Structured adsorbents for isolation of functional food ingredients

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Structured adsorbents for isolation of functional food ingredients

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Para mi familia, con cariño
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Chapter 1

General introduction
1.1 Trends in food ingredient separation

1.1.1 Background

While raw materials such as milk or milk components are valuable to prepare everyday foods (butter, yogurt, etc.), the processing of these and the side streams obtained from milk derivatives are nowadays quite valuable as well. Many of the components in whey and milk protein-derived peptides are now understood to actively contribute to specific aspects of our health [1, 2]. For instance the galacto-oligosaccharides in whey have prebiotic effects, while antihypertensive peptides may be obtained by proteolysis from milk proteins.

To isolate these compounds and use them as functional ingredients in further applications, enzyme technology and sophisticated separation techniques, such as chromatography are used. Figure 1.1 depicts a typical process in which the commercial galacto-oligosaccharides (GOS) or the antihypertensive lacto-tripeptide IPP are obtained. Chromatography in the food industry is commonly performed using a packed bed of porous particles. However, this configuration presents some drawbacks for the processing of food streams. As a result of the large volumes that need to be processed, the equipment scale needed for

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**Figure 1.1**: Typical process in which a selective adsorptive process is needed for the production of functional ingredients.
food chromatographic processes is large and cannot be compared with that in pharmaceutical processes. Besides, the process scale is growing due to the increasing awareness and consequent demand for these types of ingredients [3]. This trend coincides with the wish to positively utilize industrial food side streams which are generally large in volume (>10 m³/h) and in some cases contain only a small amount of the component of interest (g/m³). Currently, the target components are mostly separated using centrifugation, filtration and membrane technology or precipitation [4, 5] which are not sufficiently selective or need large streams of chemicals. As a result, the functional ingredients can only be obtained in the form of low-purity fractions with complex composition (e.g. TensGuard™ hydrolyzate), which complicates and limits the design and applications of end user products.

### 1.1.2 Industrial perspectives

The trend presented to separate valuable components from very large side and waste streams introduces challenges. The existing processes are too expensive to be applied on the scales that apply in the food industry. Besides, existing processes would require extensive pre-processing of the streams before they could be submitted to the separation process, for example, to avoid clogging of chromatographic columns. This would change or degrade the streams, strongly reducing their value after being processed. Thus, there is a continuing search for adsorbents that would yield a higher selectivity and a higher yield, even at these unfavorable conditions, while not requiring extensive pre-processing.

While the potential range of components to be isolated from larger food streams is wide, the new functional ingredients that are gaining more attention can be represented by two model components: carbohydrates and peptides. While the range of properties found in practice is tremendous, these two model components are both practically and industrially relevant, but also have properties that are representative of those of the two largest classes: carbohydrate and protein derived components.
1.1.3 The example of GOS and IPP

Galacto-oligosaccharides (GOS) are non-digestible, oligomerized sugars (degree of polymerization (DP) > 3) consisting of galactose monomers with a terminal glucose. GOS are considered prebiotic: they reach the colon intact and there act as nutrient for organisms that are indicated to promote a healthy gut function [6, 7] contribute to human natural defense [8–10] and improve mineral adsorption [11–13]. GOS are produced enzymatically from widely available and inexpensive, whey-derived lactose [14, 15]. However, currently galactooligosaccharides are produced in fractions that include mono- and disaccharides. This limits their use beyond the current applications as ingredient in infant foods, functional dairy products and fruit-based drinks [5]. The removal of the smaller sugars (DP = 1, DP = 2) including lactose, could extend the application to lactose-intolerant consumers, diabetic people and would reduce the caloric value. The purification of the GOS mixture would therefore upgrade the product.

Isoleucine-proline-proline (IPP) is a bioactive tripeptide that can be derived from casein. There are many bioactive peptides that have recently been identified in a wide range of proteins [4]. Peptides such as IPP were found to have antihypertensive properties [16, 17]; yet no clinical trials have been performed because of the lack of a suitable and cost-effective process to obtain IPP in a relative pure form. Currently the standard isolation process manages to obtain a peptide hydrolyzate mixture (e.g. TensGuard™) with limited purity after isolation of the peptides with membrane filtration [2, 4, 18, 19]. The development of new selective adsorptive technology, to have a purer fraction of bioactive peptides such as IPP, would lead to the development of new functional food ingredients with greater value.

1.2 Design of a chromatographic process: A multiple set of choices

Designing a chromatographic process is complex as it involves different types of factors: thermodynamic (equilibrium) interactions, adsorption kinetics, different mass transfer resistances and hydrodynamics (e.g. the pressure drop). This
results in a set of choices in the selection of a suitable adsorbent. These choices are even more important when the process will be scaled up to large volumes. Therefore, it is necessary to model different systems in order to see which process is the most optimal under several process requirements and restrictions.

### 1.2.1 Adsorptive strategy

There are two fundamental strategies in adsorption chromatography: either capturing the target component, or removing the undesired components. As a rule of thumb, the strategy in which less solute needs to be adsorbed will be chosen. However, the choice of strategy might change depending on other choices, such as the adsorbents available, the solvents needed, the throughput and the final costs of the process.

### 1.2.2 Adsorbent interaction and capacity

After choosing an adsorptive strategy, the interaction and capacity of an adsorbent for the component(s) to be adsorbed should be considered, since these have a strong influence on the throughput as they determine how much solute can be bound. This interaction is determined by the affinity of the molecules present in the liquid towards the surface of the adsorbent. The affinity can be modified by the characteristics of the solution such as ionic strength, solute concentration and the presence of other components.

The equilibrium between the adsorbed and liquid phases is summarized in the adsorption isotherm. After obtaining the affinity constant and maximum binding capacity, the adsorbent with the higher capacity and selectivity towards the target is generally chosen, however the desorption or regeneration step of the chromatographic column after every cycle also needs to be considered. In fact, the concern and studies for solvent recovery and solvent choice have grown due to stricter regulations and increased competition [20].

Furthermore, depending on the characteristics of the stream and the non-target components, a different type of interaction or adsorbent might be preferred. For instance, when the concentration of the valuable component is low compared to other components that should not be adsorbed, an adsorbent with a high
affinity and a high selectivity is required. When the concentration of the valuable component is high, an adsorbent with a high capacity is required; when the concentration of the valuable component is low and a high recovery is essential, an adsorbent with a high affinity is needed, while the capacity is less crucial.

Finally, from a desorption (regeneration) point of view, an adsorbent with moderate affinity but high selectivity is preferred as this will result in a concentrated, purified, small process stream under reasonably mild conditions (no use of solvents or pH shifts).

### 1.2.3 Structure of stationary phase vs. mass transfer performance

The stationary phase is the packing in the chromatographic column and provides the interacting material. This material can have different internal and external porosity (voidage) and has different forms. Its structure determines the mass transfer within the adsorbent which is usually the limiting step in the kinetics of adsorption in the column. These limitations are very important for the overall performance and productivity.

Conventional chromatography uses porous particles or beads, but as will be discussed in section 1.3.1, these beads are not optimal as they do not represent the best balance between convective flow (throughput) and diffusion path (velocity of transfer to the adsorbing sites). Several alternative structures exist, such as monoliths, that are already being considered for gas phase adsorption [21–24] and bioseparations [25–33] but not yet in the separation of food components. Figure 1.2 shows a packed bed of beads, a continuous monolithic column and a channeled monolith (or "honeycomb" monolith) and indicates the typical diffusion and convective paths. In monolithic columns the flow goes through the interstitial spaces between the beads and the diffusion distances are relatively small, in channeled monoliths the convective flow goes through the channels: the diffusion distance can be \textit{a priori} customized. In continuous monolithic columns, the convective flow needs to pass through very small pores, leading to large hydrodynamic resistance and large pressure drops; however the diffusion distances are extremely small.

Different mass transfer resistances and dispersion effects are present in a chromatographic column as is shown in Figure 1.3. First, the convective
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Figure 1.2: Representation of the different type of structures studied in this thesis. The continuous lines represent the direction of the convective flow; the semi-continuous lines represent the direction of diffusion into the adsorbent.

Figure 1.3: Dispersion and mass transfer effects occurring in a chromatographic packing.

Flow along the column direction induces axial dispersion (1) since there is a distribution of flow rates. Second, the transfer of the target component from the bulk liquid towards the adsorbent introduces an (external) mass transfer resistance (2). Third, the mass transfer within the adsorbent is through diffusion through the liquid in the pore (3) and over the surface of the pores (4) of the stationary phase. Finally, although the kinetics of adsorption (5) can be limiting, generally this contribution can be neglected, especially in most ion exchange and hydrophobic adsorption systems or in systems with relatively high diffusive lengths.
The axial dispersion is related to the non-uniformity of the flow produced by the tortuosity and obstacles of the stationary phase. In the case of channeled monoliths the axial dispersion depends on the hydraulic diameter of the channels: a larger diameter increases the non-uniformity of the fluid velocity over the cross section of the channels [34]. Also the homogeneity of the hydraulic diameter is important: a system in which some channels are large and others are smaller will have axial dispersion. The external mass transfer resistance depends on the flow regime and distance within the inter-adsorbent space. The intraparticle mass transfer is directly related to the length of the diffusion path inside the adsorbent.

1.2.4 Configuration

Although the most straightforward configuration of a chromatographic system is an axial packed bed, there are different configurations in which the particles can be packed such as a radial packed bed, an expanded bed or a simulated moving bed.

In a radial packed bed, the porous adsorbent is packed in between two concentric cylinders and the flow is applied radially. The radial packed bed has shown potential to up-scale packed bed systems [35], but it presents some limitations regarding the bed height related to the compaction of the adsorbent particles, and in diameter, since the thickness of the annular ring determines again the pressure drop of the bed, and a smaller thickness and larger diameter would result in a large unused space.

In an expanded bed of porous particles the particles are not tightly packed but can move freely with the flow, giving the advantage of low pressure drop and low risk of plugging. Thus this configuration is used for direct recovery of components without the need of prior removal of suspended solids [36, 37] that are usually hindered by the low interparticle space of packed beds. However, the velocities that can be applied are restricted by the requirement for a stable bed and the difficulties in operation. Besides, an expanded bed has a lower capacity per column volume associated with the high interparticle spaces.

A simulated moving bed is basically a series of chromatographic columns (either packed axial columns or packed radial columns, or in theory even
expanded beds) in which continuous process operation is obtained by switching from one to the other column in time. This configuration is used if the product is produced in large quantities [38].

1.3 Bottlenecks of conventional chromatography

1.3.1 Negative trade-off between mass transfer and pressure drop

The main bottleneck of a packed bed configuration is the trade-off between particle size and pressure drop. Large particles yield a high permeability but the diffusion length from convective flow to adsorptive sites is high; small particles will have a small diffusion length but the pressure drop is much higher (Figure 1.4). The only possibility to scale these columns up is therefore to have a wider diameter so that the superficial velocity remains small for pressure drop and mass transfer. The resulting pancake-like columns require more factory space, larger capital costs and may show suboptimal performance due to inhomogeneous flow distribution.

This trade-off between pressure drop and mass transfer is a critical bottleneck for the processing of large and diluted industrial streams. And this bottleneck is also noticeable when trying to process more concentrated or less polished streams, due to the increase in viscosity.

1.3.2 Fouling and plugging of conventional media

Besides the intrinsic problems caused by the pressure drop, other phenomena, such as fouling, plugging and bed compression, increase the already existing pressure drop with the processing time [40, 41]. These phenomena demand the dilution and filtration of the streams to maintain the capacity and resin integrity after many cycles [42]. This increase of the size of the stream has a direct impact on the efficiency and productivity of the process as well as in the capital expenses (equipment size or increased number of unit operations). Additionally, the dilution of the feed may have consequences for its use after having been subjected to the chromatographic process.
1.3.3 Costly processes

Chromatography is not only complex but also expensive. A balance between complexity and costs needs to be found. For larger streams the main costs are associated with the size of the equipment. The capital costs can increase depending on the pretreatments needed and particularly if the up-scaling is made by increasing the width of the columns. The costs of the process equipment can be estimated using the relationship between the costs and the cross-sectional surface area (or column diameter) as proposed by Guiochon et al. [43].

The resin is considered an operational cost since it has to be discarded after a number of cycles. If the amount of resin needed is large, this can represent a big expense especially if costly affinity ligands are used.
1.3.4 Current state of our understanding

The numerous bottlenecks of packed beds systems for the separation of small food ingredients suggest the need of alternative low-cost, efficient and robust interactive materials and structured stationary phases.

Activated carbon could be a feasible adsorptive material for large-scale applications, as it is inexpensive, has tunable chemistry and porosity, and possesses a large surface area (up to 2000 m²/g). Previous work has demonstrated the possibilities of using activated carbon for the enrichment or partial purification of fractions of different functional ingredients such as: oligosaccharides [15] and peptides [44–48]. However, not much research has been conducted using activated carbon for the enrichment of a specific functional compound. Besides, the regulated use of buffers on activated carbon to regulate the interaction, elution and separation of components in a real application has also not yet been explored.

Furthermore, stationary phases in which a hierarchical structure can be designed, such as channeled monoliths have been studied in other application and could have some potential to solve bottlenecks from particulated columns such us the inefficient tradeoff between pressure drop and mass transfer and the susceptibility to plugging. However, not much work has been done yet on the prediction of the behavior of channeled monoliths using liquid streams. Some research has been performed though on channeled monoliths for gas as well as liquid systems to obtain an analytical solution based on numerical simulations [49]. Yet, there are no validated models and experimental experience available for channeled monoliths in liquid systems.

Finally, there is a need to find a window of operation as comparable to membrane filtration to see which structure should be used as a function of process requirements. Although this is not a straightforward task, guidelines and methodologies are needed to find the best structure for different types of applications. While most of the work focuses on the separate analysis of the pressure drop, mass transfer or column efficiency, these aspects have not been analyzed in conjunction.
1.4 Thesis aim

The food industry needs to develop more robust, low-cost and food-grade adsorbents that are suited to selectively adsorb components with high-added value for foods, such as oligosaccharides and bioactive peptides.

The ultimate aim of this thesis is therefore to find the principles that determine the suitability of alternatively structured adsorbents for the adsorptive separation of smaller food ingredients from large streams.

To maintain a reasonable volume of the adsorption process for these streams, adsorbents need to have very large active surfaces, should allow very short contact times, a high mass transfer and short regeneration times. Besides, the adsorbents should be robust against fouling and plugging during processing to avoid the dilution of streams and pretreatments prior to the chromatographic step. Finally, since we are considering the processing of food components, mild separation methods are required to preserve the quality of the product stream.

1.5 Thesis outline

In this thesis the principles that determine the suitability of adsorbents for chromatographic separations are explored by combining practical experimental experience with model-based considerations. Low-cost adsorbents with large surface areas are needed for the purification of components from food streams such as a lacto-tripeptide from a crude hydrolyzate and oligosaccharides from a mixture of sugars. Activated carbon can be used to adsorb based on differences in hydrophobicity, is relatively low in cost and is available in different structures. It is thus a good model adsorbent material, and therefore the experimental work in this thesis mainly focusses on the comparison between the performance of an activated carbon packed bed and activated carbon channeled monoliths. The study is extended by a model-based comparison between continuous monolithic columns and different channeled monoliths. With the help of this theoretical framework, a window of operation is built for this kind of structures as a function of process conditions, adsorptive structure and column dimensions. The overall outlook of the thesis is summarized in Figure 1.5.
For the comparison between an activated carbon packed bed and channeled monolith, **chapter 2** first focuses on the development and assessment of an enrichment process using an activated carbon packed bed to separate the lacto-tripeptide IPP from a crude commercial hydrolyzate. Furthermore, different adsorptive-desorptive cycles are carried out to understand the exhaustion and fouling mechanisms in the adsorbent. **Chapter 3** reports on the performance of the activated carbon packed bed that is compared with the performance of activated carbon channeled monoliths during the loading of the commercial hydrolyzate. The chapter discusses the intrinsic permeability as well as the susceptibility to plugging of the adsorbent during the loading, which is described with empirical analogies. In **chapter 4**, the adsorption of lactose on the activated
carbon packed bed and the activated carbon channeled monolith structures is modeled with the use of methods normally used for particulated resins, in order to understand the adsorption kinetics including mass transfer and the dispersion mechanisms within these structures. **Chapter 5** proposes a method to compare different adsorbents (a packed bed of porous silica, a silica monolithic column and silica channeled monoliths) based on the dimensions of the adsorbent and column needed to comply with different restrictions and process requirements such as efficiency, pressure drop, throughput, and affinity. **Chapter 6** concludes with a general discussion summarizing the bottlenecks and opportunities of activated carbon and channeled monoliths for the separation of small food components. Besides, further possible candidate adsorbents for large scale separations of small components are discussed as well as future prospect in the screening of adsorbents.
Recovery of a bioactive tripeptide from a crude hydrolysate using activated carbon

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Abstract

Separation and purification processes of nutraceuticals, such as bioactive peptides, are usually done in a multistep process that sometimes requires a final chromatographic step using expensive resins. Activated carbon is a promising economic alternative for the resins. We report here on the application of a hydrophobic interaction on a chromatographic column packed with particles of activated carbon to isolate a lactotripeptide from a crude hydrolysate. Consecutive adsorptive–desorptive cycles were used until exhaustion of the column. Liquid chromatography–mass spectrometry results showed an enrichment of the lactotripeptide isoleucine–proline–proline with a yield of up to 80% in the third cycle and a twofold increase in purity to up to 35%. Some guidelines are given for the competitive exhaustion of the adsorbent for process optimization in order to obtain higher purity and yield.
2.1 Introduction

The production of bioactive peptides has become important in the food industry for their health benefits. For instance, the lactotripeptide isoleucine–proline–proline (IPP) reportedly has antihypertensive properties [16, 17]. These bioactive peptides are generally hydrolyzed from a foodstuff with proteases and then enriched by means of multiple-step downstream processing. The standard process combines an enrichment or purification step after a solid/liquid separation and concentration [4]. Ultrafiltration has mainly been used for the enrichment of peptides [4, 18, 19]. If specific peptides of similar size need to be purified or purity is a requirement, then an adsorptive or chromatographic step needs to be applied [50]. However, chromatographic processes are not easily scalable and often uneconomic for food products.

Proteins and peptides show differences in hydrophobicity depending on their side chains and structure [51]. Therefore, they can be separated by interaction with a hydrophobic stationary phase. The hydrophobic effect can be influenced by varying the ionic strength, pH, temperature and solvent composition [52] to improve the separation. Separation of these peptides has been reported based on their hydrophobicity using reverse-phase high-performance liquid chromatography (HPLC) [53], ion exchange resins [54] and hydrophobic interaction chromatography (HIC) [55].

Activated carbon has been used to enrich peptides with particular amino acid compositions. Many studies have been published on the use of activated carbon for debittering hydrolyzates and to lower the proportion of aromatic amino acids for nutritional reasons for patients with liver diseases [44–48]. Contrary to other stationary phases used in chromatography, activated carbon is inexpensive. In addition, activated carbon has a large surface area up to 2000 m²/g, and has tunable chemistry and porosity, which makes it an ideal candidate for a chromatographic process. However, not much research has been conducted using activated carbon for the enrichment of a specific peptide; nor are there published results on the use of different buffers on activated carbon to regulate the interaction, elution and separation of components in a real application.
The aim of this study is to report on the performance of activated carbon for the enrichment of the antihypertensive lactotripeptide, IPP, from a crude filtered hydrolysate using HIC, and on its suitability for industrial production.

### 2.2 Materials and methods

#### 2.2.1 Materials

Milk casein hydrolysate TensGuard™ S was kindly provided by DSM Food Specialties (Delft, The Netherlands). A standard of IPP was purchased from Bachem (Switzerland). Ammonium sulfate and acetic acid (both ≥99% purity) were obtained from Sigma-Aldrich (Germany) and all other chemicals were of analytical grade and obtained from Merck (Germany).

Mesoporous activated carbon particles TE7/20, batch DEC 35, with a diameter range between 250 and 500 µm were purchased from MAST Carbon International Ltd (UK). The results for the surface area and porosity of the activated carbon particles are shown in Table 2.1.

#### 2.2.2 Buffer and feed solutions

Preliminary adsorption experiments were done using three different buffers containing 10 mM sodium phosphate buffer at pH 7.5: one without additional salt, one with 2 M NaCl added and one with 0.75 M ammonium sulfate added. The buffer used for the peptide separation was prepared by mixing 10 mM of sodium phosphate buffer with 0.75 M (NH₄)₂SO₄ adjusted to pH 7.5 with 1 M NaOH. All buffers were made in Milli-Q water and filtered under vacuum using a 0.22-µm Millipore NY20 nylon filter from Sartorius.
The 15 g/L peptide hydrolysate feed solution used in the injections was prepared by dissolving the hydrolysate in the same buffer used during the adsorption step. The solution was then filtered using a 28-mm PES Sartorius Minisart HighFlow filter with pore size 0.45 µm.

### 2.2.3 Column packing and characterization

The column was packed by slowly pouring 0.6 g of dry activated carbon particles into a 5-mm diameter Tricorn from GE Healthcare (Uppsala, Sweden). A slight vibration was applied to allow the particles to rearrange and settle.

### 2.2.4 Peptide identification and quantification

**HPLC analysis**

Samples were analyzed using an Ultimate 3000 UHPLC+ system from Thermo Fisher Scientific (Sunnyvale, CA, USA) with an Atlantis dC 18 column (100 Å, 3 µm, 3.9×150 mm). The mobile phase flow rate was 0.22 mL/min. Two buffers were used for the gradients: buffer A, made with 0.1% (v/v) trifluoroacetic acid in Milli-Q water and buffer B made with 0.1% (v/v) trifluoroacetic acid in acetonitrile. The system was run for 20 min in total: 0–2 min using 100% buffer A; from 2 to 12 min, a gradient using buffer B decreased buffer A from 100% to 60%; and from 12 to 12.2 min another gradient was applied to give 100% buffer A. The temperature of the column and the sample tray was 50 °C and 10 °C, respectively. The eluent was monitored using a UV detector at 220 and 254 nm. To obtain the concentration of IPP, a standard was used to spike the corresponding peak on the chromatogram. In additional, mass spectrometry (MS) was used for peak identification and resolution of coelution conflicts in the chromatogram. The concentration, in absorbance units, of the peptides coeluting with IPP were obtained from the shoulders of the IPP peak. The concentration of these peptides was negligible and did not affect the end result. For all components, the results were processed using Chromeleon 7.0 software from Dionex (Thermo Fisher Scientific, Sunnyvale, CA, USA). To have a measure of the reproducibility of the measurements, the feed samples were measured in triplicate (relative standard deviation of peptide concentrations <2%).
MS analysis

To support the HPLC results, parts of the samples were measured by coupling the Atlantis dC 18 column on Accela UHPLC (Thermo Electron, Breda, The Netherlands) with a LTQ-Orbitrap Fourier transform mass spectrometer (Thermo Electron, Bremen, Germany) using a Fourier transform analyzer operated at 7500 resolution with a scan range of 190–2000 \( m/z \). Data were acquired in positive mode using the profile data type. Because the hydrolysate mixture contained a very limited amount of peptides, we based the identification of the peptides on the MS results only. All \( m/z \) values determined were within the specifications of the Orbitrap mass spectrometer (<2 ppm).

**Relative peptide quantification**

The mass fraction of the peptides was estimated for the feed samples by applying the Lambert–Beer law to the absorbance results (in mAU×mL) obtained by liquid chromatography (LC)–MS using the molecular weight and molar extinction coefficient. The molar extinction coefficient of each peptide was obtained by previous validated prediction [57].

**2.2.5 Column experiments**

**Explorative adsorption experiments**

The experiments were performed in an ÄKTA Explorer. The overall amount of peptides was monitored using a UV detector at three wavelengths: 215, 254 and 280 nm. Preliminary adsorption–elution tests were done by injecting a 5-mL pulse of 15 g/L of DSM TensGuard™ hydrolysate dissolved in different buffers as the mobile phase during the adsorption step. The desorption step was then done by making a gradient, at the same time gradually decreasing the salt concentration.

**Peptide separation**

Peptide separation with activated carbon was done on an ÄKTA purifier system with a flow rate of 1 mL/min. The column was first equilibrated with 10 mM
Recovery of a bioactive tripeptide from a crude hydrolysate using activated carbon

Na$_2$HPO$_4$ at pH 7.5 with 0.75 M (NH$_4$)$_2$SO$_4$ for approximately 5 column volumes (CV) before injection. Then, 5 mL of a 15 g/L hydrolysate solution was injected; 14 CV after injection, two consecutive elution gradients were applied to progressively induce peptide separation. The gradients were applied by switching from 0–33% and from 33–50% of ethanol with gradient lengths of 6 and 11 CV. The column was then cleaned for another 11 CV with 50% ethanol. After every cycle, a washing step was performed with 10 CV of peptide-free buffer solution. A UV detector at a wavelength of 280 nm was used to monitor the eluting peptides. Several 1-mL fractions were collected during some of the cycles to analyze the adsorption and elution peaks observed in the ÄKTA elution profile for each component using LC and LC–MS. The enrichment and peptide separation steps and the measured conductivity and concentrations of ethanol used are shown in Figure 2.1.

