phases [48-49] and, more recently, the Chiralpak ID, IE and IF stationary phases. [50] This step was also relevant since these highly selective CSPs are now available with particle size diameters from 3 to 20 μm allowing to broad the

scope from analytical to new preparative separations. Besides the possibility to improve selectivity and retention, the use of the before “prohibited” solvents can result in new CSP-mobile phase combinations that can additionally improve the compounds solubility allowing more favourable preparative separation performances.

In a preparative point of view, and when considering the choice of the mobile phase composition, a high selectivity of the enantiomers should not be the only goal to be aimed, as it is frequently followed at analytical scale. Besides the choice of a CSP with high loading capacity, a high solubility of the solutes in the solvent and low retention times should also be taken into account, in order to improve the preparative process performance, as it was extensively explained for the separation of chiral non-steroidal anti-inflammatory drugs. [51-52]

The separation of nadolol stereoisomers on Chiralpak® AD at both analytical and preparative scales was recently reported by Ribeiro et al. [15] using classical SMB and by Jermann et al. [25] using a three column intermittent SMB chromatographic process. However, no further work was developed to better understand and exploit the capabilities of immobilized chiral stationary phases, such as Chiralpak® IA, for both the analytical and the preparative separation of chiral APIs, particularly of nadolol stereoisomers.

This work presents the main results obtained from the complete solvent screening using the immobilized Chiralpak® IA CSP. The selection of the proper CSP-mobile phase combination for analytical and preparative operations is fully studied taking into account the screening strategy proposed by Zhang and Franco. [53-56] The selection is carried out by studying the effect of the different CSP-mobile phase combinations on the solubility, retention and resolution of nadolol stereoisomers.

4.2 Solvent screening

when choosing the solvent composition, there is a trade-off that must be settled upon between obtaining high selectivity values and obtaining short retention times. In order to confirm previously obtained results⁴ using pure methanol and methanol-acetonitrile 25:75 solvent composition, several elution pulses were carried out using one preparative column. These pulses were conducted in order to approach the real preparative and production conditions by using bigger injection volumes and high nadolol feed concentrations. The obtained results are presented in Figure 4.1, and were performed using a SMB column home-packed with Chiralpak® IA, a nadolol feed solution of 10 g/L and a loop with 1000 μ L volume.

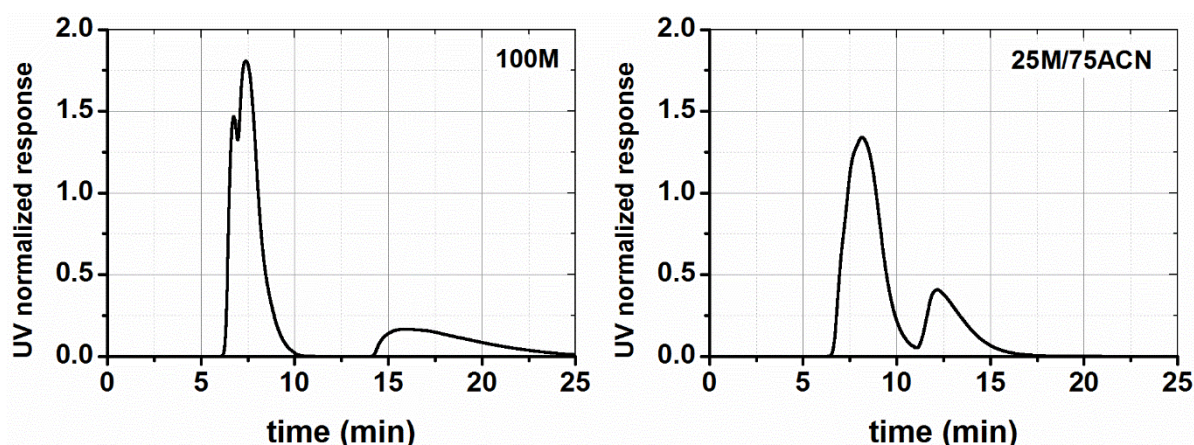


Fig. 4.1. UV normalized response to a pulse of nadolol using a mobile phase composition of 100:0.1 methanol-diethylamine (left) and 25:75:0.1 methanol-acetonitrile-diethylamine (right) in the SMB column ($C_F^T = 10.0$ g/L; $Q = 5.0$ mL/min; injection volume = 1000 μ L).

These results confirm previously published results by Arafah et al 2016 (Loading Experiments, Fig. 4, 100%methanol and 25%methanol-75%acetonitrile). The pure methanol composition allows high selectivity values between the more retained compound and the three less retained compounds. However, this solvent composition also represents an important non-linearity and also a significant increasing of the retention time for the more retained compound, when compared with the methanol-

acetonitrile 25:75 composition. The use of the former solvent composition, represents a loss in selectivity, although this loss is compensate with a strong decrease in retention time and also an important increase in linearity of the adsorption equilibrium which can lead to a significant improvement in the productivity, mainly under high feed concentrations.

4.3 Adsorption equilibrium isotherms measurements and breakthrough experiments

The equilibrium adsorption isotherms of the racemic nadolol mixture were measured experimentally using the adsorption-desorption method. The equilibrium data was experimentally measured using a SMB column, and five nadolol feed concentrations (2, 4, 6, 8 and 10 $g l^{-1}$) and a methanol-acetonitrile 25:75 solvent composition. The previously presented linear + Langmuir model was used to fit the experimental equilibrium adsorption data. The obtained results are presented in Fig. 4.2A and show a good agreement between experimental data (points) and the predicted equilibrium adsorption behaviour (lines). The obtained model parameters are: $m = 0.6947$, $Q = 5.874 \text{ } lg^{-1}$, $b_{123} = 2.449 \times 10^{-2} \text{ } gl^{-1}$ and $b_4 = 1.547 \times 10^{-1} \text{ } gl^{-1}$, with a normalized standard deviation of $SD = 0.134$. The comparison between these results obtained with the methanol-acetonitrile 25:75 solvent composition and the results previously published⁴ for the pure methanol, show that the acetonitrile-based composition represents higher linearity of the equilibrium adsorption data. Comparing the model parameters using the two solvent compositions, we can see an increase in the linear parameter from $m = 0.6887$ to a value of $m = 0.6947$. This increase in linearity is more important for the more retained compound as the obtained value of $b_4 = 1.547 \times 10^{-1} \text{ } lg^{-1}$ is clearly lower that the value of $b_4 = 4.379 \times 10^{-1} \text{ } lg^{-1}$, previously reported. Whereas the values for $b_1 = b_2 = b_3 = 2.373 \times 10^{-2} \text{ } lg^{-1}$ and $b_{123} =$

$2.449 \times 10^{-2} \text{ lg}^{-1}$ are similar. Figure 4.2B presents the experimental and the predicted values of selectivity as a function of the nadolol feed concentration. As it is presented, there is a constant (linear behaviour) value of selectivity for the three less retained compounds and a decrease in selectivity between the two more retained compounds. However, and for a feed concentration of 10 g l^{-1} , the decreases in the selectivity value from a predicted value of 1.9 to a value of 1.7, is not as strong as it was observed using the 100%methanol solvent composition, from a value of 2.1 to a value of 1.5. The increase of linearity of the equilibrium adsorption isotherm, when changing from pure methanol to 25:75 methanol-acetonitrile, as a strong impact in the selectivity between the two more retained compounds, mainly for high feed concentrations. Therefore, it is logical to predict an improvement in the preparative performance of the $(1+2+3)/4$ pseudo-binary separation.

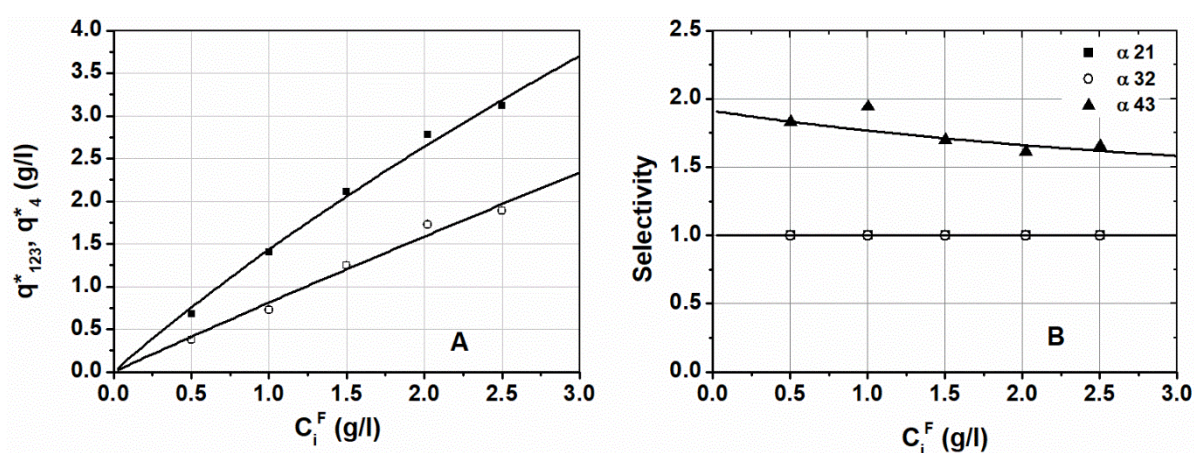


Fig. 4.2. Comparison between experimental and model results on the equilibrium adsorption isotherms for nadolol stereoisomers using methanol-acetonitrile-diethylamine 25:75:0.1 as solvent composition: Fig. 4.2A, the multicomponent equilibrium adsorption isotherms and its fitting to a linear + Langmuir competitive model, Eq(1)-Eq(2). Fig. 4.2B, the selectivity between the different nadolol stereoisomers as a function of their feed concentrations and its fitting to the same linear + Langmuir competitive model.

In order to study the fixed-bed adsorption behaviour and validate the linear + Langmuir equilibrium adsorption isotherm model different breakthrough measurements (saturation and regeneration) were performed. These breakthroughs

experiments were conducted on a SMB column, using a methanol-acetonitrile 25:75 solvent composition and a nadolol total feed concentration of 9.7 g l^{-1} . The prediction of the axial dispersion was done using a non-retained compound leading to a Peclet number of 1000, and a nadolol solution was used to predict a value of $k = 1.0 \text{ s}^{-1}$ for the mass transfer resistance coefficient. Fig. 4.3 presents the experimental results (points) obtained from the saturation and regeneration steps of the preparative column, as well as the fixed-bed adsorption behaviour (lines) predicted by the selected equilibrium adsorption isotherm model.

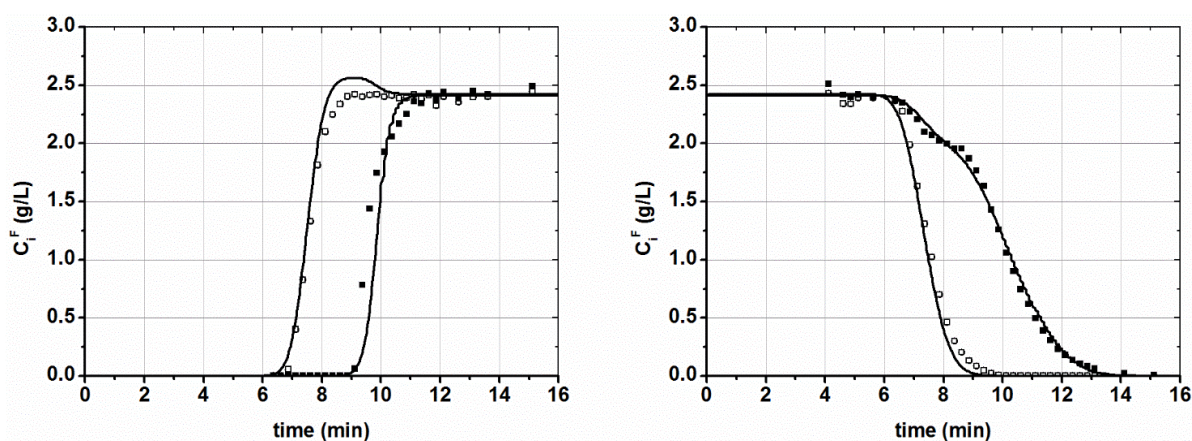


Fig. 4.3. Saturation (adsorption) and regeneration (desorption) curves for a 9.7 g l^{-1} nadolol racemic feed concentration and a methanol-acetonitrile-diethylamine 25:75:01 solvent composition. Comparison between experimental (points) and simulation (lines) results. Flow-rate: 5 mL/min . Model parameters: $\varepsilon=0.4$; $Pe=1000$; $k=1.0 \text{ s}^{-1}$, and the linear + Langmuir model parameters.

It can be notice, that there is a very good agreement between the experimental adsorption behaviour (points) and the adsorption behaviour predicted using the selected linear + Langmuir model (lines). Beyond what was concluded in the HETP measurements results analysis, this very good agreement is also an important tool to validate the predicted value for the mass transfer coefficient.

Once again, comparing obtained results presented in Fig. 4.3 and those previously published using the pure methanol solvent composition [26], it can be seen that the retention time for third compound is almost the same (slightly higher) but that there is

a strong increase in the retention of the more retained compound. Furthermore, it can be observed, for both solvent compositions, a roll-up phenomenon for the three less retained species, due to the competition of stereoisomers for adsorption under overload nonlinear conditions. However, for the methanol-acetonitrile 25:75 solvent composition this phenomenon is strongly decreased (almost inexistent), and thus it is a clear signal of more linearity. Moreover, there is also a strong decreasing in the dispersion of the more retained compound that can be observed by the less tailing in the regeneration-desorption step. These are all signals of less non-linearity of the equilibrium adsorption isotherm, as it was previously presented and discussed for the results presented in Fig. 4.2.

4.4 SMB columns efficiency

After packing the seven SMB columns with the same procedure, all the columns were tested to evaluate the packing quality by pulses and breakthroughs measurements. The dispersion and the variation of the retention time were also measured. Additionally, after packing, one SMB column was selected to perform the HETP measurements. This study was carried out by injecting a non-retained compound solution and a nadolol solution using three different flow-rates (5, 10 and 15 ml/min). The obtained results are presented in Fig. 4.4 as the high equivalent to a theoretical plate (HETP) value as a function of the superficial velocity using the pure methanol and the methanol-acetonitrile 25:75 solvent compositions. These results shows that HETP varies between 400 and 700, and there is no significant difference in the SMB columns efficiency when change from one solvent composition to the other. Thereby, it is acceptable to consider the same constant value for the mass transfer coefficient using either solvent composition.

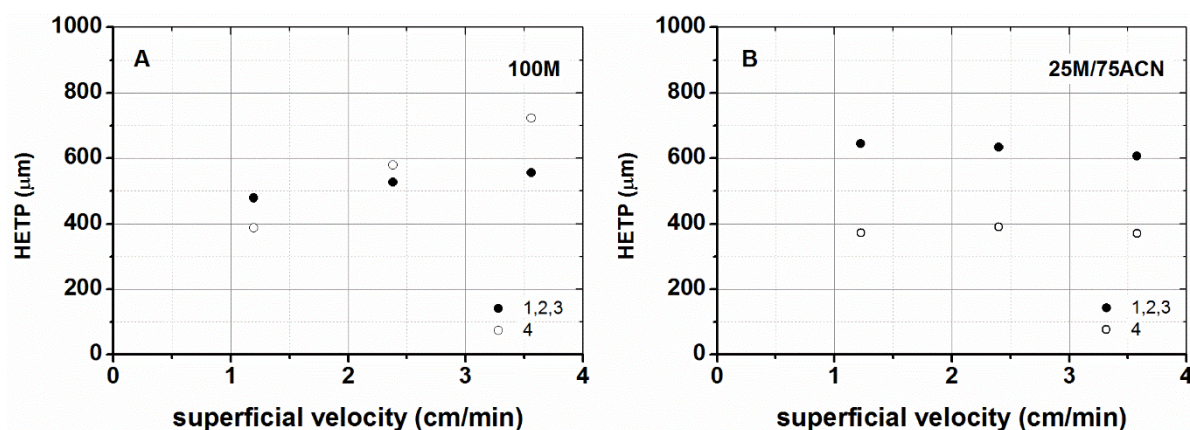


Fig. 4.4. High equivalent to a theoretical plate as a function of superficial velocity for a SMB home-packed column using a mobile phase composition of methanol-diethylamine 100:0.1 (figure A) and methanol-acetonitrile-diethylamine 25:75 (figure B). Closed circles and open circles, for the three less retained and more retained stereoisomers, respectively. ($C_F^T = 2.0 \text{ gl}^{-1}$; $Q = 5.0, 10.0$ and 15.0 mL/min ; injection volume = 100 μL).

4.5 Simulated moving bed operation

The experimental pseudo-binary separation of the nadolol stereoisomers was performed in the FlexSMB-LSRE[®] pilot unit. In order to avoid the damage of the stationary phase a maximum pressure drop of 35 bar was set inside section I, were the SMB unit internal flow-rates, and using a methanol-acetonitrile 25:75, have a maximum near $Q_I^* = 35 \text{ ml/min}$. The SMB operating conditions were predicted based in the Equilibrium Theory¹⁵ applied to nonlinear systems using the above presented linear + Langmuir equilibrium adsorption isotherms. A detailed description of the methodology applied to the definition of SMB operating conditions can be found on previously published work¹. Fig. 4.5 presents the predicted region of complete separation for a feed solution of 2 gl^{-1} , as well as the experimentally measured $\gamma_{II}x\gamma_{III}$ points for the two SMB runs.

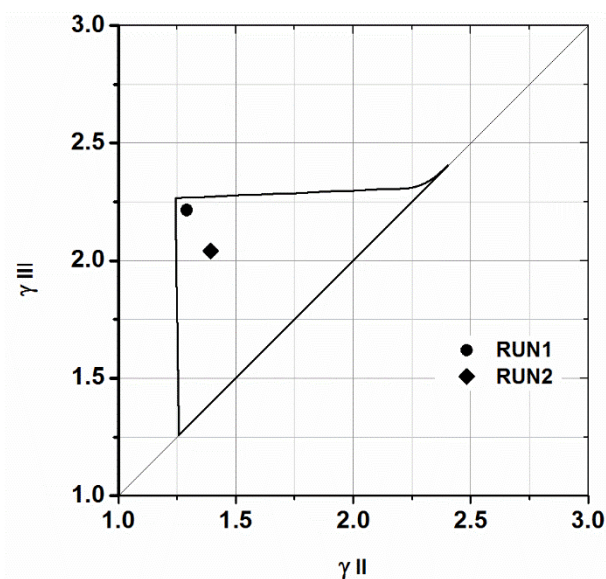


Fig. 4.5. SMB complete separation region (Equilibrium Theory) and experimental SMB operating points for the pseudo-binary separation of the more retained nadolol stereoisomer using Chiralpak® IA CSP and methanol-acetonitrile-diethylamine 25:75:0.1 solvent composition for a nadolol racemic feed concentration of 2 g l⁻¹. Points represent the experimental $\gamma_{II} \times \gamma_{III}$ values for the two SMB runs: RUN1 (closed circle) and RUN2 (closed diamond).

The unit was operated using a conventional SMB mode of operation using six columns and a [1-2-2-1] configuration. The extract flow-rate was also measured by means a Coriolis flow meter installed for control purposes at the extract stream outlet. The recycle flow-rate was monitored by the other Coriolis flow meter. The extract and raffinate flow-rates were also continuously monitored by means of the total weighted recovered mass in each outlet over each complete cycle. This procedure was repeated for all the 28 cycles of the SMB operation. The extract and raffinate collected samples were then analyzed by HPLC to evaluate the concentrations of all the four stereoisomers in the outlet streams. Fig. 4.6 present the transient evolution for the concentration of nadolol stereoisomers species in the extract and raffinate outlet streams during the SMB operation for both run (RUN1 from initial do cycle 9 and RUN2 from cycle 10 to cycle 28). The cyclic steady state was considered to be obtained when both the extract and the raffinate concentrations do not change more than 5% during

five successive cycles. At that point, several samples were collected and analyzed to obtain the internal concentration profiles, using a 6-port valve installed after the end of section IV. These samples were collected at different intervals (25%, 50%, 75% and 90%) of the switching time. In Fig. 4.7 is presented the experimental (points) and simulated (lines) internal concentration profiles at the cyclic steady state of SMB operation (cycles 20, 22, 24 and 26). It should be pointed that the presented internal profiles show two small plateaus near the extract and raffinate outlets, due to the characteristic dead volumes of the FlexSMB-LSRE unit that are also taken into account in the simulation and control software.

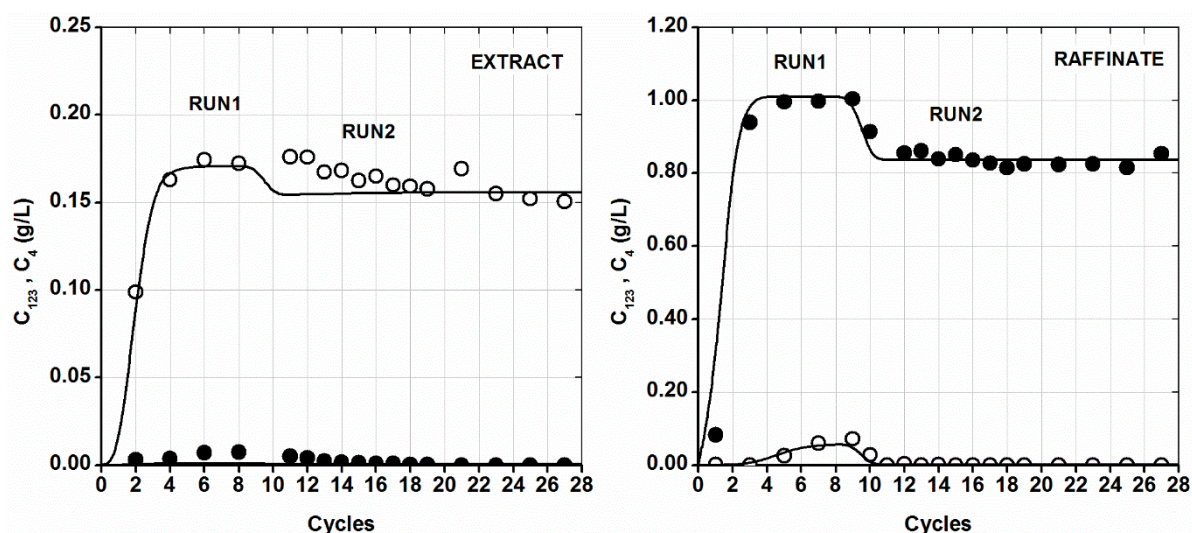


Fig. 4.6. Experimental (points) and predicted (lines) transient evolution of the nadolol stereoisomers concentration in both the extract (left plot) and raffinate (right plot) outlet streams for the two SMB runs: RUN1 and RUN2. Closed circles and open circles for the three less retained and the more retained stereoisomer, respectively. Model parameters used: bed porosity, $\varepsilon=0.4$; Peclet number, $Pe=1000$; mass transfer coefficient, $k=1.0 \text{ s}^{-1}$; and linear+Langmuir isotherm parameters.

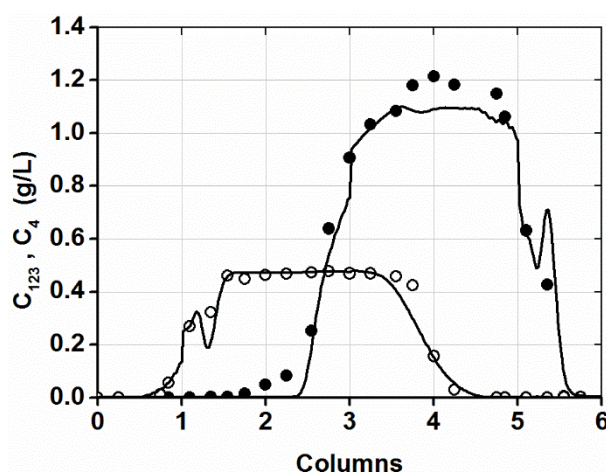


Fig. 4.7. Experimental (points) and predicted (lines) internal concentration profiles at the cyclic steady state during the SMB operation (cycles 20, 22, 24 and 26). Closed circles and open circles for the three less retained and the more retained stereoisomer, respectively. Model parameters used are the same as in Figure 4.6.

The main SMB experimental operation parameters are presented in Table 4.1: internal and external flow rates, switching time, and the corresponding γ values defined for the true moving bed (TMB) operation. A first SMB run was set (RUN1) owing the achievement of a maximum system productivity. However, this run led to both extract and raffinate outlet contaminated streams due to incomplete recovery of the more retained compound in the extract and the three less retained compounds in the raffinate. In order to have a complete separation, a second run was carried out, changing the $\gamma_{II} \times \gamma_{III}$ values to a point more inside the triangle. The second experimental results obtained for the second run (RUN2) show that for a 2 g l^{-1} feed solution, both extract and raffinate outlet streams were collected with a purity of 100%. For this second SMB run a system productivity of $0.77 \text{ g l}^{-1} \text{ hr}^{-1}$ and a solvent consumption of 9.43 l g^{-1} were obtained.

Table 4.1. SMB experimental runs for the pseudo-binary separation of the more retained nadolol stereoisomer using the FlexSMB-LSRE® pilot unit with Chiralpak® IA CSP and acetonitrile-methanol-diethylamine 25:75:0.1 solvent composition. Performance parameters experimentally measured during the whole cycle 9 for RUN1 and during the cyclic steady-state obtained during cycles 20, 22, 24 and 26 for RUN2.

RUN	C_F^T (g/l)	Internal flow-rates (ml/min)	External flow-rates (ml/min)	TMB γ values	Performance parameters
1	2.00	$Q_I^* = 34.4$	$Q_E = 20.4$	$\gamma_I = 3.5221$	$PUX = 95.9\%$
		$Q_{II}^* = 17.4$	$Q_F = 7.00$	$\gamma_{II} = 1.2920$	$PUR = 93.3\%$
		$Q_{III}^* = 24.4$	$Q_X = 16.9$	$\gamma_{III} = 2.2125$	$RCX = 93.1\%$
		$Q_{IV}^* = 13.8$	$Q_R = 10.6$	$\gamma_{IV} = 0.8181$	$PR = 0.84 \text{ g l}^{-1} \text{ h}^{-1}$
		$t^* = 1.82 \text{ min}$	$Q_{REC} = 14.0$		$SC = 9.43 \text{ l g}^{-1}$
2	2.00	$Q_I^* = 34.5$	$Q_E = 20.5$	$\gamma_I = 3.5371$	$PUX = 100.0\%$
		$Q_{II}^* = 18.2$	$Q_F = 4.9$	$\gamma_{II} = 1.3931$	$PUR = 100.0\%$
		$Q_{III}^* = 23.1$	$Q_X = 16.3$	$\gamma_{III} = 2.0422$	$RCX = 100.0\%$
		$Q_{IV}^* = 13.9$	$Q_R = 9.3$	$\gamma_{IV} = 0.8260$	$PR = 0.77 \text{ g l}^{-1} \text{ hr}^{-1}$
		$t^* = 1.82 \text{ min}$	$Q_{REC} = 14.0$		$SC = 9.62 \text{ l g}^{-1}$

Using the 100%methanol solvent composition, and using a nadolol feed concentration of 2 gl^{-1} , for the complete separation (PUX and PUR of 100%) the reported values of productivity and solvent consumption were $0.33 \text{ gL}^{-1}\text{hr}^{-1}$ and 26.1 Lg^{-1} , respectively. Present work, shows that the methanol-acetonitrile 25:75 is a better solvent selection, for the same (1+2+3)/4 pseudo-separation, since it allows better performance parameters values. This solvent composition means not only a higher productivity of $0.77 \text{ gl}^{-1}\text{hr}^{-1}$ but also a lower solvent consumption of 9.6 lg^{-1} .

4.5 Conclusions

The pseudo-binary separation of the nadolol stereoisomers was performed by simulated moving bed technology. The preparative separation was performed using the immobilized Chiralpak® IA adsorbent and a methanol-acetonitrile 25:75 solvent composition. This work stresses the fact that, a proper selection of the solvent composition can represent an important improvement on the performance of the preparative separation process. The pseudo-binary (1+2+3/4) separation of the most active stereoisomer of nadolol (RSR-nadolol) was performed using the FlexSMB-LSRE® unit. Using a feed solution of 2 gl^{-1} , the more retained component was collected

in the extract outlet stream with a recovery and purity of 100%. Comparing present results, with the ones previously published [26] for the same separation using a pure methanol solvent composition, it was observed an important increase in the performance parameters: productivity was increased from $0.58 \text{ gl}^{-1}\text{hr}^{-1}$ to $0.77 \text{ gl}^{-1}\text{hr}^{-1}$ and the solvent consumption was decreased from 15.8 lg^{-1} to 9.43 lg^{-1} . Experimental and simulation results clearly show that, in spite of presenting low selectivity and solubility values, the selection of a solvent composition that allows a decrease in the non-linearity for the adsorption behaviour, can promote an increase in the preparative separation performance, mainly, for high feed concentrations.