![Figure 2.1](image-url)

**Figure 2.1:** Final enrichment method including adsorption, elution and equilibration steps showing the overall UV signal of the peptides (solid line), conductivity (dotted line) and the percentage of ethanol used (dashed line) observed in the ÄKTA system.
2.2.6 Process evaluation

The adsorption yield of a component $i$ from the mixture ($Y_{ads,i}$) was defined as the ratio of the mass adsorbed (in kg) divided by the mass present in the feed loaded during the adsorption (in kg). The desorption yield of component $i$ ($Y_{des,i}$) was obtained by dividing the total mass eluted during desorption (in kg) by the mass adsorbed (in kg). The total process yield ($Y_i$) of one cycle was calculated by multiplying the adsorption and desorption yields. The purity of IPP (PurityIPP) was calculated from the mass of IPP desorbed divided by the sum of the mass of all the components.

\[
Y_{ads,i} = \frac{m_{ads,i}}{m_{feed,i}} \quad (2.1)
\]

\[
Y_{des,i} = \frac{m_{des,i}}{m_{ads,i}} \quad (2.2)
\]

\[
Y_i = Y_{ads,i} \cdot Y_{des,i} \quad (2.3)
\]

\[
Purity_{IPP} = \frac{m_{des,IPP}}{\sum_{i=n} m_{des,i}} \quad (2.4)
\]

where $n$ is the total number of peptides and $m_{feed,i}$, $m_{ads,i}$ and $m_{des,i}$ are the feed, adsorbed and desorbed masses (in kg), respectively, of peptide $i$. The masses to obtain the yields were calculated from the peaks obtained during the peptide separation process (presented in section 2.2.5) monitored by fraction collection and the LC–MS analysis results: $m_{ads,i}$ by subtracting the area of the peak eluted during the adsorption step to $m_{feed,i}$ and $m_{des,i}$ from the area under the desorption peak. The masses to estimate the purity were calculated from the process yield of every peptide and by multiplication with the mass feed of each component obtained according to section 2.2.4 – Relative peptide quantification. The masses for the yield calculation were obtained in extracted ion intensity units from the MS results, whereas the masses to obtain the purity were in grams. This procedure has been performed assuming that the peak intensity is proportional to the peptide concentration in the sample as in comparative LC–MS [58]. It
should be noted that purity calculations are done for a proof of concept and not to obtain an exact value.

2.3 Results and discussion

The essentials of a separation process based on HIC, using an activated carbon packed bed together with analytical techniques, are presented in section 2.3.1. In section 2.3.2, the column exhaustion mechanisms are described by observing the changes in yield and selectivity after several adsorption–desorption cycles. IPP enrichment and the process yield are evaluated in section 2.4.

2.3.1 Development of a selective adsorptive process using activated carbon

Analysis of the hydrolysate

The tripeptide IPP was enriched from a hydrolysate containing IPP. A reverse-phase HPLC column was used together with MS for peptide identification. Because of the limited availability of proper standards, we only quantified IPP. For all the other peptides, only absorption unit based concentrations (in mAU) could be obtained and relative concentrations assessed, but this was sufficient to evaluate the yields of all the components. IPP was quantified (in mg/mL) after calibration using a pure standard. Table 2.2 shows the properties of the peptides identified in the hydrolysate after LC–MS. The HPLC chromatograms from the hydrolysate are shown in Figure 2.2a and the corresponding LC–MS measurements are presented in Figure 2.2b and c. The LC chromatogram in Figure 2.2a shows a pattern that is comparable with the LC–MS chromatogram in Figure 2.2b. Figure 2.2c shows the unresolved coelution conflicts for IPP with IP, KTEIP, ATLE and TIASGEP, grouped as CoePep1 (first coeluting peptides), which form the shoulder of the peak in Figure 2.2a. The IPP concentration was calculated from the IPP peak shown in Figure 2.2a by subtracting the shoulder corresponding to CoePep1. With the resulting HPLC method, the total amount of IPP was underestimated by about 17% when compared with the corresponding LC–MS method. Because of their
Figure 2.2: Comparison of the chromatograms of the hydrolysate obtained with (a) HPLC, (b) LC–MS, and (c) enlargement of the coelution effects shown in (b). Legends show peptides in elution order. Details on identified peptides through MS masses are presented in appendix A.1.1.
Recovery of a bioactive tripeptide from a crude hydrolysate using activated carbon

**Table 2.2:** Properties from the peptides present in the hydrolysate (shown in the order of elution from the HPLC chromatogram).

<table>
<thead>
<tr>
<th>Peptides</th>
<th>Mean hydrophobic moment*</th>
<th>Mean hydrophobicity*</th>
<th>Molecular weight (g/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MA</td>
<td>1.18</td>
<td>1.85</td>
<td>220</td>
</tr>
<tr>
<td>SGEP</td>
<td>0.7</td>
<td>-1.57</td>
<td>388</td>
</tr>
<tr>
<td>TSTP</td>
<td>0.27</td>
<td>-0.95</td>
<td>404</td>
</tr>
<tr>
<td>KKNQDKTEIP</td>
<td>0.26</td>
<td>-2.35</td>
<td>1200</td>
</tr>
<tr>
<td>IPP</td>
<td>2.12</td>
<td>0.43</td>
<td>325</td>
</tr>
<tr>
<td>IP</td>
<td>2.51</td>
<td>1.45</td>
<td>228</td>
</tr>
<tr>
<td>ATLE</td>
<td>0.88</td>
<td>0.35</td>
<td>432</td>
</tr>
<tr>
<td>KTEIP</td>
<td>0.89</td>
<td>-1.03</td>
<td>587</td>
</tr>
<tr>
<td>TIASGEP</td>
<td>0.48</td>
<td>-0.1</td>
<td>674</td>
</tr>
<tr>
<td>TINTIA</td>
<td>1.81</td>
<td>0.98</td>
<td>632</td>
</tr>
<tr>
<td>EVIESPP</td>
<td>1.47</td>
<td>-0.32</td>
<td>770</td>
</tr>
<tr>
<td>TINTIASGEP</td>
<td>0.89</td>
<td>-0.04</td>
<td>1002</td>
</tr>
<tr>
<td>EINTVQQVSTAV</td>
<td>0.58</td>
<td>0.45</td>
<td>1162</td>
</tr>
</tbody>
</table>

* Calculated according to previous research [59].

noticeable and distinguishable presence in the chromatograms, in further research, we monitored the peaks corresponding to the peptides KKNQDKTEIP, IPP and another set of coeluting peptides named CoePep2 including TINTIA, EVIESPP and TINTIASGEP. The MS results were also used to confirm and analyze some of the cycles with the activated carbon column in more detail.

**HIC using activated carbon**

The target peptide IPP has a relatively high hydrophobicity: the second highest mean hydrophobic moment and the fourth highest mean hydrophobicity (Table 2.2). Therefore, its interaction with the activated carbon could be enhanced to a greater extent by reducing its solubility using (NH₄)₂SO₄ because it ranks high in the Hofmeister series [60]. Other salts like NaCl and Na₂SO₄ were also tested during explorative adsorptive experiments. Areas from the ÄKTA curves confirmed a higher overall adsorptive capacity when using (NH₄)₂SO₄. Solubility tests were done to ensure high peptide solubility comparable with the initial sample. The pH chosen was 7.5 because a range between 6.5 and 8 provides the highest dynamic binding capacity when adsorbing proteins with salts on a hydrophobic stationary phase [61].

To enhance the difference in polarities between peptides during the desorption step, an elution gradient was performed with a simultaneous
decrease in the salt concentration and increase in the ethanol concentration, as indicated in Figure 2.1. A decrease in the elution gradient slope was done to improve the resolution of the separation.

Figure 2.3 depicts the elution peaks during the adsorption and desorption steps for the different peptides. During the adsorption step, IPP is barely eluted and it seems there is a size exclusion effect: the biggest peptide EINTVQVTSTAV (1162 g/mol) was eluted directly, and KKNQDKTEIP (1200 g/mol) was partly eluted. Thus, the adsorption step already gives some resolution, i.e. between the largest peptides and the others. The subsequent desorption step then yields additional resolution: enrichment can be obtained by isolating the volume on the right-hand side of the first elution peaks (EINTVQVTSTAV, SGEP, EVIESPP and IP); i.e. the eluate after an elution volume of 60–65 mL.

![Figure 2.3: Relative peptide concentration during adsorption (0–18 mL) and desorption (50–100 mL). Data obtained via MS analysis from the 3rd adsorptive–desorptive cycle applied using the hydrolysate on the activated carbon.](image-url)
2.3.2 Multiple adsorption cycles

It is known that adsorption and desorption on activated carbon changes over the life time of the activated carbon. To understand and describe this, consecutive adsorptive–desorptive cycles were performed until a steady behavior was observed. The adsorption and desorption yields are given for some of the peptides in Figure 2.4 for the different cycles for which only HPLC measurements are considered. Only peptides showing a higher signal in the chromatograms are monitored clustering the coeluting peptides EVIESP, TINTIA and TINTIASGEP as shown in Figure 2.2b.

![Graph a](image1)
![Graph b](image2)

**Figure 2.4:** Yields of (a) adsorption and (b) desorption obtained from the HPLC data for IPP, KKNQDKTEIP and (EVIESPP + TINTIA + TINTIASGEP) along the 95 cycles applied to the column.
For the adsorption yield (Figure 2.4a), exhaustion was observed as a linear decrease ($R^2 = 0.86–0.97$) for the first 8 cycles for all the peptides monitored, followed by a power law decrease ($R^2 = 0.72–0.88$) of similar exponents -0.72 and -0.75 for the bigger peptides KKNQDKTEIP and (EVIESPP + TINTIA + TINTIASGEP), respectively, and a smaller exponent -0.5 for the smaller peptide, IPP, that can be considered almost linear ($R^2 = 0.87$). The desorption yield as shown in Figure 2.4b first increases and then after a sharp peak at 5–10 cycles, slowly decreases again. The increase in desorption yield coincides with the initial linear decrease in the adsorption yield mentioned earlier. The low desorption yield at the beginning of the process can be explained by the presence of irreversible binding sites that may be associated with the micropores; micropores feature a greater number of contact points between the peptides and the pore walls giving a much higher binding energy [62]. It is then not surprising that (micro) pores become irreversibly blocked or obstructed. The filling of these pores leads to a decreasing adsorption yield (irreversible sites are already occupied), whereas the desorption yield increases (irreversible sites are not used anymore). Further decrease of the adsorption yield reveals that the sites with the strongest interaction slowly become permanently occupied, and the sites with less interaction do not bind all of the peptides.

The decreasing desorption yield may be related to steric hindrance as a result of blockage of mesopores and interparticle spaces; as more and more peptides become adsorbed, the bed porosity is constricted, which makes desorption of the peptides that are adsorbed deeper inside the pore impossible.

Exhaustion of the activated carbon is different for different peptides. After 8 cycles, the adsorption yield of the larger peptides (KKNQDKTEIP, EVIESPP, TINTIA and TINTIASGEP) decreased faster than the adsorption yield of IPP. The adsorption yield of the large peptides decreased down to 20% after 50 cycles, whereas for di- and tripeptides (such as IPP), this value was not reached even after 95 cycles (Figure 2.4a). Thus, peptide size is a major parameter in the rate of exhaustion. To obtain more details, LC–MS analysis was performed after 3, 27 and 66 cycles (Figure 2.5 and appendix A.1.2). The smaller peptides, MA, IP and IPP, which are also more hydrophobic according to Table 2.2, had the highest adsorption yields even after 27 and 66 cycles. The gradual peptide
saturation might be due to the size exclusion effect caused by the hierarchical pore structure of the activated carbon used (Table 2.1). Several references report the important influence of the adsorbent pore size distribution on steps in the adsorption process [62–64]. Figure 2.5 shows the adsorption and desorption yield as a function of molecular weight for three of the cycles. Only peptides showing a higher signal in the chromatograms are monitored clustering the coeluting peptides EVIESP, TINTIA and TINTIASGEP as shown in Figure 2.2b. At the beginning of the bed’s life, there is a slight linear decrease ($R^2 = 0.96$) up to 632 g/mol, which is more pronounced at higher molecular weights. Toward the end of the bed’s life, there is again a linear but steeper decrease up to

![Figure 2.5: Adsorption and desorption yields as a function of molecular weight for all the peptides present in the mixture for the 3rd, 27th and 66th cycles.](image)
around 400 g/mol. This shows that there is a growing hindrance or saturation preventing the molecules from being adsorbed. This progressive exclusion can be explained by the progressive blockage and saturation of the smaller pores in the first cycles and a gradual blockage and saturation of the bigger ones. Pores might be progressively constricted by aggregating peptides and peptides occupying the surface tending to full obstruction and saturation. Consequently, this effect reduces the binding of smaller peptides with increasing cycles decreasing the desorption yield as shown in Figure 2.4b and a power law decrease for the bigger peptides, which becomes linear for the smaller peptides (e.g. IPP). This constriction effect produced in the biggest pores, and later in the interparticle spaces, is confirmed with an almost linear increase in the maximum pressure drop ($R^2 = 0.8$) observed along the different cycles.

The fact that many peptides seem to be bound irreversibly indicates that the interactions are too strong. In fact, when using HIC to separate relatively high hydrophobic substances, a less hydrophobic resin is required [60]. The irreversible binding might then be reduced with the use of a more hydrophilic activated carbon, or a more hydrophobic mobile phase, e.g. adding ethanol. In this case, the overall adsorptive capacity would be reduced, which might provide even more selectivity for the more hydrophobic components such as IPP. Furthermore, activated carbon without microporosity would lower the irreversibility of the binding and increase the yield during elution, maintaining a high adsorption and process yield along consecutive cycles.

### 2.4 Evaluation of the enrichment process

The results in the previous sections suggest that the fractionation process developed in this study possesses selectivity and therefore shows potential for the enrichment of single peptides. We focus on the enrichment of IPP through the evolution of the purity along different cycles and fractions.

The total eluate was divided into two fractions based on their retention times, one rich and one poor in IPP; the fraction poor in IPP elutes first. We used four different criteria to enrich the fraction rich in IPP: I, pooling 56.5–100 mL; II, 59.5–100 mL; III, 62.5–100 mL; and IV, 65.5–100 mL. The process yield is
compared in Figure 2.6 for the different peptides using the total elution peak and fractions I and IV for the third cycle. An overview of IPP purity as a function of the IPP process yields for the different combinations of fractions and cycles analyzed with the detailed results of LC–MS is presented in Figure 2.7. For all cycles, the later the cut-off chosen, the richer the later fraction was in IPP (Figure 2.6 and Figure 2.7). When looking at one cycle in detail (Figure 2.6) and fraction IV, all yields decreased considerably by up to 64% but the decrease for IPP was only 24%. Therefore, the IPP yield (60%) was larger than the yield of the non-target peptides (2–51%) with a consequent increase in purity, twofold from the initial sample taking into account fraction IV (Figure 2.6 and Figure 2.7).

In all cases, the overall process yield is reduced when the purity increases. Thus, we have the classic trade-off between purity and yield.

The current work was performed to obtain insight into the phenomena occurring during multiple cycle adsorption of a complex peptide mixture. Although it does not yet yield a complete process design, it provides some

![Figure 2.6: Process yield of the IPP-enriched fraction before and after fractionation on the desorptive peak of the 3rd process cycle and the collection of fractions I and IV.](image-url)
Figure 2.7: Purity vs IPP process yield for cycles 3, 27 and 66, on the activated carbon packed bed.

Information. The solvent, elution gradient, residence time and porous structure must be carefully chosen in further studies on the overall design in order to increase yields and purities.

2.5 Conclusions

The adsorption and desorption of peptides from a crude mixture on activated carbon has been studied. The adsorption yield was found to decrease monotonously with repeated cycles, whereas the desorption yield increased over the first 8 cycles and then slowly decreased with additional cycles.

The decrease in adsorption and desorption yields was shown to be strongly dependent on the molecular weight of the adsorbing peptide, which indicates that size exclusion is important, along with the hydrophobic interaction.

The purity of IPP was increased even more by splitting the eluate into just two fractions; two times the purity of the initial sample was achieved. Tuning the interaction with the activated carbon by changing the hydrophobicity of the solution or that of the activated carbon will very likely result in even greater improvements in the purity and the yield.
Overall, activated carbon was shown to be a cost-effective stationary phase that may be used in the production of enriched peptide products, e.g. from crude hydrolyzates, without any need for prior purification or processing of the hydrolysate, apart from filtration to remove particulates.

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Channeled monoliths for selective recovery of a lacto-tripeptide from a crude hydrolyzate
Abstract

Adsorption processes using a packed bed reactor are often used for the purification of neutraceuticals. However, when dealing with untreated streams, this leads to fouling and consequent increase in pressure drop. This work compares the use of channeled monoliths, "honeycomb" structures, with a packed bed, both made of the same type of activated carbon. The intrinsic permeability and performance of both structures during the adsorption of a bioactive peptide from a crude hydrolyzate were studied. Breakthrough experiments were performed on both types of systems under similar conditions. The results showed similar productivity and dynamic adsorptive capacity for both structures at comparable linear velocity and residence time, but the packed bed showed a strong pressure drop increase during column saturation, revealing plugging of the column, especially at high velocities (short residence times). The channeled monoliths did not present any significant pressure drop increase, and were able to operate at high velocities which increases the range of application for these types of processes.
3.1 Introduction

The isolation of specific, high-value components from food raw materials has been shown to be an excellent way to enhance the overall value of the materials. Examples are the isolation of lactoferrin and lactoperoxidase from milk [40], but also the separation of oligopeptides from the same source or from fermentation broths [2, 65, 66]. Prior to a chromatographic purification step, process streams generally require extensive pretreatment, such as centrifugation, filtration or a combination of both, to clarify, remove particulate material and concentrate the diluted streams. These pretreatments help to maintain the state and performance of chromatographic resins but lead to serious product losses (yield and activity of bioactive components), longer process times, and higher operational costs and capital investments.

A simpler isolation process from an untreated feedstock would adapt the capturing step such that it would not necessitate the pretreatment. This has been done in the past by using adsorption in stirred-tank processes, or with fluidized or expanded-bed chromatography [37, 67]. Chromatographic membranes give the possibility of a combination of unit operations by for instance in-situ hydrolysis and selective separation of peptides from the reacting mixture [68, 69]. Recent literature reports on the purification of crude streams using packed bed chromatography, such as the adsorption of high-value milk proteins from raw milk using cation exchange resins [40, 41] and from whey using immobilized affinity ligands on the resin [70]. However, processes involving packed beds are still restricted to certain column volumes, flow rates and factors like temperature which in turn affect the pressure drop [40]. The column is often limited to certain column volumes due to fouling, which affects the chromatographic columns by (1) particle pore blockage resulting in the reduction of the dynamic binding capacity [42] and by (2) foulants obstructing the interparticle-void volume (plugging) resulting in pressure build-up over the column. These phenomena limit the matrix lifetime with a decrease in capacity and yield over consecutive cycles [42, 71]. Bed obstruction leads to a pressure drop build-up produced by the reduction in permeability, which in turn can also lead to an additional bed compression [72] and thus even lower permeability.
This increase in pressure drop limits the range of feasible flow rates and stream viscosity and hence reduces the range of applications of packed beds.

Due to their improved balance between mass transfer and column permeability, channeled monoliths could reduce problems related to pressure drop and inter-particle accumulation. Polymeric monolith columns were used for the purification of a component from a yeast homogenate and exhibited a higher dynamic capacity than a packed bed [73]. Generally, structures with a higher porosity or even higher permeability could be less susceptible to fouling and decrease plugging problems, without a compromise in throughput, yield and productivity. These highly permeable structures with similar performance have already been suggested in chapter 5 and [73] for separations where pressure drop or fouling present a bottleneck. However, these one-piece material structures have limited adsorptive capacity for small molecules [74]. Activated carbon is a relatively low cost material with a high surface area for small (food) molecules and it was shown in chapter 2 [71] to be a potential material in chromatographic applications where typically resins are used. Besides, some researchers have already studied the use of channeled monoliths [22, 75]. The low pressure drop that can be attained with these systems has been highlighted previously in other applications, such as in heterogeneous catalysis and in gas phase reactions [21, 22, 76]. There is not a great variety of channeled monoliths, but they can be found commercially as a Cordierite substrate that can be coated, or made of activated carbon [77]. All these considerations point to (activated carbon) monoliths as a candidate for the separation of food streams.

In this chapter we assess the feasibility of channeled monoliths as an alternative to packed beds in the purification of crude hydrolyzates. We compare the intrinsic permeability, performance and plugging behavior of an activated carbon channeled monolith with an activated carbon packed bed. We first measure the permeability of these structures comparing them with established empirical equations from literature. We then use a challenging separation prone to fouling, the partial-purification of a peptide from a crude hydrolyzate developed in chapter 2 [71], to compare both structures. The comparison is based on: (1) dynamic binding capacity, productivity, and (2)
plugging behavior. The susceptibility to plugging in both structures is evaluated by using an analogy with fouling in modeled parallel channels and fouling in a membrane with permeate flow.

3.2 Theory

3.2.1 Packing pressure drop and permeability

The pressure drop in packed beds can generally be modeled by Darcy’s law under laminar flow assuming rigid particles or by the Ergun equation:

\[
\frac{\Delta P}{L} = \frac{150\mu(1 - \epsilon_b)^2 u_0}{\epsilon_b d_p^2} + \frac{1.75\rho(1 - \epsilon_b)u_0^2}{\epsilon_b d_p} \tag{3.1}
\]

The Karman–Cozeny equation (left-hand term of equation (3.1)) is also commonly used to derive and estimate the hydraulic permeability, \(B_0\), of the bed proportional to the squared of the diameter [78], as follows:

\[
B_0 = \frac{\epsilon_b^3}{150(1 - \epsilon_b)^2} \cdot d_p^2 \tag{3.2}
\]

This expression allows the calculation of an adimensional specific permeability \((B_0/d_p^2)\) which is useful to compare permeabilities of differently sized packed particles and monoliths [79].

For squared channeled monoliths, the pressure drop can be predicted using the expression from Patton et al. [23] as follows:

\[
\frac{\Delta P}{L} = \frac{2f Re \mu u}{d_p^2} = \frac{28.4\mu u_0}{a^2\epsilon_b} \tag{3.3}
\]

where \(f\) is the Fanning friction factor, and \(f \cdot Re\) corresponds to the friction in the channels, which is equal to 14.2 for square channels.

The hydraulic permeability can then be derived from the following expression (see appendix A.2.1 for the derivation):

\[
B_0 = \frac{\epsilon_b^2}{28.4(1 - \sqrt{\epsilon_b})^2} \cdot (2\delta)^2 \tag{3.4}
\]

where \(\delta\) is the characteristic length (half wall thickness) of the channeled monolith.
3.2.2 Description of column fouling in time

Analogy with parallel channels

Fouling in a chromatographic adsorbent may be modeled assuming that it consists of parallel cylindrical channels. In a packed bed the interparticle space representing the constriction to the flow is modeled by an initial channel radius $R_0$. In a channeled monolith the analogy is more straightforward as the structure does consist of a set of parallel channels.

Assuming that the liquid contains a component that tends to foul the adsorbent, it is possible to describe the fouling behavior by the decrease of the internal radius due to increase of the fouling layer as shown in Figure 3.1. This decrease can be modeled by equation (3.5).

\[
\frac{dR}{dt} = -\frac{kc}{\rho}
\]  

(3.5)

Figure 3.1: Representation of the fouling using the channel model analogy.
This relation makes use of an overall mass transfer rate coefficient \( k \) that is dependent on the predominant mass transfer mechanism. In the case of a reaction limitation, \( k \) would be a constant; in the case of mass transfer limitation \( k \) would be a function of the radius. For example, for fully developed flow through a channel, the Sherwood number would be constant, implying that \( \frac{2kR}{D} = Sh \) or \( k = \frac{k_R}{R} \) with \( k_R \) a constant, or \( k = \frac{k_R}{R^2} \) if the film mass transfer resistance would be predominant. Therefore, we have modeled the general case where \( k = \frac{k_R}{R^n} \).

Using the Hagen–Poiseuille equation for pipes and replacing the radius by the time dependent radius derived in equation (3.5), it is possible to derive an expression for \( \Delta P / \Delta P_0 \) as a function of time for the case of reaction limitation, mass transfer limitation or the general case. Therefore, this gives a general equation that describes the fouling behavior (equation (3.6)) which derivation is shown in appendix A.2.2.

\[
\frac{\Delta P}{\Delta P_0} = \left( \frac{1}{1 - \frac{\tau}{\tau}} \right)^{K_0}
\]

where \( \tau \) indicates the total time (or, column volumes) that fluid can typically pass through the column, before total blockage occurs; the value of \( K_0 \) indicates the initial rate of fouling.

**Analogy with membranes**

Due to the lack of fouling models in chromatography we also made use of an analogy with membranes, where fouling is one of the most studied phenomena, such as cake layer formation, pore blocking and combined effects. Combined models describing a two-stage fouling mechanism have been gathered in [80]. For instance, the cake-complete model assumes that fouling occurs initially through complete pore blocking with consequent deposition of aggregates blocking larger areas of the membrane, and consequent cake formation. This would result in two resistances \( K_b \) and \( K_c \), related to the pore blocking and cake formation respectively, and thus an increase in pressure drop with time following equation (3.7).

\[
\frac{\Delta P}{\Delta P_0} = \frac{1}{1 - K_b t} \left( 1 - \frac{K_c J_0^2}{K_b} ln(1 - K_b t) \right)
\]
3.3 Materials and methods

3.3.1 Materials

A milk casein hydrolyzate was kindly donated by DSM Food Specialties (Delft, The Netherlands): the commercially available TensGuard™ S. A standard of IPP (Isoleucine–Proline–Proline), the target peptide in the crude hydrolyzate, was purchased from Bachem (Switzerland). Ammonium sulfate and acetic acid were obtained from Sigma-Aldrich (Germany). All the other chemicals were obtained from Merck (Germany).

TE7/20 mesoporous activated carbon particles from MAST Carbon International Ltd (UK), batch DEC 35, with a diameter range between 250–500 µm were purchased from MAST Carbon. Additionally, MAST Carbon kindly donated two types (with different cell density) of mesoporous activated carbon monoliths in various lengths. The monoliths were prepared from a mesoporous cake made from the same material TE7/20 as the activated carbon particles.

3.3.2 Preparation of solutions

The buffer for the peptide separation was prepared by mixing 10 mM of sodium phosphate with 0.75 M ammonium sulphate, adjusted to pH 7.5 with 1 M NaOH. The buffer was then filtered over a 0.22 µm Millipore NY20 nylon filter from Sartorius used under vacuum.

The 15 g/L peptide hydrolysate feed solution was prepared by dissolving the powder in the buffer. The solution was then filtered by means of a Sartorius Stedim Minisart high flow filter with pore size 0.45 µm.

3.3.3 Column packing

The packed bed was packed by slowly pouring the dry activated carbon particles into two different columns: a glass 5/200 Tricorn and a XK 16/20 with adjustable length, both from GE Healthcare (Uppsala, Sweden). To allow the particles to rearrange and settle a slight vibration was applied during the packing. Once packed, the columns were flushed first with a flow of Milli-Q in the up-flow
direction to remove air bubbles, followed by a down-flow at approximately 1 h. The flow rate of the down-flow was chosen according to the maximum pressure drop used in the following experiment.

The monolith was in-house wrapped and glued in a polyethylene tube equipped with connections compatible with ÄKTA fittings. The air in the column was removed by flushing the buffer in the up-flow direction.

### 3.3.4 Dead volume and porosity determination

The external porosity $\varepsilon_b$ of the packed bed, was obtained for newly packed columns once the particle density $\rho_p$ was known, by measuring the weight, $m_{\text{carbon}}$, and the length of the particulated packing and using the following expression as described in literature [81]:

$$\varepsilon_b = 1 - \left( \frac{m_{\text{carbon}}}{\rho_p} \right) V_c$$

(3.8)

The particle density is the mass of the adsorbent per volume (solid and internal pore volumes) of particle and can be obtained by comparing this expression to experimental results. The experimental values for the external porosity were obtained for 4 different flow rates by performing pulse experiments with injections of 33 mL of 1 g/L Blue Dextran analyzed in the UV and RI signals of the ÄKTA.

The monolith external channel voidage was measured using a microscope (Axiovert 200 MAT, Carl Zeiss GmbH, 2× magnification) and a camera (HS4, IDT Inc.) together with a scale placed at the distance of the monoliths surface. Channels dimensions and pixel-mm calibration were analyzed using ImageJ software.

To determine the internal porosity and surface area of the activated carbon particles and monolith, standard nitrogen adsorption isotherms were determined in an Autosorb 6 (Quantachrome) equipment at 77 K. Before the experiment, the samples were out-gassed at 523 K for 4 h under vacuum ($10^{-6}$ kPa). The specific surface area was obtained using the BET method. The micropore volume was deduced from the adsorption data by means of the Dubinin–Radushkevich
method [82]. Finally, the mesopore volume was estimated by subtracting the micropore volume from the total volume adsorbed at $p/p_o = 0.95$.

### 3.3.5 Stream characterization

Particle sizes of the filtered and non-filtered peptide mixtures were measured in a Malver Mastersizer and a Zetasizer (Malver Instruments Ltd, UK). The viscosity of the solutions was measured shearing the fluid between two concentric cylinders on a Physica MCR 301 rheometer at 25 °C.

### 3.3.6 Peptide identification: HPLC analysis

Samples were analyzed using an Ultimate 3000 UHPLC+ system from Thermo Fisher Scientific (Sunnyvale, USA) with an Atlantis dC 18 column (100 Å, 3 μm, 3.9×150 mm) following the same procedure as described previously in chapter 2 [71].

### 3.3.7 Peptide separation (fouling-inducing experiment) in the activated carbon structures

The peptide separation was performed as described for the activated carbon packed bed used in chapter 2 [71], with minor modifications. In this paper, the adsorptive step/fouling-inducing experiment was applied in two different ways: using a 5 mL injection of feed applied during consecutive runs (as in chapter 2 [71]) and applying the same feed until complete saturation using the second pump of the ÄKTA. Both types of experiments were performed on an ÄKTA purifier system with a flow rate calculated to have comparable residence time when using a different packed bed or channeled monoliths. The eluting IPP was monitored and quantified as in the original method after collecting 1 mL fractions.

### 3.3.8 Pressure drop measurements

Figure 3.2 illustrates the setup used for the three different type of pressure drop measurements performed. The relative pressure drop $\Delta P/\Delta P_0$ was obtained
Channeled monoliths for selective recovery of a lacto-tripeptide from a crude hydrolyzate

by monitoring the pressure in the ÄKTA purifier. The pressure was obtained by averaging the maximum pressure drop observed for each run during adsorption-desorption experiments (Figure 3.2a) and smoothed along the different column volumes during the continuous loading of the adsorbent (Figure 3.2b). In both experiments the measurements were repeated with an empty column, in order to account for a possible pressure build-up in the filters of the column or other elements from the setup. No increase in pressure drop was observed in the extra-column elements.

Pressure drop measurements as a function of flow rate (Figure 3.2c) were also performed by using Milli-Q water as mobile phase. Two different EL-PRESS pressure sensors from Bronkhorst High-Tech (The Netherlands) were used in this case. The sensors were calibrated with a maximum pressure drop of 1–5 bar
for the packed bed and 300 mbar for the channeled monoliths. For the packed bed measurements the adjustable XK 16/20 column was used. Prior to packing, the pressure drop on the empty GE column and tubing was measured for all the different flow rates. The channeled monoliths were simply connected to the pressure sensors using compatible in-house made fittings.

3.3.9 Process evaluation

The dynamic binding capacity (DBC) was used as measure of capacity and productivity, calculated from the collected samples as shown in Figure 3.3. The experimental data obtained by measurements on the collected data was fitted to sigmoidal (adsorption curves) and peak (desorption curves) functions, which were then used, as shown in Figure 3.3, to determine the adsorbed ($m_{ads}$) and desorbed ($m_{des}$) amounts of IPP (Isoleucine–Proline–Proline), and the times derived from the calculated volumes involved in the adsorption ($t_{ads,87\%} = V_{87\%}/Q$) and desorption ($t_{des} = (V_2 - V_1)/Q$) steps.

$$m_{ads} = C_o \cdot \frac{[V_B - (V_o + V_{ext})]}{A_1} \cdot [1 - A_1]$$

$$m_{des} = C_o \cdot \int_{V_1}^{V_2} \frac{C}{C_o} dV$$

Figure 3.3: Evaluation of adsorption (left) and desorption (right) during the process. Plotted curves represent the fit of the data obtained from the collected fractions.

The DBC at 87% of adsorptive breakthrough (we used 87% since in some cases 100% saturation was not reached due to the displacement of IPP towards
the other peptides as shown in appendix A.2.3):

\[ DBC_{87\%} = \frac{m_{ads,87\%}}{m_{carbon \ (or \ V_c)}} = \frac{C_0 \cdot \left[ V_{87\%} - (V_0 + V_{ext}) - \int_{V_0+V_{ext}}^{V_{87\%}} \frac{C}{C_0} \, dV \right]}{m_{carbon \ (or \ V_c)}} \]  

(3.9)

To calculate the productivity, we evaluated the cycle time for adsorption when about 87% of the breakthrough curve was reached \( (t_{87\%}) \). This was converted in the specific productivity through:

\[ Productivity_{ads,87\%} = \frac{DBC_{87\%}}{t_{ads,87\%}} \]  

(3.10)

If we take into account the recovery of the structure and therefore the amount desorbed \( (m_{des}) \) then the productivity becomes:

\[ Productivity_{des} = \frac{m_{des}}{(t_{ads,87\%} + t_{des}) \cdot m_{carbon \ (or \ V_c)}} \]  

(3.11)

### 3.4 Results and discussion

#### 3.4.1 Permeability of the structures

Figure 3.4 shows photographs of the two monoliths used in this study. Table 3.1 summarizes the dimensions for both channeled monoliths including the resulting cell density (in cells per square inch). We can see that the overall resulting bed

![Figure 3.4: Microscope photographs of the activated carbon monoliths used in this study with two different cell densities: a) 700 cpsi and b) 1600 cpsi.](image)
Table 3.1: Structure dimensions from the structures used in this study.

<table>
<thead>
<tr>
<th>Structure</th>
<th>a (µm)</th>
<th>2δ (t_w or d_p) (µm)</th>
<th>ε_b (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(700±60) cpsi monolith*</td>
<td>560±20</td>
<td>380±20</td>
<td>0.36±0.05</td>
</tr>
<tr>
<td>(1600±200) cpsi monolith*</td>
<td>360±20</td>
<td>280±20</td>
<td>0.31±0.08</td>
</tr>
<tr>
<td>TE7/20 Particles (packed bed)</td>
<td>–</td>
<td>323.8±0.1**</td>
<td>0.34–0.35±0.01***</td>
</tr>
</tbody>
</table>

*Obtained from microscope measurements.
**Volume weighted mean obtained from particle size distribution using Mastersizer.
***Obtained from the different packed beds column packings, by weight and length measurements and using equation (3.1).

Porosities from the two monoliths are comparable to the one of the packed bed, which facilitates the direct comparison between the structures.

Figure 3.5 displays the experimental and theoretical values of the pressure drop as a function of the superficial velocity ($v_{sup}$) for both structures using water as the mobile phase. The pressure drop in the packed bed increases strongly with $v_{sup}$ and is much higher than in the channeled monoliths even though the porosity and characteristic length ($δ$) are similar. As expected, the pressure drop when using a channeled monolith with a higher cell density (and hence narrower channels) also increases slightly due to the smaller hydraulic diameter and slightly smaller porosity. Unlike for the channeled monoliths that follow a
more or less linear increase of the pressure drop with the superficial velocity, for the packed bed there is a critical velocity (at about 0.0015 m/s) at which the pressure drop increases exponentially. This increase seems to be associated with bed compaction of the bed as described in [72]. Apart from this deviation from the Ergun equation, the result in both structures agrees with the theoretical predictions (equations (3.1) and (3.3)).

When using packed beds, smaller particle diameters result in a better performance at the expense of a lower permeability (equation (3.2)). Hence, a specific permeability (permeability divided by two times the characteristic length squared \((2\delta)^2\)) is necessary for a fair comparison between different adsorbents. Figure 3.6 compares the specific permeability of the channeled monoliths with packed beds and one-piece monoliths as a function of the flow porosity. In this graph we use the existing theoretical frame for particulated packed beds and monoliths. The experimental points were calculated from the experiments with TE7/20 activated carbon particles and channeled monoliths at two cells densities in cells per square inch (cpsi). The predictions on the gray area were gathered from [79], including the Karman–Cozeny equation for packed beds also presented in equation (3.2) and the prediction for channeled monoliths obtained through equation (3.4).

**Figure 3.6:** Prediction (lines) and experimental data from this work (dots) for the specific permeability of different chromatographic structures as a function of the flow porosity \(\varepsilon_c\). This graph is an extension to channeled monoliths of the theoretical frame already existing for particulated packed beds and monoliths. The experimental points were calculated from the experiments with TE7/20 activated carbon particles and channeled monoliths at two cells densities in cells per square inch (cpsi). The predictions on the gray area were gathered from [79], including the Karman–Cozeny equation for packed beds also presented in equation (3.2) and the prediction for channeled monoliths obtained through equation (3.4).
packed beds and (non-channeled) monolithic adsorbents [79] and extend it to our systems. The data points are experimental values from this study; the lines represent predictions from equation (3.3) for channeled monoliths and from [79] for the other structures. According to Figure 3.6, the specific permeability of channeled monoliths is around two orders of magnitude larger than that of packed beds at similar porosities and about 30–50 times larger than that of other previously reported monolithic adsorbents.

### 3.4.2 Capacity and productivity

Table 3.2 summarizes the results from the measurements of porosity and surface area obtained by performing the nitrogen adsorption isotherm. The BET surface area indicates how these structures compare in terms of static binding capacity. With the micro- and mesoporosity we can not only quantify the volume of the different hierarchy of pores where the molecules can be bound, but we can also help to predict the mass transfer hindrance in the adsorbent and thus the intraparticle mass transfer. The macro and mesoporosity are involved in the intraparticle mass transfer rate while the microporosity affects the capacity. According to Table 3.2 the activated carbon particles have a higher overall porosity (in cm$^3$/g) for the packed bed, a higher microporosity and a higher BET surface area than the channeled monolith. Although the total pore volume is different, the volume of mesopores is comparable (≈0.2 cm$^3$/g).

To compare the adsorptive performance of both activated carbon packed bed and channeled monoliths during the recovery of the lacto-tripeptide IPP, we monitored the concentration of this peptide during adsorption. Figure 3.7
describes the breakthrough of the target peptide, IPP, during the adsorption (Figure 3.7a) and the following elution peak (Figure 3.7b). Table 3.3 summarizes the capacities, yields, adsorption/desorption times and subsequent productivities. For comparison we have chosen to calculate the dynamic capacity at 87% breakthrough of IPP because of the flow through present in the channeled monolith (at \( t = 0 \), \( C/C_0 > 0.1 \)). The typical industrial limit of breakthrough times is set at 10% \( C/C_0 \) especially when the product purity is more important than the yield. However, here the 87% breakthrough limit was needed for a good comparison of the dynamic capacity.

\[
\begin{align*}
\text{Figure 3.7: Comparison of a) adsorption and b) desorption steps of IPP after the loading of the filtered TensGuard™ S hydrolyzate using a packed bed and the 1600 cpsi channeled monolith at the velocities and residence times presented in Table 3.3. Empty symbols represent the packed bed and full symbols represent the channeled monolith. The dotted lines represent the fittings for the packed bed and the continuous line, the fitting for the channeled monolith.}
\end{align*}
\]

Results in Figure 3.7 and Table 3.3 show that the 1600 cpsi monolith has a slightly smaller dynamic binding capacity (DBC_{87%}) than the packed bed, but it is still in the same range. This is related to a lower overall mass transfer rate and the lower surface area (in m\(^2\)/g) of the channeled monolith (Table 3.2). The relatively low residence time relative to the rate of mass transfer explains the non-adsorbed IPP that emerges even at the beginning of the adsorption step in the channeled monolith, as shown in Figure 3.7. To reduce this effect and to increase the dynamic binding capacity (at 10% or 90%) either lower flow rates...
Table 3.3: Process conditions and evaluation parameters at 87% breakthrough from the different structures.

<table>
<thead>
<tr>
<th>Structure</th>
<th>$u$ (cm/s)</th>
<th>$t_{res}$ (min)</th>
<th>$\text{DBC}_{0.87}$ (mg adsorbent/mL column)</th>
<th>$t_{0.87}$ (min)</th>
<th>$t_{des}$ (min)</th>
<th>Recovery ($%$)</th>
<th>Productivity$_{ads}$ (mg adsorbent/h mg adsorbent)</th>
<th>Productivity$_{des}$ (mg adsorbent/h mg adsorbent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Packed bed, D = 5 mm</td>
<td>860±20</td>
<td>68</td>
<td>20.3</td>
<td>4.9</td>
<td>52</td>
<td>95</td>
<td>37</td>
<td>19.4</td>
</tr>
<tr>
<td>Packed bed, D = 5 mm</td>
<td>890±30</td>
<td>64</td>
<td>24.8</td>
<td>3.3</td>
<td>33</td>
<td>94</td>
<td>64</td>
<td>13.9</td>
</tr>
<tr>
<td>Packed bed, D = 16 mm</td>
<td>760±20</td>
<td>48</td>
<td>21.0</td>
<td>4.1</td>
<td>2</td>
<td>–</td>
<td>–</td>
<td>510.4</td>
</tr>
<tr>
<td>1600 cpsi monolith</td>
<td>1000±300</td>
<td>37</td>
<td>9.0</td>
<td>2.5</td>
<td>12</td>
<td>69</td>
<td>9</td>
<td>43.4</td>
</tr>
</tbody>
</table>

*Elution results not shown due to column breakage for excessive pressure build-up.

or longer columns could have been used, or alternatively a number of monoliths in series.

### 3.4.3 Susceptibility to plugging

One of the problems in working with a packed bed is the tendency of the packing to compact or collapse increasing the back pressure irreversibly. Consequently, chromatographic operations need strong pumps and are limited to a certain number of column volumes, after which the resin needs to be cleaned, generally by cleaning-in-place procedures. Figure 3.4 already shows that increasing superficial velocities lead to a much stronger increase in pressure drop for the packed bed when using just water. Figure 3.8 shows the influence of the feed volume on the pressure drop for the filtered hydrolysate stream for the channeled monoliths and the packed bed. Two adsorptive fouling-induced experiments were performed as previously reported by Siu et al. [42] and depicted in Figure 3.2a and b: (1) a continuous column saturation (Figure 3.8a, b) and (2) consecutive adsorptive-desorptive cycles (Figure 3.8c). Figure 3.8c illustrates the maximum relative pressure drop observed during consecutive injections of hydrolyzate followed by ethanol gradients inducing adsorption and elution steps over the packed bed and monolith. The data presented in Figure 3.8 was fitted to the fouling analogies presented in section 2.2. The best fit was determined by minimizing and averaging the sum of squared residuals. Table 3.4 summarizes the model parameters and error fits to these analogies.

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Figure 3.8: Experimental and fitted results for the relative pressure drop as a function of the column volumes of feed in the activate carbon packed beds and monoliths. Data obtained during continuous loading of the feed hydrolyzate dissolved in the buffer at pH 7.5 and subsequent column saturation at a) low and b) high linear velocities, and c) after injections of 5 mL loaded to the column after consecutive adsorptive and desorptive cycles.

According to Figure 3.8 results from the continuous loading and consecutive experiments (Figure 3.2a and b) show a higher increase in pressure drop in the packed bed compared to the increase pressure drop in the channeled monoliths that appeared to be negligible.

According to the results in Figure 3.8 and Table 3.4, the results seemed to agree well with the combined fouling model made for fouling in membranes (equation (3.7)). This model describes fouling as a two-step mechanism, in our case: pore blockage and plugging (cake formation in membranes). The high
values of $K_b$ and $K_c$ from the membrane analogy (Table 3.4) in the case of thepacked bed suggest that both mechanisms occur in the packed bed. Thus, apartfrom pore blockage there is a consequent interparticle blockage in the packedbed presenting resistance, similarly as a cake building in a membrane probablyrelated to the formation of bridges of peptide aggregates. In fact, the increaseof pressure drop in the packed bed presents two different trends (Figure 3.8b).The first trend (0 to 64 CV) coincides with the saturation of the bed with IPP(Figure 3.7a) and thus is due to the increased concentration (and hence viscosity)of the liquid phase flowing through the packed bed. The second increase (from64 to 100 CV) might be linked to a decrease in porosity [42, 71] and consequentpermeability drop.

In order to fit the analogy to parallel channels (equation (3.6)), a dynamiccorrection factor $\beta(t)$ was added (equation (3.12)) to account for the steeperincrease in pressure drop during the adsorption.

$$\frac{\Delta P}{\Delta P_0} = \left(\frac{1}{1 - \frac{t}{\tau}}\right)^{K_0 \tau} + \beta(t) \quad (3.12)$$

This steep increase in pressure drop seems to be caused by compression orhindrance in the bed coinciding with the increase in viscosity of the solutionduring saturation of the packed bed. However, this correction factor cannotbe predicted \textit{a priori} and deserves further study (e.g. calculation of a dynamicviscosity $\mu(t)$). Assuming that this correction factor does not have an influence
after the full breakthrough is reached \((C/C_0 \approx 1)\), we have fitted the model to the second part of the curve in order to predict the time of collapsing of the bed and be able to compare with the channeled monolith.

The fitted results from the analogy with parallel channels presented in Table 3.3 confirm that the time that the channeled monoliths would need to collapse is several orders of magnitude larger than the one of the packed bed. Besides, in the case of the packed bed there is a dependency of \(\tau\) with the superficial velocity, so the higher the velocity the faster the column gets blocked and the lower the value of \(\tau\) is. Figure 3.8 shows that this blockage does not happen in the channeled monolith even at very high linear velocities and after different adsorptive and desorptive cycles. This advantage of the channeled monoliths is most likely associated with the shear induced by convection of the flow (higher velocity) in the channel. As a result, the channeled monoliths are less susceptible to fouling, especially at higher flow rates. This resistance to plugging in the monolith can be associated to the larger permeability of these structures as discussed in section 3.4.1. This shows the potential of (channeled) monoliths in the separation of hydrolyzates and in applications where the life of the bed due to fouling presents and issue.

### 3.4.4 Process design considerations

In order to design a capturing process from a fouling-inducing stream, such as a peptide hydrolyzate, a comparative design assessment is needed. As already mentioned, the main limitation of this kind of processes is the maximum pressure drop allowed which should be the main constraint and criterion in both optimization and adsorbent selection. Therefore, we need to use the relations for the pressure drop and fouling susceptibility presented in earlier sections which are both dependent on the flow rate.

Figure 3.9 shows a summary of the new considerations developed here for adsorbent screening and process design. Assuming that a specific selectivity is required, the constraint is the maximum allowed pressure drop. The semi-empirical parameters \(\tau\) and \(K_0\), or \(K_b\) and \(K_c\) can be obtained from small-scale experiments, which can then be used to predict the pressure drop increase (equations (3.6) and (3.7)) and thus, restrict the flow rate and the length of
the column combining equations (3.1)–(3.4) with (3.6) and (3.7). With the flow rate and the column length it is then possible to obtain the corresponding residence time and calculate the specific productivity through an empirical model relation of productivity vs residence time. Once the productivity is obtained for the different structures, it is possible to calculate the overall costs and profits.

In section 3.4.2, we have seen that kinetic (diffusive) limitation to the adsorption can be an issue in the case of the monolith due to the rather fast flow and hence short residence times. However, due to the lower pressure drop restriction in channeled monoliths one can use longer columns which would increase the residence time and consequently decrease the flow through. In any case, the kinetic limitations of the channeled monoliths should be improved as much as possible by for instance using a higher intra adsorbent porosity or by using a small adsorbent thickness as suggested in [83] and chapter 5. In fact, in channeled monoliths the pressure drop decreases with decreasing wall thickness whereas in the packed bed the pressure drop increases with decreasing particle size. If particle size is smaller the constriction is bigger and therefore the radius $R_0$ of an equivalent cylindrical channel would be smaller. This reduces the time
to total blockage of the column, $\tau$, and decreases the column volumes that can pass through the column before reaching a certain pressure drop.

### 3.5 Conclusions

The potential of channeled monoliths in liquid chromatographic operations was assessed. We compared the plugging behavior of the commercially available channeled monoliths with that of conventional packed beds, taking into consideration their productivity and capacity. This was done using the isolation of a bioactive peptide from a crude hydrolyzate as example in casu. It was possible to extend the window of possible operating chromatographic structures at different functional permeabilities and porosities. Channeled monoliths are highly permeable, even at low porosities, with respect to packed beds and other monoliths already used in liquid chromatography.

Breakthrough curves of the target peptide in the hydrolysate showed that the monoliths used present a lower capacity and partial adsorption, slightly higher in the packed bed however having a resulting similar adsorptive productivity. Furthermore this type of monoliths shows a much lower susceptibility for plugging when fitting the evolution of the pressure drop to a semi-empirical fouling model. These results suggest the possibility for the channeled monoliths for treating unfiltered hydrolyzates loaded at higher flow rates.

Overall this study shows the potential advantages of channeled monoliths in the capturing and purification step of a relatively small component (in this case a peptide) from a hydrolyzate. Furthermore it suggests the incorporation of fouling and plugging considerations in the selection of adsorbents for industrial liquid chromatography.

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The Netherlands), Cosun Food Technology Centre (Roosendaal, The Netherlands) and DSM (The Netherlands), for their financial support and interest in this project. The authors also would like to thank DSM for providing with the hydrolyzates, MAST Carbon International Ltd (UK) for their donated carbon monoliths, the group of Catalysis Engineering (TU Delft) for their facilities and help to determine the N$_2$ isotherms and Nynke Draijer for technical support.
Lactose adsorption onto activated carbon beads and monoliths

This chapter has been submitted as:
Abstract

To study and compare the predictions from packed beds together with channeled monoliths, the adsorption of lactose onto an activated carbon packed bed and analogous squared channeled monolith has been modeled. A detailed chromatographic model was used taking internal and external mass transfer resistances into account. First, the single component adsorption isotherm parameters were obtained using frontal analysis on both adsorbents. Second, the kinetics of adsorption of lactose on both activated carbon adsorbents were estimated using the shallow bed method assuming an infinite bath. The uptake curves were fitted to the homogeneous surface diffusion model and linear driving force approximation. An intraparticle diffusion coefficient and the film mass transfer coefficient were then estimated numerically. Finally, the breakthrough curve data was fitted to the general rate model describing the full column operation. Both structures have a similar intraparticle mass transfer performance during the uptake adsorptive process; differences in performance during the overall column operation were mainly due to the higher axial dispersion in the squared channeled monoliths. Axial dispersion could be reduced by choosing a different channel geometry closer to circular.
4.1 Introduction

The increasing demand of neutraceuticals and the necessity of waste stream valorization present new challenges for the food industry in adsorbent development for the purification of small components. The conventional packed bed presents severe drawbacks at large-scale applications with unpolished streams in the food industry: large pressure drop, column plugging (chapter 3) and uneconomic processes and equipment. However, in other fields, research has been performed on alternative adsorbents to overcome the bottlenecks of particulated adsorbents. For gas separation and catalysis, structures such as parallel channeled monoliths (or ”honeycomb”-like structures), foams, fabric adsorbents and laminates were reviewed [21] and modeled [23, 24, 49, 75]. Polymeric monoliths have received much attention in the pharmaceutical field [31] for separating biomolecules with high molecular weight, and in the analytical field with polymeric [84] and silica monolithic columns [33], mainly for the separation of low molecular weight components. Attention for the recovery of valuable components from side streams has risen in the last years, and this has brought interest for new adsorbents. The drawbacks of the purification with the packed bed have been overcome with the use of membrane chromatography [85, 86] using functionalized membranes or mixed matrix membranes [87–89]; however they suffer from low capacities and high costs.

Apart from some work on the separation of sugars with supported liquid membranes [90–92] and some small-scale analytical work, there is not much research on new adsorbents for the large scale adsorptive separation of small food ingredients. Besides, to compare fairly different structured adsorbents for a certain application, an effort needs to be made to have the same interaction, porosity and surface area. To perform such comparisons there are two options: via coatings of the different structural systems or integrally manufacture the adsorbents with equivalent material. The first option has the drawback that only the volume of the coating is functional; the rest of the scaffold is inert and does not contribute to the separation or the capacity. Integral adsorbents are preferred but are only possible with relatively low-cost adsorbent material with good mechanical structural properties.
Activated carbon is a low-cost material that is available on large scales. While the surface interaction is mainly based on hydrophobic interactions, this can be modified to some degree, either by varying the starting material to be carbonized, or by chemical modification afterwards, e.g. by treatment with acid. At the same time, the material has a very large surface area, and thus potentially has a large capacity for adsorption of components. It can be prepared in mechanically stable structures, and has long been used in the food industry, and is thus safe to use.