Chapter 5 Strategies for the complete separation of the multicomponent nadolol mixture into its four pure stereoisomers

This chapter will provide strategies for the complete separation of the multicomponent nadolol mixture into its four pure stereoisomers. A screening for mobile phase composition using different columns was important to evaluate the one step separation of all nadolol stereoisomers; it is possible to separate the more retained stereoisomer from the quaternary nadolol mixture using pure alcohol or alcohol-acetonitrile solvents, but no effective separation between the three first stereoisomers. There is a need for a different separation strategy that combines achiral and chiral separation steps.

5.1 One step separation for quaternary mixture of nadolol

In Part 4, the active therapeutic component's pseudo-binary (123/4) separation was achieved by targeting the more returned component (4). However, components 1/2/3 are close to each other with a limited resolution value. This is a massive drawback for ternary separation at the preparative scale. The qualitative and quantities analysis was established in 2016 with an exhaustive screening of solvents compositions at the analytical scale.

The candidate solvents with higher resolution between component $R_{2,3}$ are presented in table 5.1 [26 and 15].

Table 5.1. Most promising solvents for separating nadolol stereoisomers on Chiralpak®IA at analytical scale: retention time (tr), resolution (R_{j,i}), all solvents were mixed with 0.1 diethylamine.

Solvent	Tr ₁ (min)	Tr ₂ (min)	Tr ₃ (min)	Tr ₄ (min)	R _{2,1}	R _{2,3}	R _{3,4}
20E/80C6	8.23	9.52	12.43	19.48	1.72	2.91	4.03
20E/80C7	7.92	9.77	12.00	20.57	2.74	2.88	5.19
10E/90ACN	5.02	5.53	6.23	9.53	1.57	1.65	6.00
10M/90ACN	4.68	4.90	5.60	6.88	1.10	2.80	3.66

Several elution chromatography was made at preparative scale using alcohol-hydrocarbon (ethanol with hexane and heptane) and alcohol-acetonitrile (methanol and ethanol with acetonitrile) mixtures. The column of preparative dimensions (30 mm ID x 250 mm L) with Chiralpak IA chiral adsorbent (20 micron) was used.

The objective of solvent screening was to evaluate solvent mixtures to succeed in the separation of all four nadolol stereoisomers in just one chiral chromatographic process.

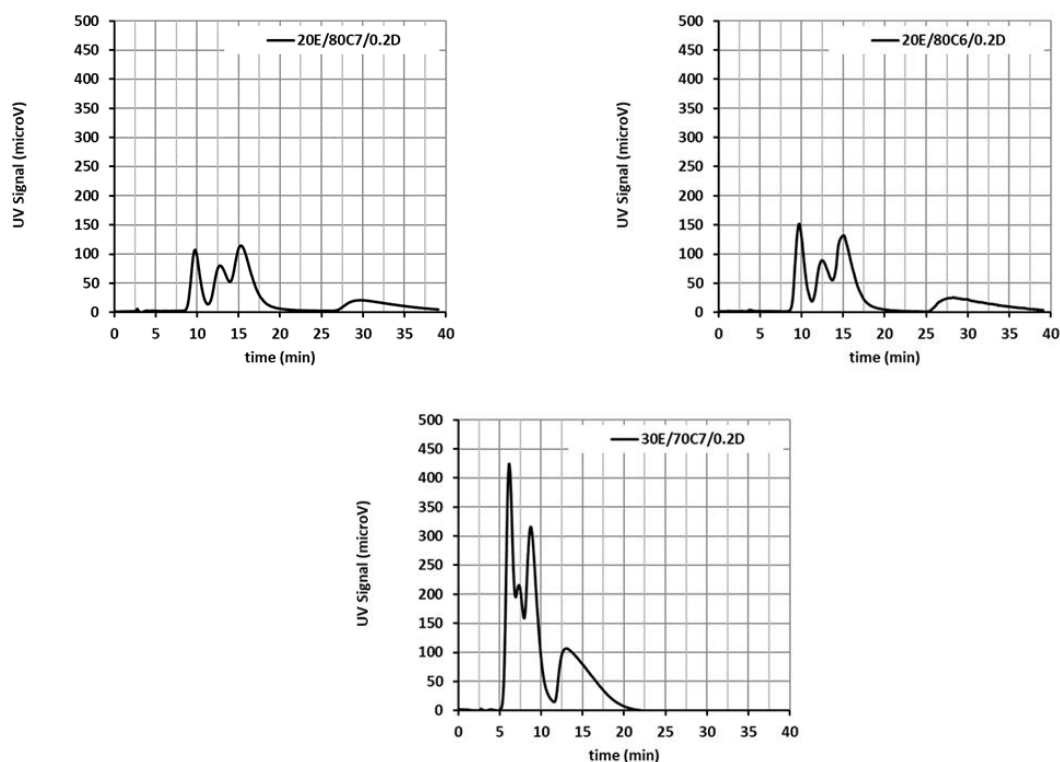


Fig 5.1. UV response to pulse of nadolol solutions on a Chiralpak®IA with preparative diameters (30D X 250 L). ($C_F^T = 10 \text{ g/L}$; $Q = 40 \text{ mL/min}$; injection volume = 10 mL).

Chromatograms show that no good resolution is achieved for the nadolol quaternary mixtures using alcohol-hydrocarbon solvents. For 20E:80C6 and 20E:80C7, stereoisomer 4 is completely resolved from the other three stereoisomers but strongly retained (40 minutes operation with very long tailing), and stereoisomers 2 and 3 elute very close to each other. Increasing ethanol content to 30E, the retention time of component 4 diminishes significantly (to 20 minutes) but with very low resolution for the three other stereoisomers.

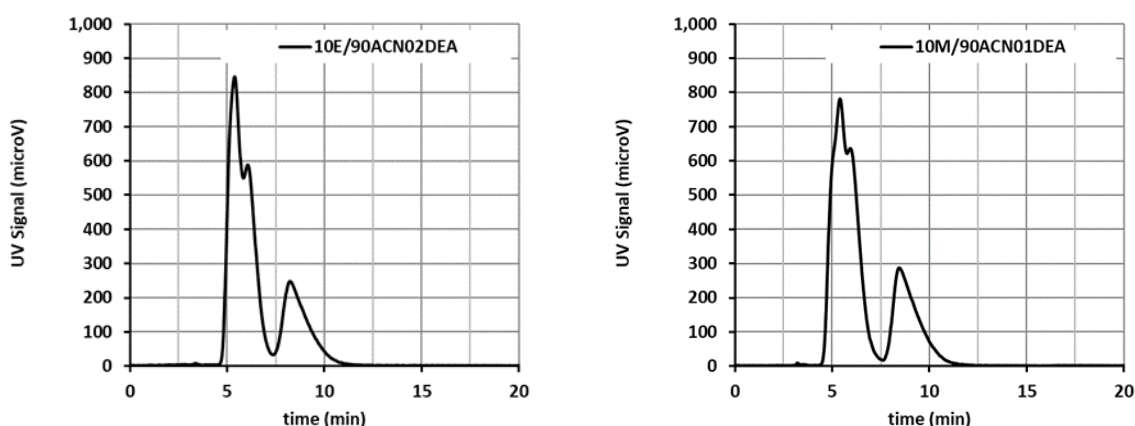


Fig 5.2. UV response to pulse of nadolol solutions on a Chiralpak®IA with preparative diameters (30D X 250 L) using ethanol/methanol added to acetonitrile mobile phase composition, ($C_F^T=10$ g/L; $Q=40$ mL/min; injection volume =10 mL).

Enough resolution was also not achieved with alcohol-acetonitrile solvents. 10E:90ACN and 10M:90ACN present less retention for the fourth stereoisomer but the first three stereoisomers elute almost together. Reducing to 5M/95ACM, resolution is better although still no resolution between the two first stereoisomers.

In resume: it is possible to separate the more retained stereoisomer from the quaternary nadolol mixture using pure alcohol [15] or alcohol-acetonitrile [26] solvents, but no effective separation between the three first stereoisomers. There is a need for a different separation strategy that combines achiral and chiral separation steps.

5.2 Strategies for the complete separation of the multicomponent nadolol mixture

Achiral C18 material does not directly discriminate chiral solutes. But nadolol is composed by four stereoisomers, being two racemates (two pairs of enantiomers). So, adequate operation using C18 material can distinguish and separate the two nadolol racemates. Additionally, chiral material can separate and identify the nadolol enantiomers of each racemate. A 20 g/L nadolol feed solution was prepared and injected in the SiliaChrom XT C18 preparative column, using a mobile phase of 40:60:0.2 ethanol:water:diethylamine and a 1000 μ L loop. The flow-rate was set to 5 mL/min. The obtained result is presented in Fig. 5.3.

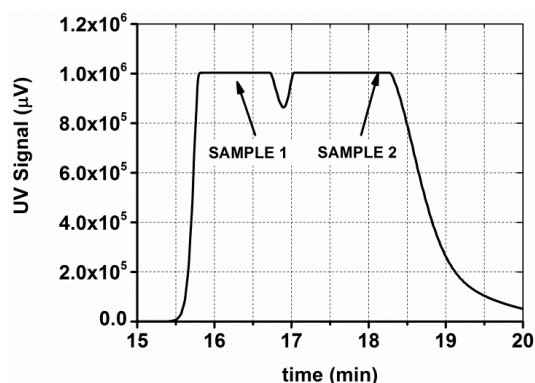


Fig. 5.3. UV response to a pulse of nadolol using a SiliaChrom XT C18 column and a mobile phase composition of ethanol:water:diethylamine (40:60:0.2). Flow-rate of 5 mL/min; $C_F^T=20$ g/L; injection loop=1000 μ L.

The SiliaChrom XT C18 preparative column succeeded to discriminate the two nadolol racemates. At the top of both peaks (UV saturated-signal plateaus at Fig. 1), two samples were collected. The collecting times were from 16'20'' to 16'35'' for sample 1 and from 18'20'' to 18'35'' for sample 2. Both collected samples were then analysed in the Knauer HPLC system equipped with the Chiralpak IA analytical column and the UV and polarimeter detectors in series. The results are presented in Fig. 5.4.

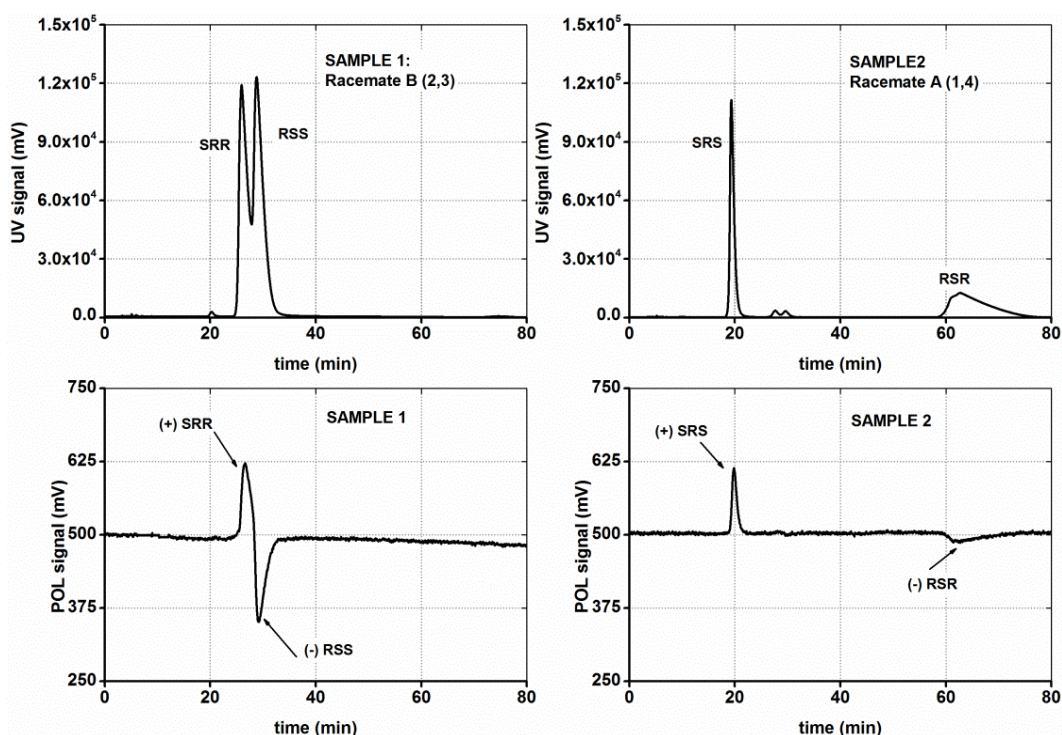
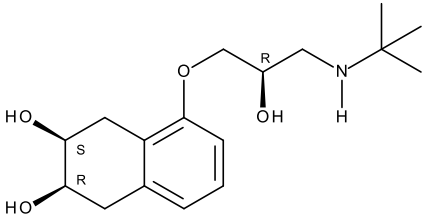
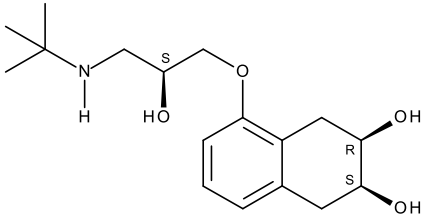
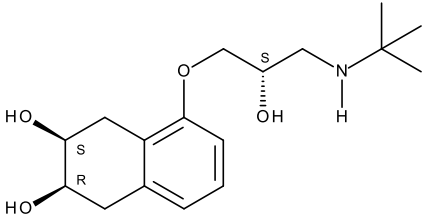
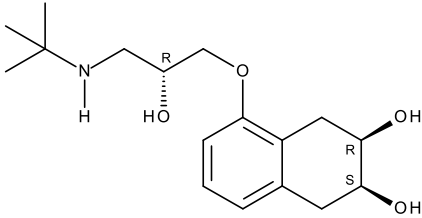


Fig. 5.4. UV (top) and polarimeter (bottom) response to a pulse of each collected sample 1 and 2 (see Fig. 5.3) using a Chiralpak IA analytical column and a mobile phase composition of ethanol:water:diethylamine (40:60:0.2). Flow-rate of 0.5 mL/min; injection loop=20 μ L.

Fig. 5.4 reinforces that the achiral C18 material can be used to perform the pseudo-binary separation of the two nadolol racemates: the nadolol racemate B (sample 1; mixture of SRR and RSS nadolol enantiomers) from the nadolol racemate A (sample 2; mixture of SRS and RSR nadolol enantiomers). Considering the elution order obtained using the Chiralpak IA chiral stationary phase, sample 1 is a mixture of the two compounds with the intermediate affinity (chiral elution order 2 and 3, racemate B) and sample 2 is a mixture of the less and the more retained compounds (chiral elution 1 and 4, racemate A), as represented in Table 5.2. Therefore, the reader must note that, using the achiral C18 material, “racemate B” is less retained than “racemate A”.

Table 5.2 Molecular structure, elution order and optical rotation signal of the four nadolol stereoisomers using Chiralpak AD [15] and Chiralpak IA [26] chiral stationary phases and ethanol-hydrocarbon solvent compositions, and in this work using Chiralpak IA and ethanol:water solvent composition.

Racemate A	
	
RSR-Nadolol	SRS-Nadolol
Elution order: 4 th ; optical rotation signal: (-)	Elution order: 1 st ; optical rotation signal: (+)
Racemate B	
	
RSS-Nadolol	SRR-Nadolol
Elution order: 3 th ; optical rotation signal: (-)	Elution order: 2 nd ; optical rotation signal: (+)

The methodology for optimizing a binary or pseudo-binary separation is based on selecting a proper combination of a stationary-phase (solid) and a mobile-phase (solvent) composition. It is a much more challenging task when dealing with a preparative separation instead of a traditional analytical separation problem. The complexity will increase if we consider using a simulated moving bed (SMB) or a fixed-bed preparative unit, in which high feed concentrations are typically used to improve the process performance. In these situations, the desired high concentrations of the different solutes inside the chromatographic column(s) will significantly enhance the mutual competition between solutes for adsorption with the stationary phase. From a preparative point of view, and when considering the choice of the solvent composition, the high selectivity of the enantiomers should not be the only goal, as is frequently the case at the analytical scale. In addition to choosing a stationary phase with high loading

capacity, the high solubility of the solutes in the solvent and low retention times should also be taken into account to improve the process performance.

Screening the solvent composition is always the first experimental step if facing a new mixture separation problem. In these “preliminary studies,” the main goal is to measure the mixture solubility covering the wider range of pure solvents, and solvent characterization of each “SMB column” is also a relevant step because the standard modeling of the SMB operation assumes that all columns are identical in terms of dimensions and that they contain equal amounts of stationary phase. The experimental packing procedure can lead to local fluctuations in the packing density of each SMB column. Therefore, characterization of each SMB column must be done to minimize the differences between columns: non-retained-compound pulses (retention), HETP (Height Equivalent to Theoretical Plate) experiments (total porosity), and/or pressure drop (bulk porosity). After column packing and characterization, the most promising solvent compositions are checked by performing elution pulse experiments using one column with the preparative dimensions. Then, the equilibrium adsorption isotherms are experimentally determined. This task is critical, and an accurate experimental method is advised to ensure the quality of the equilibrium data. Frontal analysis and perturbation methods are the most used because they are less time-consuming. However, the adsorption/desorption method provides accurate and reliable adsorption equilibrium experimental data. After that, several adsorption equilibrium models were used to fit experimental data. Breakthrough experiments are then used to collect the kinetic data and develop a dynamic fixed-bed adsorption model, validating at the same time the adsorption equilibrium isotherm data.

The adsorption equilibrium and kinetic data are mandatory to predict the separation process performance using SMB and fixed-bed technology. For SMB, this

task is based on several simulation runs in which the separation region is constructed, and the performance parameters are estimated: purity, recovery, solvent consumption, and productivity. Finally, based on these performance parameters, the decision of the better combination between solvent/solvent mixture and stationary phase must be made, followed by the definition of the initial SMB and fixed-bed operating conditions, and experimental separation performed using the real pilot/laboratory scale units. For the preparative experimental operation, two different units will be used. The FlexSMB-LSRE unit was completely built inside the LSRE group and a preparative HPLC Azura system (acquired from Knauer). The experimental results obtained from separating the nadolol stereoisomer mixture are used to validate the SMB and fixed-bed models.

Figure 5.5 presents a scheme of the two main possible strategies for complete separation of nadolol stereoisomers using the previously described methodology.

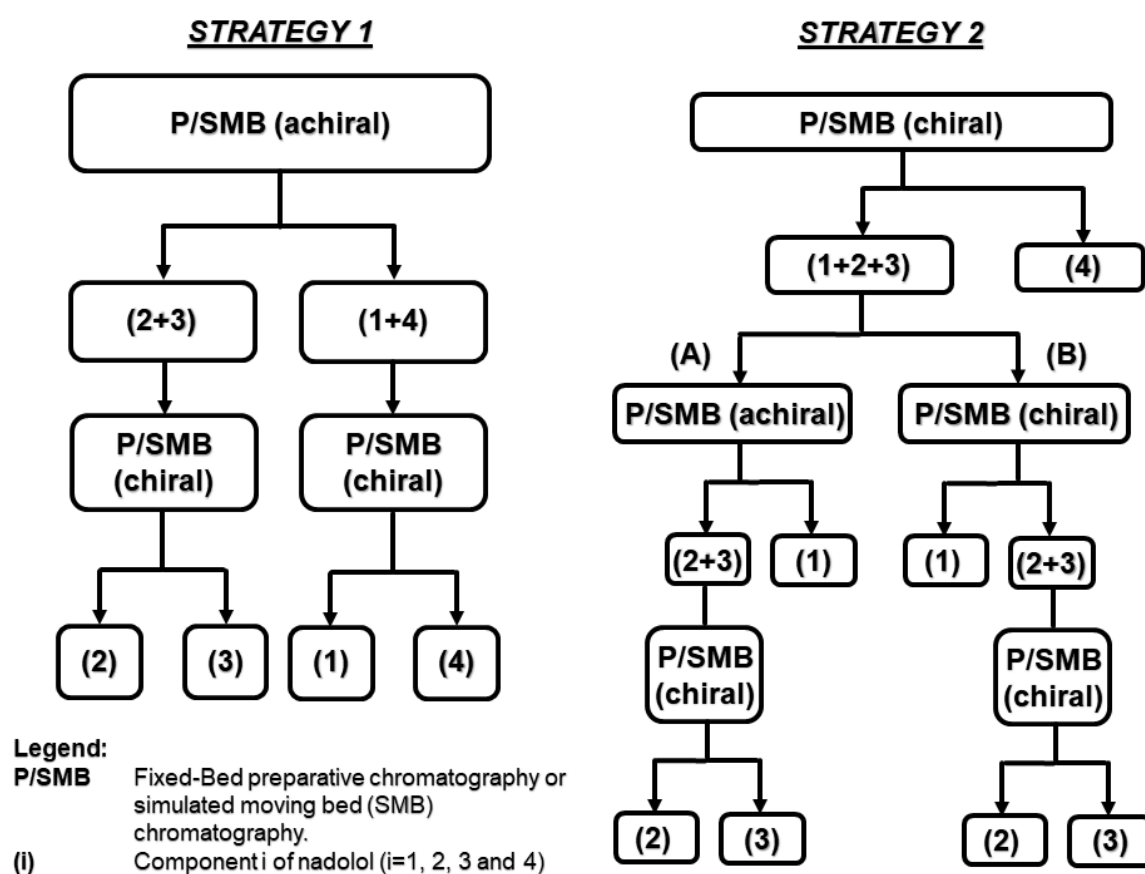


Fig 5.5. Strategies for complete separation of nadolol stereoisomers.

Strategy 1 will require three experimental operation steps. The first step is based on the fact that an achiral adsorbent, Waters XBridge C18, Waters Shield RP18, and Waters XSelect CSH, can separate the nadolol quaternary mixture in two pairs of enantiomers (two racemic mixtures). During the first preparative or simulated moving bed (P/SMB) separation, the two outlets' streams were collected and then concentrated using a rotary evaporator and lyophilizator. Then, each of the two new binary mixtures will be fed to a new P/SMB operation using a chiral adsorbent, such as the Daicel Chiralpak IA that allows the enantiomers discrimination.

Strategy 2; After a first chiral P/SMB pseudo-binary separation, i.e., the separation of the more retained stereoisomer (1+2+3/4), the remaining ternary mixture (1+2+3) will be collected, concentrated, and then completely separated using three sub-strategies (A and B).

In sub-strategy A, the ternary mixture can be fed to a P/SMB operation using an achiral adsorbent, and the more retained compound (1) is separated from the ternary mixture. Finally, the remaining binary mixture (2+3) is fed to a third P/SMB operation using a chiral adsorbent for the final binary separation (2/3). Alternatively, in sub-strategy B, the ternary mixture can be fed to a P/SMB operation using a chiral adsorbent, and the less retained compound (1) is separated from the ternary mixture. Finally, the remaining binary mixture (2+3) is fed to a third P/SMB operation using a chiral adsorbent for the final binary separation (2/3).

In this research, A two-step separation presented in strategy 1 was selected and applied to achieve complete separation of nadolol stereoisomers; the following pages will present and discuss all results obtained from strategy 1.

Chapter 6 Separation of nadolol quaternary mixtures into its pure racemates by achiral preparative chromatography

The separation of nadolol racemates under high pH reversed-phase preparative chromatography is presented for the first time.

Three Waters C18 adsorbents (XBridge, Shield and XSelect) are compared for the separation of nadolol racemates using ethanol:water:diethylamine solvent mixtures. Experimental and simulation results are presented to compare the separation performances at preparative scale using both the fixed-bed and the simulated moving bed operations. The experimental preparative separations using one commercial fixed-bed preparative HPLC Azura system equipped with one sole column of preparative dimensions (30 mm ID x 250 mm L) and one lab-scale SMB apparatus (the FlexSMB-LSRE pilot unit) equipped with six semi-preparative columns (19 mm ID x 100 mm L) are presented. Both systems use the Waters XBridge C18 adsorbent of 10 μm particle diameter. The screening of the mobile phase composition elected the 30:70:0.1 (v/v/v) ethanol:water:diethylamine solvent mixture to perform both FB and SMB preparative operations.

This chapter is based on the following publications:

Arafah, R.S., Ribeiro, A.E., Rodrigues, A.E., and Pais, L.S., Separation of nadolol racemates by high pH reversed-phase preparative chromatography, Sep. Purif. Technol. 233 article number 116018 (2020).

Arafah, R.S., Ribeiro, A.E., Rodrigues, A.E., and Pais, L.S., Separation of nadolol racemates by high pH reversed-phase fixed-bed and simulated moving bed chromatography, Sep. Purif. Technol. 305 article number 122529 (2022).

6.1 Introduction

C18 adsorbents are recently being used for different applications in fixed-bed [57-60] and simulated moving bed (SMB) [61-62] preparative separations. Waters manufacturer (USA) introduced three different types of C18 stationary phases, namely, the XBridge C18, the XBridge Shield RP18 and the XSelect CSH C18 [63, 64]. These columns are designed with the Ethylene Bridged Hybrid (BEH), the Optimum Bed Density (OBD) and the Charge Surface Hybrid (CSH) technologies. BEH technology synthesis creates particles prepared from two high purity monomers, tetraethoxysilane and bis(triethoxysilyl)ethane, resulting in a pH-resistant and mechanically-strong particle. OBD technology was specially developed for preparative separations to effectively improve the packed-bed stability and column lifetime by eliminating the bed collapse. The combination of both manufacturing technologies is referred by the manufacturer to ensure a direct scalability from analytical to preparative liquid chromatography separations. The XBridge C18 column uses a trifunctional C18 as ligand type and the pH range is 1-12. The Shield RP18 uses a monofunctional embedded polar group (carbamate) that “shields” the silica residual silanol surface from highly basic analytes, improving significantly the peak shape and resolution. The XSelect CSH C18 adsorbent uses a trifunctional C18 ligand type and is referred to improve chromatographic selectivity with the increase of solvent’s hydrophobicity. These two last adsorbents can both be used in the pH range between 1 and 11. A wide number of published results in analytical HPLC using XBridge C18 [65-70], XBridge Shield RP18 [71-77] and XSelect CSH C18 [78-80] can be found in literature. All three packings generally use alcohol:water or acetonitrile:water solvents, often with an acid or basic modifier (typically 0.1%).

Asymmetric organic synthesis and racemate resolution methods are two alternative strategies to obtain pure enantiomers [81]. Despite the large amounts of enantiomer provided by the first strategy, asymmetric synthesis is also time consuming and has the disadvantage of producing only one pure enantiomer. Taking into account that, in the earlier stages of drug discovery processes, only few quantities are necessary, racemate resolution can be the more appropriate choice since it can allow an efficient preparative separation process and provide both pure enantiomers. As referred before, the availability of all the pure enantiomers is an important step to identify new therapeutic principles and possible harmful pharmacological side effects. Among the different resolution methods, direct chromatographic resolution of enantiomers using continuous processes, such as the simulated moving bed (SMB) technology, is nowadays considered an attractive production alternative.

6.2 Selection of the achiral adsorbent

6.2.1 Loading Pluses

In a liquid chromatographic separation, the choice of the solvent composition is a crucial step. The solvent selection normally has a remarkable impact on both retention and selectivity. As referred before, due to the strong alkaline nature of nadolol, the baseline separation of nadolol racemates often needs a mobile phase composition having a pH value two units above its pKa value. A pH value near 11 can be reached by adding 0.005% of diethylamine modifier to a 30%ethanol:70%water mobile phase composition. For a pH near 12, a 0.1% of the same modifier is needed.

Fig. 6.1 presents several loading pulses of 2 and 10 g/L nadolol feed solutions, using a 30%ethanol:70%water solvent composition with a pH of 11 and 12, and for 20, 100

and 1000 μL injection loops. These pulses were carried out using both the analytical and the preparative Waters XBridge, Shield and XSelect columns.

For the XBridge C18 material there is a significant difference on the performance of the pulse separation of the nadolol racemates when using pH=12 instead of pH=11, at both the analytical and the preparative scales. Particularly, the use of lower pH introduces higher retention, lower selectivity and higher dispersion (peak tailing). This could be justified by pH=11 to be still close to the nadolol pKa (9.67).

The loss in selectivity at pH=11 is considerably overcome by using the other Waters C18 materials. Shield RP18 material at pH=11 presents a separation behaviour close to the one obtained using XBridge C18 at pH=12. XSelect CSH C18 material at pH=11 also presents improved selectivity but still maintains higher retention and peak tailing. This can justify the preference for Shield RP18 material since it achieves good separation performances using pH=11, close to ones obtained with XBridge C18 but using pH=12. Nevertheless, this conclusion should be validated at overloaded conditions (high amounts and concentrations of nadolol) as found at preparative and production scales. This validation can be obtained by measuring the adsorption equilibrium isotherms and through breakthrough experiments.

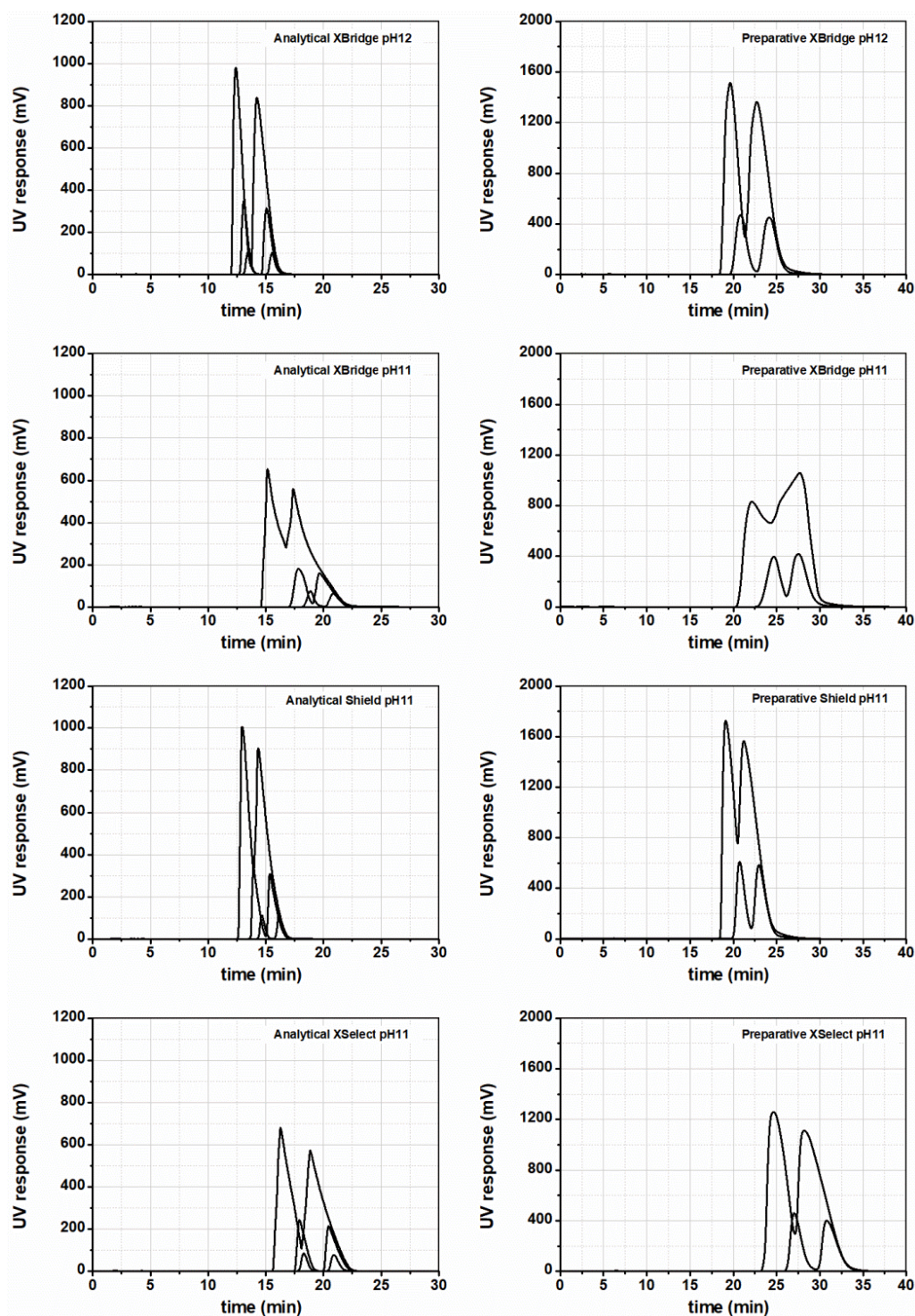


Fig. 6.1. Experimental chromatograms for the separation of nadolol racemates using 30:70:0.1 (pH=12) and 30:70:0.005 (pH=11) ethanol:water:diethylamine mobile phase compositions, for the three analytical and preparative Waters C18 columns (XBridge, Shield and XSelect). For analytical columns, feed concentrations of 2 g/L (loops of 20 and 100 μ L) and 10 g/L (loop of 100 μ L); flow rate of 0.9 mL/min. For preparative columns, feed concentrations of 2 and 10 g/L (loop of 1000 μ L); flow rate of 5.0 mL/min. UV detection at 270 nm.

6.2.2 Measurement, modeling, and fitting of adsorption equilibrium isotherms

For the present work, the following competitive linear+Langmuir (LLG) model was found to better describe the adsorption behaviour, as described in Eq. 3:

The adsorption equilibrium isotherm parameters obtained for the different C18 materials and pH values are presented in Table 6.1.

Table 6.1 Adsorption equilibrium isotherm parameters for the different Waters C18 preparative columns and pH values (at 23 °C).

C18 adsorbent, pH	m	Q (g/L)	b_B (L/g)	b_A (L/g)	SD
XBridge, pH12	1.3694	31.02	9.877×10^{-2}	1.258×10^{-1}	0.2326
XBridge, pH11	1.3594	32.19	9.667×10^{-2}	1.210×10^{-1}	0.2200
Shield, pH11	1.3829	38.46	7.373×10^{-2}	8.529×10^{-2}	0.0423
XSelect, pH11	0.7947	56.92	8.150×10^{-2}	9.551×10^{-2}	0.1201

The comparison between the experimental data and the adsorption behaviour predicted by the selected LLG model is presented in Fig. 6.2 for the XBridge C18 preparative column, using an ethanol:water (30:70) solvent composition and for both pH=12 and pH=11. The left plot presents the adsorption equilibrium isotherms and the right plot the selectivity behaviour, as a function of the feed concentration. Fig. 6.3 presents the same comparison for the three different Waters C18 materials using ethanol:water (30:70) solvent composition and pH=11.

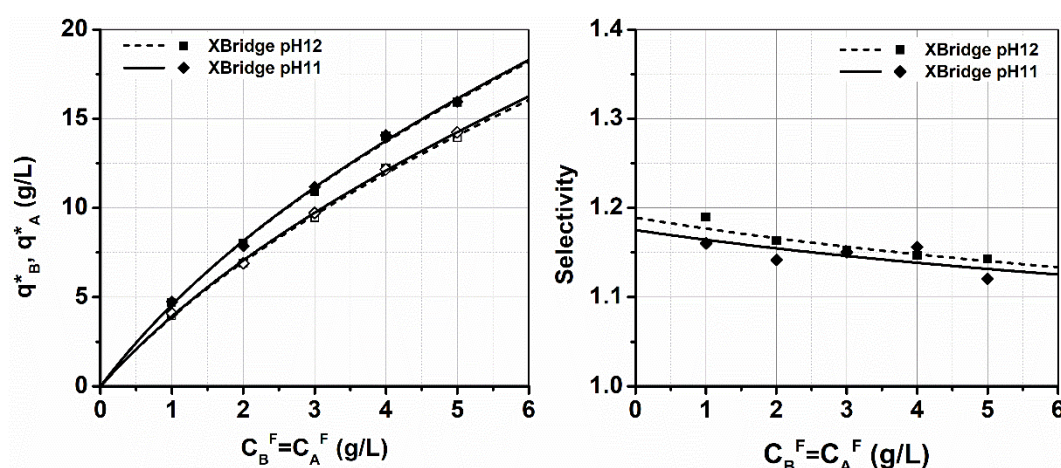


Fig. 6.2. Comparison between experimental and model results for the adsorption equilibrium isotherms (left) and selectivity (right) for the two nadolol racemates, as a function of their feed concentrations, using ethanol:water (30:70) mobile phase composition with 0.1% diethylamine (dashed line, pH=12) and with 0.005% diethylamine (solid line, pH=11), and the XBridge C18 material. Both fittings (lines) use the linear+Langmuir (LLG) competitive model parameters presented in Table 6.1.

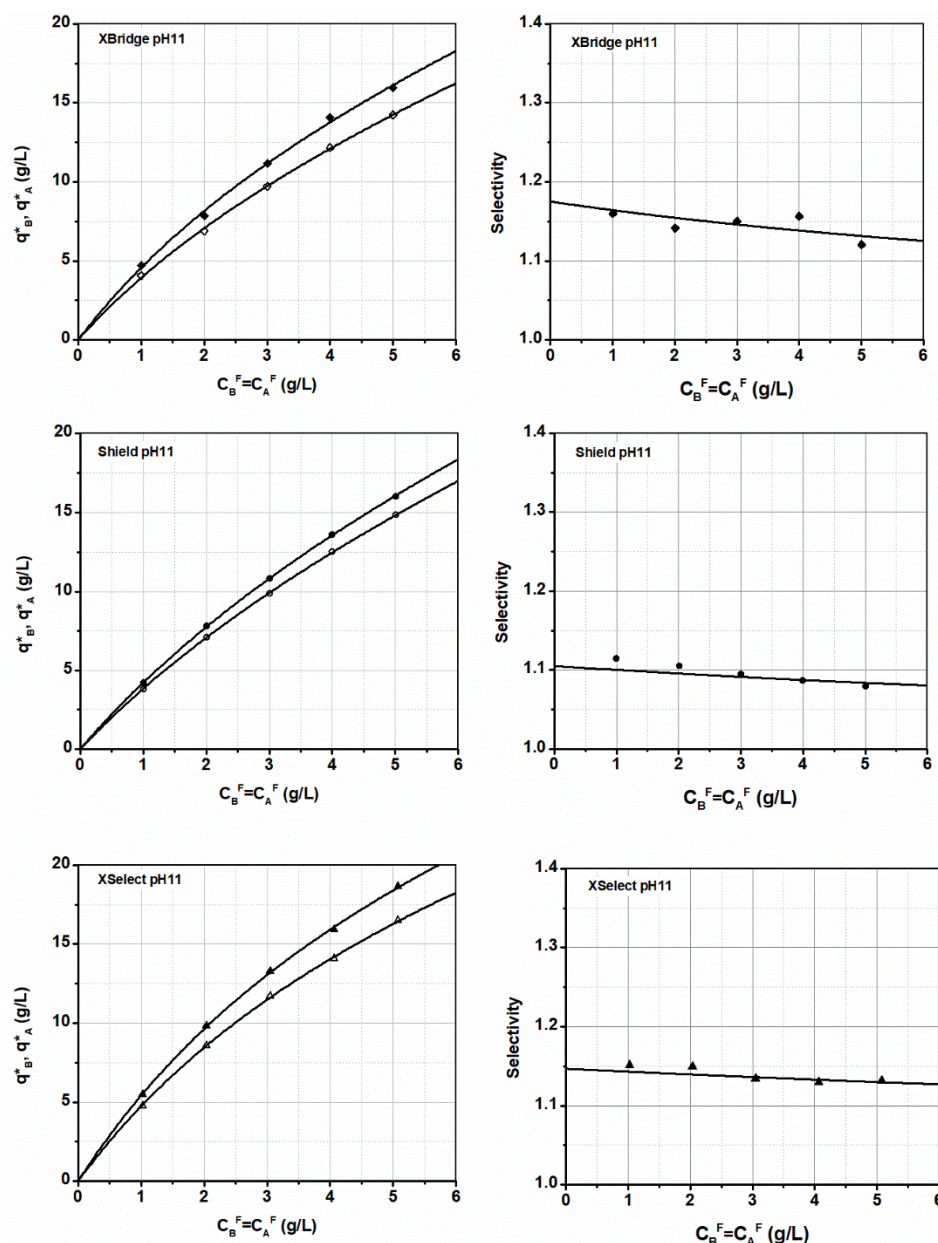


Fig. 6.3. Comparison between experimental and model results for the adsorption equilibrium isotherms (left) and selectivity (right) for the two nadolol racemates, as a function of their feed concentrations, using ethanol:water (30:70) mobile phase composition with 0.005% diethylamine (pH=11) and the three different Waters C18 preparative columns: XBridge (diamonds, upper row), Shield (circles, middle row) and XSelect (triangles, bottom row). All fittings use the linear+Langmuir (LLG) competitive model parameters presented in Table 6.1.

6.2.3 Breakthroughs experiments and simulation

For each breakthrough experiment, several outlet samples were collected at different times of the saturation and regeneration steps. These samples were analyzed by HPLC and compared with the simulated chromatograms. Using uracil as a non-

retained compound in the C18 material, the axial dispersion was predicted through a Peclet number of $Pe=5000$. From the breakthrough curves using 10 g/L nadolol feed solutions, a predicted value for the mass transfer coefficient of $k=5\text{ s}^{-1}$ proved to be adequate for all the C18 materials. Fig. 6.4 presents the obtained results using the three C18 materials with pH=11 or pH=12.

Fig. 6.2 and Fig. 6.4 show that the significant differences found at pulse chromatography for the XBridge C18 material using pH=12 versus pH=11 (see Fig. 6.1 for the loading pulses) are not observed in the comparison of the adsorption equilibrium isotherms and in the breakthrough experiments. Both saturation and regeneration curves for pH=11 and pH=12 show very similar behaviours, and only a slight decrease in selectivity is observed for pH=11. This is a nice and clear example showing that the separation performance under preparative conditions should not be evaluated based on the extrapolation of the results obtained under analytical and linear chromatographic conditions, but needs to be carefully evaluated under those preparative and overloaded conditions.

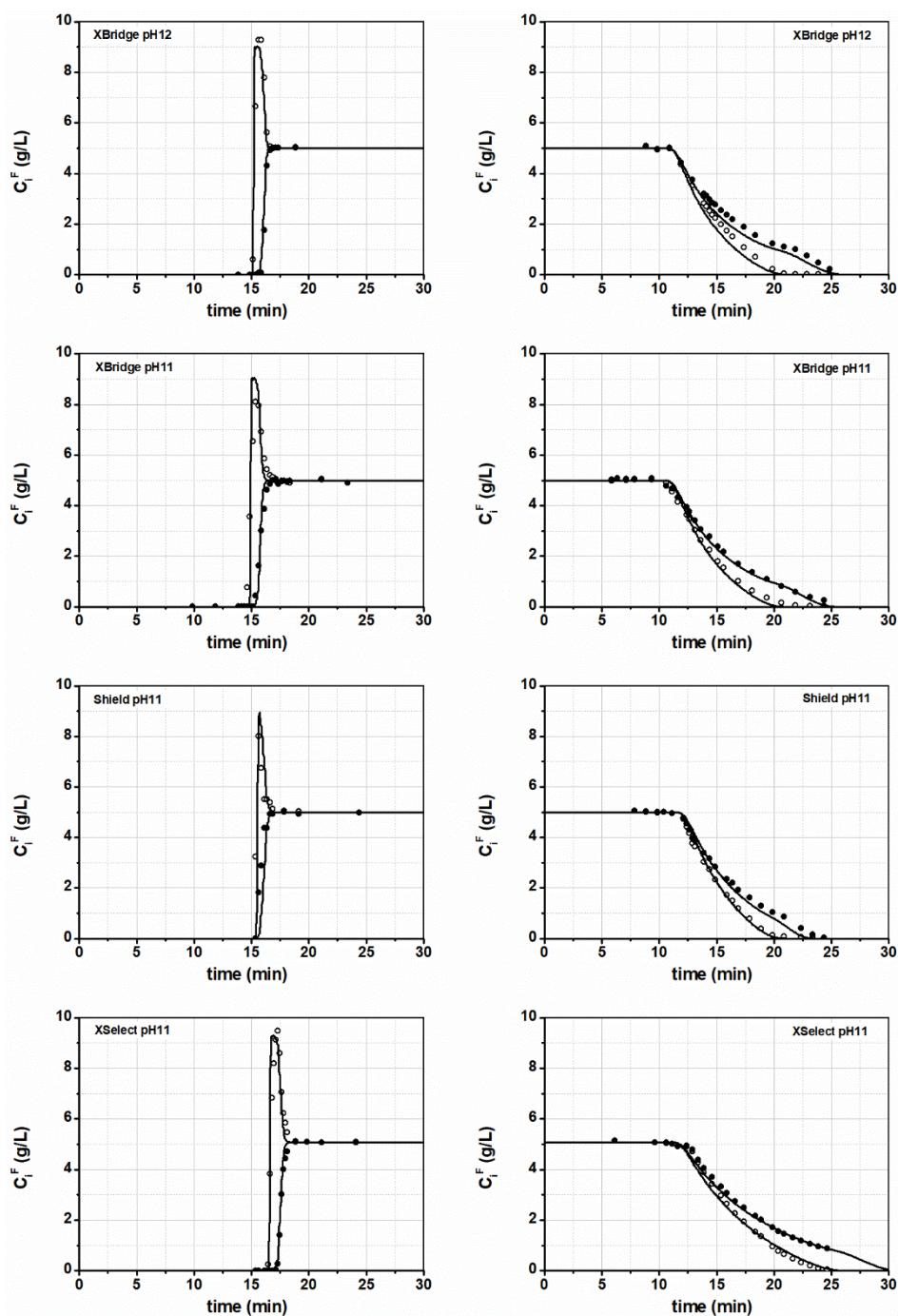


Fig. 6.4. Saturation (adsorption; left plots) and regeneration (desorption; right plots) curves for a 10 g/L nadolol feed solution using XBridge with 30%ethanol/70%water/0.1%diethylamine (pH=12), and using XBridge, Shield and XSelect with 30%ethanol/70%water/0.005%diethylamine (pH=11). Comparison between the experimental (points) and the simulation results (lines). Flow-rate of 5 mL/min; model parameters of $\varepsilon=0.4$ (bed porosity), $Pe=5000$ (Peclet number), and $k= 5 \text{ s}^{-1}$ (mass transfer coefficient); linear+Langmuir isotherm model parameters as in Table 6.1.

Comparing the different Waters C18 materials using pH=11, Fig. 6.3 and Fig. 6.4 show that Shield presents a considerable loss in selectivity. For Shield material, the less retained nadolol racemate is clearly more adsorbed, while the more retained

racemate is also less retained when compared with the behavior found using XBridge at pH=11. These results for the Shield adsorbent show that the ones obtained at pulse conditions do not maintain at overloaded conditions.

The results obtained for the XSelect material clearly confirm the higher retention behavior (observed in both Fig. 6.3 and 6.4) and a selectivity that stands clearly above the Shield material, but slightly below the one obtained with XBridge at pH=11.

The results presented in Fig. 6.2 to 6.4 and obtained under preparative and overloaded conditions justify the use of the XBridge C18 material for the preparative separation of the nadolol racemates. Although presenting a slight decrease in selectivity using XBridge C18 material with pH=11 instead of pH=12, the preparative separation performance using pH=11 seems to be not significantly affected, ensuring a longer adsorbent lifetime. These results will be further studied and validated in the next section through the simulation of the preparative separation of nadolol racemates by SMB chromatography using the three C18 materials and pH conditions.

6.2.4 Regions of SMB complete separation

Fig. 6.5 shows the separation regions obtained for the XBridge adsorbent and for nadolol feed concentrations of 2 and 10 g/L, using an 30:70 ethanol:water solvent composition with pH=11 and pH=12. These results show, once more, that there are no significant differences in the SMB performance parameters as the dimension of the triangles separation areas are similar. These results confirm that it is feasible to use XBridge C18 material with pH=11 for a longer column lifetime.

Fig. 6.6 shows the comparison of the separation regions for the three C18 adsorbents, using the same ethanol:water (30:70) solvent with pH=11 and for 2 g/L and 10 g/L nadolol feed concentrations. The SMB separation regions presented in Fig. 6.6

are in line with the results obtained for the adsorption equilibrium isotherms and the breakthrough experiments presented in Fig. 6.3 and 6.4. XBridge and XSelect present similar size SMB separation regions due to similar selectivity values, although XSelect presents considerable higher operating gamma values due to the higher retention of the nadolol racemates in this adsorbent. Fig. 6.6 also confirms the results obtained with the Shield RP18 adsorbent. Its SMB separation region is significantly smaller in the entire range of nadolol feed concentrations (2 and 10 g/L) due to the lower selectivity values found previously in Fig. 6.3 and 6.4.

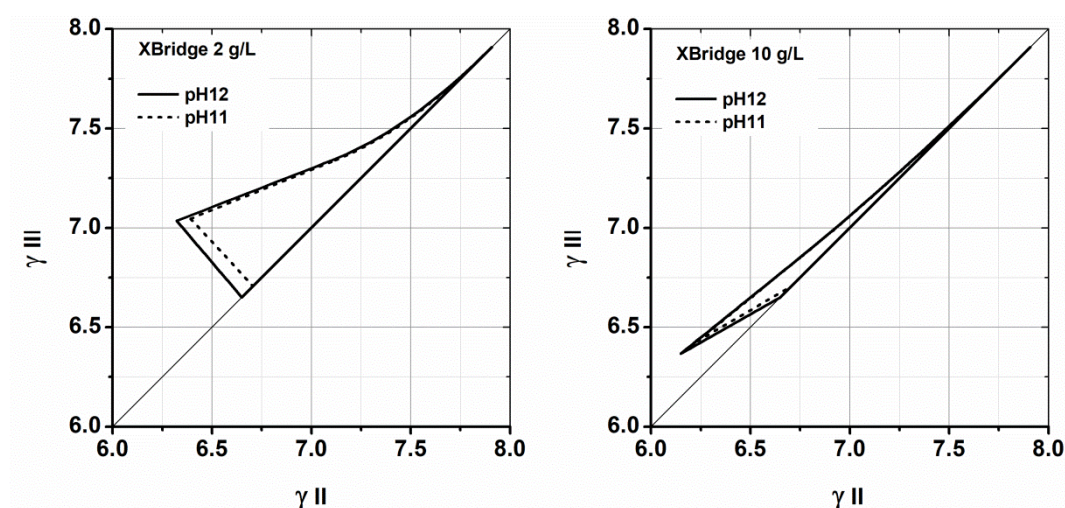


Fig. 6.5. SMB complete separation regions for the separation of nadolol racemates using the XBridge C18 material and ethanol:water (30:70) as mobile phase, with 0.1% diethylamine (pH=12, solid lines) and with 0.005% diethylamine (pH=11, dashed lines), for a nadolol feed concentration of 2 g/L (left) and 10 g/L (right). The SMB complete separation regions are predicted considering the equilibrium theory and the linear+Langmuir (LLG) model with the adsorption equilibrium isotherm parameters presented in Table 6.1.

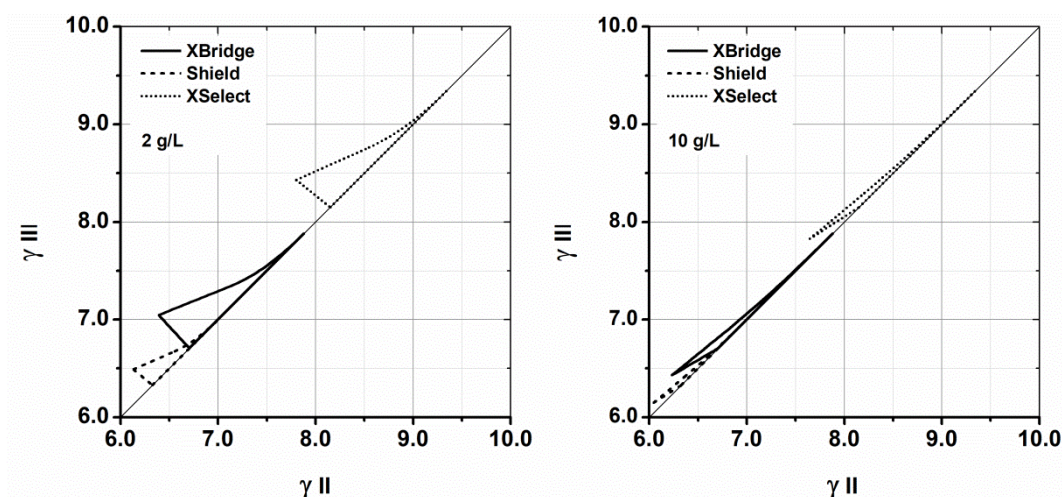


Fig. 6.6. SMB complete separation regions for the separation of the nadolol racemates using the XBridge (solid lines), Shield (dashed lines) and XSelect (dotted lines) C18 materials and ethanol:water:diethylamine (30:70:0.005) as mobile phase (pH=11), for a nadolol feed concentration of 2 g/L (left) and 10 g/L (right). The SMB complete separation regions are predicted considering the equilibrium theory and the linear+Langmuir (LLG) model with the adsorption equilibrium isotherm parameters presented in Table 6.1.

6.2.5 SMB system productivity and solvent consumption

Fig. 6.7 presents the predicted SMB system productivity and solvent consumption for the Waters XBridge C18 adsorbent, using an ethanol:water (30:70) solvent composition with pH=11 and pH=12, as a function of the nadolol feed concentration. The productivity and solvent consumption parameters were calculated at the vertex of each SMB separation regions considering the equilibrium theory model, the equilibrium adsorption isotherm parameters presented in Table 2, a maximum internal flow-rate of $Q^*_{I}=20$ mL/min at section I of the SMB unit, and safety margins of 20% for the SMB sections I and IV. The simulation results confirm that there is not a strong effect of the pH value (pH=12 versus pH=11) in the SMB system productivity and solvent consumption obtained using the XBridge C18 adsorbent.

Fig. 6.8 presents the comparison for the predicted SMB performance parameters for the three Waters C18 adsorbents using the same ethanol:water:diethylamine (30:70:0.005) solvent composition with pH=11, as a function of the nadolol feed concentration.

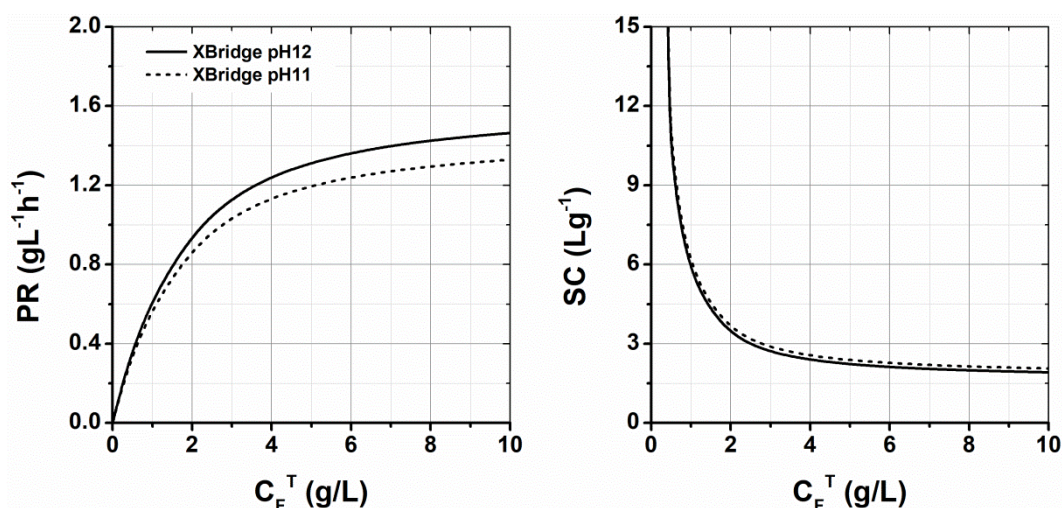


Fig. 6.7. Predictions of the SMB system productivity (left) and solvent consumption (right) as a function of the nadolol feed concentration for the XBridge C18 adsorbent. Solid line for (30:70:0.1) ethanol:water:diethylamine (pH=12) and dashed line for (30:70:0.005) ethanol:water:diethylamine (pH=11). Flow-rate in SMB section I of $Q_I^*=20$ mL/min; safety margins of 20% for SMB sections I and IV; linear+Langmuir isotherm model parameters as in Table 6.1.

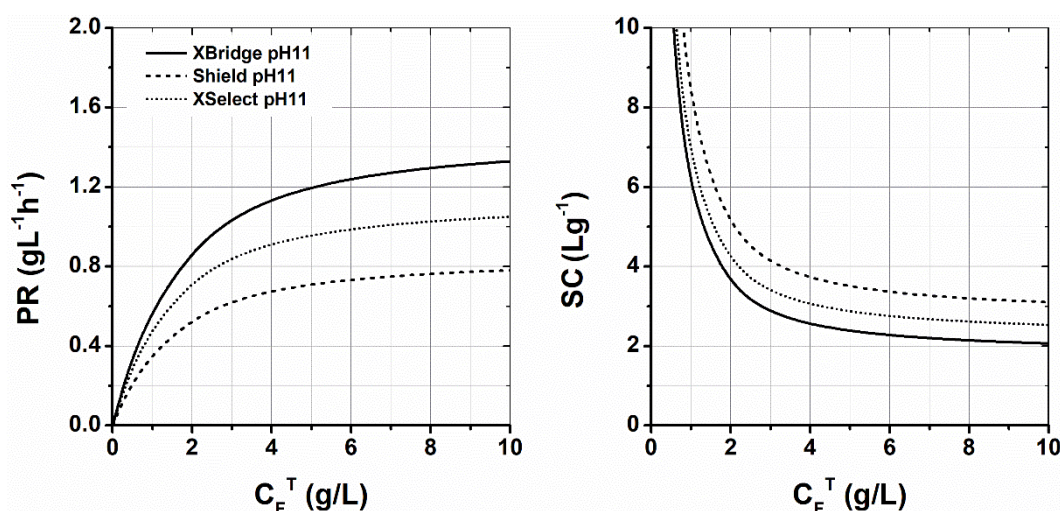


Fig. 6.8. Predictions of the SMB system productivity (left) and solvent consumption (right), as a function of the nadolol feed concentration, for the three Waters C18 adsorbents and using (30:70:0.005) ethanol:water:diethylamine (pH=11). Solid line for the XBridge, dashed line for Shield and dotted line for XSelect C18 materials. Flow-rate in SMB section I of $Q_I^*=20$ mL/min; safety margins of 20% for SMB sections I and IV; linear+Langmuir isotherm model parameters as in Table 6.1.