In previous work we tested and compared the use of channeled monoliths and packed bed columns for the separation of a bioactive peptide from a crude hydrolyzate (chapter 3) using activated carbon material. Channeled monoliths showed similar productivities to the packed bed while having much higher permeability and negligible plugging. Therefore, channeled monoliths have potential for the separation of small food ingredients and even from crude streams. However, there is not much work done and models are not available for the prediction of the behavior of channeled monoliths using liquid streams.

Some research has been done on the adsorption behavior in channeled monoliths for gas as well as liquid systems to obtain an analytical solution based on numerical simulations of the full 3-D problem [49]. This solution is in the form of the height equivalent to a theoretical plate (HETP) which is a representation of the breakthrough dynamics and dispersion within the chromatographic column. However, these models have not yet been validated with experimental data for channeled monoliths in liquid systems. A characterization of the adsorbents, together with detailed non-linear model validation is necessary for adsorbent dynamic behavior prediction and validation of the HETP equations in the linear regime.

The goal of the present work is thus to validate detailed models that can be applied for the adsorption of sugars on activated carbon particles and activated carbon channeled monoliths. These models can be used in further up-scale, or comparison and validation of the analytical HETP equation for channeled monoliths [49]. In order to have a detailed model, a step-by-step procedure is followed taken into account the characterization of the materials, the determination of the adsorption isotherms and uptake kinetics of both structures, as well as the overall performance of the systems.
4.2 Theory

4.2.1 Frontal Analysis

Frontal analysis is a dynamic measurement method to obtain adsorption isotherms describing the equilibrium of a flowing solute with a fixed stationary phase packed in a column. It is an accurate method for determining the isotherm, even if the column efficiency is low and the mass transfer kinetics are concentration dependent [93]. Frontal analysis has mainly been applied for packed bed adsorbents, but also for other configurations such as membrane adsorbers [94], polymeric monolithic columns [32, 95] and capillary-channeled polymer fibers [96].

The stoichiometric volume \( V_s \) or retention volume \( V_R \) is a thermodynamic parameter that represents the amount of solute that would saturate the column assuming the equilibrium. Depending on the symmetry of the breakthrough curve there are different methods to obtain \( V_s \) [97]. The most rigorous method, that can also be applied with non-symmetrical breakthrough curves, is the integration method in which \( V_s \) is obtained by dividing the breakthrough curve by a vertical line into two parts with equal areas (Figure 4.1a, b). This is equivalent to the use of equation (4.1) as in [32, 95–97].

\[
V_s = \int_{V_0}^{V_e} \frac{(c_j - c)}{c_j} dV
\]

(4.1)

The stoichiometric volume is used to calculate the bound concentration (equations (4.20) and (4.21)). Frontal analysis equilibrium results are then most commonly fitted to the Langmuir isotherm (equation (4.2)).

\[
q_e = q_m \frac{K \cdot c_e}{1 + K \cdot c_e}
\]

(4.2)

4.2.2 Adsorption kinetics

The adsorption kinetics of lactose by the activated carbon particles and channeled monoliths is modeled using the homogeneous surface diffusion model (HSDM). This model has been widely used to describe the adsorption of various organic compounds by particulated adsorbents [98, 99]. The HSDM
Figure 4.1: Schematic representation of the frontal analysis method applied using the step series method for symmetrical breakthrough curves a) in the packed bed as in [97] and b) non-symmetrical in the channeled monolith. c) Staircase method used for the channeled monolith. Frontal analysis methods based on [97]. The thick solid line represents the concentration of lactose in the liquid phase at the column outlet; the gray area represents the mass of solute bound to the solid phase. The stoichiometric volume is calculated with the half height method a) for the packed bed and with the integration method (equaling area A to B) b) and c) in the case of the channeled monolith.

assumes homogeneous particles and fast adsorption kinetics with an overall adsorption rate dependent on the intraparticle and external mass transfer. It is assumed that surface diffusion is the predominant intraparticle mass transfer mechanism and that this is independent of the concentration of the adsorbed component. These assumptions hold for adsorption by granular activated carbon [100] due to the high binding capacity (high $K$ or $dq/dc$). The parameters in the model are generally estimated with batch and shallow bed
Lactose adsorption onto activated carbon beads and monoliths

We have chosen to perform the measurement assuming an infinitely large bath, which implies that the concentration of lactose, \( c \), in the bulk liquid, does not vary with time. The HSDM representing the intra-adsorbent diffusion of lactose in the carbon material can be described in a packed bed by a partial differential equation with its appropriate boundary and initial conditions. We applied the same model equations to the channeled monolith with some geometrical variations and change of coordinates. We have simplified the geometry of the channels to that of a hollow cylinder as by Patton et al. [23].

The equations of the HSDM are summarized in Table 4.1. The equilibrium between the solid and the liquid assumes non-linearity in the adsorption equilibrium which may be described by the Langmuir isotherm (equation (4.2)).

**Table 4.1:** Equations of the homogeneous surface diffusion model (HSDM) applied to the activated carbon packed bed and channeled monolith.

<table>
<thead>
<tr>
<th></th>
<th>Packed bed</th>
<th>Channeled monolith</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PDE</strong></td>
<td>( \frac{\delta q}{\delta t} = \frac{1}{r^2} \frac{\delta}{\delta r} \left( D_s r^2 \frac{\delta q}{\delta r} \right) )</td>
<td>( \frac{\delta q}{\delta t} = \frac{1}{r^2} \frac{\delta}{\delta r} \left( D_s r \frac{\delta q}{\delta r} \right) )</td>
</tr>
<tr>
<td><strong>I.C.</strong></td>
<td>( t = 0 \quad q = 0 )</td>
<td>( t = 0 \quad q = 0 )</td>
</tr>
<tr>
<td><strong>B.C.</strong></td>
<td>( r = 0 \quad \frac{\delta q}{\delta r} = 0 )</td>
<td>( r = r_p \quad \frac{\delta q}{\delta r} = k_f (c_b - c_s) )</td>
</tr>
<tr>
<td></td>
<td>( r = r_i \quad \frac{\delta q}{\delta r} = -k_f (c_b - c_s) )</td>
<td>( r = r_i \quad \frac{\delta q}{\delta r} = 0 )</td>
</tr>
<tr>
<td><strong>Average loading</strong></td>
<td>( \overline{q} = \frac{\int_{r_p}^{r_i} 2 \pi r q r^2 dr}{\frac{\pi}{2} r_p^3} = \left( \frac{3}{\pi r_p^2} \right) \int_{r_p}^{r_i} q r^2 dr )</td>
<td>( \overline{q} = \frac{\int_{r_p}^{r_i} 2 \pi r q r^2 dr}{\pi (r_i^2 - r_p^2)} = \frac{2}{r_i^2 - r_p^2} \int_{r_p}^{r_i} q r^2 dr )</td>
</tr>
</tbody>
</table>

PDE: partial differential equation; I.C.: initial conditions; B.C.: boundary conditions

For comparison, an analytical solution of the linear driving force model (LDF) presented by equation (4.13), shown in in equation (4.14) was also used [101, 102]. This relation lumps the different mass transfer resistances into an overall mass transfer coefficient \( k \).

\[
\frac{\delta \overline{q}}{\delta t} = k (q^* - \overline{q}) \quad (4.13)
\]

\[
\frac{q}{q^*} = 1 - e^{-kt} \quad (4.14)
\]
External mass transfer by definition plays a role during the initial phase of the adsorbent uptake. Therefore, several researchers have proposed to estimate the kinetics from the initial slope of the adsorption curve [102, 103] and then use equation (4.15).

\[ F = \frac{q}{q^*} = \frac{3k_f c_b}{r_p q^*} t \]  

(4.15)

### 4.2.3 Column operation

The entire breakthrough curves describing the column operation, regardless of the internal structure (packed bed or channeled monolith) were modeled using the general rate model (GRM) with adaptation of the model using cylindrical coordinates for the channeled monolith. The GRM is described in this case by equations (4.16)–(4.19). In order to assess the external and intraparticle mass transfer the equations of the HSDM (equations (4.3)–(4.12)) have also been included for the complete simulation of the column.

\[ \epsilon b \frac{\delta c}{\delta t} + u \frac{\delta c}{\delta z} = \epsilon b D_L \frac{\delta^2 c}{\delta z^2} - (1 - \epsilon b) k_f a_{spec} (c - c_s) \]  

at \( t = 0 \), \( c = 0 \) \hspace{1cm} (4.16)

\[ z = 0 \quad u(c - c_0) = D_L \frac{\delta c}{\delta z} \]  

(4.18)

\[ z = L \quad \frac{\delta c}{\delta z} = 0 \]  

(4.19)

### 4.3 Materials and methods

#### 4.3.1 Materials

D-lactose monohydrate, Blue dextran and 95% ethanol were purchased from Sigma-Aldrich (St. Louis, USA).

Mesoporous activated carbon particles TE7/20, batch DEC 35, with a particle diameter range between 250–500 µm were purchased from MAST Carbon International Ltd (UK). Channeled monoliths with 7 mm wide channels were kindly donated by MAST Carbon International Ltd (UK) in different lengths. The channeled monoliths have the same material properties as
Lactose adsorption onto activated carbon beads and monoliths described in chapter 3. The monoliths were cut into the right length when needed and in-house wrapped into polyethylene housings.

Table 4.2 and Table 4.3 summarize the material characteristics from the two different structures used in this study. These characteristics have been obtained as in chapter 3.

### Table 4.2: Structure dimensions from the structures used in chapter 3.

<table>
<thead>
<tr>
<th>Structure</th>
<th>a (µm)</th>
<th>2δ (tw or 2rp) (µm)</th>
<th>εb</th>
<th>ρs (kg/m³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TE7/20 particles (packed bed)</td>
<td>–</td>
<td>323.8±0.1**</td>
<td>0.34–0.35±0.01***</td>
<td>486.2±0.3</td>
</tr>
<tr>
<td>(700±60) cpsi monolith*</td>
<td>560±20</td>
<td>380±20</td>
<td>0.36±0.05</td>
<td>490±60</td>
</tr>
</tbody>
</table>

*Obtained from microscope measurements.
**Volume weighted mean obtained from particle size distribution using Mastersizer.
***Obtained from the different packed beds column packings, by weight and length measurements and using equation (3.8).

### Table 4.3: Nitrogen isotherm results from the TE7/20 activated carbon particles and the 700 cpsi monolith (chapter 3).

<table>
<thead>
<tr>
<th>Structure</th>
<th>BET surface area (m²/g)</th>
<th>Micropores (&lt;2nm)**</th>
<th>Pore volume</th>
<th>Total at $P/P_0 = 0.95$</th>
</tr>
</thead>
<tbody>
<tr>
<td>TE7/20 activated carbon particles</td>
<td>1450</td>
<td>78.6 v/v%</td>
<td>21.4 v/v%</td>
<td>0.77 cm³/g</td>
</tr>
<tr>
<td>700 cpsi monolith</td>
<td>734</td>
<td>60.3 v/v%</td>
<td>39.7 v/v%</td>
<td>0.51 cm³/g</td>
</tr>
</tbody>
</table>

$p$ and $P_0$ are the partial and the saturated vapor pressures of $N_2$ (in mmHg).

Pore diameter according to the International Union of Pure and Applied Chemistry (IUPAC) [56].

### 4.3.2 Single-component adsorption isotherm determination by frontal analysis

The single-component adsorption isotherms of the activated carbon particles and monolith were determined by frontal analysis using the step series method and staircase methods as in [97]. To perform this method in the packed bed, a 5/20-mm Tricorn column (GE Healthcare, Belgium) was packed by gently adding some activated carbon, applying slight vibration, 0.6 g of dry TE7/20 activated carbon particles resulting in a packing height of about 16 mm. Two wrapped monoliths were used with two different lengths of 264 and 204 mm. The methods were run by equilibrating the column first with Milli-Q water and then applying stepwise concentration increases of lactose, first through a by-pass and then by switching
the valves to feed the column. The external dead volume from the by-pass switch to the column, $V_{\text{ext}}$, and the void volume were determined as in chapter 3. The step concentrations were implemented until saturation of the column at low flow rates: 1 mL/min for the packed bed as recommended in [97] for coarse particles, for the channeled monoliths 0.25 mL/min in the case of the channeled monoliths. After saturation each step of the step series method or at the end of the staircase method, the columns were cleaned with a desorbing step of about 15 column volumes using 50% ethanol with consequent equilibration with water, necessary after equilibration with the sugar. The different step concentrations during the adsorption of lactose, as well as the elution and equilibration were programmed, run and monitored using an ÄKTA purifier (GE Healthcare, Belgium) equipped with a P-900 pump, a M-925 mixer coupled to a RI-102 detector (Shodex, Japan). To convert the monitored concentration into mass units per volume, a calibration curve was made injecting different steps of lactose at known concentrations. All measures were done at room temperature (about 25 °C).

The mass of sugar adsorbed at equilibrium $q_{e,j}$ per mass of carbon at equilibrium with a $j^{\text{th}}$ step of concentration $c_j$ was calculated, in the step series method, from the stoichiometric volume at equilibrium according to equation (4.20):

$$q_{e,j} = \frac{c_j \cdot ((V_{s,j} - V_{0,j}) - (V_0 + V_{\text{ext}}))}{m_{\text{carbon}}}$$

(4.20)

For the case of the monoliths, the staircase method was also applied to validate the results found with the step series method due to a higher uncertainty in the results at high concentrations. In this case $q_{e,j}$ was calculated using equation (4.21).

$$q_{e,j} = q_{e,j-1} + \frac{(c_j - c_{j-1}) \cdot ((V_{s,j} - V_{0,j}) - (V_0 + V_{\text{ext}}))}{m_{\text{carbon}}}$$

(4.21)

### 4.3.3 Shallow bed adsorption

The shallow bed method was applied similarly as in [27]. To perform this experiment in the packed bed, a high resolution glass column 20 mm from Knauer (Germany) was packed with about 0.2 g of TE7/20 activated carbon particles obtaining a packing height of about 2–3 mm. The wrapped monoliths were previously cut in short lengths of about 50 mm (representing about 0.5 g
activated carbon). The experiments were performed in the ÄKTA purifier as for the frontal analysis method. During the experiment a 0.3 g/L lactose solution was fed at a superficial velocity of about $2 \cdot 10^{-3}$ m/s, in the range of literature values [104, 105]. The feed solution was recirculated to a 10 L bottle to assume an infinite bath. To obtain the amount of lactose adsorbed in the bed a desorption step with 5% ethanol was performed. A blank with only lactose was carried out to obtain the elution peak of ethanol. The elution peak of the desorbed lactose was obtained by subtraction of the ethanol peak signal to the overall RI signal. The resulting lactose peak was then integrated and the amount lactose calculated from the calibration curve. Figure 4.2 shows a representation of the shallow bed method together with the experimental derivation of the uptake kinetic curves.

**Figure 4.2:** Shallow bed adsorption method adapted from [102]. a) Experimental setup used in the shallow bed method. b) Graphical calculation of the uptake curve from the desorbed sugars previously loaded during different times.
4.3.4 Simulation and parameter estimation

The models in section 4.2.2 and 4.2.3 were implemented for simulation and parameter estimation using the software package gPROMS from Process Systems Enterprise Limited (London, UK). The discretization methods used were the centered finite difference and the backward finite difference methods, with fourth and second order approximation, respectively, for the radial and axial coordinated respectively using a grid of 200 points for both. The parameters were estimated using a least-squares fitting procedure provided by the software.

4.4 Results and discussion

4.4.1 Determination of adsorption isotherms by frontal analysis

Figure 4.3 shows the normalized breakthrough curves obtained during frontal analysis measurements as described in section 4.3.2. While the packed bed breakthrough curves are symmetrical, the channeled monolith shows a concentration dependency and early breakthrough even at a lower flow rate. This early breakthrough is caused by the high dispersion in the column and the small residence time chosen in the experiments. Nevertheless, the
stoichiometric volume found from these breakthrough curves is independent of the value of the rate coefficient of mass transfer or of the axial dispersion when using the integration method described in section 4.2.1. Since the packed bed showed symmetric breakthrough curves, we used both the half-height and integration methods. Figure 4.4 shows the resulting experimental isotherms of lactose on a packed bed column packed with TE7/20 activated carbon particles and a 700 cpsi activated carbon monolith fitted to the Langmuir isotherm. Table 4.4 gives a compilation of the fitted parameters from both regressions.

![Graph](image)

**Figure 4.4:** Adsorption isotherm of lactose on a: a) packed bed of TE7/20 activated carbon packed particles and b) activated carbon monolith. Both isotherms obtained by Frontal analysis with the step series method. In the case of channeled monoliths some more points were added to the fit with the staircase method and after equilibrium with the shallow bed method.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Langmuir isotherm parameters</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$K$ (l/g)</td>
<td>$q_m$ (mg/g)</td>
</tr>
<tr>
<td>Packed bed</td>
<td>3.1±0.4</td>
<td>335±7</td>
</tr>
<tr>
<td>700 cpsi monolith</td>
<td>1.6±0.2</td>
<td>180±6</td>
</tr>
</tbody>
</table>

**Table 4.4:** Parameters from the Langmuir fit from the isotherm of lactose on the activated carbon particles and monolith.

According to the Langmuir parameters, the saturation at equilibrium of the activated carbon particles is about twice higher than that of the carbon monolith. This ratio coincides with the ratio of the surface areas of the two structures given
in Table 4.3, and indeed both structures give the same value of area used per molecule: about 2.5 nm$^2$ per molecule. The results in the case of the packed bed were reproducible, when obtaining duplicated data points. Thus we could neglect possible irreversible adsorption. In the case of the channeled monolith there was a higher uncertainty at high concentrations probably because the difference between the stoichiometric volume and the error in the dead volume ($V_0 + V_{ext}$) determination was of similar order of magnitude. The inaccuracy of the method with systems exhibiting very low sorption was already pointed out in [97]. Therefore, to mitigate this uncertainty, and improve the fit, we carried out experiments on a second column, but this time with the staircase method to have more data points and as the same time study the possibility of reduced binding due to previous irreversible binding. The staircase method by itself is not affected by the irreversible binding as it does not make use of adsorption-desorption steps but sums up the accumulated bound solute with each stepwise increase of concentration. Results in Figure 4.4 show that these results fit well with the previous results using the step series method.

### 4.4.2 Determination of adsorption kinetics

The shallow bed method was used for determining the adsorption kinetics; Figure 4.5 and Table 4.5 summarize the results as well as the model predictions by HSDM and LDF model. The film mass transfer coefficients $k_f$ obtained in Table 4.5 have the same order of magnitude as correlations from literature presented in Table 4.6. These ranges were used for initialization during the fit procedure as well as constraints for $k_f$ in the parameter estimation for the fit of the shallow bed data to the HSDM (initialization and constraints shown in appendix A.3).

Figure 4.5 shows that both the HSDM and LDF models fit reasonably well with the experimental data as can be seen by the weighted residual plots in appendix A.3. Since the overall maximum capacity at the equilibrium is twice as high in the packed bed as in the monolith, the results are in good agreement with the results for the BET surface area presented in Table 4.3 and the isotherms in Figure 4.3.
Figure 4.5: Uptake curves of lactose on: a) a TE7/20 activated carbon packed and b) a 700 cpsi activated channeled monolith. Two sets of experimental data using two different columns together with the modeling fits are represented for both structures.

Table 4.5: Kinetic parameters estimated from the datasets of uptake curves obtained from the shallow bed adsorption experiments on two activated carbon packed beds and two 700 cpsi channeled monoliths.

<table>
<thead>
<tr>
<th>Structure</th>
<th>HSDM solution (equations (4.3)–(4.12))</th>
<th>Initial slope (equation (4.15))</th>
<th>LDF (equations (4.13)–(4.14))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$k_{f,1}$ (m/s)</td>
<td>$D_{s,1}$ (m$^2$/s)</td>
<td>$k_{f,2}$ (m/s)</td>
</tr>
<tr>
<td>Packed bed</td>
<td>5.4 · 10^{-5}</td>
<td>8.1 · 10^{-13}</td>
<td>1.3 · 10^{-5}</td>
</tr>
<tr>
<td>700 cpsi monolith</td>
<td>6.8 · 10^{-5}</td>
<td>8.5 · 10^{-12}</td>
<td>3.25 · 10^{-5}</td>
</tr>
</tbody>
</table>

Table 4.6: $k_f$ values from the columns used in the shallow bed experiments. Values calculated using correlations for the Sherwood number used in literature.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Sherwood correlation</th>
<th>$k_f$ (m/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Packed bed</td>
<td>Kataoka et al. [106]</td>
<td>4.5 · 10^{-5}</td>
</tr>
<tr>
<td></td>
<td>Carberry [107]</td>
<td>3.6 · 10^{-5}</td>
</tr>
<tr>
<td></td>
<td>Wilson and Geankoplis [108]</td>
<td>6.6 · 10^{-5}</td>
</tr>
<tr>
<td>700 cpsi monolith</td>
<td>Patron et al. [23]</td>
<td>1.8 · 10^{-5}</td>
</tr>
<tr>
<td></td>
<td>Hawthorn [109]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Skelland [110]</td>
<td>9.6 · 10^{-6}</td>
</tr>
</tbody>
</table>

According to Table 4.5 the results for the overall mass transfer coefficient and the film mass transfer coefficient for both packed bed and monolith are comparable. Therefore, the concentration dependency observed in the channeled monolith breakthrough curves presented in Figure 4.3 is probably caused by the lower capacity and a higher dispersion in the column. The LDF approximation
for monoliths [23] yielded large errors in estimating the parameters due to interdependencies between the parameters.

### 4.4.3 Model validation of the breakthrough

Figure 4.6 shows some plots from the experimental and the fitted breakthrough curves for the packed bed and the monolith. The experimental breakthrough curves of both structures were fitted to the general rate model (GRM). During the parameter estimation, we fitted the axial dispersion and refitted the surface diffusion. The surface diffusion and constraints (presented in appendix A.3) were chosen considering the results obtained via the fit to the HSDM model. To translate the $k_f$ values to the new columns, we chose the Sherwood number correlation that gave a closer value of $k_f$ to the values obtained in the previous section via the initial slope (equation (4.15)) and fit to HSDM. In the case of the packed bed this is the correlation used by [107] and in the case of the channeled monolith the correlation from Hawthorn [23, 109]. The obtained parameters are shown in Table 4.7. The values obtained for the coefficient of axial dispersion with the GRM are higher than the ones obtained with literature correlations. For the case of the packed bed a one to two orders of magnitude lower axial dispersion was expected [24]. In the case of channeled monolith we...

![Figure 4.6](image_url)
Table 4.7: Predicted parameters from the parameter estimation from the breakthrough curves on both packed bed and monolith.

<table>
<thead>
<tr>
<th></th>
<th>Packed bed</th>
<th>700 cpsi monolith</th>
</tr>
</thead>
<tbody>
<tr>
<td>$D_s$ (m$^2$/s)</td>
<td>8.7 · 10$^{-12}$</td>
<td>1.9 · 10$^{-5}$</td>
</tr>
<tr>
<td>$D_L$ (m$^2$/s)</td>
<td>2.4 · 10$^{-12}$</td>
<td>1.3 · 10$^{-4}$</td>
</tr>
</tbody>
</table>

obtained axial dispersion that was around 3 orders of magnitude higher than the value obtained assuming mass transfer in circular coated tubes [34] according to Taylor’s analysis which is a generally applicable correlation for channeled monoliths also in gas applications.

In fact, axial dispersion is induced by the combination of axial diffusion and inhomogeneity of concentration in the radial direction. Previous authors have mentioned [49] that for liquid systems the Golay approximation which is used for coated tubular columns [111] underestimates the dispersion along squared channeled monoliths. The flow profile in these squared channels does not have a parabolic profile. The assumption of parabolic profile and thus, for instance applicability of height to a theoretical plate equations, can only be used in the limit of an infinitely long column [112], so when the mean residence time is sufficiently large. According to Ahn and Brandani [49] only rectangular channels with an aspect ratio of 10 were not affected by the limiting dispersion occurring for typical (short) columns. The inhomogeneous concentration profile, together with the relatively low residence time and lower capacity are responsible of concentration dependency observed. Therefore, to have a lower dispersion and have a good performance in terms of dispersion, channeled monoliths need to be configured as long columns, with smaller channel diameters or with different geometries (e.g. rectangular channels with high aspect ratio or with hexagonal shape). In fact, this should not be a problem since these structures have a very high permeability (chapter 3).

4.5 Conclusions

The adsorption of lactose on an activated carbon particulated packed bed and an activated carbon channeled monolith was studied. The parameters involving
the equilibrium and mass transfer for the adsorption of lactose on an activated carbon packed bed and channeled monolith were determined by combining a variety of experimental methods for the equilibrium and the kinetic behavior, with modeling the overall, non-linear behavior.

The shallow bed and frontal analysis methods were used to determine the equilibrium and kinetic parameters; the homogeneous surface diffusive model was used assuming surface diffusion as the main intraparticle mass transfer limitation. The resulting film mass transfer coefficients showed a good agreement with correlations found in literature. The surface diffusion coefficients of both structures revealed a similar intraparticle mass transfer performance during the uptake adsorptive process.

Taking into account the overall column performance and the estimated isotherm and kinetic parameters, the general rate model was combined with the experimental breakthrough curves produced from the overall column operation in both structures. The higher axial dispersion achieved in the channeled monoliths compared to expected is responsible for the difference in performance between both structures.

These modeling results can be used for adsorbent design predictions and optimization where the goal is to screen different adsorbents and study the possibilities of using channeled monoliths in food-liquid industrial applications. While the channeled monoliths were found to perform well, the results suggest that they should be used in systems with considerable length, different channel geometry (non-squared) and smallest hydraulic diameter, to reduce the effects of the axial dispersion. Their very high hydraulic permeability makes this eminently possible.

Acknowledgements

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The authors also would like to thank MAST Carbon International Ltd (UK) for their donated carbon monoliths.
Comparison of structured adsorbents for the adsorptive isolation of food ingredients from large streams

This chapter has been submitted as:
Abstract

We present guidelines for the configuration of industrial scale chromatographic separation of small molecules. We compared the performance of different axial packed beds, channeled monoliths and a continuous monolith assuming silica as base material. The calculated mass transfer rates were used to calculate the height of a theoretical plate (HETP). The HETP and pressure drop relations as a function of velocity were used to calculate the resultant velocity and packing length for different conditions (efficiency, pressure drop, affinity constant and throughput). The specific productivity of channeled monoliths can be up to 2.5 orders of magnitude higher than that of a packed bed. This implies that at large scales (in which the pressure drops need to be limited, and the flow rate is high), channeled monoliths are preferred since they may reduce the equipment size up to 100 times and the required resin volume up to 1000 times. Accordingly, we demonstrate the potential of channeled monoliths in chromatographic processes but also draw a window pointing out the feasible configurations to use with the highest productivity for a given set of process requirements.
5.1 Introduction

The food industry has an increasing need for large-scale selective separation processes. This is not only to satisfy a growing health awareness and market for functional foods and neutraceuticals, but also to process and add value to low value side streams and waste streams. Most of these streams are typically large in volume (>10 m$^3$/h), and contain only a low concentration (order of g/m$^3$) of target molecules, which typically have a molecular mass of 400–1200 Da. While chromatographic processes offer the required resolution, it is a challenge to find cost-effective systems with reasonable equipment size and process times.

Fractionation or enrichment of food ingredients using chromatography is normally done using a packed bed. The conventional packed bed of spherical particles presents many drawbacks for processing large streams, such as the pressure drop that becomes limiting when using viscous streams, while streams with suspended solids or components that cause fouling may result in blockage of the column. To minimize this, short and wide (pancake-like) columns or big particle diameters are used in practice; with the exception of radial flow chromatographic configurations in which the width-to-length ratio can be reduced up to a certain extend while keeping the same performance [35]. Hence, standard axial chromatography leads to expensive columns or low productivities and efficiencies due to the resulting long diffusive lengths (particle diameters). The resulting chromatographic process cost is generally not compatible with the relatively low economic value of food products (e.g. compared with pharmaceuticals). Therefore, the food industry needs more efficient adsorbents to reduce cycle times, pressure drop and equipment cost while maintaining or improving the productivity and column efficiencies.

Over the last half century, new chromatographic media have emerged. The so called monoliths, one-piece porous structures with interconnected pores or channels have found already their place in different applications. In the pharmaceutical sector generally polymeric continuous monolithic rods, and an up-scaled version of these monolithic rods to be run in radial flow mode, have been evaluated [113]. In the field of High Performance Liquid Chromatography (HPLC) polymeric and silica structures have been applied for analysis purposes [31, 114–
reducing the analysis times while yielding higher resolutions. In catalysis and gas (preparative) applications, structures in the shape of "honeycombs" (or channeled monoliths) and foams have been used, and these structures usually consist of a metallic or ceramic support in which a catalyst is either immobilized or on which a washcoat is applied on its inner surface.

Non-particulate adsorbents leading to more optimal adsorption are discussed in literature for gas-phase adsorption [21–24] and for heterogeneous catalysis [117]. The main advantage of these structures is a lower pressure drop combined with a higher mass transfer rate. In the case of channeled monoliths, mass transfer may still be a limitation with thick walls or low cell densities, but monoliths with very high cell densities and thin walls have been made. Indeed, these materials show an excellent combination of low pressure drop and high productivity [83].

Nevertheless, few researchers have developed theory-based guidelines for finding the best structure for different types of applications, even though many studies emphasize the importance of the adsorbent structure for the mass transfer performance and thus efficiency of adsorptive columns [22–24, 118]. Most of the work focuses on the separate analysis of the pressure drop, mass transfer or column efficiency, but these aspects have not been analyzed in conjunction.

The aim of this work is therefore to set up a comparative methodology to provide a simple set of guidelines for the selection of the optimal structure for chromatographic separation of small molecules at a specific production scale based on sizing, productivity and efficiency.

5.2 Theory

We make a comparison between the performance of a packed bed of porous particles, a channeled monolith and a continuous monolithic column. Figure 5.1 displays the different structures considered. All three structures are assumed to consist of the same material, silica, with the same internal porosity. To compare the adsorbent performance, equal pressure drop and equal efficiencies are imposed. The structure and corresponding mass transfer contributions will be combined to calculate the height to a theoretical plate (HETP). The HETP and pressure drop relations as a function of velocity will be used to calculate the
Comparison of structured adsorbents for the isolation of food ingredients from large streams

resultant velocity and packing length after fixing the above mentioned conditions. We based all calculations based on a linear isotherm $q_i = K \cdot C_i$ since generally in the food industry target components are present in low concentrations.

### 5.2.1 Column productivity

The specific productivity $Prod_i$ is defined as the mass of a certain target component separated $i$ per total column volume. If we assume that the concentration of the target component is equal for all three cases, the productivity for all the systems becomes proportional to the ratio between the superficial velocity and length of the column as follows:

\[
\text{Specific productivity} = \frac{Prod_i}{V} = \frac{Q \cdot c_i}{V} = \frac{u_o \cdot c_i}{L} \propto \frac{u_o}{L} \tag{5.1}
\]

In this equation the length $L$ is defined as:

\[
L = N_{\text{plates}} \cdot HETP \tag{5.2}
\]

**HETP equation**

The height equivalence of a theoretical plate is the space or height needed to reach equilibrium between two phases: in chromatography, the stationary phase
and the mobile phase. This equilibrium results in a concentration distribution ratio equal to \( q_i/C_i = K_i \) over a column with a height of HETP (when assuming a linear isotherm). It is therefore a measure of the rate of transfer: if the transfer is very fast, the height will be small, while slow mass transfer will result in a considerably larger HETP.

The height equivalent to one theoretical plate (HETP) has been widely used for both particulate and monolithic columns \([119, 120]\), and for other systems \([121]\). Assuming instantaneous adsorption at the surface of the adsorbent, the following equation can be used \([120, 122]\):

\[
\text{HETP} = 2 \frac{D_L}{u} + 2 \frac{\epsilon_b}{1 - \epsilon_b} \left( \frac{k_1}{1 + k_1} \right)^2 \frac{1}{k_K} u
\]

(5.3)

The first term represents the limitation through axial dispersion; the second is the contribution caused by limitation in mass transfer.

Expressions for the HETP for channeled monoliths have been derived for rectangular channels with different aspect ratios \([49]\) and for square channels \([75]\) included a corrected wall thickness to account for the volume that is left on the corners of the squared channel.

\[
\text{HETP} = 2 \left( D_m + K F D_s \right) u + \frac{2K F}{3(1 + K F)^2} \cdot \frac{w_c^2}{D_s} \cdot u + \tilde{\bar{C}}_M \frac{d_H^2}{D_m} u
\]

(5.4)

The dimensionless resistance to mass transfer in the mobile phase \( \tilde{\bar{C}}_M \) has been determined and defined for rectangular channels as a function of the aspect ratio \( \alpha \) \([123]\). Using the analytical solutions and approximation by Ahn and Brandani \([49]\), the expression for \( \tilde{\bar{C}}_M \) for squared channels becomes:

\[
\tilde{\bar{C}}_M = 0.083 \left( \frac{K F}{1 + K F} \right)^2 + 0.017 + 0.063 \left( \frac{K F}{1 + K F} \right)
\]

(5.5)

The HETP is often used in the dimensionless form by dividing it by the particle diameter, thus subtracting the effect of the diffusional distance when comparing columns with particles of different diameters. We used the reduced plate height \( h (h = \text{HETP}/\delta) \) using in this case the characteristic length of the structures. In order to use equation (5.3) in dimensionless form, we defined a Péclet number or reduced velocity, \( Pe \) as: \( Pe = u \cdot \delta / D_m \).

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Comparison of structured adsorbents for the isolation of food ingredients from large streams

Chapter 5

Calculation of the different mass transfer contributions of the adsorbents

Mass transfer may be limited at three stages influenced by the pore hierarchy inside the adsorbent:

(i) mass transfer limitation due to transfer from the channels (or, in a packed bed, from the interstitial fluid) into the bigger pores (external)

(ii) mass transfer limitation from diffusion inside the bigger pores (intraparticle)

(iii) mass transfer limitation from diffusion inside the smaller pores.

In order to generalize the HETP concept and compare it to other systems, the overall mass transfer coefficient $k$ corrected for the equilibrium over the two phases by taking $kK$, is rewritten as:

$$\frac{1}{kK} = \frac{1}{k_f a_{spec}} + \frac{1}{k_{macro}} + \frac{1}{k_{meso}} = \frac{1}{k_{ext}} + \frac{1}{k_{intra}}$$  \hspace{1cm} (5.6)

The mass transfer coefficients of the different adsorbents presented in Figure 5.1 are calculated assuming the same base structure of silica consisting of bimodal pores as in [29], macropores constituting the external void volume and mesopores within the intraskeleton. The substitution of the overall mass transfer coefficient obtained by equation (5.6) in equation (5.3) leads to the three contributions in equation (5.7). To calculate the HETP, we neglect the mass transfer within the mesopores, which is the same in each system and is small compared with the other resistances.

$$HETP = H_{ax} + H_{ext} + H_{intra}$$  \hspace{1cm} (5.7)

The resistances in the continuous silica monolith can be calculated using an analogy with packed beds when making a dimensionless analysis [26, 29], hence its reduced plate height $h$ can be calculated similarly as for a packed bed, with an equivalent particle diameter accounting for dispersion (ddisp) [28, 29].

External mass transfer

The external mass transfer coefficient $k_f$ for transfer from the bulk into the macropores can be obtained from the following equation:

$$Sh = \frac{k_f d}{D_m}$$  \hspace{1cm} (5.8)
The Sherwood number $Sh$ and the system diameter $d$ for three systems are given in Table 5.1.

**Table 5.1:** Parameters used to calculate the external mass transfer resistance in the different structures.

<table>
<thead>
<tr>
<th>Structure</th>
<th>$Sh$</th>
<th>$a_{spec}$ $(m^2/m^3)$</th>
<th>$d$ $(m)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Packed bed of silica particles</td>
<td>$Sh = 1.85 \left( \frac{1-\epsilon_b}{\epsilon_b} \right)^{0.33} Re^{0.33} Sc^{0.33}$</td>
<td>$a_{spec} = \frac{6}{d_p}$</td>
<td>$d = d_p$</td>
</tr>
<tr>
<td>Continuous silica monolith</td>
<td>$Sh = 1.85 \left( \frac{1-\epsilon_b}{\epsilon_b} \right)^{0.33} Re^{0.33} Sc^{0.33}$</td>
<td>$a_{spec} = \frac{6}{d_{disp}}$</td>
<td>$d = d_{disp} = 3 \mu m$</td>
</tr>
<tr>
<td>Channeled monolith</td>
<td>$3.66^*$</td>
<td>$a_{spec} = \frac{2r_o}{r_o^2 - r_i^2}$</td>
<td>$d = 2r_i$</td>
</tr>
</tbody>
</table>

*Assumption of fully developed flow in the channels. The inlet effect was neglected due to the high column length-to-channel diameter ratio considered.

**Intra-particle mass transfer**

The intra-particle mass transfer resistance is related to the diffusion through the macropores (Table 5.2). The macropore diffusion coefficient was obtained using the linear driving force (LDF) approximation already applied earlier for monoliths [23].

**Table 5.2:** Macropore diffusion coefficients used to calculate the intraparticle mass transfer resistance.

<table>
<thead>
<tr>
<th>Structure</th>
<th>$k_{macro}$</th>
<th>$R^2$</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Packed bed of silica particles</td>
<td>$\frac{15D_e}{R^2}$ $(5.9)$</td>
<td>$\frac{d_p^2}{2}$</td>
<td>[124]</td>
</tr>
<tr>
<td>Continuous silica monolith</td>
<td>$\frac{15D_e}{R^2}$ $(5.10)$</td>
<td>$\left( \frac{d_{disp}}{2} \right)^2$</td>
<td></td>
</tr>
<tr>
<td>Channeled monolith</td>
<td>$\frac{4D_e}{R^2}$ $(5.11)$</td>
<td>$\frac{1}{2} \left( \frac{r_o}{r_i} - 1 \right) \left( r_o^2 - r_i^2 \right) - \frac{1}{r_i(r_o - r_i)} \cdot \left[ \frac{1}{2} \left( \frac{r_o^4}{r_i^4} - \frac{4r_o}{3} \right) \left( r_o^3 - r_i^3 \right) + \frac{r_i^2}{r_o^2} \right]$</td>
<td>[23]</td>
</tr>
</tbody>
</table>
Diffusion and dispersion coefficients

Table 5.3 summarizes the different diffusion and dispersion coefficients used in the calculation of the mass transfer resistances.

Table 5.3: Equations used to calculate the diffusional parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Equation</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular diffusivity</td>
<td>$D_{m,A} = 7.4 \cdot 10^{-8} \sqrt{\frac{\psi_B M B T}{\eta_B V_{A,6}}}$ (5.12)</td>
<td>[125]</td>
</tr>
<tr>
<td>Effective diffusivity</td>
<td>$D_e = D_p + (1 - \epsilon_p) KD_s$ (5.13)</td>
<td>[119]</td>
</tr>
<tr>
<td>Axial dispersion coefficient</td>
<td>Packed bed and silica monolith: [ DL = \gamma_1 D_m + \gamma_2 D_p u ] (5.14)</td>
<td>[126]</td>
</tr>
<tr>
<td></td>
<td>Channeled Monolith: [ DL = D_m + \frac{1}{192} u_0^2 d_p^2 D_m ] (5.15)</td>
<td>[24, 34]</td>
</tr>
<tr>
<td>Pore diffusivity</td>
<td>$D_p = \frac{\epsilon_p D_m K_p}{\tau_{intra}}$ (5.16)</td>
<td>[29]</td>
</tr>
<tr>
<td>Hindrance factor</td>
<td>$K_p = 1.03 \cdot e^{-4.5 \cdot \lambda_m}$ (5.17)</td>
<td>[127]</td>
</tr>
</tbody>
</table>

The axial dispersion coefficient, $D_L$, used to calculate the HETP of the packed bed of silica particles and of the continuous silica monolith is calculated assuming parallel molecular diffusion and eddy diffusion [126]. The axial dispersion in the channels of the channeled monolith is evaluated according to Taylor’s analysis [24, 34]. The effective diffusivity in the pores located inside the stationary phase skeleton ($D_e$) is defined assuming parallel contribution of pore diffusion and surface diffusion [119]. The surface diffusion coefficient is estimated at $10^{-6}$ cm$^2$/s as in [119]. The molecular diffusivity is calculated assuming an arbitrary average molecular weight (420 Da) in the range of small food ingredients, such as oligosaccharides [128] and bioactive oligo-peptides [4, 16], and assuming to have a spherical adsorbate with a density close to the density of a glucose molecule.
5.2.2 Pressure drop

For the pressure drop in a silica packed bed the Ergun equation was used [39]:

\[
\frac{\Delta P}{L} = \frac{150 \mu (1 - \epsilon_b)^2 u_0}{\epsilon_b^2 d_p^2} + \frac{1.75 \rho (1 - \epsilon_b) u_0^2}{\epsilon_b^3 d_p}
\] (5.18)

The pressure drop through the channels in a channeled monolith is calculated using the following expression [23]:

\[
\frac{\Delta P}{L} = \frac{2f \text{Re} \mu u}{d_H^2} = \frac{28.4 \mu u_0}{a^2 \epsilon_e}
\] (5.19)

where \( f \) is the Fanning friction factor, and \( f \cdot \text{Re} \) corresponds to the friction in the channels equal to 14.2 for the case of square channels [23].

The pressure drop in the continuous monolithic column is calculated from the Kozeny–Carman equation, normally used for a packed bed, using an equivalent particle diameter \( d_{\text{perm}} \) of 15 \( \mu \text{m} \) as obtained by experimental data [29].

\[
\frac{u_0}{d_{\text{perm}}^2} = \frac{\epsilon_b^3}{180 \mu (1 - \epsilon_b)^2} \frac{\Delta P}{L}
\] (5.20)

5.3 Results and discussion

5.3.1 Structural characteristics of the stationary phases

The performance of a packed bed of beads, a foam or a continuous monolith column and a channeled monolith is compared. For a fair comparison, silica is chosen as adsorbent material for all different structures (Figure 5.1 and Figure 5.2). The characteristics for this base material, including their equivalence to a packed bed when considering dispersion and permeability [28, 29], were obtained from literature and are summarized in Table 5.4. The

<table>
<thead>
<tr>
<th>( d_{\text{macro}} )</th>
<th>( d_{\text{meso}} )</th>
<th>( \epsilon_T )</th>
<th>( \epsilon_b )</th>
<th>( \epsilon_p )</th>
<th>( F )</th>
<th>( \tau )</th>
<th>( d_{\text{disp}} )</th>
<th>( d_{\text{perm}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.9 ( \mu \text{m} )</td>
<td>12.5 nm</td>
<td>0.862</td>
<td>0.597</td>
<td>0.658</td>
<td>0.16</td>
<td>1.71</td>
<td>3 ( \mu \text{m} )</td>
<td>15 ( \mu \text{m} )</td>
</tr>
</tbody>
</table>
Comparison of structured adsorbents for the isolation of food ingredients from large streams

**Figure 5.2:** Structure of the packing at the same characteristic length \( \delta \) from: a) packed bed, b) continuous silica monolith and c) channeled monoliths.

Specific outer surface area is the surface of the particles in a packed bed, or the surface of the channels inside a channeled monolith. In general, smaller particles, and smaller channels, will give smaller diffusion paths and lower external mass transfer limitation, but this is at the cost of a larger pressure drop. Since the continuous monolith column forces the feed to flow through the macropores, it has a very high (outer) surface area. Thus, it has a higher capacity and higher specific area available for mass transfer and adsorption than in the other structures, but also a much higher pressure drop, since all fluid has to flow through the pore structure. Figure 5.3 compares the specific outer surface area \( (a_{\text{spec}}) \) and pressure drop (as \( \Delta P/(u_0 \cdot L) \)) of the different structure configurations, as a function of the characteristic length \( \delta \). The pressure drop was made non-dimensional with the superficial velocity and the column length, which is equivalent to plotting the inverse of the permeability of the structures. The pressure drop in the packed bed was modeled for this representation.
Figure 5.3: Comparison of a) the external specific surface area and b) pressure drop divided by the length and velocity (using equations (5.19) and (5.20), assuming no bed compaction) for the packed bed, continuous monolithic columns and channeled monoliths at different characteristic lengths.

assuming non-compaction of the bed in the range studied, and thus following the Kozeny–Carman equation (equation (5.20)). For channeled monolith the equation from Patton et al. [23] is used. For a certain characteristic length (particle radius or half thickness of the lamella between channels) the packed bed has a higher specific surface area than the channeled monoliths, but at the cost of a higher pressure drop (lower permeability). Channeled monoliths show a much lower pressure drop and higher permeability than the packed bed covering a wider range of applications at different characteristic length \( \delta \).

Channeled monoliths appear to be less restricted by process conditions (such as pressure drop and flow rate) and may give also less practical problems such as column blockage (as seen in chapter 3). But, to increase the external surface area and compete with the packed bed, channeled monoliths need to use small adsorbent wall thicknesses and high channel porosity, which is in turn translated into high cell densities with high channel voids. This is in line with previous results [24]. The continuous silica monolith, at the difference of the packed bed, has the same specific surface area as a packed bed of 3–5 \( \mu m \) while having a higher permeability of a packed bed of 15 \( \mu m \) \( d_p \) [28, 29, 119].
5.3.2 Mass transfer performance and contributions to mass transfer of the different structures

To calculate the productivity \((Prod_i)\), we need the HETP relation as a function of the velocity. Since the resulting performance of these structures depends on their dimensions such as voidage and characteristic length, the HETP should be made dimensionless, into a reduced plate height of a theoretical plate \(h\) [29] by dividing it over the characteristic length \(\delta\) of the system. Figure 5.4 shows these values obtained from the different (internal and external) mass transfer resistances and axial dispersion involved (equation (5.3)) and through the equation defined by Ahn and Brandani [49] (equation (5.4)). The reduced plate height is defined for different \(K\) present in the adsorption isotherm \((q_i = K_i c_i)\), reported to be an influencing parameter [119], as a function of the reduced velocity or Péclet number \(Pe\), that accounts for the flow rate \(u\) and characteristic length \(\delta\) of the diffusion process. Although a wide range has been plotted for \(Pe\), showing an optimal reduced velocity with the classical van Deemter pattern, real applications do not operate below the optimal value, to avoid impractical small particle sizes and slow processes. Accordingly, when comparing the structures, a larger optimal \(Pe\) (larger \(u\) and \(\delta\) implies that the structure operates at its optimal (better overall mass transfer rate) while keeping a larger velocity and diffusion path which are beneficial in chromatographic process.

At high Péclet numbers the packed bed and the continuous silica monolith present a lower reduced plate height \(h\) and thus a higher system efficiency than the channeled monoliths (Figure 5.4). Channeled monoliths with a smaller channel voidage \((\epsilon_b)\) show a higher efficiency, which is in line with other studies with monoliths [24, 83]. With \(\epsilon_b = 0.1\), the efficiency of channeled monoliths is close to the one of the packed bed or even higher (lower \(h\)) for \(K = 100\) and Péclet values of 0.5 – 30. The optimal \(Pe\) is also bigger for lower channeled monolith voidage becoming equal to the optimal \(Pe\) of the packed bed. The mass transfer performance of a continuous silica monolithic column overlaps with the one of the packed bed, and in fact they are modeled with the same equations (but with much smaller characteristic length).

Predictions for the reduced plate height incorporating the axial dispersion and mass transfer resistances (Figure 5.4a and b) follow the same trend but are
Figure 5.4: Reduced height equivalent to a theoretical plate as a function of the reduced velocity of the mobile phase for a) $K = 1$ and b) $K = 100$ using equation (5.3), and for c) $K = 1$ and d) $K = 100$ using equation (5.4) according to Ahn and Brandani [49].

slightly underestimated when compared with the predictions from the equation from Ahn and Brandani [49] (Figure 5.4c and d). This observation was also reported [49] since these new 3D predictions for channeled monoliths incorporate all the dimensions in the system including also diffusion in the corners of the channels and axial diffusion in the solid phase. Indeed, Figure 5.4 shows different results depending on the porosity for the two different approaches. For further calculations on potential use of channel monolith using of large streams, it is
therefore more complete to use equation (5.4). However, equation (5.3) is still a good approximation and the advantage is that it is easier to differentiate and study the different mass transfer contributions individually.

Figure 5.5 compares the different contributions to the overall reduced plate height $h$, calculated through equation (5.3), for all the structures. This comparison is done to identify the main limiting resistances within each structure. It is well-known for a packed bed [104], and can be seen from Figure 5.5a that at smaller Péclet numbers ($Pe < 1$) the main limiting contribution is the axial dispersion whereas at high Péclet numbers ($Pe > 30$) the controlling mechanism is the intraparticle mass transfer resistance. Channeled monoliths with small voidage (Figure 5.5b) behave as a packed bed
in terms of mass transfer contributions, whereas at higher monolith voidage (Figure 5.5d) the axial dispersion becomes the main limitation at both low and high Péclét numbers.

The packed bed and the continuous silica monolith have a smaller external mass transfer contribution than the channeled monoliths: the smaller the channel voidage is the smaller is the external contribution to the plate height. This is understandable, since at small channel voidage ($\epsilon_b = 0.1–0.5$) the system becomes closer to being a packed bed ($\epsilon_b \approx 0.4$). Bigger hydraulic diameters of the channels drastically affect the external mass transfer and axial dispersion being significantly higher than in the other structures.

### 5.3.3 Comparison of chromatographic performance: specific productivity and separation performance

This section relates to the specific productivity calculated according to the procedure explained in section 5.2.1. The specific productivity ($\text{kg}/(\text{m}^3 \cdot \text{s})$) is particularly important in large scale chromatographic processes involving big volumes of feed streams, which might lead to unfeasibly large and uneconomic systems. A system with larger characteristic length will feature slower mass transfer and a higher HETP. This implies that a longer column is needed with a lower flow velocity to achieve a certain efficiency ($N_{plates}$). Obviously, this reduces the productivity.

Figure 5.6 illustrates the decrease in specific productivity with the typical size of the internal structure for different monolith channel voidage, packed bed and silica monolithic column. Thus, the finer the internal structure is, the more productive the system can be, since diffusive transfer is faster. The continuous silica monolith presents the highest specific productivity (since it has a given a structure size $\delta$ of 3 $\mu$m), followed by the packed bed and channeled monoliths when comparing at the same characteristic length scale of the internal structure. Different pressure drops do not give different results; the specific productivities remain constant for the monolith and for the packed bed at high Péclét numbers. According to the HETP curve and equation (5.3), at high flow rates the axial dispersion can be neglected: the HETP is then linear with the velocity and thus the resulting length for a fixed number of plates is proportional to the velocity.
Figure 5.6 shows that there is not much influence of the affinity factor $K$ in the productivity of the packed bed whereas the productivity in channeled monolith changes considerably. At high $K$ values the productivity is bigger for smaller channels, while the differences between differing void fractions are bigger than at low values of $K$. Unlike at low $K$, a high affinity increases the intraparticle diffusion rate (equations (5.9), (5.11), and (5.13)), which is the main limiting mass transfer resistance at low porosities (Figure 5.5) opposite to the limiting axial and external diffusion observed at high porosities.