From the three studied Waters C18 materials, the XBridge adsorbent presents the better behaviour for both the SMB system productivity and solvent consumption. As explained in the previous results, the lower SMB performances presented by the XSelect and the Shield adsorbents can be justified by the higher retention of the

nadolol racemates and by the significant lower selectivity values, respectively. Therefore, the XBridge C18 adsorbent can be considered as the more appropriate material for the preparative separation of nadolol racemates and can operate at pH=11, avoiding higher pH values (up to 12) and ensuring longer adsorbent lifetime.

6.2.6 Prediction of the SMB operation for the preparative separation of nadolol racemates

The simulation of the SMB operation using a conventional four-zone SMB mode with a [1-2-2-1] column configuration was carried for each C18 adsorbent and a nadolol feed concentration of 10 g/L. The SMB model used in simulation took into account both the axial dispersion and the mass transfer resistances, and the linear+Langmuir (LLG) model to describe the adsorption equilibrium isotherms. The model parameters used were the ones raised in the experimental measurements carried out in this work and presented in Table 6.1 and Fig. 6.2 to 6.4.

Table 6.2 presents the SMB operating conditions, including the internal and external SMB flow rates, the SMB switch time interval, and the corresponding γ values for the equivalent true moving bed (TMB) operation, calculated at the vertex point of each separation region for a 10 g/L nadolol feed concentration (see Fig.6.6, right plot). The predictions of the outlet streams purities and the SMB system productivity and solvent consumption parameters are also presented in Table 6.2, and are in line with the ones presented in Fig. 6.8. The small contaminations in the extract outlet streams are due to the SMB configuration (6 columns) and the estimated axial dispersion and resistance to mass transfer included in the simulation of the SMB operation.

Fig. 6.9 shows the predicted transient evolution of the nadolol racemates' average concentration in both the extract and the raffinate outlet streams during the first 25 cycles of SMB operation, for XBridge, Shield and XSelect C18 materials. Also

presented are the predicted internal concentration profiles of the two nadolol racemates after the SMB cyclic steady-state was achieved (cycle 25). Fig. 6.8 again validates the use of the XBridge C18 adsorbent for the separation of nadolol racemates with better SMB system performances than the ones obtained with Shield and XSelect materials for the same solvent and pH conditions.

Table 6.2. SMB operating conditions and predicted performance parameters for the separation of nadolol racemates using the three different C18 adsorbents and an ethanol:water:diethylamine (30:70:0.005) solvent composition (pH=11). Nadolol feed concentration of 10 g/L; model parameters of $\varepsilon=0.4$ (bed porosity), $Pe=5000$ (Peclet number), and $k=5\text{ s}^{-1}$ (mass transfer coefficient); linear+Langmuir (LLG) isotherm model parameters as in Table 2. The SMB performance parameters are calculated after achieved the SMB cyclic steady-state (cycle 25).

C18 adsorbent	Internal flow rates (mL/min)	External flow rates (mL/min)	TMB γ values	SMB performance parameters
XBridge	$Q_I^* = 20.00$	$Q_E = 7.40$	$\gamma_I = 9.4579$	$PUX = 99.4\%$
	$Q_{II}^* = 13.83$	$Q_F = 0.38$	$\gamma_{II} = 6.2333$	$PUR = 100.0\%$
	$Q_{III}^* = 14.21$	$Q_X = 6.17$	$\gamma_{III} = 6.4303$	$PR = 1.33\text{ gl}^{-1}h^{-1}$
	$Q_{IV}^* = 12.60$	$Q_R = 1.61$	$\gamma_{IV} = 5.5890$	$SC = 2.06\text{ lg}^{-1}$
	$t^* = 5.930\text{ min}$	$Q_{REC} = 12.60$		
Shield	$Q_I^* = 20.00$	$Q_E = 6.64$	$\gamma_I = 8.3937$	$PUX = 98.4\%$
	$Q_{II}^* = 15.00$	$Q_F = 0.22$	$\gamma_{II} = 6.0449$	$PUR = 100.0\%$
	$Q_{III}^* = 15.22$	$Q_X = 5.00$	$\gamma_{III} = 6.1489$	$PR = 0.78\text{ gl}^{-1}h^{-1}$
	$Q_{IV}^* = 13.36$	$Q_R = 1.86$	$\gamma_{IV} = 5.2732$	$SC = 3.10\text{ lg}^{-1}$
	$t^* = 5.327\text{ min}$	$Q_{REC} = 13.36$		
XSelect	$Q_I^* = 20.00$	$Q_E = 7.24$	$\gamma_I = 11.2160$	$PUX = 99.3\%$
	$Q_{II}^* = 14.15$	$Q_F = 0.30$	$\gamma_{II} = 7.6454$	$PUR = 100.0\%$
	$Q_{III}^* = 14.45$	$Q_X = 5.85$	$\gamma_{III} = 7.8272$	$PR = 1.05\text{ gl}^{-1}h^{-1}$
	$Q_{IV}^* = 12.76$	$Q_R = 1.69$	$\gamma_{IV} = 6.7921$	$SC = 2.53\text{ lg}^{-1}$
	$t^* = 6.927\text{ min}$	$Q_{REC} = 12.76$		

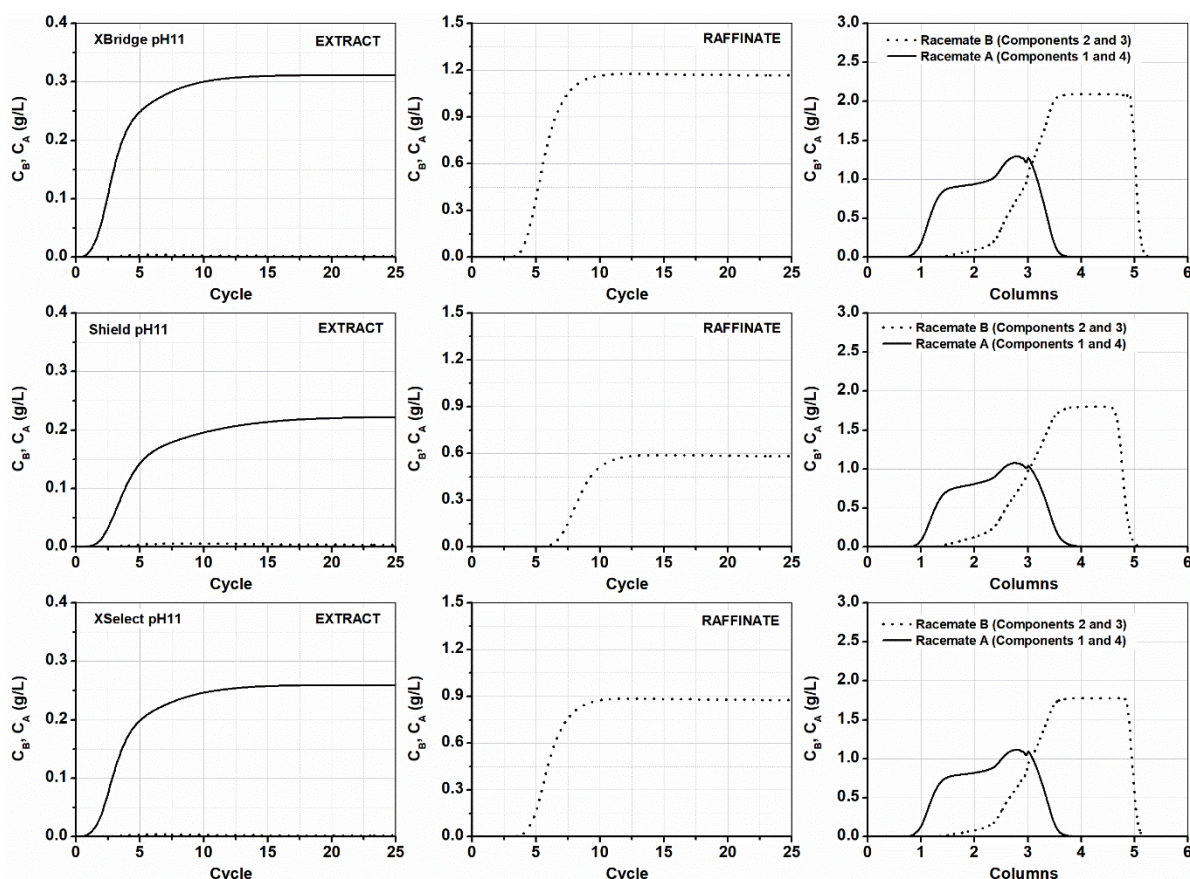


Fig. 6.9. SMB predicted transient evolution of the nadolol racemates' average concentrations in the extract (left) and raffinate (middle) streams for the three C18 adsorbents (XBridge, Shield and XSelect) and an ethanol:water:diethylamine (30:70:0.005) solvent composition (pH=11). SMB predicted internal concentration profiles at cyclic steady state (right; cycle 25). Dashed line for the less-retained racemate B (stereoisomers 2 and 3) and solid line for the more-retained racemate A (stereoisomers 1 and 4). Nadolol feed concentration of 10 g/L; model parameters of $\varepsilon=0.4$ (bed porosity), $Pe=5000$ (Peclet number), and $k=5\text{ s}^{-1}$ (mass transfer coefficient); linear+Langmuir (LLG) isotherm model parameters as in Table 6.1.

The Waters XBridge C18 adsorbent was concluded to be the better solution for the preparative separation of nadolol racemates, using an ethanol:water: diethylamine (30:70:0.005) solvent composition (pH=11) and under preparative and simulated moving bed (SMB) chromatography.

6.3 Simulated moving bed operation

6.3.1 Screening of solvent composition and evaluation of SMB column efficiency

A large set of preliminary studies were carried out to validate the solvent composition. Several pulses of nadolol were performed using a Waters XBridge C18 column (19 mm ID x 100 mm L) packed with C18 absorbent with a particle size diameter of 10 μm . These experiments were performed using the preparative HPLC system, using a 2 g/L of nadolol feed solution prepared in the same solvent. A flow-rate of 5 mL/min and an injection volume of 1000 μL were used.

Some of the most representative experimental results are presented in Fig. 6.10 for three ethanol:water:0.1diethylamine compositions, 30E70W, 35E65W and 40E60W.

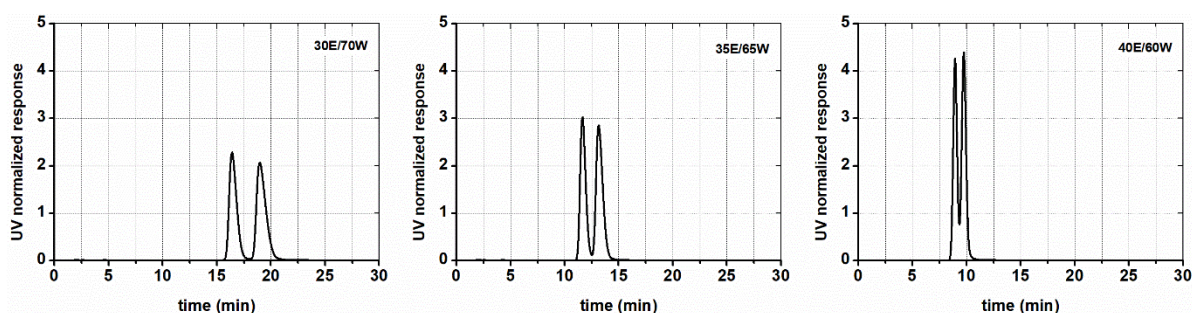


Fig 6.10. Pulse experiments of nadolol using three different ethanol:water:0.1diethylamine solvent compositions: 30E70W, 35E65W and 40E60W. $C_F^T = 2$ g/L; loop of 1000 μL ; $Q = 5$ mL/min.

These results confirm that the 30E70W composition represents a good situation in terms of resolution and confirm the previous published results for nadolol racemates retention using this adsorbent [28].

The same Waters XBridge C18 column was used to measure the influence of the superficial velocity, $U_0 = Q/A$, upon the Height Equivalent to a Theoretical Plate, $HETP = \sigma^2 L / \mu_1^2$ (in the former equations, Q is the flow-rate, A the column section area, L the column length, σ the peak variance and μ_1 the first moment of the

chromatographic peak). The HETP results obtained for 30E70W and 40E60W compositions are presented in Fig. 6.11. These results show that, when using 40E60W, the HETP values remain constant (around 30 μm) for the two racemates as the flow-rate increases. For the 30E70W, the HETP value increases with the increase of the flow-rate (around 40 to 60 μm). This means a better chromatographic efficiency when using the 40E60W, being more significant at high flow-rates. However, this must be confirmed by measurements of the adsorption behaviour using high nadolol concentrations.

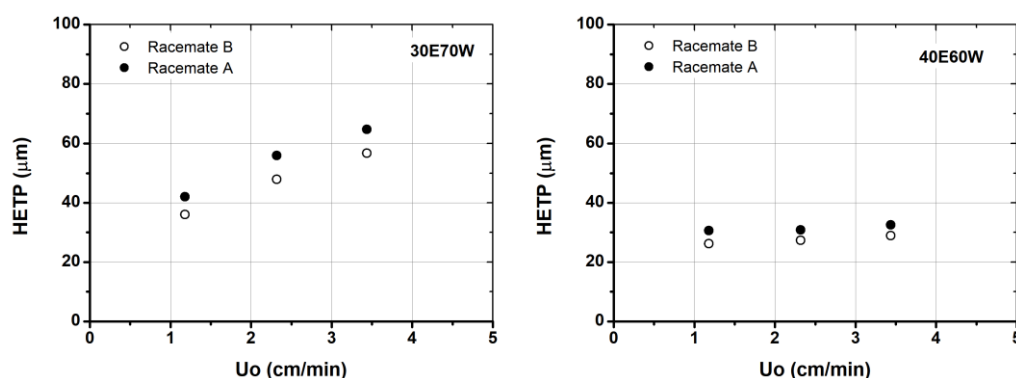


Fig. 6.11. HETP as a function of superficial velocity (U_o) for a Waters XBridge C18 preparative column (19 mm ID x 100 mm L) and a mobile phase composition of 30%ethanol:70%water:0.1% diethylamine (left) and of 40%ethanol:60%water:0.1% diethylamine (right). Open and closed circles for the less and the more retained nadolol racemate, respectively. $C_F^T = 2$ g/L; loop of 1000 μL ; $Q = 5, 10$ and 15 mL/min.

6.3.2 Measurement of the adsorption equilibrium isotherms

The competitive linear + Langmuir model was found to better describe the experimental adsorption behaviour using the 30E70W composition and the competitive Langmuir model was found to better describe for the 40E60W composition. Table 6.3 presents the model equations and the estimated parameters using the Levenberg-Marquardt algorithm for both solvent compositions.

Table 6.3. Selected adsorption equilibrium isotherm and estimated parameters obtained by fitting the experimental data to the competitive linear + Langmuir and Langmuir models. Average room temperature of 23°C. Nadolol racemate A is more retained than racemate B.

Mobile Phase Composition	Theoretical Model	Estimated Parameters
30E70W	Linear + Langmuir eq(3)	$m = 1.3694$ $Q = 31.02 \text{ g/L}$ $b_B = 9.877 \times 10^{-2} \text{ L/g}$ $b_A = 1.258 \times 10^{-1} \text{ L/g}$ $(SQ = 0.3245; SD = 0.2326)$
40E60W	Langmuir eq(2)	$Q = 99.56 \text{ g/L}$ $b_B = 1.534 \times 10^{-2} \text{ L/g}$ $b_A = 1.725 \times 10^{-2} \text{ L/g}$ $(SQ = 0.1287; SD = 0.1356)$

q_i^* and C_i are, respectively, the solid and the liquid concentrations of racemate i ($i=A, B$), and m , Q , b_B and b_A are the adsorption equilibrium isotherm parameters. SQ is the sum of square of the residues and SD is the corrected standard deviation.

The experimental results (points) and the predicted adsorption behaviour (lines) obtained for the 30E70W and 40E60W mobile phase compositions are presented in Fig. 6.12. The left plot presents the adsorption equilibrium isotherm and the right plot the A/B selectivity behaviour.

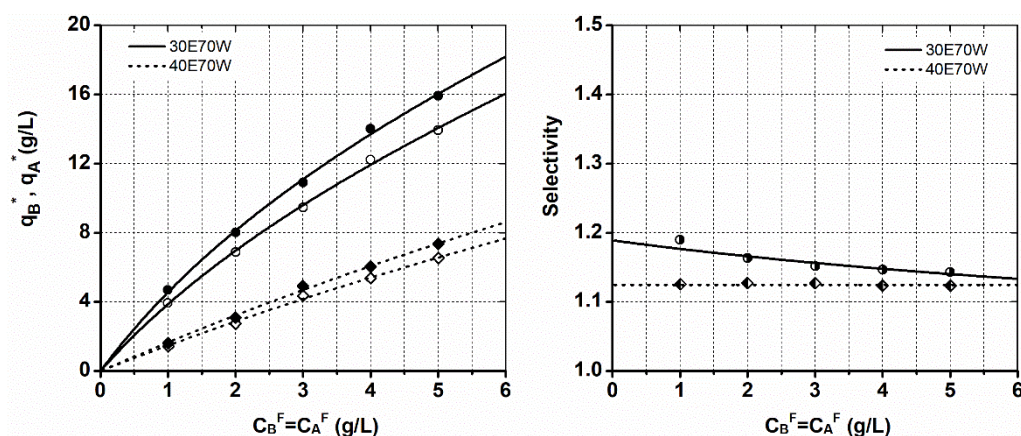


Fig. 6.12. Comparison between experimental (points) and model (lines) results for the adsorption equilibrium isotherms of nadolol racemates using two solvent compositions: 30%ethanol:70%water (solid lines) and 40%ethanol:60%water (dashed lines). Left figure presents the adsorption equilibrium isotherms and its fitting to a linear + Langmuir (30E70W) and to a Langmuir (40E60W) models. Right figure presents the selectivity of racemates A/B as a function of the nadolol feed concentration.

These results show that, for high feed concentrations such as 10 g/L ($C_B^F = C_A^F = 5 \text{ g/L}$), there are no significant differences in selectivity for both mobile phase compositions (1.12 for 40E60W and 1.13 for 30E70W). Nevertheless, the 40E60W composition allows higher adsorption isotherm linearity for high feed concentrations, that may be advantageous for the preparative separation.

6.3.3 Fixed-bed behaviour (frontal chromatography)

The model equations used for fixed-bed chromatography (breakthrough) using the LDF model. The simulation of the fixed-bed adsorption behaviour requires the estimation of the mass transfer resistance coefficient (k) and the Peclet number (Pe) that accounts with the axial dispersion effect. The mass transfer coefficient was estimated by fitting the model to the experimental data using a 10 g/L of nadolol feed solution. The Peclet number was estimated with the same experimental methodology and a 10 g/L of uracil solution, used as the non-retained compound. The estimated values of $Pe = 5000$ and $k = 5 \text{ s}^{-1}$ show very good agreement with the experimental data, representing a situation of low resistance to mass transfer, and validates the high column efficiency shown previously by the HETP experiments.

The experimental results (points) and the predicted adsorption behaviour (lines) obtained with the selected adsorption isotherms models for 30E70W and 40E60W solvent compositions are both presented in Fig. 6.13. There is a very good agreement between experimental (points) and simulation (lines) using the linear + Langmuir and Langmuir adsorption equilibrium isotherm models for saturation and regeneration steps for both the 30E70W and the 40W60W compositions. These results are a good validation of the selected models and reinforce previous conclusions for the adsorption equilibrium isotherm measurements. Also shows that the 40E60W composition, notwithstanding the decrease in selectivity, presents a decrease in the roll-up phenomena, a decrease in the retention and less dispersion (tailing) for the two

racemates in the regeneration step. Knowing that the use of a solvent composition with high alcoholic content will also promote nadolol solubility, all these behaviours may have a positive impact in the productivity and solvent consumption at the preparative scale.

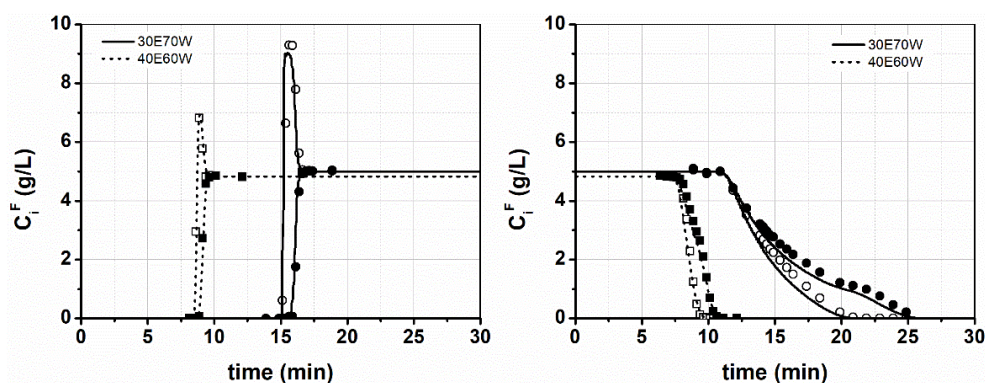


Fig. 6.13. Saturation (adsorption; left) and regeneration curves (desorption; right) for a 10 g/L nadolol feed concentration and 30%ethanol:70%water (30E70W) and 40%ethanol:60%water (40E60W) mobile phase compositions. Comparison between experimental (points; circles for 30E70W and squares for 40E60W) and simulation results (lines; solid for 30E70W and dashed for 40E60W). Flow-rate: 5 mL/min. Model parameters: $\varepsilon = 0.4$, $Pe = 5000$, $k = 5 \text{ s}^{-1}$, and the linear + Langmuir (for 30E70W) or Langmuir (for 40E60W) model parameters.

6.4 Regions of complete separation for SMB operation and performance parameters

The performance of the SMB operation is normally predicted by defining the region of complete separation. Within this region, both extract and raffinate outlet streams are collected pure. The region is obtained by plotting γ_{III} as a function of γ_{II} , where $\gamma_j = v_j/u_s$ is the ratio between the interstitial velocities of the liquid and the solid in section j of the equivalent true moving bed concept (counter-current operation). The vertex of this region represents geometrically the furthest point from the diagonal $\gamma_{III} = \gamma_{II}$ and so, it represents the optimal operation conditions in terms of productivity and solvent consumption still achieving complete separation (100% pure racemates at both the extract and the raffinate outlet streams). Fig. 6.14 presents the predicted regions of

complete separation using nadolol feed concentrations of 2 and 10 g/L for both the 30E70W and the 40E60W mobile phase compositions.

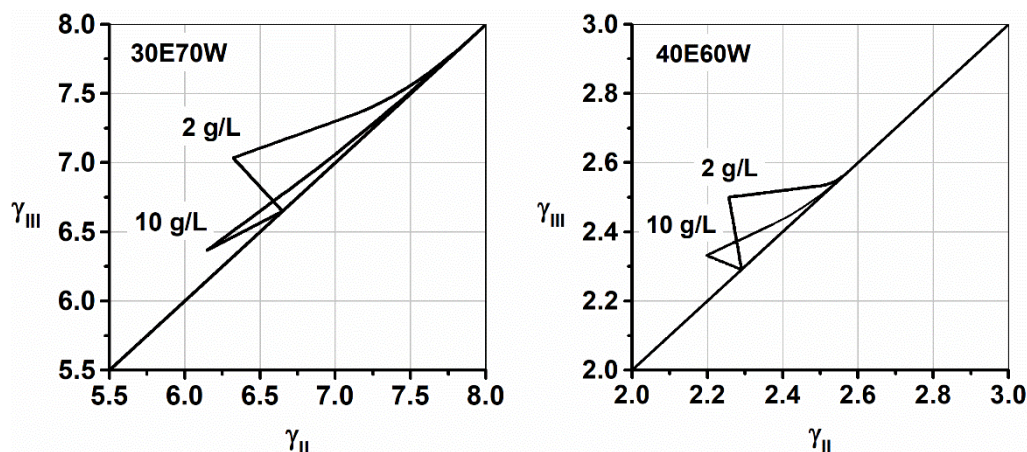


Fig. 6.14. SMB regions of complete separation for the pseudo-binary separation of nadolol racemates for 30E70W (left plot) and 40E60W (right plot) solvent compositions and for nadolol feed concentrations of 2 and 10 g/L.

As expected, the increase in the feed concentration leads to higher productivities but to smaller triangle separation regions and, consequently, harder experimental SMB operating conditions. In this case, the 40E60W presents very small complete separation areas, meaning that the SMB unit will be quite more difficult to operate using this composition.

The SMB performance parameters are summarized in Table 2.2. Fig. 6.15 presents the prediction of SMB productivity and solvent consumption for the two solvent compositions, as a function of the nadolol feed concentration, for a maximum flow-rate of 20 mL/min in section I, a safety margin of 20% in both sections I and IV, and at the vertex points of the correspondent complete separation regions. Fig. 6.14 shows that there is no significant differences in SMB productivity and solvent consumption for a nadolol feed concentration of 2 g/L. However, for high feed concentrations, such as 10 g/L, the 40E60W composition presents better SMB productivity performance, despite its smaller separation region and, so, harder SMB operating conditions.

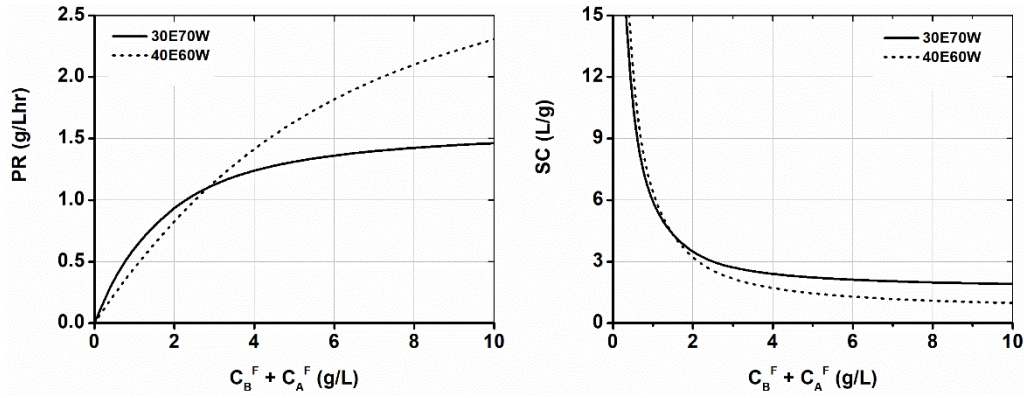


Fig. 6.15. Prediction of SMB productivity (left) and solvent consumption (right) for the two solvent compositions, as a function of the nadolol feed concentration. Solid line for the 30E70W, dashed line for the 40E60W solvent composition.

6.5 SMB operating conditions

The experimental SMB preparative pseudo-binary separation of the nadolol racemates was performed using a 2 g/L feed solution. For this feed concentration, similar performance parameters are predicted for the 30E70W and the 40E60W solvent compositions. Avoiding the small region of complete separation associated with 40E60W, the 30E70W was the selected solvent composition to perform the SMB separation. The system was initially stabilized in order to identify the maximum allowed flow-rate in section I due to pressure drop limitations. Since this section represents the maximum local pressure inside the unit, a maximum pressure drop of 75 bar was imposed in the lab-scale FlexSMB®-LSRE unit, corresponding to a flow-rate in section I of $Q_I^* = 5 \text{ mL/min}$. This represents a very low experimental maximum flow-rate, due to the hardware characteristics of the pilot unit, the use of a 10 μm adsorbent, and the high viscosity of the mobile phase. Even knowing that, under these conditions, very low productivity will be obtained, the system was run to validate the SMB separation methodology.