While in terms of mass transfer rates systems with smaller internal structure dimensions are obviously superior, these columns require very large pressure gradients, and therefore can only be very short. This implies that they have low capacity over the length of the column. Thus, to capture sufficient components from the feed between two regeneration steps, these columns having small lengths must be extended in width, to allow for sufficient capacity. For the systems having small structure size, this results in extreme dimensions, with column widths of more than 10 times its length. This is different with the channeled monolith columns, as they have low hydraulic resistance, and allow much longer columns.
5.3.4 **Column dimensions screening as a function of industrial process conditions**

The chromatographic throughput $Q \ (m^3/s)$ is directly linked to the column cross-sectional area and thus determines the aspect ratio $(D/L)$ of the column. For any given combination of $N_{plates}$ and $\Delta P$, the velocity and length are determined as well as the column diameter for a given throughput.

Figure 5.7 compares the resulting $D/L$ ratio as a function of $Q/\Delta P$ for two pressure drop limits fixed represented by two lines: at 0.5 bar (upper line) and 5 bar (lower line). We only show results for systems with 100 theoretical plates, since we did not observe significant differences when using more or less plates. To also reduce the amount of variables involved, we chose the most optimal, yet feasible characteristic length for the channeled monolith. This is, according to literature, assumed to be 3 $\mu$m [83]. Since we could not find references for axial columns showing typical $D/L$ limits, according to our industrial chromatographic knowledge, we assumed a $D/L$ upper limit of 5.

![Figure 5.7: The $D/L$ ratio at $N_{plates} = 100$ for the different structure configurations as a function of the industrial permeability requirements for two pressure drops: at 0.5 bar (upper line) and at 5 bar (lower line). Solid lines represent the packed bed, dashed lines the channeled monoliths ($\delta = 3 \mu$m) and dotted lines the continuous silica monolith. In industrial practice, systems with $D/L > 5$ are generally ineffective and therefore not used.](image-url)
Figure 5.7 shows that for all the conditions considered the structures with low to medium permeabilities (the continuous silica monolith, the packed beds with $d_p = 5–50 \, \mu m$ and the channeled monoliths with $\epsilon_b$ between 0.1 and 0.5) require generally a $D/L$ higher than 5. These systems only show reasonable $D/L$ values for applications that require low $Q/\Delta P$, such as in the analytical or pharmaceutical field or other high-value, low-volume applications. Indeed this is the main application area for these types of systems.

While the $D/L$ ratio is determined by the throughput of the column, the volume of the column is determined by the required capacity, i.e., the amount of resin that is needed to capture the target component. Figure 5.8 compares the overall column volume and volume of resin in the column, plus the $D/L$ ratios relative to their maximum for two $K$ values. A different number of plates ($N_{plates}$), different flow rate ($Q$) or different maximum pressure drop ($\Delta P$) yields a similar picture when looking at relative values.

When combining Figure 5.7 and Figure 5.8, one can see that for all conditions (requiring $D/L < 5$), the resin and column volumes needed for the feasible channeled monoliths ($\epsilon_b \approx 0.9$) is much lower than for the packed beds with feasible particle diameters ($d_p \approx 500 \, \mu m$), especially at lower affinities. At low affinities ($K = 1$), the channeled monoliths show the best combination of column and resin volumes and lowest $D/L$ at intermediate size channel porosities, $0.9 > \epsilon_b > 0.5$, although according to Figure 5.7 slightly above the $D/L$ limit. This is not the case at high $K$ values (Figure 5.8b) where channeled monoliths require a resin volume that increases monotonously with the porosity, analogously to packed beds. These trends influenced by the affinity constant are in good agreement with the ones found in Figure 5.6 representing the specific productivity.

### 5.3.5 Window of design

All characteristics discussed in the previous sections can be compiled into a single window of design that indicates the best structure for a given set of process requirements. Figure 5.9 shows the window of design covering a wide range of efficiencies ($N_{plates} = 50–500$) that are typical of this kind of processes. The channeled monoliths having a small characteristic structure size show a
Figure 5.8: Relative comparison of column performance and required column dimensions for the different structures in this study for any fixed set of process requirements \((Q, \Delta P, N_{plates})\) and for a \(K\) value of a) 1 and b) 100.

higher specific productivity than the packed bed for all combinations of channel porosity \(\epsilon_b\) and particle diameters \(d_p\) considered and under almost all conditions. The continuous silica monolith is not shown, since it requires high \(D/L\) values even at the low throughputs considered. For low throughputs \((Q/\Delta P < 2 – 4 \text{ cm}^4/\text{(kg} \cdot \text{s}))\), a packed bed can compete, depending on the conditions, with a channeled monolith with \(\epsilon_b = 0.5\), but for higher throughputs and particularly low affinities, the channeled monolith is the undisputed optimal configuration. At higher affinities, the packed bed might compete with channeled monoliths (especially if \(K >> 100\), the characteristic length in the monolith is much higher than 3 \(\mu m\) and the throughput is low).
5.4 Conclusions

Several different adsorbent structures have been compared for the design of an adsorptive system for industrial scale isolation of components from large streams: (i) axial packed beds with different particle diameters, (ii) continuous silica monoliths and (iii) channeled monoliths having flow channels, with between those channels, porous walls with varying thicknesses. The systems were evaluated with a number of criteria:

1. The mass transfer characteristics were determined with the height equivalence of a theoretical plate (HETP), which is directly related to the minimum length of a column required for sufficient removal of the component from the feed. In this HETP, contributions from axial
dispersion and from mass transfer limitations were identified outside and inside the porous matrix. Generally, the packed beds and the monolith without flow channels showed the smallest HETP.

2. By setting limits on the pressure drop that has to be applied for a given throughput, column length-to-width ratios could be determined and were within realistic industrial boundaries only for a few systems; mostly for the channeled monoliths with high porosities and for packed beds with large particle sizes.

3. The required volumes of the resin and of the total column were compared, and found most optimal for the channeled monoliths, especially for systems in which the resin has a low affinity for the target component. By combining all criteria, a window of design could be constructed, that shows that for most applications a channeled monolith with a larger channel porosity ($\varepsilon_b = 0.9$) performs best; however a channeled monolith with somewhat lower porosity ($\varepsilon_b = 0.5$) performs better in terms of the required resin and total column volumes.

It is therefore clear that the different criteria for large-volume adsorptive processes with small generally lower-valued target components (e.g. in the food industry), compared with low-volume high-value systems (e.g. pharmaceutical applications), point towards the use of hierarchically structured adsorption media such as channeled monoliths. Not only do they have the best combination of throughput, capacity and transfer rates but also a higher permeability.

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General discussion
6.1 Introduction

This thesis explores the principles that determine the suitability of adsorbents for the adsorptive separation of small food ingredients from large streams as an alternative to commonly used expensive packed bed resins. Besides experimental data, emphasis is put on providing model-based tools or guidelines.

In chapter 2, we developed a partial purification process using activated carbon to separate the lacto-tripeptide IPP from an hydrolyzate (1.5% w/w). The purity of the initial mixture was doubled, with a recovery of IPP of about 80% in the first cycles of adsorption. The separation process developed in this chapter was repeated along several adsorption-desorption cycles until complete exhaustion of the activated carbon packed bed column. The evolution of the adsorptive and desorptive yields revealed not only the presence of irreversible sites but also a pore blockage effect due to size exclusion effects.

Chapter 3 compares the performance of fresh, activated carbon columns with channeled monoliths with respect to the productivity, the pressure drop and fouling of the columns. The plugging behavior was found to be due to inter-particle blockage and the presence of two fouling trends. This was not observed in the channeled monoliths that presented a negligible susceptibility to plugging while presenting similar productivity.

In chapter 4, we modeled the adsorption of lactose on the two activated carbon structures by estimation of the isotherm and mass transfer diffusion parameters. We found that the activated carbon packed bed and channeled monolith had similar intraparticle and external mass transfer rates. The main differences in performance were associated to the axial dispersion and to the difference in the characteristic length of the columns.

In chapter 5, we proposed a model-based methodology for adsorbent selection as a function of the process requirements. This methodology was used to compare the conventional packed bed, monolithic columns with channeled monoliths and build a window of design for a large scale chromatographic process. We estimated the benefits of using channeled monoliths on a large scale from a process design point of view. A general conclusion is that channeled monoliths show potential for the separation of food ingredients.
Chapter 6

General discussion

In the current chapter, we will first discuss the bottlenecks of using chromatographic systems such as those based on activated carbon, including the exhaustion of the column caused by irreversible adsorption that was observed in chapter 2. This discussion involves potential solutions to overcome this irreversible adsorption, as well as a suggested method to assess the mechanisms behind column exhaustion and fouling by exploring the adsorption kinetics. As an example for this method we used the exhausted activated carbon from chapter 2. Furthermore, using the conclusions from Chapter 3 and Chapter 5 for channeled monoliths, we will draw conclusions for the design and application of these structures. Other possible adsorbents considered but not used in this thesis will be discussed for further considerations. Finally, some thoughts will be given for future prospects in the selection of adsorbents.

6.2 Bottlenecks vs opportunities of activated carbon

While this thesis did not deal exclusively with active carbon as chromatographic material, it was the material that was found to have the widest applicability, combining good characteristics for large scale applications. As such it deserves more attention in the design of chromatographic systems for food streams, and therefore we will discuss the findings of this thesis on active carbon, and extend the discussion with further considerations.

Activated carbons are generally considered low-costs adsorbents as they can be derived from very low-value sources such as agricultural waste, e.g. nutshells, coconut husk, peat and are much less inexpensive than the usual chromatographic resins (e.g. ion exchange resins). This makes activated carbon especially interesting for large-volume applications. Furthermore, activated carbon is known for having a high surface area, relatively small pore sizes and therefore high capacity for small molecules. It is therefore possible to use higher concentrations and therefore, in some cases, reduce the volume of feed that needs to be processed. However, since the selectivity of activated carbon is mainly based on relative hydrophobicity, the product purities that can be obtained are generally not very large. In addition, the activated carbon has limited reusability. Therefore, all these aspects will be discussed for the mentioned potential application.
6.3 Purification potential

6.3.1 State of the art

The adsorption of organic molecules on activated carbon is affected by its pore texture (pore size distribution and surface area), surface chemistry, mineral matter content and finally by the size and inner surface accessibility of the adsorbing molecules [129]. Due to its chemical heterogeneity, originating from the use of different raw materials, and variation in the carbonization and activation processes, the affinity and selectivity of the activated carbon to components from aqueous solutions are understood only in general terms. While in general the presence of oxygen containing groups on the carbon surface influences its hydrophobicity, other structure-function relations are more complex. These are reviewed in literature [130].

Activated carbon has been shown to be a selective adsorbent in the purification of oligosaccharides from mixtures of mono- and disaccharides. It has a higher affinity towards the sugars with higher degree of polymerization [15] which have an hydrophobic character due to the increasing number of CH groups. Therefore, activated carbon has enabled the purification of fructooligosaccharides [131] and galactooligosaccharides [5] with purities up to 97% and considerable yields when desorbing the sugars with controlled concentrations of ethanol. Activated carbon adsorption compares well to other separation techniques such as nanofiltration, size exclusion chromatography and yeast treatment [5]. Especially for concentrated streams and products, and in situations where large volumes have to be treated, an activated carbon column may be preferred. With dilute streams and in applications where resin cost is not an issue, other methods, such as size exclusion chromatographic resins may be a better choice [5]. As in this thesis the research question was on the processing of larger volumes, here activated carbon is a very suited adsorptive material.

In chapter 2 we reviewed the limited literature regarding the use of activated carbon in order to enrich fractions of peptides. In this chapter we also demonstrated that it is possible to make use of the interactions between activated carbon, the molecules and the buffer conditions in the same way as by conventional materials used in hydrophobic interaction chromatography. In this
way and by optimizing the gradient used during elution, the purity during the fractionation can be increased.

### 6.3.2 Enhancing the product purity

Activated carbon is not as specific as an adsorption medium based on the use of affinity ligands; however it can yield a purity suitable enough for food enrichment. One may enhance the purity that can be obtained by modifying the surface of the carbon (e.g. its hydrophobicity), the interaction between the solvent (water) and the solutes in the solution or by using different porosities.

To show the influence of the chemistry (e.g. acidity) and textural characteristics of different activated carbons on their selectivity for a case as the purification of oligosaccharides (degree of polymerization < 2), we estimated the Langmuir parameters from the adsorption isotherm. Table 6.1 summarizes (from chapter 3 and from literature [132]) the properties of different activated carbon adsorbents, as well as the pH at which they were assessed. Table 6.2 gathers the results from the adsorption isotherm of three sugars using the different carbons. Figure 6.1 shows the selectivities of adsorption of maltotriose compared to glucose and lactose.

#### Table 6.1: N\textsubscript{2} isotherm result parameter from different commercial activated carbon used for sugar isotherm determination (according to [132] and chapter 3.)

<table>
<thead>
<tr>
<th>Activated carbon</th>
<th>BET surface area m\textsuperscript{2}/g</th>
<th>$\frac{p}{p_0}$ &lt; 0.5</th>
<th>Micropores (&lt;2 nm)</th>
<th>Pore volume v/v %</th>
<th>Mesopores (&gt;2 nm)</th>
<th>cm\textsuperscript{3}/g Total at $\frac{p}{p_0} = 0.95$</th>
<th>pH\textsuperscript{**}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Darco</td>
<td>660</td>
<td>36.7</td>
<td>63.3</td>
<td>0.71</td>
<td></td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td>CNR-115</td>
<td>1834</td>
<td>73</td>
<td>27.0</td>
<td>1.01</td>
<td></td>
<td>7.2</td>
<td></td>
</tr>
<tr>
<td>RGC-30</td>
<td>1520</td>
<td>39.5</td>
<td>60.5</td>
<td>1.14</td>
<td></td>
<td>6.3</td>
<td></td>
</tr>
<tr>
<td>TE7/20</td>
<td>1499</td>
<td>78.6</td>
<td>21.4</td>
<td>0.77</td>
<td></td>
<td>9–11</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{p and p\textsubscript{0} are the partial and vapor pressures of N\textsubscript{2} (in mmHg).}

\textsuperscript{approximate values considering reduced aging of the activated carbons.}

As seen in Table 6.2, the Darco activated carbon shows a much lower surface area compared to the other activated carbons. This results in a lower adsorptive capacity for the different sugars. It is possible to lower the interaction of the
sugars with the activated carbon by acidic surface modification. An acid activated carbon has more oxygen containing surface groups and thus has a less hydrophobic character. Tables 6.1–6.2 and Figure 6.1 together show that the acid washed activated carbons (Darco, CNR-115 and RGC-30) have a lower affinity for the sugars. Remarkably, the affinity towards the smaller sugars (glucose) is reduced more strongly by the acid groups than the affinity towards the bigger sugars. Thus, the selectivity of maltotriose relative to glucose (seen in Figure 6.1) is higher using the acid washed activated carbon compared to the more basic (more hydrophobic), which indicates that this modification can improve the purity of the adsorbed components.

While the isotherm represents the equilibrium behavior on the activated carbon, desorption may yield different values than just the adsorption. We have therefore run ad- and desorption batch experiments with a commercial Vivinal® GOS sugar mixture containing the target galactooligosaccharides, on three activated carbons that differ in their surface modification. We used two types of commercial acid washed (AW) activated carbons: AW1 (C5510) washed

Table 6.2: Langmuir parameters of glucose, lactose and maltotriose determined by single-component isotherm determination experiments.

<table>
<thead>
<tr>
<th>Activated carbon</th>
<th>Glucose $K_d$ (l/g) $q_{max}$ (mg/g)</th>
<th>Lactose $K_d$ (l/g) $q_{max}$ (mg/g)</th>
<th>Maltotriose $K_d$ (l/g) $q_{max}$ (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Darco, Norit*</td>
<td>0.106±0.012 92±2.7</td>
<td>0.94±0.31 117.7±2.5</td>
<td>2.05±1.0 133.2±3.1</td>
</tr>
<tr>
<td>CNR-115**</td>
<td>0.026±0.011 342±94</td>
<td>2.23±0.52 189±15</td>
<td>6.0±2.2 282±37</td>
</tr>
<tr>
<td>RGC-30***</td>
<td>0.102±0.014 246±16</td>
<td>35±51 49±16</td>
<td>153±15 268.2±3.7</td>
</tr>
<tr>
<td>TE7/20***</td>
<td>0.29±0.18 203±87</td>
<td>2.31±0.33 362.4±9.9</td>
<td>15.4±2.0 218.5±2.2</td>
</tr>
</tbody>
</table>

Source: *[15], ‡batch estimations, ‡‡frontal analysis

Figure 6.1: Comparison of selectivities for different activated carbons.
with phosphoric and sulfuric acid and AW2 (C4386 or Norit SX Plus) washed with hydrochloric acid, both from Sigma-Aldrich. Figure 6.2 shows (a) the adsorption yield of the different sugars and (b) the ads- and desorbed yields of GOS with the resulting GOS purity after loading the GOS mixture on three activated carbons with difference in hydrophobicity. Figure 6.2 confirms the observation from Figure 6.1. The acid washed activated carbon presents a lower adsorption yield for the sugars but particularly for the smaller sugars (Figure 6.2a). Although the differences are not so big, this result suggests again that the more hydrophilic surfaces due to acid washing of the activated carbon, have a higher effect on the adsorptive capacity of the smaller sugars. Besides this reduction, the bigger sugars (GOS) have a higher yield of adsorption and desorption caused by the reduced interaction of the more hydrophilic surface. This is an interesting result since we not only see a purity increase but also a higher desorption yield which is important for activated carbon reusability.

As we have seen in chapter 2, ethanol can be used for the recovery by using a gradient during the desorption step of the separation process. Another option is to add ethanol during the loading in order to have an influence on the adsorption of the different components. In both cases, the use of ethanol can be optimized and by tuning the polarity of the mobile phase it is possible to further increase the purity. Desorption at different ethanol concentrations was already shown in literature to have an effect on the recovery of sugars with
different degree of polymerization [5, 131]. To see the effect of ethanol during the adsorption step, we ran different experiments using the AW1 activated carbon with loading with Vivinal® GOS dissolved in different ethanol concentrations (Figure 6.3). Figure 6.3 shows that with around 10% ethanol or higher, the breakthrough curves become steeper and the adsorbed components are displaced during the adsorption. Eventually, these steeper and displaced breakthrough curves enable the elution of small sugars first while increasing the dynamic binding capacity of the bigger sugars at standard industrial conditions (e.g. \( C/C_0 \approx 10\% \)). Consequently, the adsorption using more hydrophilic carbon using ethanol during the adsorption could also lead to an increased yield and purity in this application.

![Figure 6.3](image_url)

**Figure 6.3:** Breakthrough curves obtained after loading of Vivinal® GOS dissolved in four different ethanol concentrations on a AW1 activated carbon.

Furthermore, the temperature was shown to have an effect in the purity of the enrichment process of oligosaccharides. We performed the separation process
of the commercial Vivinal® GOS at different temperatures with the experimental set-up as shown in Figure 6.4. Figure 6.5 indicates that a higher purity can be achieved at higher temperatures.

According to the breakthrough curves during the adsorption step, this trend occurs due to a higher displacement effect of the small sugars by the sugars with higher degrees of polymerization.

The use of higher temperatures is convenient since oligosaccharides are generally produced at temperatures around 60 °C [133]. Therefore, the separation with activated carbon could be done directly after the conversion without any cooling step though a separation step to retain the enzymes would still be necessary (e.g. UF).

6.3.3 Adsorbent reusability

Activated carbon needs to be regenerated and reactivated after several adsorption-desorption cycles [62, 64, 134–136]. In chapter 2 this phenomenon was indeed observed with peptide adsorption. Figure 6.6 illustrates the hypothetical mechanism of the exhaustion process while exposing the active carbon to 100 loading cycles as explained in chapter 2. The 10 first cycles result
Figure 6.5: Adsorption and desorption curves of Vivinal® GOS at different temperatures. The column was loaded with a 67 g/L feed solution, then equilibrated with water and finally, the adsorbed sugars were desorbed with an elution gradient of 0-10% ethanol.
in initial irreversible adsorption (step 1). During these cycles the adsorption yield decreases while the elution yield increases because of the gradual saturation of the irreversible sites. Consecutively, the adsorption leads to gradual pore blockage (step 2 and 3) which is due to a size exclusion effect that was not yet observed during the first 10 cycles. Subsequently, packed bed blocking and clogging of the interparticle spaces of the column takes place. This clogging has been confirmed and modeled in chapter 3. The two models in

Figure 6.6: Hypothetical graphical explanation of the steps during the exhaustion (described in chapter 2) of the activated carbon during several adsorptive and desorptive cycles after loading of the peptide hydrolyzate.
chapter 3, that fitted the evolution of the packed bed pressure drop, assume a two-step saturation mechanism: a first step with pore blockage and saturation, and a second step leading to "cake" formation in the interstitial spaces (according to the model that uses an analogy to membrane fouling) and with the increase in constriction of the "channel" (when using the model that follows an analogy to fouling on the walls of equivalent channels).

Fouling or exhaustion of activated carbon may be prevented or reduced at its origin by manipulation of the interaction or the stationary phase. As mentioned in chapter 2, peptide irreversible adsorption can be reduced by weakening the interactions with a more hydrophilic (acidic) activated carbon, more hydrophobic mobile phase (e.g. adding ethanol) or reduction of the ionic strength (lower salt concentration). Figure 6.2 shows that the commercial acid washed activated carbons have a higher desorption yield (give less irreversible adsorption) than the non-acid washed carbon presented in the experimental chapters of this thesis. Apart from the surface chemistry, the pore size in relation to the molecular weight of the fouling components was shown to be an important parameter.

The shallow bed method used in chapter 4 was used to determine the parameters describing the uptake kinetics of any type of adsorbent: total capacity at equilibrium ($q^*$) external film mass transfer coefficient ($k_f$) and surface diffusion ($D_s$). While it can be used in the screening for finding suitable adsorbents, it is also suited to characterize the consequences of fouling by many adsorption-desorption cycles.

The shallow bed method was applied on repacked activated carbon used after 10 and 95 cycles according to the method in chapter 4. Figure 6.7 illustrates the adsorption in time, derived from the experimental data and the corresponding fit obtained after application of the shallow bed method on fresh and 10-cycle exhausted activated carbon, while Table 6.3 shows the fit parameters obtained after parameter estimation using the homogeneous diffusion model (equations (4.3)–(4.7)) and using the initial slope of the uptake curve (as shown in equation (4.10)). Results using the 95-cycle exhausted activated carbon are not shown due to the low adsorption and the resultant unreliable uptake curve.
Table 6.3: Fitted parameters of fresh and 10-cycled used activated carbon using the shallow bed method used in chapter 4.

<table>
<thead>
<tr>
<th></th>
<th>(k) (s(^{-1}))(^a)</th>
<th>(D_\text{s}) (m(^2)/s)(^b)</th>
<th>(k_f) (m/s)(^b,c)</th>
<th>(q_f) (kg/m(^3))(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh activated carbon</td>
<td>1.4 (\times) 10(^{-3})</td>
<td>1.6 (\times) 10(^{-12})</td>
<td>1.1 (\times) 10(^{-5})</td>
<td>40.0</td>
</tr>
<tr>
<td>Activated carbon after 10 cycles</td>
<td>3.2 (\times) 10(^{-4})</td>
<td>5.9 (\times) 10(^{-14})</td>
<td>1.1 (\times) 10(^{-6})</td>
<td>17.2</td>
</tr>
</tbody>
</table>

\(^a\) Linear driving force approximation (equations (4.13)–(4.14))

\(^b\) Homogeneous diffusion model fit (equations (4.3)–(4.7))

\(^c\) Fit to the initial slope of the uptake curve and use of (equation (4.15))

Table 6.3 shows that after the 10\(^{th}\) cycle there is a decrease in surface diffusion; in line with the findings in [136]. This proves that after 10 cycles there is an increase in hindrance in diffusion by pore blockage. The reduction in the film mass transfer coefficient might be caused by an increase in particle diameter as a result of the saturation of the surface of the adsorbent particle. These results confirm the hypothesis that was earlier laid out in Figure 6.6. This indicates that the shallow bed technique can be used for assessing the degree of fouling, not only in packed bed structures but also in other structures such as channeled monoliths.

Several regeneration techniques have been developed and used over the past decades. Thermal volatilization and oxidation at high temperature (800–850 °C) of adsorbed organics has been the most common technique in regeneration.
and reactivation of activated carbon. This method evidently presents some disadvantages such as cost of the process [138], loss of carbon and treatment of the exhaust gases. Chemical regeneration with inorganic chemicals or organic solvents [139–143] is a more efficient method than the thermal method [144], but the solvents used in the regeneration still need further treatment. Multiple regeneration technologies have emerged to try to reduce the disadvantages of the other two methods: electro-thermal [145], electrochemical [146, 147], using superheated steam [148], using ozone [149], supercritical fluid extraction [150], microwave assisted [151–153], biodegradation [154], catalytic oxidation [155] and ultrasound [156–159], either alone or combined with other techniques [160].

6.4 Applicability of channeled monoliths

6.4.1 Processing of large streams

To process large streams, it is important that the hydrodynamic resistance in the column is low, and that the packing will not block easily. According to chapters 3 and 5, channeled monoliths have the advantage of a large permeability which reduces the need of the high diameter-to-length column ratios that are needed for packed beds. However, as was shown in chapters 3 and 5 the efficiencies of these structures are still low compared to packed beds (Figure 5.4) resulting in early breakthrough and low dynamic binding capacity (Figure 3.7) when using relatively high velocities or thick channel walls. To overcome this disadvantage the design of channeled monoliths should focus on increasing the residence time and lowering the diffusive time which means:

- small characteristic lengths: by manufacturing thinner walls between the channels, or applying thin coatings on inert channeled monoliths
- small channel diameters
- longer columns or columns in series

Although the manufacturing of small walls presents some challenges, already some work has been done on achieving high cell density monoliths with small
channels and thicknesses up to 6 µm [83]. Hence, this should encourage the production of this type of stationary phases. Regardless of possible manufacturing breakthroughs, the application of thin coatings might be the current easiest option. Nonetheless, the unused support volume should be minimized to the micron range in order to have negligible volume increase and have competitive capacities. Since the monolith support does not need porosity, it should be feasible to manufacture high cell density materials with enough mechanical strength.

6.4.2 Selective separation of an untreated feedstock

Channeled monoliths dedicate a larger volume to convection (flow-through) than other structures. Consequently, as shown in chapter 3, channeled monoliths are less susceptible to plugging and can handle higher concentrations and column volumes than packed beds, and even unfiltered feedstock. Preliminary tests with the raw stream from the TensGuard™ S hydrolyzate in a channeled monolith showed no increase in pressure drop or flow decrease. The same experiment in a packed bed resulted in a decrease in flow rate caused by the increased column resistance due to plugging. The low susceptibility shown with channeled monoliths suggests their suitability for the isolation of components from raw streams. Thus, it is possible to skip a solid-liquid separation unit operation such as ultrafiltration that are needed for instance to separate the enzymes from a hydrolyzed product. This reduces the costs and complexity of the process.

6.4.3 Selective separation during synthesis

Selective and continuous removal of a synthesized product from a reaction mixture enhances the reaction and overall product yield. This was shown in the separation of oligosaccharides from mono- and disaccharides with activated carbon in a continuous batch process [15, 131]. Channeled monoliths could be ideal systems for this, considering their proven low obstruction by fouling, absence of pretreatments and low pressure drop.

To reduce the mass transfer effects increase the productivity and reduce the size of equipment in catalysis or biocatalysis, enzymes may be immobilized in a
packed bed reactor. This has already been studied for the enzymatic production of GOS [161]. This could as well be performed using channeled monoliths, which has long been described in literature mainly in chemical catalysis [162, 163]. Thus, a catalytic channeled monolith with adsorptive channels would form a simultaneous synthesis and removal system, reducing the diffusion paths and increasing at the same time the conversion into one single unit operation.

6.4.4 Electrical adsorbent regeneration

Channeled monoliths have shown to be good conductors of electricity and are electrically heatable [164, 165]. By the transformation of electricity into heat it is possible to desorb bound organic compounds [166]. In this way, channeled monoliths can be regenerated by electricity [166]. This has advantages compared to normal heating in view of the lower costs and regeneration speed compared to other heating methods [167].

6.5 Alternative adsorbents

This thesis was restricted to the use of channeled monoliths and discusses the use of foams or continuous monolithic columns. However, other options may be considered as they might have potential benefits to help overcoming some of the bottlenecks already discussed for food applications.

6.5.1 Ordered porous structures

Alternative ordered porous structures, such as zeolites, mesoporous silicates, or metalorganic frameworks (MOFs), because of their tunable functionality and structure have potential in the separation of functional food ingredients. These materials can also be made into stationary phases such as channeled monoliths. Figure 6.8 illustrates some of these ordered mesoporous structures. Zeolites can be found or synthesized in different forms and have shown good adsorptive properties: in terms of high capacity, selectivity and specificity. Zeolites have been widely applied in the chemical industry, in water treatment [170] and food industrial applications like the separation of small sugars [171, 172] and
3.4 Electrical adsorbent regeneration

Channeled monoliths have shown to be good conductors of electricity and are electrically heatable (Garcia-Gomez et al., 2010a, b). By the transformation of electricity into heat it is possible to desorb bound organic compounds (Gadkaree and Tyndell, 2000). This way, channeled monoliths can be regenerated by electricity (Gadkaree and Tyndell, 2000). This has advantages compared to normal heating due to the lower costs and regeneration speed compared to other heating methods (Sanchez Liarte, 2009).

4. Other possible suitable adsorbents

This thesis was restricted to the use of channeled monoliths and discusses the use of foams or continuous monolithic columns. Though other options should be considered as they have potential benefits to help overcoming some of the bottlenecks already discussed for food applications.

4.1 Ordered porous structures

Alternative ordered porous structures, such as zeolites, mesoporous silicates, or metal-organic frameworks (MOFs), because of their tunable functionality and structure have also potential in the separation of functional food ingredients. Besides these materials can be part of stationary phases such as channeled monoliths. Figure 6.8 illustrates some of these ordered porous structures.

Mesoporous silica
Zeolites
Metal-organic frameworks

Figure 6.8: Overview of some ordered porous structures. Illustration shows a Zeolite LTA framework [168], an hexagonal pore of molecular sieve MCM-41 [169] and crystals of metal-organic framework.

the separation of oligosaccharides [173]. Zeolites can be extruded [174] or synthesized in-situ into channeled monoliths with wall thicknesses of up to 6 µm [83, 174]. The main disadvantage perhaps is the limitation of the pore size which is about 13 Å, and the very low diffusivity in these small pores.

Hierarchical mesoporous silica materials (e.g. MCM-41, SBA-15) can be tailored into a variety of porous architectures, wider range of pores 20–100 Å, maintaining very high surface areas and narrow pore size distributions. Therefore, these materials have been considered and reviewed as promising adsorbents for the efficient separation of functional food ingredients [175]. The suitability of these materials for a real food application still needs to be assessed.

Metal-organic frameworks (MOFs) are another type of ordered porous structure consisting of metal ions (used as connectors) and organic ligands (used as linkers). Their structure can be changed by changing the building blocks. This flexibility may make them suitable for a range of applications [176]. The combination of extremely large surface area and pore volumes together with their tuneable functionality makes them suitable for both selective...
separations and catalytic processes. Yet, these structures are very recent and therefore their production costs and robustness need to be further investigated.

6.5.2 Channel geometries

The performance of channeled monoliths can be improved by using different geometries besides the most commonly used squared channel. For instance, hexagonal channels have been suggested to be the most effective and practical \[23\]. The hexagonal shape would reduce the inconvenience of squared channels, which have a larger local characteristic length in the four corners of the solid phase as described for rectangular and squared channel in \[49\] and used in chapter 5. Hence, a practical comparison on the dynamics of adsorption with different channeled monolith geometries is recommended.

6.5.3 Structured adsorbents

There are several other types of adsorbents that have already been used for the separation of food proteins and present particularly pressure drop advantages over packed beds: polymeric foam structures, hollow fibers \[96, 177, 178\], coated fibers and mixed matrix membranes \[87–89\]. Most of these have relatively low surface area and capacity which is even more important in the separation of small molecules. Nevertheless, it is important to keep tract on the developments of new materials with higher surface areas because those could make the difference for an optimal performance of one type of structured adsorbent.

6.5.4 Grafting technology

Grafting fibers onto adsorbent surfaces is a recent technology that increases the surface area of adsorbents (e.g. foams) while reducing cycle times \[179\]. Figure 6.9 represents the most important candidate, which is the use of grafted carbon nanofibers. The internal mass transfer in these systems can be almost excluded while the external mass transfer is reduced considerately. However, these systems will always require a mechanical support, which, if inert, does not
Figure 6.9: Principle of grafted textiles, with carbon nanofibers as an example, and in comparison with conventional porous particles [179].

contribute to the adsorptive capacity. Thus, also here the question of a suitable carrier is important.

6.6 Future prospects in the design of chromatographic processes

Chromatography research tends towards modeling and consequent prediction of adsorption and chromatographic processes. The drive for this comes probably from the stronger restrictions in terms of production size, increasing demand of products or stronger need for treatment of waste streams. In addition, the time for developing a process is continually decreasing. The predictions from models
can eventually be used in a multicriteria decision model including all the aspects involved in the process, which enables the quick and reliable development of processes. Alternatively, a less ambitious aim would be to assemble a toolbox with the corresponding models at hand and model different case scenarios which would still help in faster development of chromatographic processes.

The first efforts at the molecular level of predicting adsorption behavior have been already undertaken through thermodynamic models, such as UNIQUAC/UNIFAC, NRTL, etc., which have potential in a priori prediction of thermodynamic interactions at different conditions (temperature, pH, etc.) [180–182] and with different effects such as stoichiometry, equilibrium, steric effects and binding affinity.

Regarding adsorption kinetics, efforts have been made regarding prediction of pore diffusion parameters. However, the rates of surface diffusion cannot be predicted beforehand from the elemental chemistry, porosity and properties of the target components. Managing to do this would reduce time needed to find the parameter values from experimentation, e.g. in chapter 4, for the prediction of the kinetics of adsorption.

Next, the accuracy and applicability of the pressure drop equations should be extended, towards the effects of compaction [72], packing methods, and fouling (as seen in chapter 3). The prediction of these effects would enable us to accurately predict the pressure drop over those columns.

Finally, once it is possible to make quantitative estimations of thermodynamic, mass transfer and pressure drop parameters, it is possible to screen for the best adsorbents considering the different parameters of the process such as efficiency (number of plates), pressure drop and throughput as has been done in chapter 5.

### 6.7 Conclusions

Robust, cost-effective adsorbents with low pressure drop, faster mass transfer and high throughput are needed for the separation of small functional ingredients from large streams. Activated carbon is a low-cost adsorbent with high capacity for small molecules and channeled monoliths are known to present low pressure
drops. So either alternatives or a combination of both could be an option in the separation of small food molecules.

This thesis showed the potential of channeled monoliths in the field of chromatographic separation of small molecules. We showed the strengths of these types of structures: high permeability, low susceptibility to plugging, possibility of improving the adsorbent performance due to the non-negative trade-off between mass transfer as in the packed bed and possibilities of reducing the size of the equipment needed. Therefore, channeled monoliths could extend the application range of adsorptive beds for instance to streams of higher concentration or to untreated streams that contain particulate matter.

Nevertheless, to make a difference relative to packed beds, channeled monoliths design with high cell densities and short channeled wall thicknesses need to be developed, to enable high-throughput adsorption of components with high selectivity and capacity.
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Appendix
A.1 Appendix chapter 2

A.1.1 Identified peptides through MS masses

![Graph a](image1.png)

MA: m/z 221

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![Graph b](image2.png)

SGEP: m/z 389

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<td>30</td>
</tr>
<tr>
<td>18</td>
<td>25</td>
</tr>
</tbody>
</table>

![Graph c](image3.png)

SGEP: m/z 389

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Relative abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>95</td>
</tr>
<tr>
<td>4</td>
<td>90</td>
</tr>
<tr>
<td>6</td>
<td>85</td>
</tr>
<tr>
<td>8</td>
<td>80</td>
</tr>
<tr>
<td>10</td>
<td>75</td>
</tr>
<tr>
<td>12</td>
<td>65</td>
</tr>
<tr>
<td>14</td>
<td>35</td>
</tr>
<tr>
<td>16</td>
<td>30</td>
</tr>
<tr>
<td>18</td>
<td>25</td>
</tr>
</tbody>
</table>
Appendix

TSTP: m/z 405

KKNQDKTEIP: m/z 400 + 600
KTEIP: m/z 294

ATLE: m/z 433
EVIESPP: m/z 385 + 770

TINTIASGEP: m/z 501 + 1002
Figure A.1: Identified peptides through MS masses. Supplementary of Figure 2.2.
A.1.2 Adsorption and desorption yields LC–MS

Figure A.2: Adsorption and desorption yields of cycles 3, 27 and 66 calculated from LC–MS for all the components present in the initial mixture. Supplementary of Figure 2.5.
**A.2 Appendix chapter 3**

A.2.1 Determination of the specific permeability – derivation of equation (3.4)

The permeability in a channeled monolith can be derived by equation (3.3) and becomes:

\[ B_0 = \frac{\epsilon_b \cdot a^2}{28.4} \]  

(A.1)

To obtain the dimensionless form of the permeability we divide both terms of the previous equation by \((2\delta)^2\), \(\delta\) being the cell size of the monolith (channel radius plus half the thickness of the channel wall). To make the right-hand side of the equation independent of the characteristic length \(\delta\) we can express it as a function of the porosity and the channel width \(a\) by using the definition of external channel porosity of one cell:

\[ \epsilon_b = \frac{V_{\text{void cell}}}{V_{\text{cell}}} = \frac{A_{\text{void cell}}}{A_{\text{cell}}} = \frac{a^2}{(a + 2\delta)^2} \]  

(A.2)

From this relation we obtain an equation of second degree:

\[ 4\epsilon_b \cdot \delta^2 + 4a \epsilon_b \cdot \delta + a^2(\epsilon_b - 1) = 0 \]  

(A.3)

Using the positive root obtained the specific permeability can be expressed as:

\[ \frac{B_0}{(2\delta)^2} = \frac{\epsilon_b \cdot a^2}{28.4 \left[ 2 \cdot \frac{a}{\sqrt{\epsilon_b}} \left( \frac{1}{\sqrt{\epsilon_b}} - 1 \right) \right]^2} = \frac{\epsilon_b}{28.4 \left( \frac{1}{\sqrt{\epsilon_b}} - 1 \right)^2} \]  

(A.4)
A.2.2 Derivation of the fouling model equation used in the analogy with parallel channels (equation (3.6))

To derive the descriptive model of fouling in the chromatographic column we assume that the packing consists of a set of parallel channels with length $L$ and radius $R$. We assume that the liquid contains a component (e.g. protein) that can adsorb to the surface and slowly obstruct the channel. Assuming a constant flow rate, and that the reduction in radius due to fouling is compensated by a larger pressure gradient, we expect that the rate of fouling is dependent on the concentration in the liquid:

$$J = -kc$$  \hspace{1cm} (A.5)

$$JA \, d\, t = \rho \, dV$$  \hspace{1cm} (A.6)

Substituting $J$ by equation A.5 we obtain:

$$kc2\pi RL \, d\, t = -\rho 2\pi L \, dr$$  \hspace{1cm} (A.7)

Rearranging:

$$\frac{dR}{dt} = \frac{kc}{\rho}$$  \hspace{1cm} (A.8)

The rate of fouling can be limited by the kinetics of the adsorption or by the mass transfer. If the adsorption is reaction limited, $k$ is probably a constant. If it is limited by mass transfer, it is not a constant, but determined by the external mass transfer resistance. This resistance can be correlated with the Sherwood number which would be constant assuming fully-developed flow as follows:

$$Sh = \frac{2kR}{D_m} = cte$$  \hspace{1cm} (A.9)

The case of reaction limitation ($k = constant$)

If $k$ is a constant,

$$R = R_0 - \frac{kct}{\rho}$$  \hspace{1cm} (A.10)

the pressure drop over this channel is given by the Poiseuille equation:

$$\Delta P = \frac{8\mu L \Phi}{\pi R^4}$$  \hspace{1cm} (A.11)
which means for the pressure applied:

\[
\Delta P = \frac{8\mu \Phi}{\pi R^4} = \frac{8\mu \Phi}{\pi} \left(\frac{1}{R_0 - k_c t / \rho}\right)^4 = \frac{8\mu \Phi}{\pi R_0^4} \left(\frac{R_0}{R_0 - k_c t / \rho}\right)^4
\]  
(A.12)

After linearization and using equation (A.11):

\[
\left(\frac{\Delta P_0}{\Delta P}\right)^{\frac{1}{4}} = 1 - \frac{t}{\tau}
\]  
(A.13)

with \( \tau = \frac{\rho R_0^2}{k_c} \), being a time constant, dependent on the tendency of fouling \( \left(k_c / \rho\right) \) and the geometry \( (R_0) \).

**The case of mass transfer limitation \((k = k_c / R)\)**

In many cases, e.g. when it is mass transfer limited, \( k \) will not be a constant. If we assume that \( k = k_c / R \), we obtain

\[
\frac{dR}{dt} = \frac{k_c}{\rho R}
\]  
(A.14)

\[
R \frac{dR}{dt} = -\frac{k_c}{\rho} \, dt
\]  
(A.15)

\[
R^2 - R_0^2 = -\frac{2k_c t}{\rho}
\]  
(A.16)

\[
R = \sqrt{R_0^2 - \frac{2k_c t}{\rho}}
\]  
(A.17)

which gives

\[
\Delta P = \frac{8\mu \Phi}{\pi} \left(\frac{1}{R_0^2 - \frac{2k_c t}{\rho}}\right)^2 = \Delta P_0 \left(\frac{1}{1 - \frac{2k_c t}{\rho R_0^2}}\right)^2
\]  
(A.18)

After linearization and using equation (A.11):

\[
\left(\frac{\Delta P_0}{\Delta P}\right)^{\frac{1}{2}} = 1 - \frac{t}{\tau}
\]  
(A.19)

with \( \tau = \frac{\rho R_0^2}{2k_c} \), and since \( k = k_c / R \) this is equal to \( \tau = \frac{\rho R_0}{2k_c} \), almost the same definition as above, but with the difference that the power has shifted from \( \frac{1}{4} \) to \( \frac{1}{2} \). We may thus expect that other dependencies will generate linearization with other powers.
The general case \( k = \frac{k_R}{R^n} \)

So, in general, if we have \( k = \frac{k_R}{R^n} \), we will find

\[
\frac{\Delta P}{\Delta P_0} = \left( \frac{1}{1 - \frac{t}{\tau}} \right)^{4(n+1)}
\]  \( \text{(A.20)} \)

with \( \tau = \frac{\rho R^{n+1}}{(n+1)k_RC} \) and therefore this gives a general equation that describes the fouling behavior.

The initial rate of fouling, \( K_0 \) or initial slope at \( t = 0 \), is in fact equal to \( \frac{4}{\tau(n+1)} \).

Substituting this value we obtain:

\[
\frac{\Delta P}{\Delta P_0} = \left( \frac{1}{1 - \frac{t}{\tau}} \right)^{K_0\tau}
\]  \( \text{(A.21)} \)
A.2.3 HPLC results from the multicomponent breakthrough of the different peptides after loading the hydrolyzate.

Figure A.3: HPLC results from the multicomponent breakthrough of the different peptides after loading the hydrolyzate. The loading was made on the activated carbon packed bed at a linear velocity of 890 cm/h. Supplement of section 3.3.9.
A.3 Appendix chapter 4

A.3.1 Parameter estimation for uptake curves using the homogeneous surface diffusion model (HSDM)

Packed bed

1\textsuperscript{st} set of data:

Table A.1: Initialization and constraints for the parameter estimation using the HSDM for the packed bed (1\textsuperscript{st} set of data).

\begin{tabular}{llllll}
Parameter & Optimal estimate & Initial guess & Lower bound & Upper bound \\
\hline
$D_s$ (m\textsuperscript{2}/s) & $8.053 \cdot 10^{-13}$ & $1.000 \cdot 10^{-12}$ & $1.000 \cdot 10^{-14}$ & $1.000 \cdot 10^{-9}$ \\
$k_f$ (m/s) & $5.358 \cdot 10^{-5}$ & $3.600 \cdot 10^{-5}$ & $7.000 \cdot 10^{-6}$ & $7.000 \cdot 10^{-5}$ \\
\end{tabular}

2\textsuperscript{nd} set of data:

Table A.2: Initialization and constraints for the parameter estimation using the HSDM for the packed bed (2\textsuperscript{nd} set of data).

\begin{tabular}{llllll}
Parameter & Optimal estimate & Initial guess & Lower bound & Upper bound \\
\hline
$D_s$ (m\textsuperscript{2}/s) & $1.082 \cdot 10^{-12}$ & $1.000 \cdot 10^{-12}$ & $1.000 \cdot 10^{-14}$ & $1.000 \cdot 10^{-9}$ \\
$k_f$ (m/s) & $7.259 \cdot 10^{-5}$ & $3.600 \cdot 10^{-5}$ & $7.000 \cdot 10^{-6}$ & $7.300 \cdot 10^{-5}$ \\
\end{tabular}

Channeled monolith

1\textsuperscript{st} set of data:

Table A.3: Initialization and constraints for the parameter estimation using the HSDM for the channeled monolith (1\textsuperscript{st} set of data).

\begin{tabular}{llllll}
Parameter & Optimal estimate & Initial guess & Lower bound & Upper bound \\
\hline
$D_s$ (m\textsuperscript{2}/s) & $8.450 \cdot 10^{-12}$ & $1.000 \cdot 10^{-10}$ & $1.000 \cdot 10^{-30}$ & $1.000 \cdot 10^2$ \\
$k_f$ (m/s) & $6.761 \cdot 10^{-5}$ & $8.000 \cdot 10^{-4}$ & $1.000 \cdot 10^{-20}$ & $1.000 \cdot 10^0$ \\
\end{tabular}
2nd set of data:

**Table A.4:** Initialization and constraints for the parameter estimation using the HSDM for the channeled monolith (2nd set of data).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Optimal estimate</th>
<th>Initial guess</th>
<th>Lower bound</th>
<th>Upper bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>$D_s$ (m$^2$/s)</td>
<td>$1.181 \cdot 10^{-11}$</td>
<td>$1.000 \cdot 10^{-10}$</td>
<td>$1.000 \cdot 10^{-30}$</td>
<td>$5.000 \cdot 10^{-9}$</td>
</tr>
<tr>
<td>$k_f$ (m/s)</td>
<td>$3.253 \cdot 10^{-5}$</td>
<td>$9.600 \cdot 10^{-6}$</td>
<td>$5.000 \cdot 10^{-6}$</td>
<td>$8.000 \cdot 10^{-5}$</td>
</tr>
</tbody>
</table>

**A.3.2 Parameter estimation for the breakthrough curves using the general rate model (GRM)**

**Packed bed**

$C_0 = 2$ g/L

**Table A.5:** Initialization and constraints for the parameter estimation using the GRM for the packed bed for $C_0 = 2$ g/L.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Optimal estimate</th>
<th>Initial guess</th>
<th>Lower bound</th>
<th>Upper bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>$D_s$ (m$^2$/s)</td>
<td>$1.918 \cdot 10^{-5}$</td>
<td>$5.800 \cdot 10^{-8}$</td>
<td>$2.000 \cdot 10^{-16}$</td>
<td>$1.000 \cdot 10^{-2}$</td>
</tr>
<tr>
<td>$k_f$ (m/s)</td>
<td>$8.692 \cdot 10^{-12}$</td>
<td>$1.000 \cdot 10^{-12}$</td>
<td>$1.000 \cdot 10^{-13}$</td>
<td>$5.000 \cdot 10^{-11}$</td>
</tr>
</tbody>
</table>

**Figure A.4:** a) Absolute residual for the outlet concentration in the packed bed and b) 95% confidence ellipsoid for the estimated parameters using a feed concentration of 2 g/L.
Channeled monolith

\( C_0 = 2 \, \text{g/L} \)

**Table A.6:** Initialization and constraints for the parameter estimation using the GRM for the channeled monolith for \( C_0 = 2 \, \text{g/L} \).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Optimal estimate</th>
<th>Initial guess</th>
<th>Lower bound</th>
<th>Upper bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>( D_s ) (m(^2)/s)</td>
<td>( 1.278 \times 10^{-4} )</td>
<td>( 3.000 \times 10^{-7} )</td>
<td>( 5.000 \times 10^{-25} )</td>
<td>( 1.000 \times 10^{-3} )</td>
</tr>
<tr>
<td>( k_f ) (m/s)</td>
<td>( 2.435 \times 10^{-12} )</td>
<td>( 9.000 \times 10^{-12} )</td>
<td>( 1.000 \times 10^{-12} )</td>
<td>( 1.500 \times 10^{-11} )</td>
</tr>
</tbody>
</table>

**Figure A.5:** a) Absolute residual for the outlet concentration in the channeled monolith and b) 95% confidence ellipsoid for the estimated parameters using a feed concentration of 2 g/L.
Nomenclature
Nomenclature

Roman letters

\(a\) \hspace{1cm} \text{Side of the channeled monolith squared channel (\(\mu\text{m}\))}

\(a_{\text{spec}}\) \hspace{1cm} \text{External surface of the channels: } a_{\text{spec}} = 2r_i/(r_o^2 - r_i^2)

\((\text{m}^2\cdot\text{m}^{-3})\)

\(B_0\) \hspace{1cm} \text{Hydraulic permeability (\(\mu\text{m}^2\))}

\(C\) \hspace{1cm} \text{Outlet concentration (g\cdot L}^{-1})

\(c_b\) \hspace{1cm} \text{Bulk concentration of lactose, equal to the feed concentration. (g\cdot L}^{-1})

\(c_e\) \hspace{1cm} \text{Equilibrium concentration (g\cdot L}^{-1})

\(C_{0,i}\) \hspace{1cm} \text{Feed concentration of a certain component } i \text{ (g\cdot L}^{-1})

\(c_{0,j}\) \hspace{1cm} \text{Feed concentration of lactose (g\cdot L}^{-1})

\(C_M\) \hspace{1cm} \text{Dimensionless resistance to mass transfer in the mobile phase}

\(c_s\) \hspace{1cm} \text{Surface concentration (g\cdot L}^{-1})

\(D\) \hspace{1cm} \text{Column diameter (m)}

\(d\) \hspace{1cm} \text{System diameter in definition of Sherwood number (\(\mu\text{m}\))}

\(d_{\text{disp}}\) \hspace{1cm} \text{Equivalent dispersion particle diameter (\(\mu\text{m}\)) [29]}

\(D_e\) \hspace{1cm} \text{Effective diffusive coefficient of the target molecules inside}

\text{the stationary phase (m}^2\cdot\text{s}^{-1})

\(d_H\) \hspace{1cm} \text{Hydraulic diameter of the channel (\(\mu\text{m}\))}

\(D_L\) \hspace{1cm} \text{Axial dispersion coefficient (m}^2\cdot\text{s}^{-1})

\(D_m\) \hspace{1cm} \text{Molecular diffusivity (m}^2\cdot\text{s}^{-1})

\(D_p\) \hspace{1cm} \text{Pore diffusivity (cm}^2\cdot\text{s}^{-1})

\(d_p\) \hspace{1cm} \text{Particle diameter (\(\mu\text{m}\))}

\(d_{\text{perm}}\) \hspace{1cm} \text{Equivalent permeability particle diameter (\(\mu\text{m}\)) [29]}

\(D_s\) \hspace{1cm} \text{Surface diffusion coefficient (m}^2\cdot\text{s}^{-1})

\(F\) \hspace{1cm} \text{Phase ratio: } (1 - \epsilon_b)/\epsilon_b

\(f\) \hspace{1cm} \text{Friction factor (–)}

\(h\) \hspace{1cm} \text{Reduced height or contribution to one theoretical plate (–)}

\text{HETP} \hspace{1cm} \text{Height equivalent to one theoretical plate (m)}

\(J_0\) \hspace{1cm} \text{Initial flux in membranes, equivalent to the superficial velocity}

\text{ (m}\cdot\text{s}^{-1})