The critical values of γ_I and γ_{IV} were fixed using $\gamma_I = \frac{1-\varepsilon}{\varepsilon} K_A \beta$ and $\gamma_{IV} = \frac{1-\varepsilon}{\varepsilon} K_B / \beta$, where K_i is the initial slope of the adsorption equilibrium isotherm ($K_i = m + Q b_i$ for the

linear + Langmuir model and $K_i = Q b_i$ for the Langmuir model), and β is the safety margin, considered 20%, that is, $\beta = 1.2$. The switching time value was fixed using $t^* = \frac{\varepsilon V_C}{Q_I^*} (\gamma_I + 1)$ giving $t^* = 23.79 \text{ min}$. The final SMB operating conditions were then defined using the separation region obtained using the Triangle Theory methodology. A pair of $(\gamma_{II}; \gamma_{III})$ values inside the triangle was chosen and the corresponding internal SMB flow-rates Q_j^* in sections II, III and IV were estimated using $Q_j^* = \frac{\varepsilon V_C}{t^*} \gamma_j^*$ ($j = II, III, IV$), where $\gamma_j^* = \gamma_j + 1$. The inlet, outlet and recycle flow-rates were then calculated through $Q_E = Q_I^* - Q_{IV}^*$; $Q_X = Q_I^* - Q_{II}^*$; $Q_F = Q_{III}^* - Q_{II}^*$; $Q_R = Q_{III}^* - Q_{IV}^*$; $Q_{REC} = Q_{IV}^*$. Table 3 summarizes the initial experimental operating conditions used for the SMB operation. Fig. 6.16 shows the separation region (triangle) predicted for the 30E70W solvent composition using a feed concentration of 2 g/L and the proposed $\gamma_{III} \times \gamma_{II}$ operating point (open circle) that represents the initial SMB operating conditions presented in Table 6.4.

Table 6.4. Initial operating conditions used for the SMB preparative separation of nadolol racemates.

Column, packing parameters and feed concentration		
$N_j = [1,2,2,1]$	$d_p = 10 \mu m$	
$D_C = 1.9 \text{ cm}$	$\varepsilon = 0.4$	
$L_C = 10 \text{ cm}$	$C_F^T = 2 \text{ g/L}$	
SMB initial operating conditions		
$\gamma_I = 9.4891$	$Q_I^* = 5.00 \text{ mL/min}$	$Q_E = 1.88 \text{ mL/min}$
$\gamma_{II} = 6.5000$	$Q_{II}^* = 3.58 \text{ mL/min}$	$Q_X = 1.42 \text{ mL/min}$
$\gamma_{III} = 7.0000$	$Q_{III}^* = 3.81 \text{ mL/min}$	$Q_F = 0.24 \text{ mL/min}$
$\gamma_{IV} = 5.5416$	$Q_{IV}^* = 3.12 \text{ mL/min}$	$Q_R = 0.70 \text{ mL/min}$
$t^* = 23.79 \text{ min}$		$Q_{REC} = 3.12 \text{ mL/min}$

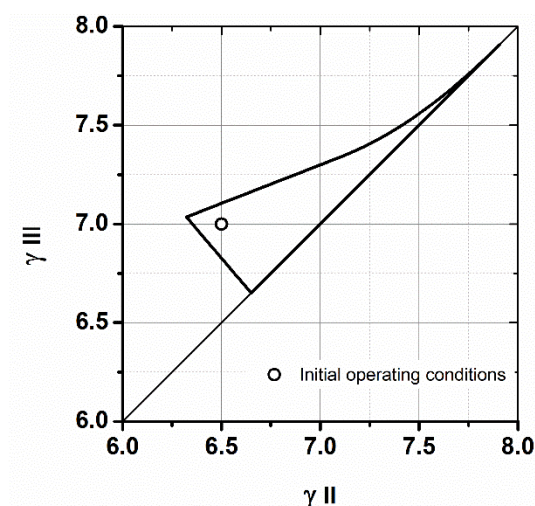


Fig. 6.16. SMB region of complete separation (triangle) and experimental SMB operating point (open circle) for the pseudo-binary separation of nadolol racemates using a feed concentration of 2 g/L and a 30%ethanol:70%water solvent composition.

6.6 SMB experimental operation and performance

During the SMB experimental operation, both raffinate and extract outlet streams were collected and weighted at the end of each full cycle (six rotations) in order to monitor the total recovered mass. In addition, two Coriolis flow-meters were used, one positioned at the extract outlet stream to measure the correspondent extract flow-rate, another located immediately before section II to measure the flow-rate inside this section. These experimental measurements allow the validation of the SMB operating conditions and the accurate flow-rates observed inside and outside the unit. All samples were analysed by analytical HPLC using the same solvent composition and the same Waters XBridge C18 adsorbent but with a particle size diameter of 5 μm . The cycle steady state was considered to be achieved when the average concentrations at the extract and the raffinate outlet streams do not vary more than 5% after ten full cycles, and both the recoveries and the purities of outlet streams were validated to be no less than 99.9%. At that moment, the SMB internal concentration profiles were measured by collecting samples at 50%, 75% and 95% of each switching time, by means of a six-port valve located at the end of section IV.

The average racemate concentrations at the extract and the raffinate products were measured and the performance parameters of recovery, purity, productivity and solvent consumption were calculated using equations presented in Table 2.2. A summary of the experimental SMB operating conditions and results can be found in Table 6.5.

Table 6.5. Experimental SMB operating conditions and results for the separation of a 2 g/L nadolol feed concentration and a 30%ethanol:70%water solvent composition.

Experimental SMB operating conditions		
$Q_E = 2.05 \text{ mL/min}$	$Q_I^* = 5.05 \text{ mL/min}$	$\gamma_I = 9.5932$
$Q_X = 1.34 \text{ mL/min}$	$Q_{II}^* = 3.71 \text{ mL/min}$	$\gamma_{II} = 6.7824$
$Q_F = 0.18 \text{ mL/min}$	$Q_{III}^* = 3.89 \text{ mL/min}$	$\gamma_{III} = 7.1599$
$Q_R = 0.89 \text{ mL/min}$	$Q_{IV}^* = 3.00 \text{ mL/min}$	$\gamma_{IV} = 5.2930$
$Q_{REC} = 3.00 \text{ mL/min}$		
$t^* = 23.79 \text{ min}$		
Outlet concentrations and performance parameters		
$\bar{C}_A^X = 0.1343 \text{ g/L}$	$PU_X = PU_R = 100\%$	$PR = 0.13 \text{ g/(L. hr)}$
$\bar{C}_B^R = 0.2022 \text{ g/L}$	$RC_X = RC_R = 100\%$	$SC = 6.19 \text{ L/g}$

Fig. 6.17 presents the experimental (points) and the predicted (lines) transient evolution (24 full cycles) of the nadolol racemate concentrations in the extract and raffinate outlet streams. Fig. 6.18 presents the experimental (points) and predicted (lines) cycle steady state internal concentration profiles in the six columns of the SMB unit (measured at cycles 20 and 22). The internal concentration profiles presented in Fig. 6.18 show two small plateaus near the extract and raffinate outlets that are due of the dead volumes of the FlexSMB-LSRE unit, taken in account in the simulation software. Despite the operational difficulties due to high pressure drops and low internal flow-rates, Fig. 6.17 and 6.18 show a very good agreement between the experimental results and the predictions using the transient SMB model. This experiment also validates the capacity to complete separate (100% purities and recoveries) the two nadolol racemates using an achiral C18 adsorbent by SMB chromatography.

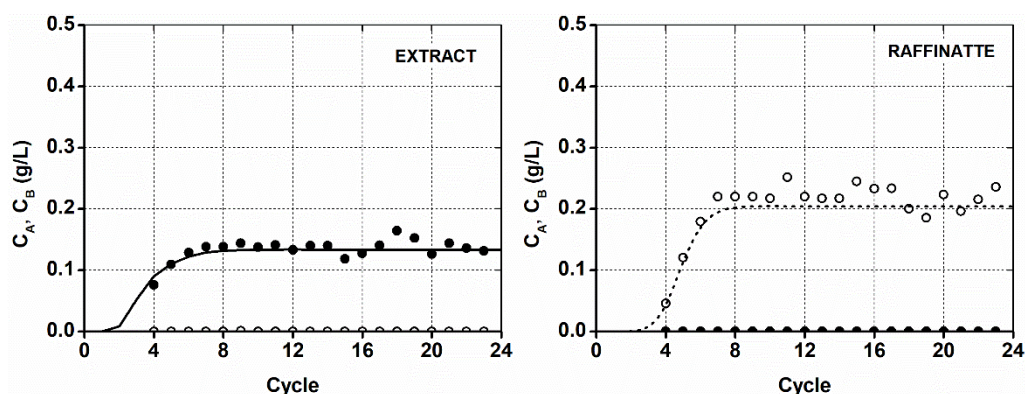


Fig. 6.17. Experimental (points) and predicted (lines) transient evolution (24 full cycles) of the nadolol racemate concentrations in the extract and raffinate outlet streams. Closed circles for the more retained racemate A, open circles for less retained racemate B. Model parameters of $\varepsilon = 0.4$, $Pe = 5000$, $k = 5 \text{ s}^{-1}$, and the linear + Langmuir isotherm model parameters as in Table 6.3.

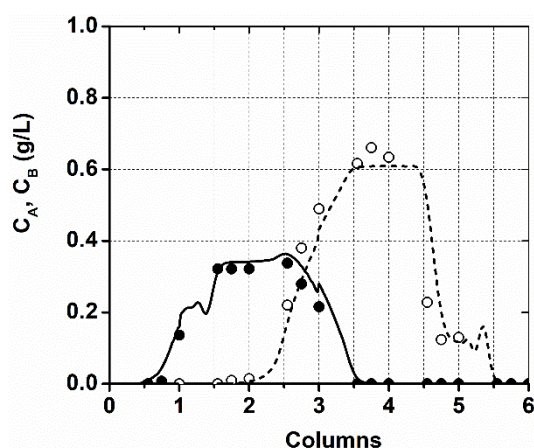


Fig. 6.18. Experimental (points) and predicted (lines) internal concentration profiles of the nadolol racemates at cycle steady state. Closed circles for the more retained racemate A, open circles for less retained racemate B. Model parameters of $\varepsilon = 0.4$, $Pe = 5000$, $k = 5 \text{ s}^{-1}$, and the linear + Langmuir isotherm model parameters as in Table 6.3.

6.7 Fixed-bed preparative separation

The use of fixed-bed (FB) preparative separation was also validated and characterized for the separation of nadolol racemates. Different ethanol:water:diethylamine solvent compositions were studied (solvent screening). The amount of diethylamine added in each solvent mixture was established in order to achieve a pH value near 12, referred by the column manufacturer as the upper safety limit for the C18 adsorbent, but ensuring its capacity to resolve the nadolol racemates with a pKa of 9.67. Additionally, the fixed-bed flow-rate was chosen in order to operate

with a system pressure drop around 150 bar. Six ethanol:water:diethylamine solvent compositions were tested using a nadolol feed concentration of 9 g/L. All feed solutions were prepared using the same solvent composition used as mobile phase. The Azura preparative HPLC system was equipped with one Waters XBridge preparative OBD C18 column (dimensions of 30 mm ID x 250 mm L) and a 10 mL feed injection loop.

6.7.1 Fixed-bed experimental operation and performance

The experimental results obtained for three consecutive pulses using six different ethanol:water:diethylamine (E:W:DEA) compositions are presented in Fig. 6.19 and the correspondent FB performance parameters can be found in Table 6.6. For each run, the injection time interval (time between two successive feed injections) was adjusted (reduced) till eliminating the need for a second waste fraction between the elution of two consecutive feed bands and to ensure that the column is continuously used to separate the two racemates. This means that, for each feed injection, there is only one waste fraction between the two product fractions, ensuring the desired purities. The fraction of the more retained racemate is then contiguous to the fraction of the less retained racemate coming out from the next injection.

As expected, increasing the ethanol content in the solvent mixture significantly decreases the retention time, together with a decrease in the baseline resolution. The best solvent composition arises from the trade-off between retention time (and time between two successive injections) and the recovery of pure fractions, ensuring a minimum purity standard. The experimental results presented in Table 6 show that the higher productivity and lower solvent consumption are obtained for a solvent composition between 30-35%ethanol:70-65%water (runs 4 and 5). Fig. 6.20 presents the HPLC analysis of the first, waste and second fractions collected at run 4.

Considering the first feed injection period, the first fraction (product fraction B) was experimentally collected from 11.8 to 13.3 min, the waste fraction (mixture of racemates A and B) was collected from 13.3 to 15.3 min, and the second fraction (product fraction A) from 15.3 to 18.9 min. The first fraction is 100% pure racemate B and the second fraction is 99.9% pure racemate A.

Figs. 6.21 and 6.22 present the effect of solvent composition (ethanol content in a ethanol:water mixture) in flow-rate, injection time interval (time between two successive feed injections), recovery, productivity and solvent consumption of the FB operation.

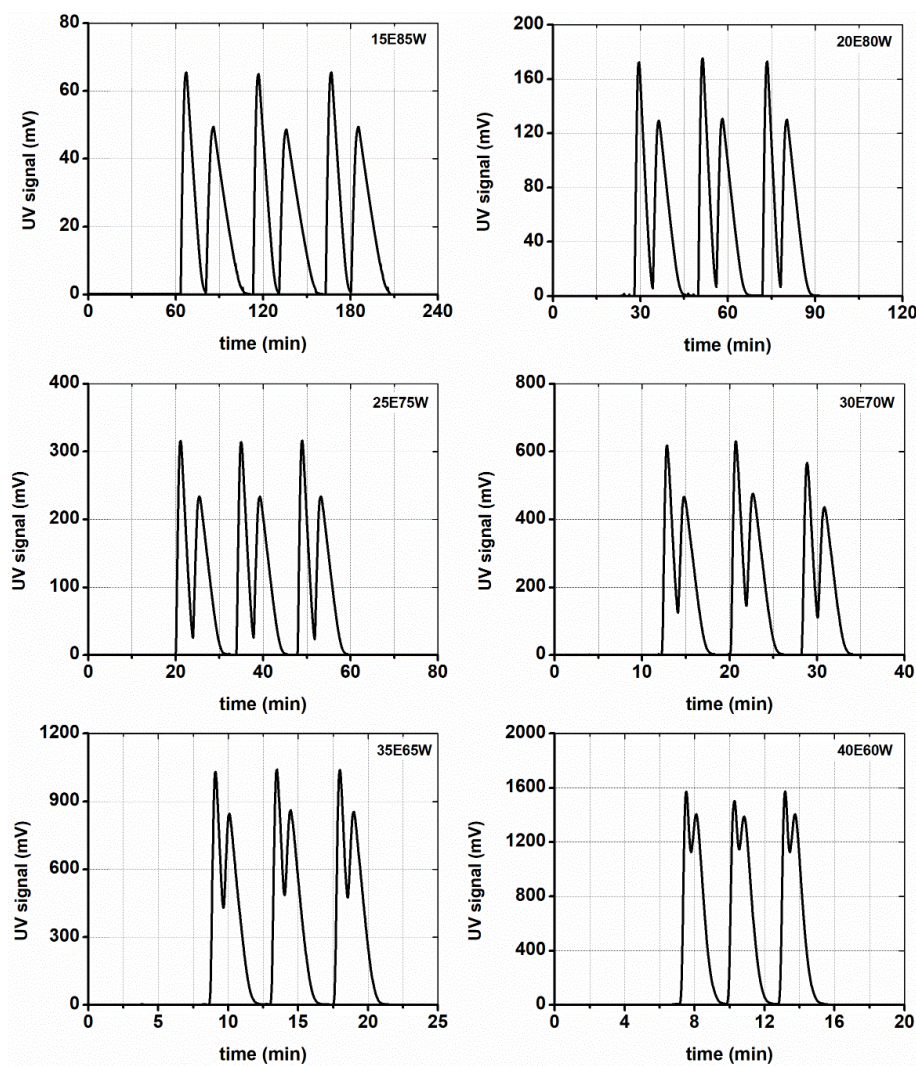


Fig. 6.19. Experimental FB chromatograms of three consecutive pulses of nadolol racemates using different ethanol:water solvent compositions. Nadolol feed concentration of 9 g/L, injection volume of 10 mL. Flow-rate and injection time interval according to Table 6.

Table 6.6. Experimental FB performance parameters for different ethanol:water solvent compositions.

RUN	E:W:DEA %(v/v/v)	pH	Q (mL/min)	ΔP (bar)	Δt_{inj} (min)	RC (%)	PU_B (%)	PU_A (%)	PR (g/L.hr)	SC (L/g)
1	15:85:0.30	11.9	54	154	47.4	96.1	100	99.4	0.62	29.6
2	20:80:0.25	11.8	50	153	18.5	94.3	100	99.7	1.56	10.9
3	25:75:0.15	11.8	42	155	12.5	89.7	100	99.9	2.19	6.50
4	30:70:0.15	11.9	38	153	7.1	60.8	100	99.9	2.62	4.93
5	35:65:0.15	11.9	35	154	4.8	40.7	100	99.8	2.59	4.59
6	40:60:0.15	11.9	32	153	3.8	17.7	100	99.8	1.42	7.63

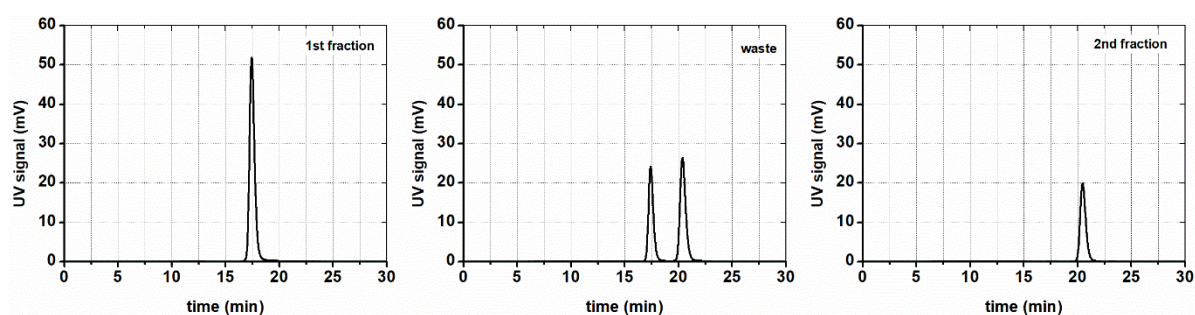


Figure 6.20. HPLC analysis of the three collected fractions of run 4.

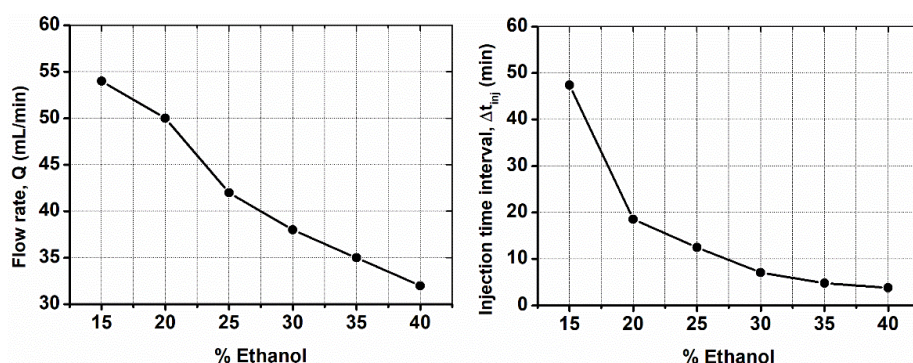


Fig. 6.21. Effect of the ethanol content in the flow-rate (left plot) and in the injection time interval (time between two consecutive feed injections, right plot) for the FB preparative separation of nadolol racemates.

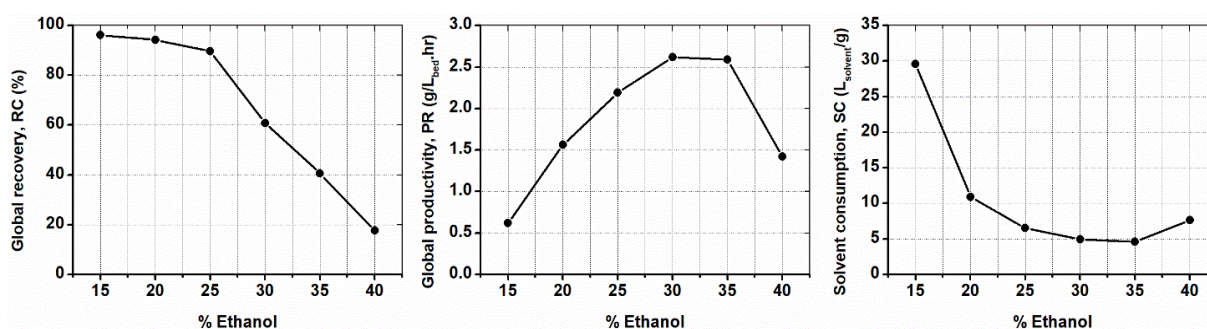


Fig. 6.22. Effect of the ethanol content in recovery (left plot), system productivity (middle plot) and solvent consumption (right plot) for the FB preparative separation of nadolol racemates.

The results presented in Fig. 6.21 clearly show that the increase of the ethanol content (in the range of 15 to 40% of ethanol in water) leads to a significant decrease in the operating flow-rate due to the increase of solvent viscosity and system pressure drop (in order to keep system pressure drop around 150 bar). The increase of the ethanol content also decreases the injection time interval (the time between two consecutive feed injections) due to the significant decrease in retention. This effect explains the significant increase of system productivity and the decrease in solvent consumption as showed in Fig. 6.22. However, the decrease in retention also implies an important decrease in resolution and, consequently, a significant decrease in recovery. For ethanol contents higher than 25% FB recovery starts to decrease significantly. In this way, a trade-off between retention and recovery (resolution) must be achieved. For the FB preparative separation of nadolol racemates, a good compromise is obtained using a 30 to 35% of ethanol content when both productivity and solvent consumption are optimized.

6.7.2 Validation of the estimated adsorption equilibrium isotherm model and kinetic data for FB simulation

The equilibrium and kinetic data estimated using one SMB column were validated in the FB preparative column. Although presenting different dimensions (19 mm ID × 100 mm L versus 30 mm ID × 250 mm L) both columns are packed with the same C18 material (Waters XBridge C18 adsorbent of 10 µm particle size diameter). The validation was carried out for the FB operation using the solvent composition of 30%ethanol:70%water (Fig.6.19, run 4), since this was the solvent composition used in the SMB column experiments, including the measurement of the adsorption equilibrium isotherms, breakthrough experiments and estimation of mass transfer resistance and axial dispersion. The same adsorption model parameters were used to

predict the three consecutive pulses in the FB preparative column. The results are presented in Fig. 6.23 and show a very good agreement between the experimental and simulated chromatograms, validating the use of these model parameters to predict both the SMB and the FB operations.

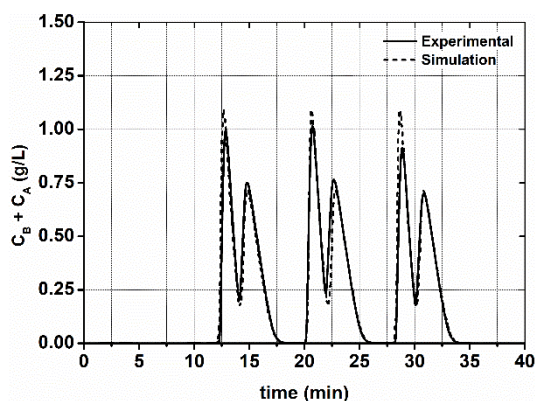


Fig. 6.23. Experimental (solid line) and simulated (dashed line) FB chromatograms of three consecutive pulses of a 9 g/L nadolol feed solution, using a 30:70 ethanol:water solvent composition (run 4 of Fig. 6.19). Model parameters of $\varepsilon = 0.4$, $Pe = 5000$, $k = 5 \text{ s}^{-1}$, and the linear + Langmuir isotherm model parameters as in Table 6.3.

6.8 Optimisation of FB operation

The FB operation was optimised by simulation for the selected mobile phase composition (30%ethanol:70%water) and using the model parameters validated in the previous section. The flow-rate was fixed to 38 mL/min considering the maximum system pressure drop around 150 bar, and the total feed concentration to 9 g/L considering it close to the solubility limit. The optimisation was then carried out by studying the effect of the feed injection volume, V_{inj} , in the FB performance parameters, ensuring both product fractions A and B 99.99% pure. Fig. 6.24 and 6.25 show how the feed injection volume influences the injection time interval (time between two consecutive feed injections), the equivalent flow-rates (as defined in Section 3.2.1), the recovery, system productivity and solvent consumption.

Contrary to SMB, FB operation with high feed injection volumes (equivalent to high feed flow-rates) implies big waste fractions and low recoveries. It should be underlined that the presence of a waste fraction (low recoveries) represents the waste of part of the feed or the need of its reuse as feed, increasing the cost of the separation process. For the FB separation of nadolol racemates under the conditions studies, Fig. 6.24 and 16 show that the productivity of racemates A and B are higher for feed injection volumes between 10 and 20 mL (roughly, 6-12% of the column volume). As the recoveries significantly decrease for feed injections volumes higher than 5 mL, a 10 mL injection volume can be chosen as the best choice to achieve high productivities with acceptable recoveries. Additionally, a 10 mL feed injection volume also presents a solvent consumption close to the minimum. Fig. 6.26 presents the simulated chromatograms of nadolol racemates and purities under these best FB operating conditions and the indication of the successive product and waste fractions. In short, nadolol feed is injected in the FB column each $\Delta t_{inj} = 7.10 \text{ min}$ and B, W and A fractions are collected at the time periods of $t_{f_B} \in [11.70 + 7.10(n_i - 1); 13.45 + 7.10(n_i - 1)]$, $t_{f_W} \in [13.45 + 7.10(n_i - 1); 15.00 + 7.10(n_i - 1)]$, and $t_{f_A} \in [15.00 + 7.10(n_i - 1); 18.80 + 7.10(n_i - 1)]$, respectively, and where $n_i = 1, 2, 3, \dots$ is the number of the feed injection.

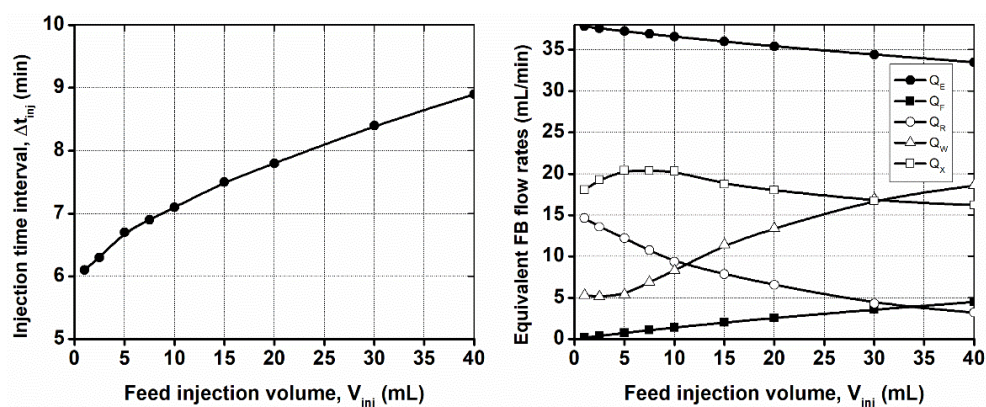


Fig. 6.24. Effect of the feed injection volume in the injection time interval (time between two consecutive feed injections, left plot) and in the equivalent flow-rates (right plot) for the FB preparative separation of nadolol racemates.

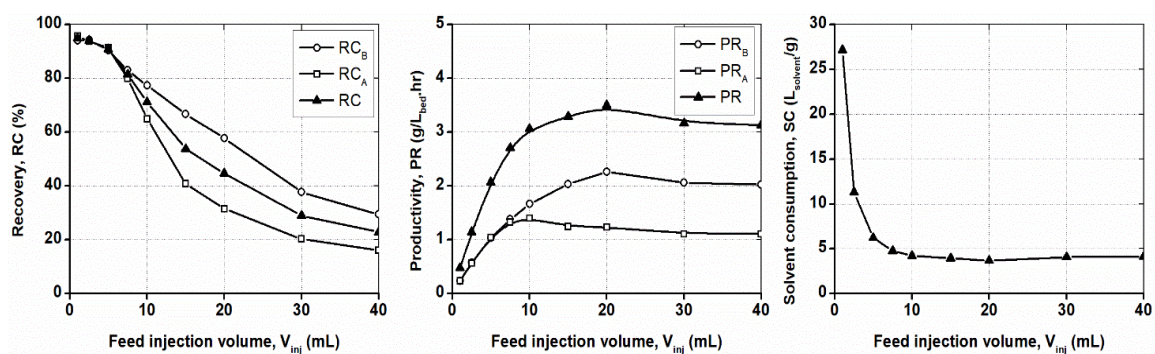


Fig. 6.25. Effect of the feed injection volume in recovery (left plot), system productivity (middle plot) and solvent consumption (right plot) for the FB preparative separation of nadolol racemates.

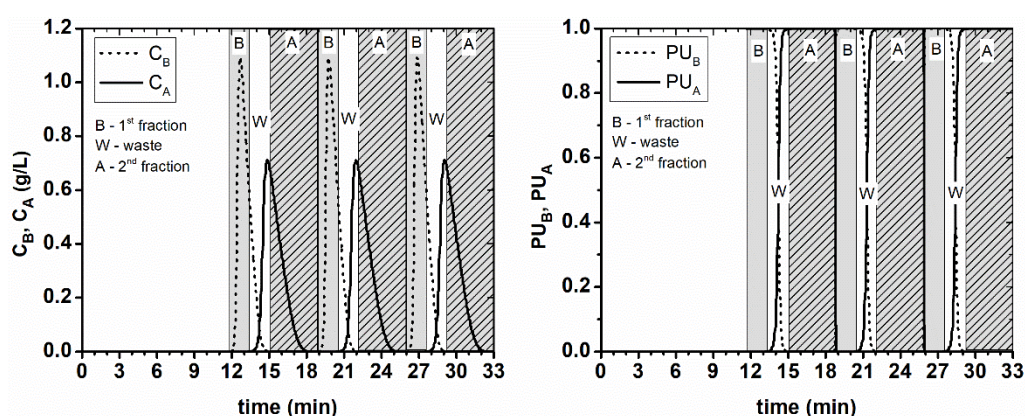


Fig. 6.26. Simulated FB chromatograms of three consecutive pulses (left plot, dashed line for the less retained racemate B, solid line for more retained racemate A) and purities of B and A (right plot, dashed line for purity of B, solid line for purity of A) of a 9 g/L nadolol feed solution, using a 30:70 ethanol:water solvent composition. Flow-rate of 38 mL/min, feed injection volume of 10 mL, and injection time interval of 7.1 min. Model parameters of $\varepsilon = 0.4$, $Pe = 5000$, $k = 5 \text{ s}^{-1}$, and the linear + Langmuir isotherm model parameters as in Table 6.3.

6.9 SMB and FB performance comparison

The performance values obtained experimentally in this work for the SMB operation may mislead the reader when selecting the better technology. It is well known that the continuous SMB has advantages when compared to the discontinuous FB operation, namely the continuous injection of feed and the simulated counter-current contact between the solid and the liquid phases that maximizes the mass transfer driving force, leading to an increase of the system productivity and a decrease of the solvent consumption. Additionally, SMB operating at complete separation regions presents full recoveries of the injected feed (100%), while FB operation presents better system productivities with the presence of waste fractions and low recoveries. It must be clarified that the SMB and FB experimental results obtained in this work cannot be directly compared. As referred before, due to the specific hardware limitations of the lab-scale SMB-LSRE unit, the use of a 10 μm C18 adsorbent material and ethanol:water solvents imposed a maximum flow-rate of 5 mL/min, leading to low productivity performance. To establish a possible comparison, the simulation of the SMB operation can be carried out under equivalent conditions to the optimised FB operation.

The following equivalences are used:

- (i) The same total adsorbent amount (column volume) is used in both situations: for FB, $D = 3$ cm, $L = 25$ cm; for SMB, as it is composed by six columns, simulation uses the same diameter and a length of one sixth, that is, $D_c = 3$ cm, $L_c = 25/6$ cm. SMB operation uses a 1-2-2-1 configuration (six columns).
- (ii) The critical values of γ_I and γ_{IV} for SMB operation are fixed the same way as previously, through $\gamma_I = \frac{1-\varepsilon}{\varepsilon} K_A \beta$ and $\gamma_{IV} = \frac{1-\varepsilon}{\varepsilon} K_B / \beta$, using $\beta = 1.2$ (see Section 3.1.6). It should be underlined that the use of a safety margin of 20%

represents a secure situation of non-contamination at SMB sections I and IV that can lead to lower productivity and higher solvent consumption performances. It means that, using lower β values, could still ensure complete separation with higher productivity and lower solvent consumption.

- (iii) The SMB operating conditions are defined using the complete separation region considering the Triangle Theory methodology, that is, the pair of $(\gamma_{II}; \gamma_{III})$ values are considered at the vertex point of the 9 g/L triangle.
- (iv) The SMB flow-rates can be established considering different equivalence situations:
 - (a) The flow-rate at SMB section I, Q_I^* (maximum internal flow-rate in SMB operation), is equal to the FB flow-rate, Q^{FB} ;
 - (b) The flow-rate at SMB section I, Q_I^* , is defined in order to keep the same average flow-rate for both FB and SMB operations, that is, $\langle Q^* \rangle = (Q_I^* + 2Q_{II}^* + 2Q_{III}^* + Q_{IV}^*)/6 = Q^{FB}$ (SMB operates with a 1-2-2-1 configuration);
 - (c) The SMB feed flow-rate, Q_F , is equal to the equivalent FB feed flow-rate (same throughput; that is, the same mass of nadolol fed into the system per unit time);
 - (d) The solvent used in SMB operation, $Q_E + Q_F$, is equal to the solvent used in FB operation, Q^{FB} .
- (v) The SMB switching time is calculated through $t^* = \frac{\varepsilon V_C}{Q_I^*}(\gamma_I + 1)$ and the other SMB internal flow-rates are calculated using $Q_j^* = \frac{\varepsilon V_C}{t^*} \gamma_j^*$ ($j = II, III, IV$), where $\gamma_j^* = \gamma_j + 1$. The other inlet and outlet flow-rates are calculated through $Q_E = Q_I^* - Q_{IV}^*$; $Q_X = Q_I^* - Q_{II}^*$; $Q_F = Q_{III}^* - Q_{II}^*$; $Q_R = Q_{III}^* - Q_{IV}^*$.

The simulated SMB results are compared with the ones obtained by simulation of the FB operation under optimised operating conditions as presented in the previous section. Table 6.7 summarizes the FB and the SMB operating conditions and the performance parameters estimated by simulation.

All SMB runs present the same γ_j values (ratios between fluid and solid interstitial velocities in the equivalent true moving bed concept), ensuring the operation at optimised conditions, i.e., at the $(\gamma_{II}; \gamma_{III})$ vertex point of the complete separation triangle, and ensuring thorough cleaning of solid and liquid in sections I and IV. The SMB switch time interval, t^* (time between the switch of the inlet and outlet stream positions at SMB operation, and related to the solid velocity in the equivalent true moving bed concept), is defined depending on the SMB liquid internal flow-rates and in order to keep constant the γ_j values. In that way, solvent consumption is constant for all SMB runs, as it depends on the feed concentration and the ratio $(\gamma_I - \gamma_{IV})/(\gamma_{III} - \gamma_{II})$. Ensuring complete separation, the system productivity increases with the increase of the feed flow-rate. Being the feed concentration and the γ_j values constant, also the product $PR \times t^*$ is constant.

Table 6.7 shows the main advantages of SMB operation when compared with FB chromatography. The SMB optimised operating conditions are achieved for full recovery of the feed, while that do not occurs with the optimised FB operation. This means that SMB is a cleaner process and that FB operation introduces the waste or the need to reuse the waste fraction, increasing the cost of the operation particularly when the cost of feed or feed reuse are considerable. SMB also presents significant lower values of solvent consumption (in this study, 46% of the solvent consumption at FB operation), as the unit includes sections I and IV to continuously clean and reuse solvent and adsorbent.

Considering SMB and FB operating with the same throughput, that is, the same mass of feed per unit time, $(Q_F C_{A+B}^F)^{SMB} = (Q_F C_{A+B}^F)^{FB}$, SMB achieves a system productivity 40% higher than FB operation. Considering SMB and FB operating with the same inventory of solvent used per unit time, $(Q_E + Q_F)^{SMB} = (Q_E + Q_F)^{FB} = Q^{FB}$, SMB presents a system productivity 2.2 times higher than FB. Nevertheless, it should be pointed out that, under these circumstances, SMB operates with higher internal flow-rates, which introduces the challenge to use the appropriate hardware (columns, pumps, valves, tubing and fittings) for system pressure drop. In fact, preparative and industrial SMB and FB apparatus use larger column diameters (low length to diameter ratios) and larger adsorbent particle size materials.

Table 6.7. FB and SMB operating conditions and performance parameters for the separation of nadolol racemates. Solvent composition of 30:70 ethanol:water, 9 g/L nadolol feed solution, model parameters of $Pe = 5000$; $k = 5 \text{ s}^{-1}$; $\varepsilon = 0.4$, and linear + Langmuir model parameters as in Table 6.3.

FB operation		SMB operation			
Column dimensions					
$D = 3\text{ cm}$	D_c, cm	3			
$L = 25\text{ cm}$	L_c, cm	25/6			
	N_j	[1,2,2,1]			
FB-SMB equivalence:		$Q_I^* = Q^{FB}$	$< Q^* > = Q^{FB}$	$Q_F = Q_F^{FB}$	$Q_E + Q_F = Q^{FB}$
Operating conditions					
$V_{inj} = 10\text{ mL}$	γ_I	9.4891			
$\Delta t_{inj} = 7.1\text{ min}$	γ_{II}	6.1555			
	γ_{III}	6.3945			
	γ_{IV}	5.5416			
	t^*, min	3.2519	2.3836	1.9989	1.2979
Flow-rates, $Q\text{ (mL/min)}$					
$Q = 38.000$	Q_I^*	38.000	51.842	61.820	95.210
$Q_E = 36.592$	Q_E	14.301	19.511	23.266	35.832
$Q_F = 1.408$	Q_F	0.866	1.181	1.408	2.169
$Q_R = 9.366$	Q_R	3.090	4.216	5.027	7.742
$Q_W = 8.296$	-	-	-	-	-
$Q_X = 20.338$	Q_X	12.077	16.476	19.647	30.259
Performance parameters: $PU\text{ (}\%), RC\text{ (}\%), PR\text{ (g/(L.hr))}, SC\text{ (L/g)}$					
$PU_B = 99.99$	PU_R	100			
$PU_A = 99.99$	PU_X	100			
$RC_B = 77.32$	RC_R	100			
$RC_A = 64.98$	RC_X	100			
$RC = 71.15$	RC	100			
$PR = 3.062$	PR	2.646	3.609	4.304	6.629
$SC = 4.213$	SC	1.947			

6.10 Conclusions

The separation of the two nadolol racemates was carried out using three different Waters C18 adsorbents; the XBridge C18, the Shield RP18 and the XSelect CSH C18, at both analytical and preparative scales.

The identification of all the four nadolol stereoisomers and the capacity of C18 adsorbents to discriminate the two nadolol racemates under reversed-phase chromatography was validated for each C18 adsorbent, using an ethanol:water solvent mixture with diethylamine as basic modifier. The need for a high-pH operation was also identified and justified by the strong alkaline nature of nadolol.

This work presented extensive experimental and simulation results concerning the separation of nadolol racemates by fixed-bed (FB) and simulated moving bed (SMB) achiral preparative chromatography. The two alternative separation processes were carried out using a Waters XBridge C18 10 μm adsorbent and were both optimised in terms of solvent composition and pH (screening of ethanol:water:diethylamine mixtures) considering the need of an high pH reversed-phase operation due to the high basic nature of nadolol. Both processes presented similar better solvent compositions of 30-40%ethanol in water and 0.10-0.15% of diethylamine basic modifier to ensure a pH close to 12.

The optimisation of both the SMB and the FB operating conditions was carried out through simulation, based on equilibrium (adsorption isotherms) and kinetic (mass transfer resistance and axial dispersion) data, estimated by frontal (breakthrough) chromatography. The competitive Langmuir or linear + Langmuir models proved to well describe the adsorption equilibrium behaviour and the C18 adsorbent used allowed high column efficiency with low mass transfer resistances and axial dispersion. In this way, the optimisation of SMB operating conditions can be safely carried out using the

Triangle Theory methodology, although more precise simulation strategies can be used to follow the transient evolution of SMB operation or the presence of column and equipment dead volumes.

The experimental operation of both SMB and FB processes was implemented for the separation of a nadolol quaternary mixture into two pure racemates and well predicted by simulation. Additional simulation results were carried out to optimise the FB operation by studying the effect of the feed injection volume in its operating conditions and performance parameters.

SMB and FB operations were compared in terms of its performance parameters of recovery, system productivity and solvent consumption, and using different equivalence conditions, namely, to operate with the same feed mass flow-rate (throughput), the same solvent flow-rate, and the same internal flow-rates, potentially limited by system pressure drop. SMB globally presents better operating performances than FB, being a cleaner process by allowing a more efficient use of the adsorbent and solvent. Nevertheless, this work also emphasises the need of productive and industrial SMB apparatus working at low pressure drops which can be achieved by efficient flow distributors for columns with larger diameters and/or efficient larger particle size adsorbent materials.

Together with the former work [28], these two publications fully validate a novel strategy for the complete separation of nadolol stereoisomers by using a first achiral separation step with a C18 adsorbent to effectively produce pure nadolol racemates from the original quaternary nadolol mixture. The pure products obtained in this work can now be further purified by using standard chromatographic binary chiral steps to separate each racemate into its pure enantiomers. In this way, this work presents for

the first time a successful and efficient way to completely separate a multicomponent nadolol feed into its four enantiomers at SMB and FB preparative scales.

Chapter 7 Separation of nadolol racemates into its pure enantiomers by chiral preparative chromatography

In this chapter, the complete separation of all the stereoisomers of nadolol is presented by experimental preparative FB and SMB chiral chromatographic separations of the two SMB products streams obtained from previous achiral step. One commercial fixed-bed preparative HPLC Azura system equipped with one single column of preparative dimensions (30 mm ID x 250 mm L) and one lab-scale SMB apparatus (the FlexSMB-LSRE[®] pilot unit) equipped with six semi-preparative columns (19 mm ID x 100 mm L) were used. Both systems use the Daicel Chiralpak[®] IA adsorbent of 20 µm particle diameter. The screening of the mobile phase composition elected for the chiral separation of racemate B (racemic mixture of stereoisomers 2 and 3) the 25:75:0.1 (v/v/v) methanol:acetonitrile:diethylamine composition and for the chiral separation of racemate A (racemic mixture of stereoisomers 1 and 4) the 5:95:0.1 (v/v/v) methanol:acetonitrile:diethylamine composition.

A large set of experimental, modelling and simulation results are presented, including pulses, measurement and modelling of the adsorption equilibrium isotherms, and its validation through breakthroughs measurements. The modelling and simulation steps allowed the prediction and the optimization of both the FB and SMB operating conditions.

This chapter is based on article draft to be submitted.

7.1 Introduction

Preparative chiral separations using adsorption-based processes are traditionally hard and complex tasks since they are based on a solute-adsorbent-solvent composition compromise optimization that allows the better preparative separation process performance parameters, usually productivity, solvent consumption, recovery and purity. When leading with multicomponent chiral mixtures there is a clear increase in complexity due to the rise of adsorption competition between solute molecules for the chiral adsorbent, under high concentrations, mandatory for the industry wanted high operation performances.

An extensive work has been development inside our group in order to identify and implement a global strategy for the complete separation by means of simulated moving bed chromatography of all the components present in nadolol chiral quaternary mixture. The analytical separation of nadolol stereoisomers by high performance liquid chromatography was firstly reported by McCarthy in 1994 [14], using a tris-3,5-dimethylphenyl carbamate (Chiralpak® AD, 10 µm) stationary phase and a 20%ethanol:80%hexane:0.3%diethylamine mobile phase composition. The preparative chiral liquid chromatography separation of nadolol stereoisomers was first reported by Ribeiro *et al.* in 2013 [15]. In that work, was presented an improvement of the analytical separation by a significant decreasing in the analysis time, when using a 30%ethanol:70%heptane:0.3%diethylamine mobile phase composition. Additionally, is also presented the pseudo-binary preparative separation, by SMB, of the RSR-nadolol stereoisomer, referred in several works [4-6], as the sole nadolol molecule owning the active principle of the pharmaceutical drug. The preparative separation was achieved by means of a Chiralpak® AD adsorbent with a particle size diameter of 20 µm, and a 80%ethanol:30%heptane:0.3%diethylamine solvent composition. It is important to

notice that the marketing of pharmaceutical drugs based in mixtures of enantiomers still remains an important drawback for the pharmaceutical industry. Nowadays, pharmaceutical legislation for healthy medicines is continuously pushing the industry to produce more safer drugs.

In the present work, we implemented the second step of the global strategy selected for the complete separation of the four nadolol stereoisomers and previously presented in last our published work [29]. In this second step, the two feed solutions for SMB and FB operation were obtained using the two outlet streams collected during the SMB operation with the achiral adsorbent used in the first step. After the first achiral step, racemate B containing a racemic mixture of nadolol stereoisomers 2 and 3, was collected in the extract outlet steam, and racemate A, a racemic mixture of nadolol stereoisomers 1 and 4, was collected in the raffinate outlet stream. After SMB operation, the mass present in both racemic solutions was obtained by solvent evaporation using a rotary evaporator and freeze-drying equipment. The two obtained nadolol solids were then used to prepare new feed solutions for SMB and FB operations. After the screening for the solvent composition, we concluded that for the separation of racemate A, the solvent composition is a 25%methanol:75%acetonitrile mixture, that is the same solvent already used to separate the more retained stereoisomer (4) from the mixture of (1+2+3) in the previous work presented by Arafah et al. in 2016. We also concluded, from the solvent screening, that for the more hard chiral separation of racemate B, a binary separation of stereoisomer 2 from 3, the better values for preparative performance parameters are obtained using a 5:95 or 10:90 %methanol:%acetonitrile composition. Then, and for the three chiral separations, the experimental measure and modelling of equilibrium adsorption isotherms is presented. The validation of the selected isotherm models was done by

modelling of the adsorption behaviour using fixed-bed, by means of breakthroughs experimental measurements, both for saturation and regeneration steps. In this fixed-bed studies, the dispersion and mass transfer resistances were estimated by means of Peclet and Stanton numbers that allows better fitting of the fixed-bed adsorption behaviour using the previous selected equilibrium adsorption isotherm model. The final selection of solvent composition to be used on the SMB separation for racemate B separation, a 5%methanol:95%acetonitrile mixture, was done by comparison of productivity and solvent consumption. The final results presented that are presented in this work clearly define a complete and global strategy that can be used for the complete preparative separation of all the four stereoisomers present in the nadolol quaternary mixture. After this work, the four pure stereoisomers can now be used by the pharmaceutical industry for running important studies to clearly identify the individual pharmacological and therapeutical properties for each nadolol stereoisomer.

7.2 Screening of solvent composition for (1/2/3/4) separation

The first objective of solvent screening was to identify the solvent mixtures that better succeed in the separation of all four nadolol stereoisomers in just one chiral chromatographic step. Different solvent mixtures were extensively studied, based on the former findings at analytical scale [15, 26], namely, alcohol-hydrocarbon (ethanol with hexane and heptane) and alcohol-acetonitrile (methanol and ethanol with acetonitrile) mixtures. For this objective, the preparative Azura HPLC equipment equipped with a chiral column of preparative dimensions, packed with Chiralpak® IA chiral adsorbent was used. The main experimental solvent screening results obtained for 10 g/L nadolol quaternary mixture solution, using an injection volume of 10 mL and a flow-rate of 40 mL/min, are presented in Fig. 7.1. These pulses were obtained using three different ethanol:hydrocarbon mixtures, namely 20%ethanol:80%heptane

(20E:80C7), 20%ethanol:80%hexane (20E:80C6) and 30%ethanol:70%heptane (30E:70C7), from left to right, respectively.

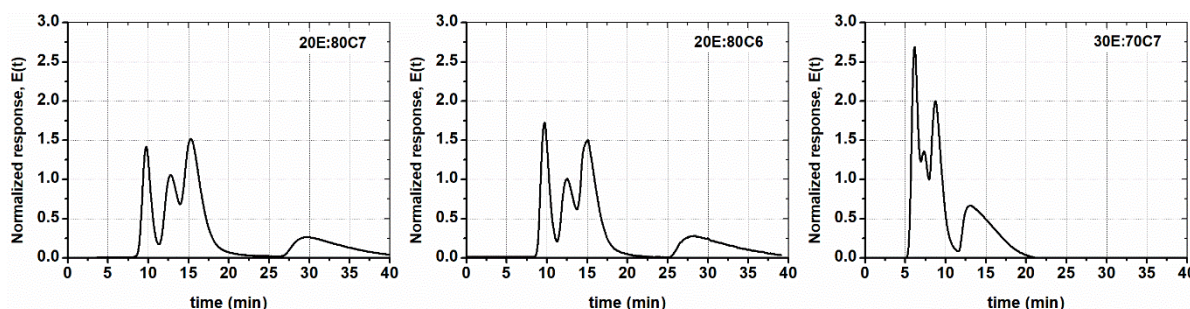


Fig. 7.1. Pulse experiments of nadolol using three different ethanol/hydrocarbon solvent compositions: 20E:80C7, 20E:80C6 and 30E:70C7. $C_F^T = 10$ g/L; loop of 10 mL; $Q = 40$ mL/min.

Chromatograms presented in Fig. 7.1, show that no good resolution is achieved for the nadolol quaternary mixtures using alcohol-hydrocarbon solvents. For 20E:80C6 and 20E:80C7, stereoisomer 4 is completely resolved from the other three stereoisomers but strongly retained (40 minutes operation with very long tailing), and stereoisomers 2 and 3 elute very close to each other. Increasing ethanol content to 30E, the retention time of component 4 decrease significantly, from 40 to 20 min, but imposing a very low resolution for the other three stereoisomers. The next step for solvent screening was to study the separation the quaternary mixture using different alcohol-acetonitrile solvent mixtures. The main results obtained can be found in Fig. 7.2, using 10%ethanol:90%acetonitrile (10E:90ACN), 10%methanol:90%acetonitrile (10M:90ACN) and 5%ethanol:95%acetonitrile (5E:95ACN) solvent compositions. These results show that it is not possible to obtain enough resolution between all the four nadolol stereoisomers using alcohol-acetonitrile solvents. Both the 10E:90ACN and 10M:90ACN compositions presents less retention for the forth stereoisomer compared with the former alcohol:hydrocarbon solvents, but the first three stereoisomers elute together. Reducing to 5M/95ACN, resolution between the two more retained stereoisomers is improved although the two first stereoisomers co-elute.

Additionally, and more relevant for preparative (high concentration) separations, it must be noticed that the solubility of nadolol has an important decrease using 5% methanol content and instead of a total (quaternary) concentration of 10 g/L a 2.5 g/L had to be used.

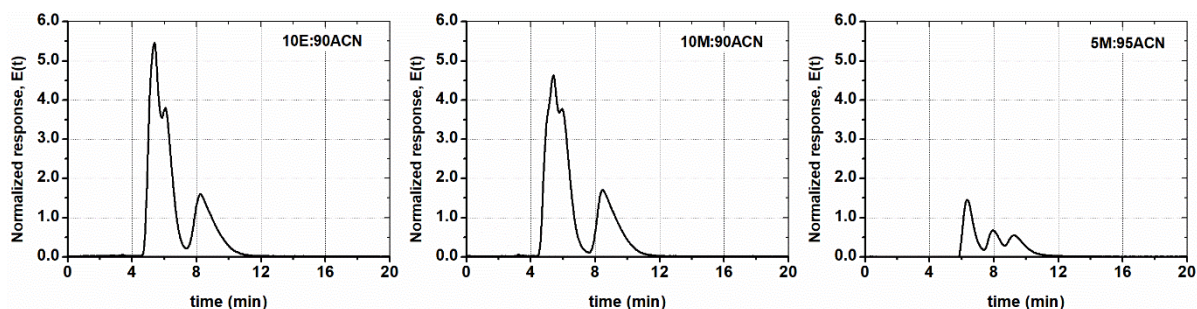


Fig. 7.2. Pulse experiments of nadolol using three different alcohol/acetonitrile solvent compositions and concentrations: 10E:90ACN and $C_F^T = 10$ g/L, 10M:90ACN and $C_F^T = 10$ g/L, 5M:95ACN. $C_F^T = 2.5$ g/L; loop of 10 mL; $Q = 40$ mL/min.

Finally, from the screening study for simultaneous quaternary preparative separation, it can be concluded that is possible to separate only the more retained stereoisomer from the quaternary nadolol mixture using pure alcohol. This preparative pseudo-binary separation (1+2+3)/(4) using pure methanol was already implemented by Ribeiro *et al.* in 2013, and then improved by Arafah *et al.* in 2016, using an 25%methanol:75%acetonitrile solvent composition. However, no effective separation between the three first nadolol stereoisomers was achieved. To get the final goal of the complete separation of nadolol stereoisomers, there is a need to implement a different separation strategy that combines achiral and chiral separation steps. The global strategy includes a first achiral separation of the two nadolol racemates (2+3)/(1+4), followed by a second step composed by two parallel chiral binary separations of the two stereoisomers present in each one of the two nadolol racemates (2)/(3) and (1)/(4).

7.2.1 Screening of solvent composition for racemate A chiral separation

Due to results obtained in previous section, several pulses were performed using the Azura system in order to study the better solvent composition for (1)/(4) chiral separation. The same solvent compositions as used for (1/2/3/4) were tested in order to characterize resolution between both enantiomers. The obtained results, using a racemate A concentration of 10 g/L, prepared in the three different solvent compositions, are presented in Fig. 7.3.

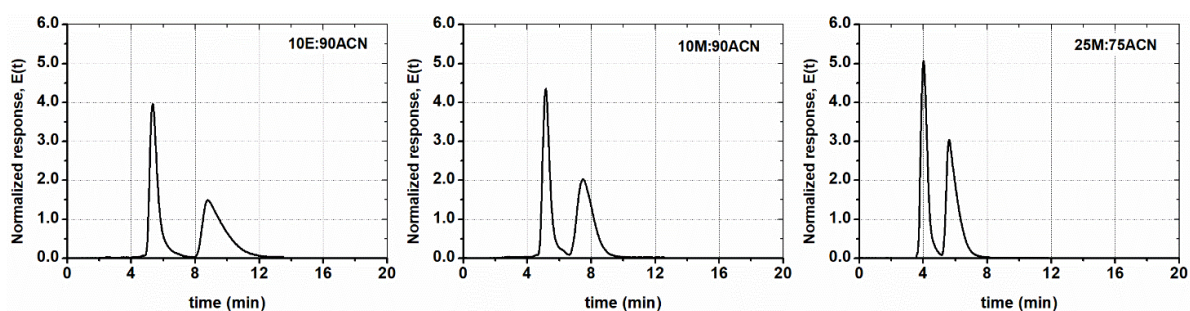


Fig. 7.3. Pulse experiments of nadolol racemate A (racemic mixture of stereoisomers 1 and 4) using three different alcohol/acetonitrile solvent compositions: 10E:90ACN, 10M:90ACN and 25M:75ACN. $C_F^T = 10$ g/L; loop of 10 mL; $Q = 40$ mL/min.

As expected, the (1)/(4) separation is the easier situation when compared with the (2)/(3) separation, since the fourth nadolol stereoisomer is significantly more retained in Chiralpak[®] IA adsorbent than the other three nadolol stereoisomers. Both compositions of 10E:90ACN and 10M:90ACN succeed to well resolve the two nadolol enantiomers. However, the 25M:75ACN composition represents the less retention without losing baseline resolution. Also, using high alcohol content is possible to increase solubility that will be important for preparative conditions. The 25M:75ACN composition was already the selected solvent composition for the pseudo-binary (1+2+3)/(4) separation reported by Arafah *et al.* in 2019.

7.2.2 Screening of solvent composition for racemate B chiral separation

A similar procedure to the one used in section 3.2 for screening of the solvent composition for the chiral separation of racemate B was performed using the Azura unit. Several alcohol:hydrocarbon, and alcohol:acetonitrile compositions were studied. The main obtained results are presented in Fig. 7.4 for a 20%ethanol:80%heptane and in Fig.7.5 for pure acetonitrile, 5-10%ethanol:95-90%acetonitrile and 5-10%methanol:95-90%acetonitrile. Due to low solubility values of the racemate B in lower ethanol content solvent mixtures, concentrations of 2.5 and 5 g/L were used.

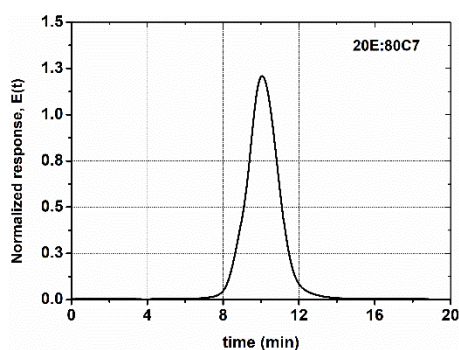


Fig. 7.4. Pulse experiment of nadolol racemate B, a racemic mixture of stereoisomers 2 and 3, using a solvent composition of 20E/80C7 and $C_F^T = 2.5$ g/L a loop of 10 mL and a $Q = 40$ mL/min.