\(K\) \hspace{1cm} \text{Equilibrium constant from the Langmuir isotherm (L} \cdot \text{g}^{-1})
**Nomenclature**

$k$ Overall mass transfer coefficient. In chapter 4, from the LDF approximation [101, 102] (s$^{-1}$)

$k_1$ Retention factor at infinite dilution: $(1 - \varepsilon_b / \varepsilon_b) \cdot (\varepsilon_p + (1 - \varepsilon_p)K)$

$K_b$ Pore blocking constant (s$^{-1}$)

$K_c$ Cake filtration constant (s·m$^{-2}$)

$k_f$ Film mass transfer coefficient (s$^{-1}$)

$K_p$ Hindrance factor for pore level diffusion (–)

$L$ Column length (m)

$m$ Amount of target component (IPP) (g)

$m_{\text{carbon}}$ Mass of adsorbent (particles or channeled monolith) (g)

$n$ Dependency of the time for blockage with the radius (–)

$N_{\text{plates}}$ Number of plates (–)

$p, p_0$ Pressure and vapor pressure of N$_2$ at 77 K (Pa)

$Pe$ Péclet number or reduced velocity: $Pe = u \cdot \delta / D_m$ (–)

$Prod_i$ Specific productivity (kg·m$^{-3}$·s$^{-1}$)

$q$ Adsorbed phase concentration (mg·g$^{-1}$)

$Q$ Flow rate (m$^3$·s$^{-1}$)

$\bar{q}$ Average adsorbent phase concentration (mg·g$^{-1}$)

$q_e$ Adsorbed amount in equilibrium (mg·g$^{-1}$)

$q_m$ Maximum adsorbed amount following the Langmuir isotherm (mg·g$^{-1}$)

$r_0, r_i$ Outer radius of an equivalent hollow cylinder used to describe intraparticle mass transfer in the channeled monolith following the LDF used by Patton et al. [23] (µm)

$R_0$ Initial inner radius of the equivalent channel with the same fouling behavior during loading (µm)

$Re$ Reynolds number (–)

$r_p$ Radius of spherical stationary phase particles (µm)

$Sh$ Sherwood number: $Sh = k_f \cdot \delta / D_m$ (–)

$T$ Mobile phase temperature (K)

$t$ Time of adsorption or desorption step (s)

$t_{res}$ Residence time (s)
Nomenclature

$t_w$  
Channeled monolith wall thickness ($\mu$m)

$u$  
Linear or interstitial velocity of the mobile phase (m·s$^{-1}$)

$u_0$  
Superficial velocity (m·s$^{-1}$)

$v$  
Linear or interstitial velocity of the mobile phase (m·s$^{-1}$)

$V$  
Process volume (m$^3$)

$V_0$  
Column void volume (mL)

$V_C$  
Packed bed or monolith column volume (m$^3$)

$V_e$  
Breakthrough curve exhaustion volume at saturation (mL)

$V_{ext}$  
Extra-column volume (mL)

$V_s$  
Breakthrough curve stoichiometric volume (mL)

$w_c$  
Corrected adsorbent wall thickness ($\mu$m)

Greek letters

$\alpha$  
Aspect ratio

$\gamma_1/\gamma_2$  
Geometrical constants (in equation (5.14)) equal to 0.7 and 0.5, respectively [126]

$\delta$  
Characteristic length ($\mu$m)

$\Delta P$  
Column pressure drop (Pa)

$\epsilon_b$  
External bed porosity: interparticle, interskeleton (equal to $\epsilon_{macro}$) [26, 29] or channel void [24]

$\epsilon_p$  
Wall, particle or intraskeleton porosity (–)

$\epsilon_T$  
Total column porosity (–)

$\lambda_m$  
Ratio between adsorbate molecule and average pore diameters (–)

$\mu$  
Viscosity of the fluid (Pa·s)

$\rho$  
Density of the fluid (kg·m$^{-3}$)

$\rho_p$  
Particle density (kg·m$^{-3}$)

$\rho_s$  
Density of the dry adsorbent (particle or channel walls) (kg·m$^{-3}$)

$\tau$  
Total time until total blockage of the column during loading (column volumes, CV)

166
Nomenclature

\( \tau_{\text{intra}} \)  
Intraparticle or intraskeleton tortuosity (–)

\( \psi_B \)  
Constant accounting for the solute-solvent interaction (equal to 2.6 in the case of water) (–)

Subscripts

87\%  
At 87\% column saturation

A  
Related to the solute

ads  
Related to adsorption

ax  
Related to axial dispersion

B  
Related to solvent

des  
Related to desorption

ext  
Related to external mass transfer

intra  
Related to intraparticle mass transfer

j  
Step concentration in the step series method during FA

macro  
Related to macropores (bigger pores)

meso  
Related to mesopores (smaller pores)
Summary
Summary

Separation and purification of functional ingredients from raw or waste streams are often done via processes that include a chromatographic step using a packed bed of resin particles that have affinity for the ingredients to be separated. A column packed with these particles presents numerous bottlenecks when dealing with untreated or large streams: a trade-off between mass transfer and hydraulic permeability, a high pressure drop and susceptibility to plugging and fouling. The large equipment (column diameters) and volume of resin needed for a moderate pressure drop and a high capacity, poses problems of elevated costs and complex operation. Other technologies such as radial flow chromatography and polymeric resins membranes may represent an improvement in other applications (e.g. pharma or fine chemicals), but at this point their capacity and costs do not seem to be feasible for the separation of small molecules from larger food streams.

The aim of the research discussed in this thesis was to find the principles that determine the suitability of different structured adsorbents, such as monoliths, for the selective adsorption and recovery of high-added value food ingredients of relatively low molecular weight, such as oligosaccharides and bioactive peptides. To ensure a cost-effective process and high capacity for small molecules, we demonstrated the feasibility of using activated carbon, and compared its adsorptive and hydraulic performance in two different structures: porous particles and channeled monoliths (”honeycomb” structures). Furthermore, we assessed the feasibility and window of operation of monoliths in terms of adsorbent and column volume required, compared to packed beds.

To demonstrate the isolation of bioactive peptides from crude mixtures with activated carbon, we used activated carbon to recover a lacto-tripeptide IPP from a commercial hydrolyzate (1.5% w/w) in chapter 2. The purity of the initial crude mixture was doubled in the isolate, to up to 35% with a recovery of IPP of about 80% in the first cycles of adsorption. This was repeated over many consecutive adsorption-desorption cycles until the activated carbon packed bed column was exhausted. This exhaustion was found to be caused not only by the occupation of irreversible sites but also by pore blockage. Finally, guidelines were
given for the competitive exhaustion of the adsorbent for process optimization in order to obtain higher purity and yield.

In chapter 3 we showed the benefits of using channeled monoliths for processing untreated streams. We compared the use of channeled monoliths with a packed bed, both made of the same type of activated carbon, for the adsorption of the lactotripeptide IPP from a crude hydrolyzate. The results showed similar productivity and dynamic adsorptive capacities at comparable linear velocities and residence times, but the packed bed showed a strong pressure drop increase during continuous loading of the column and the same consecutive adsorption-desorption cycles as studied in chapter 2. This indicated the occurrence of pore blockage and plugging of the column. These fouling mechanisms were confirmed with two semi-empirical model analogies: one analogous to membrane fouling and another using an analogy with a set of parallel channels. The strong pressure drop increase was even more noticeable at high velocities (and short residence times). These trends were not observed in the channeled monoliths: no significant pressure drop increase was found here, and high velocities were eminently feasible.

In chapter 4 the adsorption of lactose onto a bed of activated carbon particles and activated carbon channeled monoliths was described with a detailed chromatographic model, taking into account the different mass transfer resistances. First, the single component adsorption isotherm parameters were obtained using frontal analysis on both adsorbents. Second, the kinetics of adsorption of lactose on both activated carbon adsorbents were estimated using the shallow bed method, assuming an infinite bath. The uptake curves were fitted to the homogeneous surface diffusion model and the linear driving force approximation. The estimation of the intraparticle diffusion coefficient and the film mass transfer coefficient showed a similar intraparticle mass transfer performance during the uptake adsorptive process. Fitting of the breakthrough data to the general rate model describing the full column operation showed differences in performance during the overall column operation. These differences could be related to higher axial dispersion in the squared channeled monoliths. The difference between the experimentally-derived axial dispersion
and the expected assuming tubular coated tubes, suggested that the squared shape was responsible for the inhomogeneity of the flow.

In chapter 5, we presented guidelines for the configuration of industrial scale chromatographic separation of small molecules. A window was identified that defines the feasible configurations to use for the highest productivity for a given set of process requirements. The performance of different axial packed beds, channeled monoliths and a continuous monolith assuming silica as base material were compared by means of HETP (height equivalent of theoretical plates) and pressure drop relations. The relations as a function of velocity were used to calculate the resultant velocity and packing length for different conditions (efficiency, pressure drop, affinity constant and throughput). The specific productivity of channeled monoliths was shown to be up to 2.5 orders of magnitude higher than that of a packed bed. Therefore, at large scales (in which the pressure drops need to be limited, and the flow rate is high), channeled monoliths are preferred since they may reduce the equipment size up to 100 times and the required adsorbent volume up to 1000 times.

Finally, in chapter 6 we discussed the suitability of activated carbon regarding its re-usability and purification potential in the separation of small food ingredients. The suitability of channeled monoliths for certain applications was also highlighted. Finally, other suitable adsorbents were suggested, and some future prospects in the selection of adsorbents were given.
Samenvatting

Om functionele voedingsingrediënten te winnen uit product- of reststromen zijn meestal meerdere processtappen nodig, en vaak is één van die stappen een chromatografisch proces. Hierbij wordt materiaal gebruikt dat sterke affiniteit heeft voor de component die gewonnen moet worden. Meestal wordt een gepakte kolom van deeltjes van dit materiaal gebruikt. Voor processen op grote schaal en/of stromen met onopgeloste componenten is een gepakte kolom echter niet altijd wenselijk. De kolom kan snel verstopt raken, en de drukval over de kolom kan erg hoog worden. Voor voldoende capaciteit worden vaak kolommen gebruikt met een grote diameter en slechts een beperkte hoogte. Deze kolommen die qua vorm enigszins lijken op een pannenkoek zorgen voor complexe bedrijfsovering en hoge proceskosten. In de farmaceutische en fijnchemicaliën sector worden radiale kolommen en membranen gebruikt als alternatief, maar in de levensmiddelenindustrie, waar de stromen groter zijn en de marges op de producten lager, bieden deze technieken geen uitkomst.

Het doel van dit onderzoek was te bepalen of gestructureerde materialen, zoals monolieten, geschikt zijn voor selectieve adsorptie en desorptie van componenten uit product- of reststromen uit de levensmiddelensector. De aandacht was hier op kleine moleculen zoals oligosachariden en bioactieve peptiden. Vanwege de grote productstromen en de beperkte marges op het eindproduct, is het belangrijk dat het proces een hoge capaciteit heeft en bovendien goedkoop is. In dit proefschrift is actief kool als adsorptiemateriaal gebruikt, dat aan deze eisen voldoet, en bovendien door zijn hydrofobicititeit affiniteit heeft voor een verscheidenheid aan componenten. Het conventionele adsorptieproces met een gepakte kolom van deeltjes is vergeleken met het nieuwe proces waarbij kanaalvormige monolieten (zogenaamde honingraat structuren) gebruikt werden.

In hoofdstuk 2 is aangetoond dat het lactotripeptide IPP inderdaad geïsoleerd kan worden met behulp van actieve kool uit een commercieel mengsel van gehydrolyseerd eiwit. De zuiverheid van IPP kon verdubbeld worden (tot 35%). Het proces bestond uit opeenvolgende adsorptie-desorptie cycli. De opbrengst van IPP was bij de eerste cyclus 80%, maar nam na verloop
van tijd af. Dit werd veroorzaakt door uitputting van het actieve kool. Dit werd
niet alleen veroorzaakt door irreversibele binding tussen componenten in de
voeding en het actieve kool, maar ook door verstopping van de poriën. De
zuiverheid en opbrengst zouden verder verhoogd kunnen worden door de
polariteit van zowel de oplossing als het actieve kool op elkaar af te stemmen.

In hoofdstuk 3 zijn de voor- en nadelen van het gebruik van kanaalvormige
monolieten besproken voor de verwerking van een productstroom. Ook hier is als
voorbeeld de isolatie van IPP uit een mengsel van gehydrolyseerd eiwit gekozen.
Het gebruik van kanaalvormige monolieten is vergeleken met een gepakt bed,
beiden van dezelfde soort actieve kool. Gelijke langstroomsnelheid en verblijftijd
lieten een vergelijkbare productiviteit en dynamische adsorptie capaciteit zien;
echter met een gepakte kolom is de toename in de drukval over de kolom veel
groter. Dit duidt op blokkering van de poriën en verstopping van het gepakt bed
in de kolom. Ter duiding zijn twee semi-empirische modellen gebruikt. Het eerste
model is analoog aan een model dat bij membraanscheidingen wordt gebruikt
om vervuiling van het membraan te beschrijven. Het andere model gaat uit van
een aantal evenwijdige kanaaltjes, wier diameter langzaam kleiner wordt door
adsorptie. De stijging van de drukval in een gepakt bed was zelfs nadrukkelijker
aanwezig bij hoge vloeistofsnelheid (en dus korte verblijftijd), terwijl dit in
kanaalvormige monolieten niet werd waargenomen; hoge langstroomsnelheden
waren geen probleem.

Hoofdstuk 4 beschrijft de adsorptie van lactose op een gepakt bed en op
kanaalvormige monolieten (beiden bestaand uit actief kool) via een gedetailleerd
model voor chromatografische processen. Het model houdt rekening met de
verschillende massatransportweerstanden. Eerst is de adsorptie-isotherm bepaald
voor verschillende componenten; daarna is de kinetiek van adsorptie bepaald.
Hiervoor is de zogenaamde ’ondiepe bed’ methode gebruikt. De adsorptiesnelheid
is beschreven met twee verschillende modellen. Het blijkt dat de diffusie in actief
kool voor zowel het gepakte bed als voor de kanaalvormige monolieten een
zelfde snelheid laat zien; verschillen in de doorbraakcurven kunnen verklaard
worden door de relatief hoge axiale dispersie in de kanaalvormige monolieten.
Het is vooral de vierkante vorm van de kanaaltjes die resulteert in deze hoge
axiale dispersie. Het systeem zou dus verbeterd kunnen worden door gebruik te maken van een andere kanaalgeometrie.

**Hoofdstuk 5** geeft richtlijnen om de meest geschikte configuratie te kiezen voor een chromatografisch proces op industriële schaal, gebruikmakend van de verschillende adsorptiematerialen. Een raamwerk is opgesteld dat aangeeft welke configuratie de hoogste productiviteit geeft bij een gegeven combinatie van vereisten aan het proces, waarbij een vergelijking is gemaakt tussen verschillende gepakte bedden, kanaalvormige monolieten en monolieten zonder kanalen, allen vervaardigd van hetzelfde silica. De vergelijking gaat uit van het principe van HETP (hoogte equivalent van theoretische schotels) en relaties voor hydrodynamische drukval. Het was mogelijk om door een vereiste efficiëntie en doorzet voor het proces te stellen, en daarnaast een maximale drukval, en een gegeven affiniteitsconstante voor het adsorptiemateriaal aan te nemen, de langstroomsnelheid en de benodigde lengte van de verschillende systeemconfiguraties te berekenen. Deze berekeningen lieten zien dat de specifieke productiviteit van kanaalvormige monolieten tot 2.5 keer hoger is dan de productiviteit van een gepakt bed kolom. Vooral op grotere schaal, waar de drukval over een kolom limiterend wordt maar toch hoge debieten gewenst zijn, heeft het gebruik van kanaalvormige monolieten voordelen. Dit kan uiteindelijk leiden tot apparatuur die 100 keer kleiner is en een benodigd adsorbentvolume dat mogelijk 1000 keer kleiner kan zijn.

In het **laatste hoofdstuk** is tenslotte de mate waarin actief kool geschikt is voor het winnen van kleine componenten besproken. Hierbij is het hergebruik van actief kool en de capaciteit van belang. Ook de geschiktheid van kanaalvormige monolieten voor verdere toepassingen wordt nader besproken. Tenslotte worden suggesties gedaan voor andere geschikte adsorbentia en wordt een toekomstperspectief gegeven over de selectie van adsorbentia voor verschillende scheidingsvraagstukken.
Resumen

La separación y purificación de ingredientes funcionales a partir de flujos de materia prima y residual se realiza frecuentemente mediante procesos que incluyen una etapa cromatográfica utilizando un lecho fijo de resinas, en forma de esferas, con afinidad por los ingredientes a separar. Este tipo de columnas presenta varios impedimentos cuando están involucrados largos flujos o flujos sin tratar: una relación contraproducente entre transferencia de masa y permeabilidad hidráulica, un alta caída de presión y susceptibilidad a la obstrucción y el ensuciamiento (fouling). Los grandes equipos (y consiguientes diámetros de columnas) y el volumen de resina necesarios para una moderada caída de presión y alta capacidad de adsorción, plantean problemas en cuanto a los altos costes y complejo funcionamiento de los mismos. Otras tecnologías como la cromatografía de flujo radial y el uso de membranas poliméricas pueden presentar ventajas en otro tipo de aplicaciones (ej. farmacéutica y química fina), pero hasta ahora la capacidad y los costes no parecen ser factibles para la separación de moléculas pequeñas a partir de los grandes flujos de la industria alimentaria.

El objetivo de este proyecto fue encontrar principios para determinar la idoneidad de diferentes adsorbentes estructurales (ej. monolitos), para la adsorción y recuperación selectivas de ingredientes alimentarios de alto valor añadido y bajo peso molecular como los oligosacáridos y péptidos bioactivos. Para garantizar la rentabilidad del proceso y gran capacidad de adsorción para moléculas de pequeño tamaño, se demostró la viabilidad del uso de carbón activo y comparó su comportamiento adsorbente e hidráulico en dos estructuras diferentes: esferas porosas y monolitos con canales de flujo paralelo (de "panal de abeja", honeycomb). Además, se evaluó la viabilidad y la operatividad de los monolitos en cuanto a volumende adsorbente y de columna requerido en comparación con el lecho fijo de esferas empacadas.

Para probar la separación de péptidos bioactivos de mezclas crudas con carbón activado, se utilizó carbón activo para la recuperación del lacto-tripéptido IPP de un hidrolizado comercial (al 1.5% p/p), lo que se describe en el capítulo 2. La pureza de la mezcla inicial se duplicó en la fase separada hasta un 35% con
una recuperación de IPP de un 80% en los primeros ciclos de adsorción. La separación se repitió durante varios ciclos consecutivos de adsorción-desorción hasta el agotamiento de la columna empacada con carbón activo. Se encontró que el agotamiento fue producido no sólo por la ocupación de los sitios irreversibles sino también por el bloqueo de los poros. Finalmente, se proponen unas pautas en relación al agotamiento competitivo del adsorbente con el objetivo de optimizar el proceso y así obtener mayor pureza y rendimiento.

En el capítulo 3 se mostraron las ventajas de utilizar monolitos con canales para procesar flujos no pretratados. Para ello, se comparó el uso de monolitos con canales con un lecho fijo de esferas empacadas, ambas compuestas del mismo tipo de carbón activo, para la adsorción del lactotripéptido IPP de un hidrolizado sin pretratar. Los resultados mostraron productividades similares y capacidades dinámicas de adsorción a velocidades lineales y tiempos de residencia comparables, pero el lecho de esferas empacadas mostró un incremento fuerte de la caída de presión durante la alimentación continua de la columna y los mismos ciclos consecutivos de adsorción-desorción estudiados en el capítulo 2. Esto pone de manifiesto el bloqueo de poros y taponamiento de la columna. Estos mecanismos de ensuciamiento (fouling) se confirmaron con dos modelos semi-empíricos utilizando analogías: uno análogo al fouling en membranas y otro utilizando la analogía con canales en paralelo. El fuerte incremento en la caída de presión se manifestó en mayor medida a altas velocidades (cortos tiempos de residencia). Estas tendencias no se observaron en el caso de los monolitos con canales: no se observó un incremento de caída de presión significante, y las altas velocidades fueron notablemente factibles.

En el capítulo 4 se obtuvo con detalle el modelo cromatográfico que describe la adsorción de lactosa en una columna de partículas de carbón activo y monolitos de canales de carbón activo incluyendo las diferentes resistencias de transferencia de masa. En primer lugar, los parámetros de la isoterma de adsorción de un solo componente se obtuvieron mediante análisis frontal en ambos adsorbentes. En segundo lugar, se estimó la cinética de adsorción de lactosa en ambos adsorbentes de carbón activo mediante el método de lecho corto (shallow bed), asumiendo un baño infinito. Las curvas de adsorción en función del tiempo se ajustaron al modelo de difusión superficial homogénea y a la aproximación de fuerza
impulsora lineal. Mediante la estimación del coeficiente de difusión dentro de la partícula y el coeficiente de transferencia de masa externa se mostró un comportamiento similar en cuanto a la transferencia de masa durante el proceso de adsorción. El ajuste de los datos de la curva de ruptura (breakthrough curve) al modelo relacional general de cromatografía (general rate model) que describe la completa operación en la columna mostró diferencias en el comportamiento durante el proceso. Estas diferencias podrían estar relacionadas con una mayor dispersión axial dentro de los canales de los monolitos de geometría cuadrada. La diferencia que se encontró entre los valores experimentales y teóricos asumiendo una columna tubular recubierta con una capa de adsorbente, sugiere que la diferencia puede ser producida por el flujo no homogéneo causado por la forma cuadrada de los canales.

En el capítulo 5, se presentaron unas pautas para la configuración de separaciones cromatográficas de moléculas de pequeño tamaño a escala industrial. Estas directrices se basan en una ventana de posibilidades que define cuáles son las configuraciones factibles con la mayor productividad en función de determinados requerimientos del proceso. Para obtener estas directrices, se comparó el comportamiento de diferentes lechos fijos, monolitos de canales y monolitos continuos asumiendo que están compuestos de sílica y utilizando la altura equivalente de plato teórico y la caída de presión. Estas funciones relacionadas con la velocidad de flujo se utilizaron para calcular la velocidad y longitud de columna necesarias en función de las diferentes condiciones (eficiencia, caída de presión, constante de afinidad y rendimiento). La productividad específica de los monolitos de canales demostró poder llegar a ser hasta 2.5 órdenes de magnitud mayor que el lecho fijo. Por lo tanto, cuando se manejan grandes escalas en cromatografía (en las cuales la caída de presión tiene que limitarse, y el flujo volumétrico es alto), los monolitos de canales resultan ser la opción más adecuada ya que pueden reducir el volumen del equipo hasta 100 veces y requerir un volumen de adsorbente hasta 1000 veces menor.

Finalmente, como discusión general de esta tesis, en el capítulo 6 se trató la idoneidad del carbón activo como adsorbente teniendo en cuenta las posibilidades de reutilización y purificación en la separación de compuestos alimentarios de
pequeño tamaño. También se discutió la idoneidad de los monolitos de canales para ciertas aplicaciones. Para terminar, se sugieren otros adsorbentes aptos para este tipo de aplicación, y se plantean nuevas perspectivas para el futuro en cuanto a la selección de adsorbentes.
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Con cariño, with love, hartelijk,
Marta
About the author

Marta Rodríguez-Illera was born in Palencia, Spain, on the 14th of August 1984. She followed a bilingual primary and secondary education in the French Lycée français of Alicante, Spain, where she graduated with a double diploma in 2002. In the same year she began her long-cycle degree of Chemical Engineering that she followed both in the University of Alicante (first 3 years), and in the Universidad Politécnica de Valencia. She was following the "Processes" specialization and graduated in 2008. During this study, she performed an MSc thesis at the University of Leeds in the UK in 2007, entitled: "Evaluating the aerodynamic performance of a dry powder inhaler using Computational Fluid Dynamics" and an internship in Future Fibres in Valencia, Spain. After graduation she was hired by Future Fibres at the R&D department. In 2009, she traveled to the Netherlands for an internship granted with the Leonardo scholarship at Nizo Food Research, Ede, the Netherlands. She was working on waste stream valorization and isolation, characterization and testing functionality of Rubisco extracted from green leaves. In October 2009 she started her work as a PhD student at the Food Process Engineering Group (FPE) of Wageningen University within the framework of the Institute for Sustainable Process Technology (ISPT). The result of her work is described in this thesis. Currently, Marta works at the Food Process Engineering Group of Wageningen University as a PostDoc as part of the project "Valorization of raw materials" run by the Top Institute of Food and Nutrition (TIFN).
## Overview of completed training activities

### Discipline specific activities
- Introduction to gPROMS Process Systems Enterprise 2010
- Advanced course of gPROMS Process Systems Enterprise 2010
- Downstream Processing BSDL 2010
- Biorefinery for Biomolecules (VLAG) 2012
- ACAM – Advanced Computer Aided Modelling (OSPT) 2012
- MSS2010 Slovenia – Summer School and Symposium (Oral presentation) 2010
- NPS 2011, Arnhem – Conference (Poster presentation) 2011
- FoodBalt 2012 – Conference (Oral presentation) 2012
- ECCE 2013, the Hague – Conference (Oral presentation) 2013

### General courses
- Business training (ISPT) 2009
- Techniques for Writing and Presenting a Scientific Paper (WGS) 2010
- Working in projects (ISPT) 2010
- PhD week (VLAG) 2010
- PhD Competence Assessment (WGS) 2010
- Workshop: Cultural awareness (ISPT) 2011
- Teaching and supervising Thesis students (DO) 2012
- Career perspectives (WGS) 2013

### Optional courses and activities
- Study tour USA 2010
- Study tour Finland & Baltic States 2012
- Organization study tour 2012 2011–2012
- Food process engineering symposium 2009–2012
- Future of chromatography (ISPT) 2010
- Food sector meeting (ISPT) 2010
- Researchers day (ISPT) 2009–2010
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Cover: idealized internal structure of activated carbon together with the 3D molecule of the lactotripeptide Isoleucine-Proline-Proline.