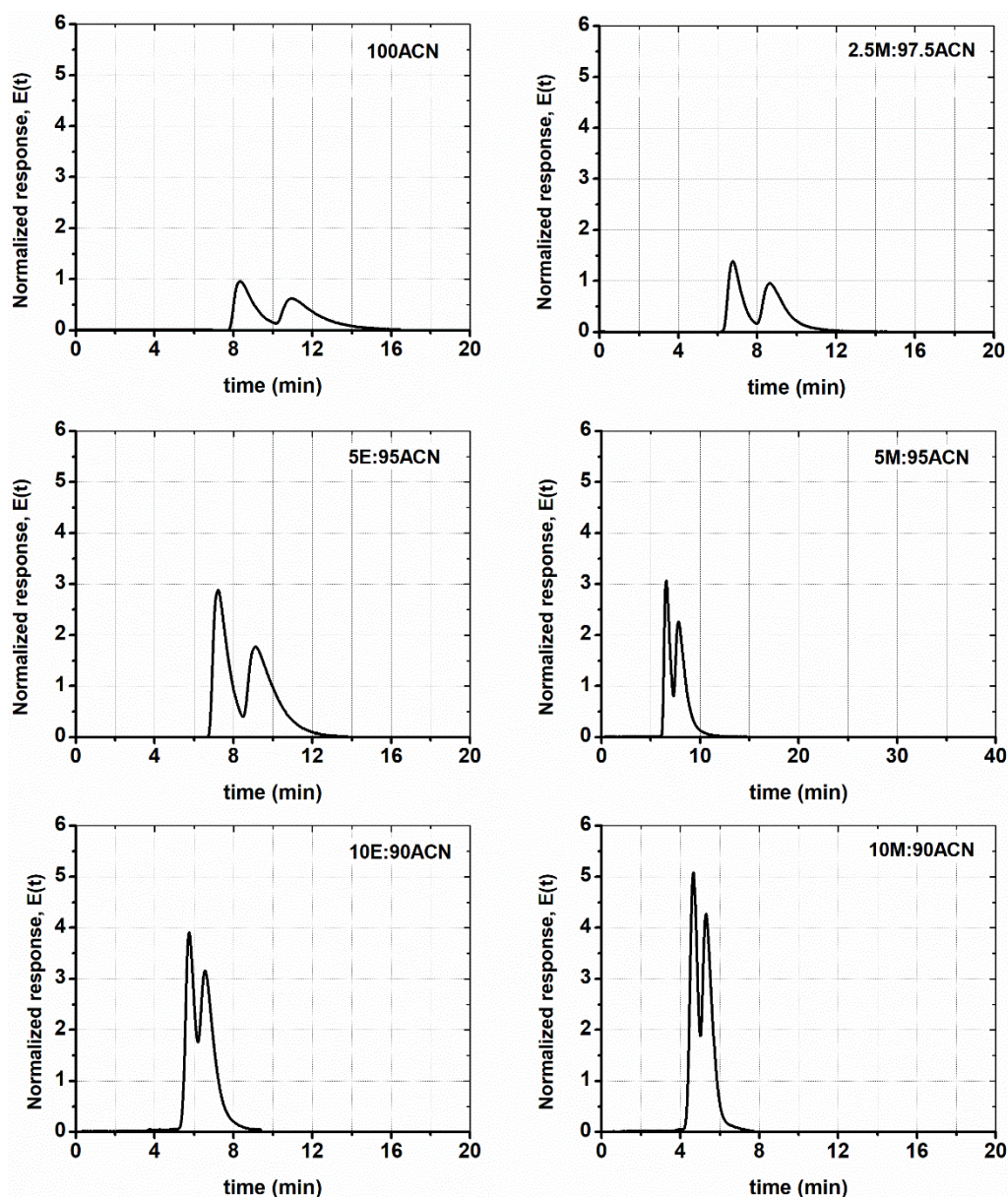


Fig. 7.5. Pulse experiments of nadolol racemate B (racemic mixture of stereoisomers 2 and 3) using several solvent compositions and concentrations. $C_F^T = 2.5$ g/L for 100%acetonitrile (100ACN) and 2.5%methanol:97.5%acetonitrile (2.5M:97.5ACN) and $C_F^T = 5$ g/L for the other four solvent compositions a loop of 10 mL and $Q = 40$ mL/min.

The preparative separation of the (2)/(3) nadolol racemate B is clearly more difficult than the preparative separation of the (1)/(4) racemate A. The more promising solvent mixture is based on an alcohol-acetonitrile solvent mixture with low alcohol content, as it was already referred by Arafah *et. al*, in 2016 at analytical scale (See Table 7.1). Different alcohol (methanol and ethanol) contents were studied using concentrations near nadolol solubility for each solvent mixture. Pure acetonitrile present good (2)/(3)

resolution but strong retention and tailing, and low solubility limit (2.5 g/L). The composition of 2.5M:97.5ACN allows better column efficiency but still presents significant solubility constraints. The 5 to 10% alcohol in acetonitrile mixtures allows the best conditions for an effective preparative separation of nadolol (2) and (3) enantiomers, methanol:acetonitrile presenting a better compromise between resolution and retention than ethanol:acetonitrile.

7.3 Measurement and modelling of the equilibrium adsorption isotherms

The equilibrium adsorption isotherms parameters were obtained using one SMB column through fixed-bed experimental operation, and then fitted to a linear + Langmuir competitive adsorption model. The adsorption equilibrium model parameters estimated for racemate A, are presented in Table 7.1 and the ones obtained for racemate B are presented in Table 7.2.

Table 7.1. Estimated parameters for nadolol racemate A (racemic mixture of stereoisomers 1 and 4) equilibrium adsorption isotherm model using the 25%methanol:75%acetonitrile (25M:75ACN) solvent composition and a temperature of 23°C. M is the number of experimental points, N is the number of estimated parameters, m, Q, b_1 and b_4 for the linear + Langmuir model; SQ is the sum of the square of the residues and SD is the corrected standard deviation, defined by Eqs. 4 and 5.

Solvent composition	M	N	m	Q (g/L)	b_1 (L/g)	b_4 (L/g)	SQ	SD
25M:75ACN	10	4	0.7991	14.13	1.108×10^{-2}	7.244×10^{-2}	0.0001	0.0032

Table 7.2. Estimated parameters for nadolol racemate B (racemic mixture of stereoisomers 2 and 3) equilibrium adsorption isotherm models using the two solvent compositions of 5%methanol:95%acetonitrile (5M:95ACN) and 10%methanol:90%acetonitrile (10M:90ACN) for a temperature of 23°C. M is the number of experimental points, N is the number of estimated parameters, m, Q, b_2 and b_3 for the linear + Langmuir model; SQ is the sum of the square of the residues and SD is the corrected standard deviation, defined by Eqs. 4 and 5.

Solvent composition	M	N	m	Q (g/L)	b_2 (L/g)	b_3 (L/g)	SQ	SD
5M:95ACN	8	4	1.733	4.211	2.966×10^{-2}	1.715×10^{-1}	0.0045	0.0336
10M:90ACN	10	4	1.573	1.479	1.109×10^{-1}	6.053×10^{-1}	0.0060	0.0316

For racemate A, a very good agreed between experimental points and the adsorption behaviour predicted by the linear + Langmuir model both for the equilibrium adsorption isotherm as for the experimental and predicted selectivity using the same model, as it can be confirmed in Fig. 7.6. Farther, and in the same Fig., a comparison between the present results for racemate A and the results obtained in 2019 [27] is presented for the pseudo-binary separation (1+2+3)/(4), using the same 25%methanol:75%acetonitrile solvent composition and using the same Chiralpak® IA adsorbent. As can see observed, and as it was expected due to the less competition for the adsorption in the stationary, higher solid concentration for the same concentration, is obtained for both enantiomers when present in racemate A than when they were present in the quaternary mixture. A higher selectivity is also observed, being more significant for higher feed concentrations.

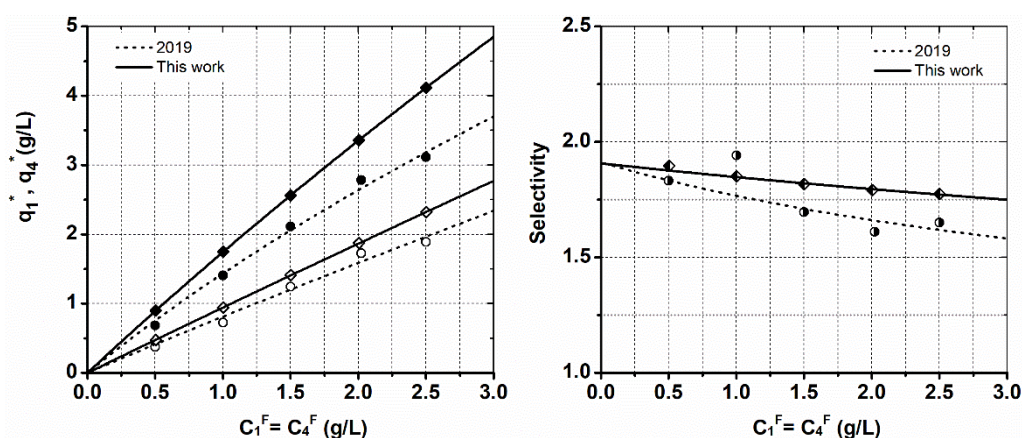


Fig. 7.6. Comparison between experimental (points) and model (lines) results for the adsorption equilibrium isotherms of nadolol quaternary mixture (pseudo-binary separation of the more retained stereoisomer of nadolol from the racemic mixture of the four stereoisomers, data obtained and published in 2019) and nadolol racemate A using the same 25%methanol:75%acetonitrile mobile phase composition. Left figure presents the adsorption equilibrium isotherms and its fitting to a linear + Langmuir. The right figure presents the predicted and the experimental selectivity between stereoisomer 4 and 1 (this work) and stereoisomer 4 and the co-eluting mixture of stereoisomers 1, 2 and 3 as a function of the nadolol feed concentration.

For racemate B, the experimental and predicted values of the equilibrium adsorption isotherms and the correspondent obtained and predicted selectivity, are presented in

Fig. 7.7, for 5%methanol:95%acetonitrile (up) and for 10%methanol:90%acetonitrile (down). Comparing results for both compositions, it is possible to see that both solvent compositions lead to similar adsorption results. However, it can be stressed out that the 10M:90ACN composition represents a situation of smaller selectivity between enantiomers 2 and 3, more relevant when using racemic feed concentrations higher than $C_2^F + C_3^F = 2$ g/L. Therefore, it is expected that this solvent composition could represent more harder conditions for preparative separation of racemate B than the 5M:95ACN composition.

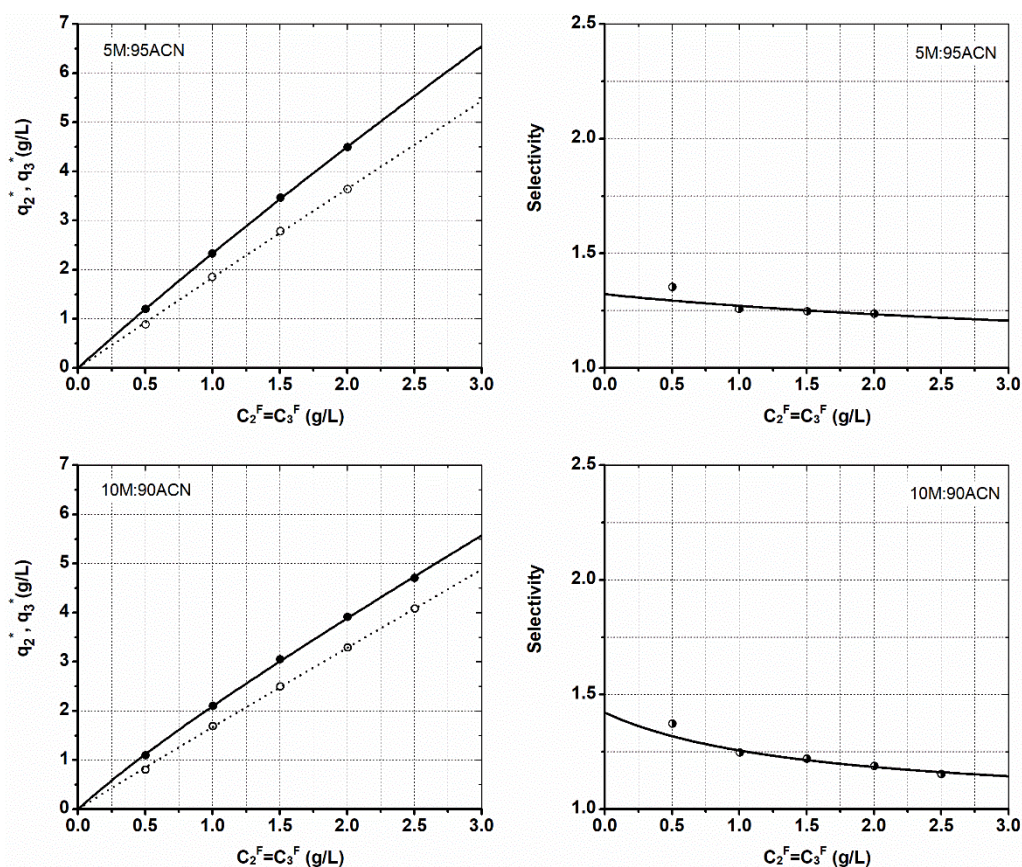


Fig. 7.7. Comparison between experimental (points) and model (lines) results for the adsorption equilibrium isotherms nadolol racemate B (stereoisomers 2 and 3) using two different solvent compositions: 5%methanol:95%acetonitrile and 10%methanol:90acetonitrile. Left figure presents the adsorption equilibrium isotherms and its fitting to a linear + Langmuir. The right figure presents the predicted and the experimental selectivity between stereoisomer 3 and 2, as a function of the nadolol feed concentration.

7.4 Fixed-bed experimental operation and performance

The experimental results obtained by recovering and analysing the samples at different times of the saturation and regeneration steps (points), and the predicted fixed-bed behaviour (lines), are presented in Fig. 7.8 for racemate A and in Fig. 9 for racemate B.

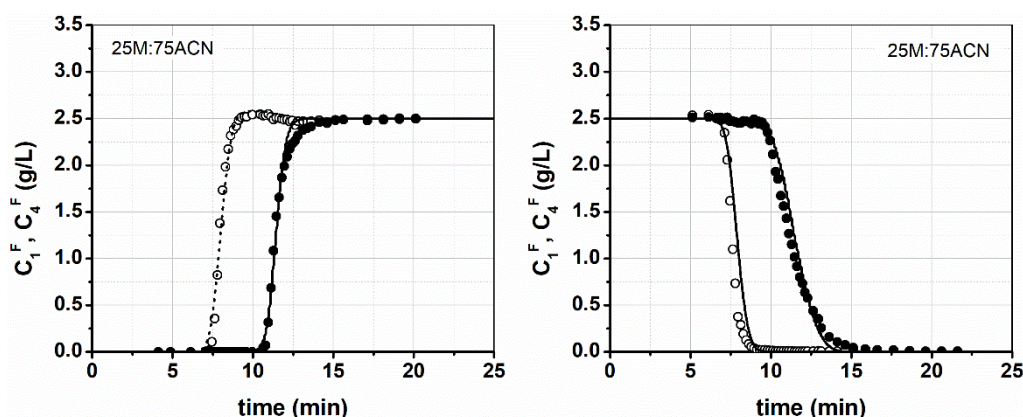


Fig. 7.8. Saturation (adsorption; left) and regeneration curves (desorption; right) for a 5 g/L nadolol racemate A (racemic mixture of stereoisomers 1 and 4) feed concentration and a 25%methanol:75%acetonitrile (25M75ACN) solvent composition. Comparison between experimental (points) and simulation results (lines). Flow-rate: 5 mL/min. Model parameters: $\varepsilon = 0.4$, $Pe = 1000$, $k = 1 \text{ s}^{-1}$, and the linear + Langmuir model parameters (see Table 7.1).

It can be concluded that there is a very good agreement between experimental points and the breakthroughs adsorption behaviour for both racemates and for all the studied solvent compositions, validating the selection of the linear + Langmuir model. The obtained results for fixed-bed reinforces the previously presented results for the equilibrium adsorption isotherms. A better experimental situation is predicted from the higher selectivity observed for the preparative separation of enantiomers 1 and 4, present in racemate A, when compared with the preparative separation of enantiomers 2 and 3, present in racemate B. The presented results for racemate B, once more predicts easier experimental conditions for the preparative separation using the 5M:95ACN solvent composition. This procedure, was also relevant for the prediction of the kinetic data, i.e., the axial dispersion using a non-retained compound leading to

a Peclet number of $Pe=1000$. Also, the nadolol racemate solutions were used to predict the mass transfer resistance value of $k=1\text{ s}^{-1}$.

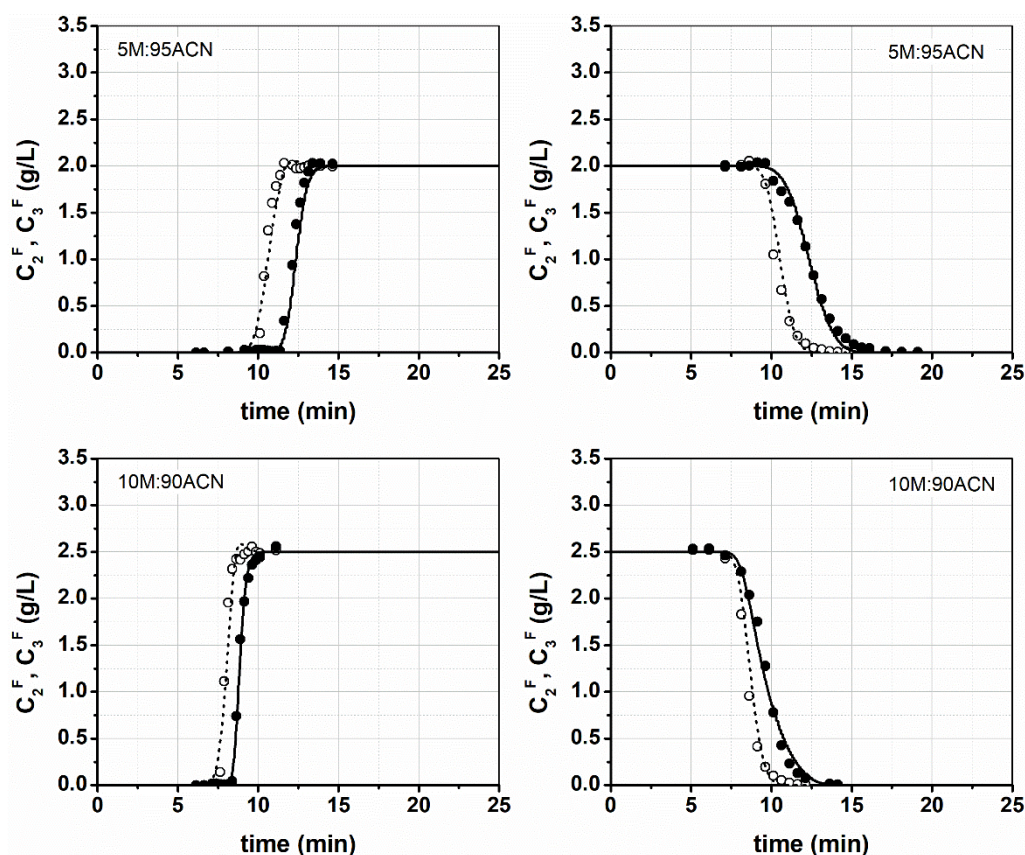


Fig. 7.9. Saturation (adsorption) and regeneration (desorption) curves for 5 g/L nadolol racemate B feed concentration (racemic mixture of stereoisomers 2 and 3). Comparison between experimental (points) and simulation (lines) results for two different solvent compositions: (top, 5M95ACN) 5%methanol:95%acetonitrile and (bottom, 10M90ACN) 10%methanol:90acetonitrile. Flow-rate: 5 mL/min. Model parameters: $\varepsilon = 0.4$, $Pe=1000$, $k=1\text{ s}^{-1}$, and the linear + Langmuir model parameters (see Table 7.2).

After the validation of the equilibrium adsorption isotherm using the linear + Langmuir model by means of frontal chromatography, and also the estimation of Pe and k values, the next experimental step can be both fixed-bed and simulated moving bed separation of both racemates as it is presented in section 7.5 and 7.6, respectively. For the preparative chiral separation of racemate A, either using FB or SMB chromatography, the selected solvent composition was 25%methanol:75%acetonitrile, while for racemate B, the elected composition was 5%methanol:95acetonitrile.

The fixed-bed operation was performed both racemate A and racemate B chiral separation using the Azura Knauer preparative HPLC system. equipped with one Chiralpak® IA column from Daicel of preparative dimensions (30 mm ID x 250 mm L) packed with the same 20 µm particle size diameter adsorbent as the SMB columns. In order to preserve both hardware system and the chiral stationary phase, a system pressure drop around 150 bar was set, limiting the flow-rate at 40 mL/min for both racemate A and racemate B separations. For each binary chiral mixture, a set of three consecutive pulses was accomplished and two fraction were collected at different times. These fractions were then analysed using the analytical Knauer HPLC system equipped with a Chiralpak® IA analytical column. All feed solutions were prepared using the same solvent as the mobile phase.

In Fig. 7.10, are presented the experimental FB chromatogram of three consecutive pulses of nadolol racemate A using a total feed concentration of 5 g/L (stereoisomer 1 + stereoisomer 4) using a 25%methanol:75%acetonitrile solvent composition. Additionally, is also presented in the same Fig. the two chromatograms obtained by HPLC analysis of the two collected fractions.

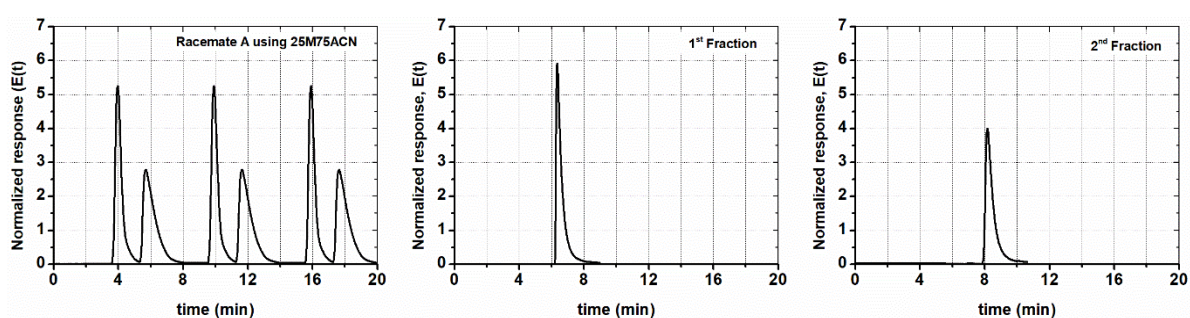


Fig. 7.10. Experimental FB chromatogram of three consecutive pulses of nadolol racemate A with a feed concentration of 5 g/L using a 25%methanol:75%acetonitrile (25M75ACN) solvent composition (left) and the HPLC analysis of the two collected fractions (middle and right).

For this separation an injection time interval of 6 min allows the complete separation between the more retained enantiomer of one injection and the less retained

enantiomer of next injection. Considering the first feed injection, the first fraction (enantiomer 1) was collected from 3.35 min to 4.16 min, the waste fraction was collected from 4.16 and 5.33 min and the second fraction (enantiomer 4) was collected from 5.33 to 7.15 min. The experimental performance parameters of recovery, purity, global productivity and solvent consumption were determined using the methodology and equations defined in previous work [29]. Due to the experimental baseline resolution between enantiomers a purity value of 100% was obtained for both fractions, the global productivity was 2.26 g/(L.hr) and a solvent consumption of 6.0 L/g.

In Fig. 7.11, are presented the experimental FB chromatogram of three consecutive pulses of nadolol racemate B using a total feed concentration of 5 g/L (stereoisomer 2 + stereoisomer 3) using a 5%methanol:95%acetonitrile solvent composition. Similar to racemate A separation, are also presented the two chromatograms obtained by HPLC analysis of the two collected fractions.

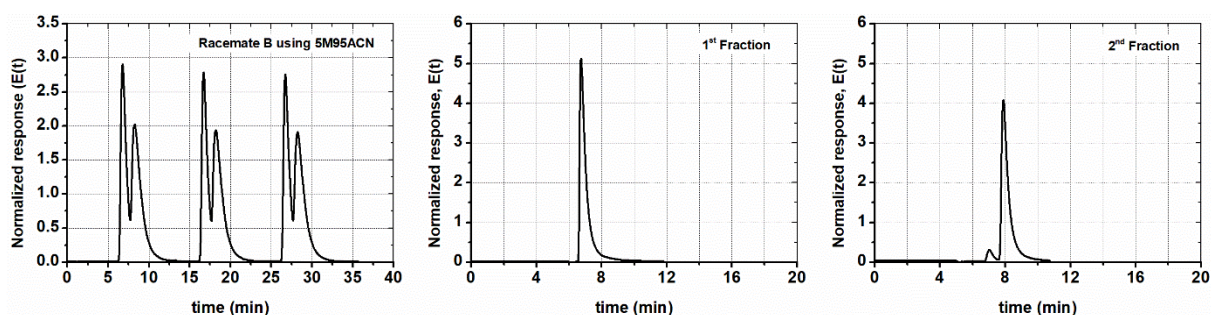


Fig. 7.11. Experimental FB chromatogram of three consecutive pulses of nadolol racemate B with a feed concentration of 5 g/L using a 5%methanol:95%acetonitrile (5M95ACN) solvent composition (left) and the HPLC analysis of the two collected fractions (middle and right).

For separation of racemate B, an injection time interval of 10 min allowing the complete separation between the more retained enantiomer of one injection and the less retained enantiomer of next injection. Considering the first feed injection, the first fraction (enantiomer 2) was collected from 6.51 min to 7.00 min, the waste fraction was collected from 7.00 and 7.45 min and the second fraction (enantiomer 3) was collected

from 7.45 to 9.15 min. The purity of 100% was obtained for the first fraction while a value of 94.1% was obtained for the purity of the second fraction, the global productivity was 1.28 g/(L.hr) and a solvent consumption of 10.6 L/g.

7.5 SMB experimental operation and performance

7.5.1 Regions of complete separation for SMB operation

Results presented in each plot of Fig. 7.12 shows two separation regions predicted using the Equilibrium Theory (solid line) and the SMB model (dashed line), that considers the rotation of the inlet and outlet streams of the SMB unit and a purity of +99% for both extract and raffinate streams. These triangles were determined for a racemic feed concentration of 2 g/L (sum of both stereoisomers present in each racemate).

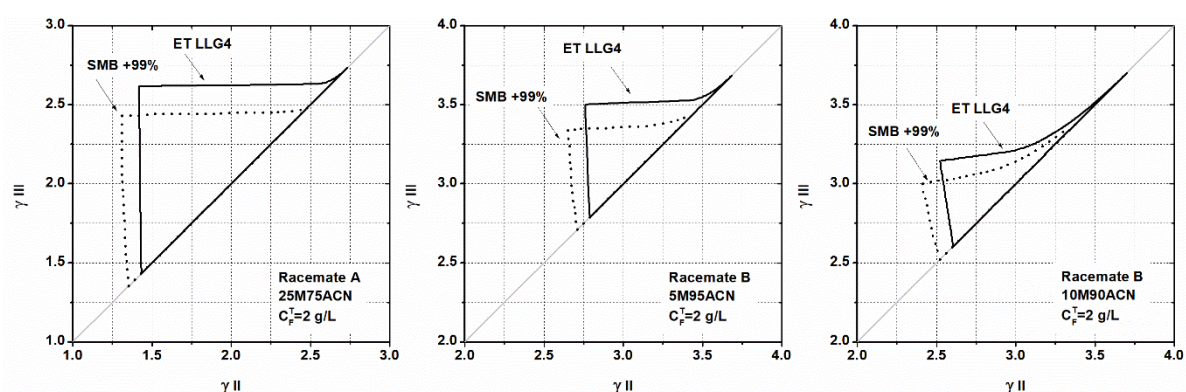


Fig. 7.12. SMB regions of complete separation for the pseudo-binary separation of nadolol racemate A for 25M75ACN (left plot) and racemate B for 5M95ACN and 10M90ACN (middle and right plot) solvent compositions and for nadolol feed concentrations of 2 g/L. Separation regions obtained with the Equilibrium Theory adapted for a linear + Langmuir isotherm model (solid line) and with the SMB transient model and a purity above 99.0% for both extract and raffinate outlet streams (dashed line).

As it can be observed, the triangles obtained with the “real SMB approach” predicts slightly lower $\gamma_{II} \times \gamma_{III}$ ratios when compared with the triangles obtained with the Equilibrium Theory. In specific cases, leading with hardware difficulties for flow-rates setting, this behaviour can represent an extra problem when fixing the best operation

conditions for maximizing the SMB productivity if the real SMB approach is not considered. Once more it is possible to see that the bigger triangle obtained for racemate A separation will represent easier operation conditions when compared with both triangles obtained for the racemate B separation. For SMB operation for racemate B separation, better operation conditions, represented as a bigger triangle, are obtained with a solvent composition of 5M95ACN.

7.5.2 SMB performance parameters

Fig. 7.13 presents the predicted SMB system productivity and solvent consumption as a function of the feed concentration, for racemate A separation (top plots) and for racemate B (bottom plots). Considering a total feed concentration of 2 g/L, better performance parameters are predicted for the racemate A separation, as already expected by the regions of complete separation. For racemate B, and again for a total feed concentration of 2 g/L, is possible to see that the 5M95ACN solvent composition predicts both higher productivity and smaller solvent composition. So, the final decision was made for the use of this solvent composition on the separation of racemate B stereoisomers.

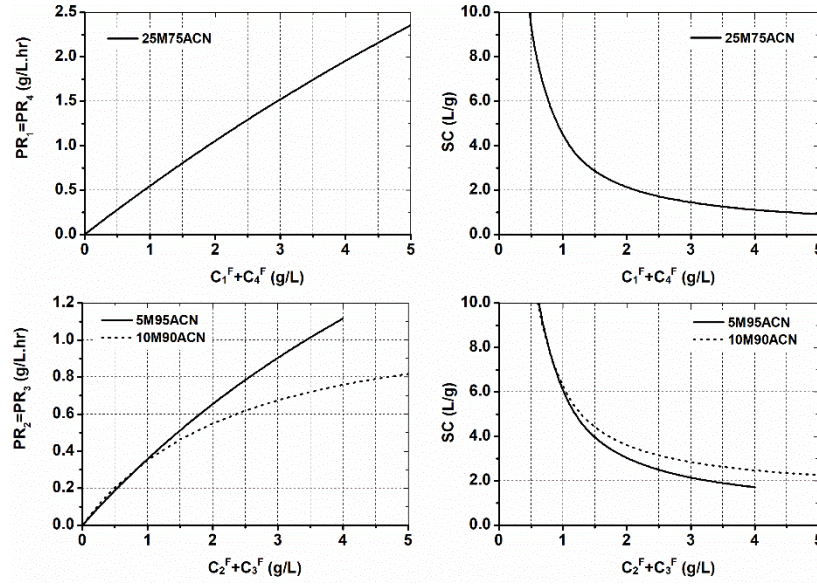


Fig. 7.13. Predictions of the SMB productivity (left) and solvent consumption (right) obtained for an internal flow-rate inside SMB section I of 20 mL/min, a safety margin of 50% and the linear + Langmuir equilibrium adsorption isotherm parameters presented in Table 1. Predictions obtained for preparative separation of racemate A (top) using 25%methanol:75%acetonitrile (25M75ACN) and racemate B using 5%methanol:95%acetonitrile (5M95ACN, solid line) and 10%methanol:90%acetonitrile (10M90ACN, dashed line).

7.5.3 SMB initial operating conditions

The SMB unit was initially stabilized in order to identify the maximum allowed flow-rate in section I due to hardware limitations, mainly due to valves and pump heads. Since the flow-rate inside this section normally imposes a total maximum pressure inside the unit, a maximum pressure drop of 75 bar was imposed in the lab-scale FlexSMB®-LSRE unit, corresponding to a flow-rate in section I of $Q_I^* = 20 \text{ mL/min}$. For both 25M75ACN and 5M95ACN solvent compositions. Several simulation runs were performed using a conventional four-zone SMB mode using a 1-2-2-1 six columns configuration.

The critical values of γ_I and γ_{IV} were fixed using $\gamma_I = \frac{1-\varepsilon}{\varepsilon} K_A \beta$ and $\gamma_{IV} = \frac{1-\varepsilon}{\varepsilon} K_B / \beta$, where K_i is the initial slope of the adsorption equilibrium isotherm ($K_i = m + Qb_i$ for the linear + Langmuir model), and β is the safety margin, considered 50%, that is, $\beta = 1.5$. The switching time value was fixed using $t^* = \frac{\varepsilon V_C}{Q_I^*} (\gamma_I + 1)$ giving. The final SMB

operating conditions were then defined using the separation region obtained using the Triangle Theory methodology. A pair of $(\gamma_{II}; \gamma_{III})$ values inside the triangle was chosen and the corresponding internal SMB flow-rates Q_j^* in sections II, III and IV were estimated using $Q_j^* = \frac{\varepsilon V_C}{t^*} \gamma_j^*$ ($j = II, III, IV$), where $\gamma_j^* = \gamma_j + 1$. The inlet, outlet and recycle flow-rates were then calculated through $Q_E = Q_I^* - Q_{IV}^*$; $Q_X = Q_I^* - Q_{II}^*$; $Q_F = Q_{III}^* - Q_{II}^*$; $Q_R = Q_{III}^* - Q_{IV}^*$; $Q_{REC} = Q_{IV}^*$. For both separations was decided to use a feed concentration of 2 g/L and the proposed $\gamma_{III} \times \gamma_{II}$ operating point (open circle) inside the triangle predicted with the Equilibrium Theory and near the vertex, that represents the initial SMB operating conditions presented in Table 7.3.

Table 7.3. Initial operating conditions used for the SMB preparative separation of nadolol racemate A using a 25%methanol:75%acetonitrile solvent composition and for the separation of nadolol racemate B using a 5%methanol:95%acetonitrile solvent composition. Both separations performed using a total racemate feed concentration of 2 g/L, and previsions obtained for a safety margin of 50% and a 20 mL/min flow-rate inside section I of the SMB unit.

Column, packing parameters and feed concentration		
$N_j = [1,2,2,1]$	$d_p = 10 \mu m$	
$D_C = 1.9 \text{ cm}$	$\varepsilon = 0.4$	
$L_C = 10 \text{ cm}$	$C_F^T = 2 \text{ g/L}$	
Racemate A: 25M75ACN		
$\gamma_I = 4.1010$	$Q_I^* = 20.00 \text{ mL/min}$	$Q_E = 12.33 \text{ mL/min}$
$\gamma_{II} = 1.4500$	$Q_{II}^* = 9.37 \text{ mL/min}$	$Q_X = 10.35 \text{ mL/min}$
$\gamma_{III} = 2.5800$	$Q_{III}^* = 12.49 \text{ mL/min}$	$Q_F = 3.22 \text{ mL/min}$
$\gamma_{IV} = 0.9557$	$Q_{IV}^* = 7.67 \text{ mL/min}$	$Q_R = 5.19 \text{ mL/min}$
$t^* = 3.5336 \text{ min}$		$Q_{REC} = 7.67 \text{ mL/min}$
Racemate B: 5M95ACN		
$\gamma_I = 5.5242$	$Q_I^* = 20.00 \text{ mL/min}$	$Q_E = 11.24 \text{ mL/min}$
$\gamma_{II} = 2.8000$	$Q_{II}^* = 11.65 \text{ mL/min}$	$Q_X = 8.35 \text{ mL/min}$
$\gamma_{III} = 3.4500$	$Q_{III}^* = 13.64 \text{ mL/min}$	$Q_F = 1.99 \text{ mL/min}$
$\gamma_{IV} = 1.8579$	$Q_{IV}^* = 8.76 \text{ mL/min}$	$Q_R = 4.88 \text{ mL/min}$
$t^* = 4.5194 \text{ min}$		$Q_{REC} = 8.76 \text{ mL/min}$

7.5.4 SMB experimental operation and performance

After start of the SMB operation, both extract and raffinate outlet streams were collected and weighted at the end of a complete cycle in order to measure the recovered mass. For each outlet stream one sample was collected and analysed by

analytical HPLC using the same solvent composition and an analytical Chiralpak® IA column with a particle size diameter of 5 µm. The SMB unit uses two Coriolis flow-meters, the first is positioned at the extract outlet stream to measure the correspondent extract flow-rate, the second is positioned immediately before section II to measure the flow-rate inside this section. With these experimental flow-rates and recoveries is possible to control the operating conditions inside and outside the SMB unit. The procedure is continuously repeated until the stereoisomers average concentration at the extract and raffinate outlet streams do not vary more than 5% during at least 10 successive cycles and the recoveries and purities are no less than 99%. The average racemate concentrations at the extract and the raffinate products were measured and the performance parameters of recovery, purity, productivity and solvent consumption were calculated using equations presented in Table 2.1. A summary of the final experimental SMB operating conditions and results can be found in Table 7.4.

Table 7.4. Experimental SMB operating conditions and results for the separation of a 2 g/L nadolol feed concentration of racemate A using 25%methanol:75%methanol and racemate B using 5%methanol:95%metahnlol solvent composition, respectively.

Racemate A: 25M75ACN		
$Q_E = 12.35 \text{ mL/min}$	$Q_I^* = 20.02 \text{ mL/min}$	$\gamma_I = 4.1061$
$Q_X = 10.65 \text{ mL/min}$	$Q_{II}^* = 9.37 \text{ mL/min}$	$\gamma_{II} = 1.3898$
$Q_F = 3.12 \text{ mL/min}$	$Q_{III}^* = 12.49 \text{ mL/min}$	$\gamma_{III} = 2.1856$
$Q_R = 4.82 \text{ mL/min}$	$Q_{IV}^* = 7.67 \text{ mL/min}$	$\gamma_{IV} = 0.9562$
$Q_{REC} = 7.67 \text{ mL/min}$		
$t^* = 3.5336 \text{ min}$		
Outlet concentrations and performance parameters		
$\bar{C}_4^X = 0.2904 \text{ g/L}$	$PU_X = PU_R = 100\%$	$PR = 1.81 \text{ g/(L.hr)}$
$\bar{C}_1^R = 0.6601 \text{ g/L}$	$RC_X = RC_R = 100\%$	$SC = 2.47 \text{ L/g}$
Racemate B: 5M95ACN		
$Q_E = 11.18 \text{ mL/min}$	$Q_I^* = 19.94 \text{ mL/min}$	$\gamma_I = 5.8133$
$Q_X = 9.00 \text{ mL/min}$	$Q_{II}^* = 10.94 \text{ mL/min}$	$\gamma_{II} = 2.7386$
$Q_F = 1.53 \text{ mL/min}$	$Q_{III}^* = 12.47 \text{ mL/min}$	$\gamma_{III} = 3.2604$
$Q_R = 3.71 \text{ mL/min}$	$Q_{IV}^* = 8.76 \text{ mL/min}$	$\gamma_{IV} = 1.9930$
$Q_{REC} = 8.76 \text{ mL/min}$		
$t^* = 4.5194 \text{ min}$		
Outlet concentrations and performance parameters		
$\bar{C}_3^X = 0.1801 \text{ g/L}$	$PU_X = PU_R = 100\%$	$PR = 0.87 \text{ g/(L.hr)}$
$\bar{C}_2^R = 0.3692 \text{ g/L}$	$RC_X = RC_R = 100\%$	$SC = 4.25 \text{ L/g}$

Fig. 7.14 and Fig. 7.15 presents the experimental (points) and the predicted (lines) transient evolution, 20 full cycles for racemate A and 25 full cycles for racemate B, of the nadolol racemate concentrations in the extract and raffinate outlet streams. The cyclic steady state for the separation of racemate B was considered to be obtained after 15 complete cycles.

Despite the operational difficulties due to high pressure drops, the final 10 cycles presented in both separations show a very good agreement between the experimental results and the predictions using the transient SMB model. This experiment also validates the capacity to complete separate (100% purities and recoveries) both nadolol racemate A and B, using the chiral IA adsorbent by SMB chromatography.

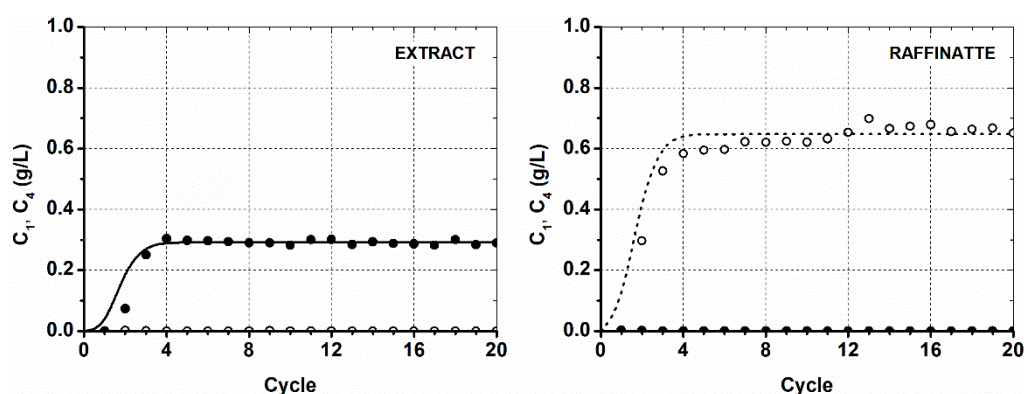


Fig. 7.14. Experimental (points) and predicted (lines) transient evolution (25 full cycles) of the nadolol stereoisomers 1 and 4 concentrations in the extract and raffinate outlet streams, present in racemate A fed to the FlexSMB unit. Closed circles for the more retained stereoisomer 4, open circles for less retained stereoisomer 1. Model parameters of $\varepsilon = 0.4$, $Pe = 1000$, $k = 1 \text{ s}^{-1}$, and the linear + Langmuir isotherm model parameters as in Table 1 using 25M75ACN solvent composition.

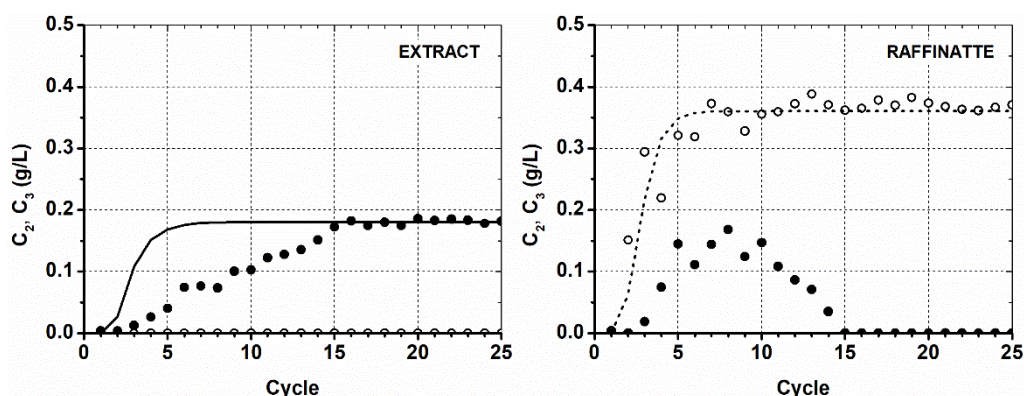


Fig. 7.15. Experimental (points) and predicted (lines) transient evolution (25 full cycles) of the nadolol stereoisomers 2 and 3 concentrations in the extract and raffinate outlet streams, present in racemate B fed to the FlexSMB unit. Closed circles for the more retained stereoisomer 3, open circles for less retained stereoisomer 2. Model parameters of $\varepsilon = 0.4$, $Pe = 1000$, $k = 1 \text{ s}^{-1}$, and the linear + Langmuir isotherm model parameters as in Table 7.1 using 5M95ACN solvent composition.

As it can be observed in Fig. 7.15, that refers to the separation of racemate B, and by the profile for the average concentrations in the raffinate outlet stream, it was observed a strong contamination between cycles 3 and 14, by the more retained stereoisomer 4 inside section IV of the SMB unit. Additionally, there is no contamination inside section I, since the extract outlet stream is clean. In cycle 5, after noticing the contamination, it was decided to decrease the flow-rate of raffinate around 1 mL/min, and to increase the extract flow-rate in the same magnitude. As it can be confirmed by the decreasing average concentration of the more retained stereoisomer after cycle 5, this action was enough to clean completely section IV of the SMB, allowing 100% of purity for both outlet streams. In order to better understand this experimental behavior, it was decided to confirm the two experimental situations (before and after the changing of raffinate and extract flow-rates) by plotting the initial and the final $\gamma_{II} \times \gamma_{III}$ points inside the separation regions obtained using the ET and SMB models. Although no contamination was observed during the SMB operation for racemate A separation, is possible to see a movement of the point representing the initial experimental conditions already presented in Table 7.3, from the inside of the triangle defined using the ET model to the inside of the triangle defined using the transient SMB model. For the

racemate B chiral separation, using the FlexSMB[®] unit, we can conclude that the ET model is not enough to accurately describe the adsorption behavior. In this situation, the transient model, which takes into account both mass transfer resistances and axial dispersion, is needed to predict the transient evolution of stereoisomers [82-83].

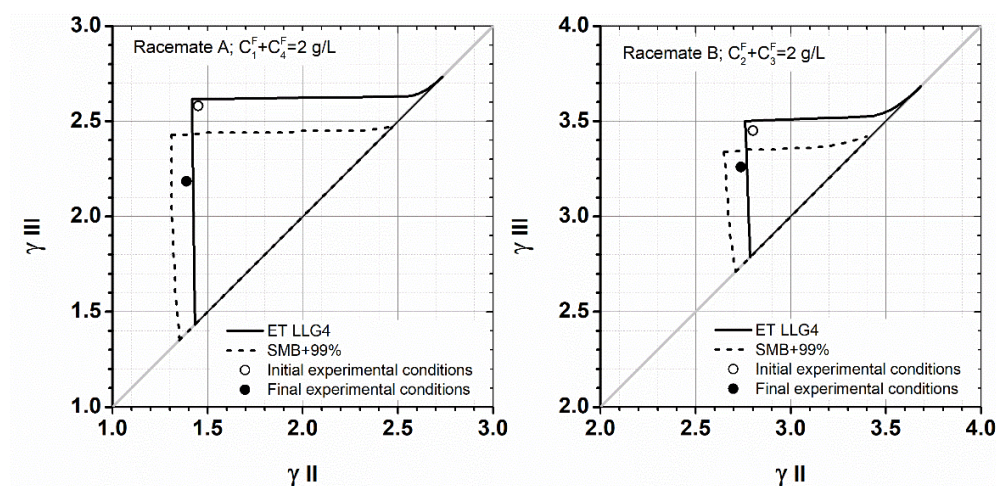


Fig. 7.16. SMB regions of complete separation (triangles) predicted using the Equilibrium Theory (solid line) and SMB transient model (dashed line) for the binary separation of nadolol racemate A (left plot, 25M75ACN) and nadolol racemate B (right plot, 5M95ACN) using a feed concentration of 2 g/L. The initial SMB operating conditions are represented by the open circle and the final SMB operating conditions are represented by the closed circle.

7.6 Conclusions

The screening of solvent composition for nadolol stereoisomers preparative complete separation using the Chiralpak[®] IA chiral stationary phase was presented. It was concluded that the complete separation of all the four nadolol stereoisomers requires the implementation of a strategy that combines a first achiral production step (2+3)/(1+4) followed by a second production chiral step. The second production step must be based on two parallel chiral separation using two different solvent compositions. For racemate A, was identified a 25%methanol:75%acetonitrile composition, whereas for racemate B, a 5%methanol:95%acetonitrile composition was elected. The equilibrium adsorption behaviour was well described by a competitive

linear + Langmuir model. This isotherm model was validated by means of breakthroughs measurements.

The experimental chiral separation of both nadolol racemates using SMB technology was carried out using the FlexSMB-LSRE unit. For racemate A, and using a total feed concentration of 2 g/L, both nadolol stereoisomer (1 and 4) were recovered 100% pure in the outlet streams with a global system productivity of 1.81 g/(L.hr) and a solvent consumption for 2.47 L/g. For racemate B, and using a total feed concentration of 2 g/L, both nadolol stereoisomer (2 and 3) were recovered 100% pure in the outlet streams with a global system productivity of 0.87 g/(L.hr) and a solvent consumption for 4.25 L/g.

Together with the former work [29], these two publications fully validate a strategy for the complete separation of the four nadolol stereoisomers. The strategy is based on a first achiral separation step with a C18 adsorbent to effectively produce pure nadolol racemates from the original quaternary nadolol mixture. The pure nadolol products (racemate A and racemate B) obtained in the first achiral step are then further purified by using two parallel binary chiral steps using the Chiralpak® IA adsorbent to separate each racemate into its pure stereoisomers. Finally, the present work validates for the first time a successful strategy to complete separate the multicomponent nadolol chiral mixture into its four pure stereoisomers at SMB and FB preparative scales.

Chapter 8 Conclusions and suggestions for future work

The main goal of this work was to identify and implement the main experimental strategy for the complete separation of nadolol quaternary mixture into its pure four stereoisomers. The main obtained results and conclusions are the following:

The pseudo binary preparative separation of nadolol stereoisomers is performed by simulated moving bed chromatography (SMB). Using the Chiralpak IA adsorbent, a 25:75:0.1 (v/v/v) methanol-acetonitrile-diethylamine solvent composition was selected to perform the experimental SMB separation and compare it with the previous results obtained using pure methanol. Using a 2 g/L total feed concentration of an equimolar mixture of the four stereoisomers of nadolol, the more retained component was fully recovered (100% purity and 100% recovery), with a system productivity of 0.77 g/(L·h) and a solvent consumption of 9.62 L/g. (See chapter 4).

The selected and implemented methodology to perform the complete separation of the four nadolol stereoisomers is based on two main steps. The first step is an achiral preparative separation of the two racemates of nadolol (two pairs of enantiomers). The second step is composed of two parallel preparative binary chiral separations of the racemates of nadolol, previously obtained on the first step. (See chapter 5).

The implementation of the first achiral step was done with the separation of nadolol racemates by high pH reversed-phase fixed-bed and simulated moving bed chromatography. Both systems use the Waters XBridge C18 adsorbent of 10 µm particle diameter. (See chapter 6).

For the first achiral step, an extensive study was carried to select the most promising achiral adsorbent and the more favourable solvent composition. The screening of the mobile phase composition elected the Waters XBridge C18 adsorbent and a 30:70:0.1 (v/v/v) ethanol:water:diethylamine solvent mixture to perform both FB and SMB

preparative operations. For FB, using a feed concentration of 9 g/L of an equimolar mixture of the two nadolol racemates, both were recovered almost pure (at least 99.9%), with a global system productivity of $3.06 \text{ g}_{\text{feed}}/(\text{L}_{\text{bed}}.\text{hr})$ and a solvent consumption of $4.21 \text{ L}_{\text{solvent}}/\text{g}_{\text{feed}}$. For SMB, the pilot unit's pressure drops limits imposed a maximum internal flow-rate of only 5 mL/min and, for a nadolol feed concentration of 2 g/L, both racemates were recovered 100% pure, with a system productivity of $0.13 \text{ g}_{\text{feed}}/(\text{L}_{\text{bed}}.\text{hr})$ and a solvent consumption of $6.19 \text{ L}_{\text{solvent}}/\text{g}_{\text{feed}}$. Additional simulation results showed that a SMB preparative unit can perform the 9 g/L nadolol racemate separation with a system productivity of $3.61 \text{ g}_{\text{feed}}/(\text{L}_{\text{bed}}.\text{hr})$ and a solvent consumption of only $1.95 \text{ L}_{\text{solvent}}/\text{g}_{\text{feed}}$ using the same average internal flow-rate as in FB operation. Even better SMB productivities can still be obtained using the same feed or solvent flow-rates as in FB operation if the internal SMB flow-rates are allowed and not limited by the system pressure drop.

Concerning the second production step and for racemate A separation, a 25%methanol:75%acetonitrile solvent composition was elected, whereas for racemate B, a 5%methanol:95%acetonitrile was the selected composition. The experimental chiral separation of both nadolol racemates using SMB technology was carried out using the FlexSMB-LSRE unit. For racemate A and using a total feed concentration of 2 g/L, both nadolol stereoisomer (1 and 4) were recovered 100% pure in the outlet streams with a global system productivity of $1.81 \text{ g}/(\text{L}.\text{hr})$ and a solvent consumption for 2.47 L/g. For racemate B and using a total feed concentration of 2 g/L, both nadolol stereoisomer (2 and 3) were recovered 100% pure in the outlet streams with a global system productivity of $0.87 \text{ g}/(\text{L}.\text{hr})$ and a solvent consumption for 4.25 L/g. (See chapter 7).

Future work can include the use of different SMB modes of operation, such as the asynchronous port shift (Varicol), variable feed concentration (ModiCon) or Japan Organo (JO) operation for ternary mixtures, among others, in order to study the improvement of preparative performance parameters of productivity, recovery and solvent consumption. The implementation of some of these non-conventional SMB modes of operation will imply the change of LSRE-FlexSMB unit hardware and software.

Another suggestion is the extension of the developed methodology to similar chiral pharmaceutical multicomponent mixtures. It will be also interesting to apply this methodology to the preparative separation of multicomponent mixtures of extracts of natural products.

From the participation of several international conferences, crystallization arose several times as a suggestion for a final (after SMB) step preparative chiral separation of a pure (one enantiomer) solution.

Annexes

Publications in international journals

Arafah, R.S., Ribeiro, A.E., Rodrigues, A.E., and Pais, L.S., Improving the performance of nadolol stereoisomers' preparative separation using Chiralpak IA by SMB chromatography, *Chirality* 31 62-71 (2019) DOI: 10.1002/chir.23034.

Arafah, R.S., Ribeiro, A.E., Rodrigues, A.E., and Pais, L.S., Separation of nadolol racemates by high pH reversed-phase preparative chromatography, *Sep. Purif. Technol.* 233 article number 116018 (2020).

Arafah, R.S., Ribeiro, A.E., Rodrigues, A.E., and Pais, L.S., Separation of nadolol racemates by high pH reversed-phase fixed-bed and simulated moving bed chromatography, *Sep. Purif. Technol.* 305 article number 122529 (2022).

Arafah, R.S., Ribeiro, A.E., Rodrigues, A.E., and Pais, L.S., Separation of nadolol racemates into its pure enantiomers by fixed-bed and simulated moving bed chiral chromatography (to be submitted *Sep. Purif. Technol.*).

List and oral and poster communications in national and international conferences (01/10/2018-30/11/2022)

Arafah, R., Ribeiro, A.E., Rodrigues, A.E., and Pais, L.S., Separation of Nadolol Racemates by High pH Reversed-Phase Simulated Moving Bed Chromatography, in CHEMPOR 2018 13th International Chemical and Biological Engineering Conference, Aveiro, Portugal, 2-4 October, 2018 (oral).

Arafah, R., Ribeiro, A.E., Rodrigues, A.E., and Pais, L.S., Separation of Nadolol Racemates by High pH Reversed-Phase Preparative Fixed-Bed Chromatography: Comparison of C18 Materials, in CHEMPOR 2018 13th International Chemical and Biological Engineering Conference, Aveiro, Portugal, 2-4 October, 2018 (poster).

Ribeiro, A.E., Arafah, R., Rodrigues, A.E., and Pais, L.S., Strategies for the Global Multicomponent Separation of Nadolol Stereoisomers by Chiral and Achiral Fixed-Bed and Simulated Moving Bed Chromatography, in SPICA 2018 17th International Symposium on Preparative and Industrial Chromatography and Allied Techniques, Darmstadt, Germany, October 7-10, 2018 (oral and poster).

Arafah, R., Ribeiro, A.E., Rodrigues, A.E., and Pais, L.S., High pH Reversed-Phase Preparative Chromatographic Separation of Nadolol Racemates using C18 Adsorbents, in XXIV Encontro Luso-Galego de Química, Porto, Portugal, 21-23 November, 2018 (oral).

Arafah, R., Ribeiro, A.E., Rodrigues, A.E., and Pais, L.S., Enantioseparation of the Four Nadolol Stereoisomers by Fixed-Bed and Simulated Moving Bed Chromatography, in 31st International Symposium on Chirality, Bordeaux, France, 14-17 July, 2019 (poster).

Arafah, R., Ribeiro, A.E., Rodrigues, A.E., and Pais, L.S., Multicomponent separation of nadolol stereoisomers combining different preparative technologies and chiral and achiral-chiral strategies, in 12th European Congress of Chemical Engineering, Florence, Italy, 15-19 September, 2019 (poster).

Arafah, R., Ribeiro, A.E., Rodrigues, A.E., and Pais, L.S., Separation of Nadolol Racemates by High pH Reversed-Phase Fixed-Bed and Simulated Moving Bed Chromatography, in 11th National Meeting on Chromatography, Sociedade Portuguesa de Química, Caparica, Portugal, 9-11 December, 2019 (oral).

Arafah, R., Ribeiro, A.E., Rodrigues, A.E., and Pais, L.S., Complete separation of the quaternary mixture of nadolol stereoisomers using preparative and simulated moving bed chromatography, in CHISA 2021 24th International Congress of Chemical and Process Engineering, 15-18 March, 2021 (oral, virtually).

Arafah, R., Ribeiro, A.E., Rodrigues, A.E., and Pais, L.S., Combining preparative fixed-bed and simulated moving bed chromatography at normal-phase and high pH reversed-phase to achieve complete separation of the quaternary mixture of nadolol stereoisomers, in CHISA 2022 25th International Congress of Chemical and Process Engineering, Prague, Czech Republic, 21-25 August, 2022 (oral).

Arafah, R., Ribeiro, A.E., Rodrigues, A.E., and Pais, L.S., Fixed-Bed and Simulated Moving Bed Chromatography Using Achiral and Chiral Adsorbents for the Complete Preparative Separation of a Quaternary Mixture, in SPICA 2022 18th International Symposium on Preparative and Industrial Chromatography and Allied Techniques, Lisbon, Portugal, October 11-14, 2022 (oral).

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