Department of Biotechnology and Chemical Technology

# Industrial Scale Chromatographic Separation of Valuable Compounds from Biomass Hydrolysates and Side Streams

Pia Saari

Aalto University

DOCTORAL DISSERTATIONS

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# Industrial Scale Chromatographic Separation of Valuable Compounds from Biomass Hydrolysates and Side Streams

**Pia Saari**

Doctoral dissertation for the degree of Doctor of Science in Technology to be presented with due permission of the School of Chemical Technology for public examination and debate in Auditorium KE2 (Komppa Auditorium) at the Aalto University School of Chemical Technology (Espoo, Finland) on the 17th of June 2011 at 12 noon.

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**Abstract**

Carbohydrates are composed of a number of various monosaccharides, glucose being the most abundant. Some of the monosaccharides are valuable compounds used in the food and pharmaceutical industries. They can be separated from biomass hydrolysates e.g. by chromatographic methods.

In this thesis, chromatographic separation of valuable compounds using ion exchange resins was studied on an industrial scale. Of special interest were rare monosaccharides in biomass hydrolysates. A novel chromatographic separation process was developed for fucose, starting from pre-processed spent sulfite liquor. The core of the process consists of three chromatographic separations with different types of ion exchange resins. Chromatographic separation of galactose was tested with three biomass hydrolysates; lactose, gum arabic and hemicellulose hydrolysates. It was demonstrated that also galactose can be separated from complex carbohydrate mixtures. A recovery process for arabinose from citrus pectin liquid residual and for mannose from wood pulp hydrolysate were also developed and experimentally verified.

In addition to monosaccharides, chromatographic separation of glycinebetaine from vinasse was examined with a hydrogen form weak acid cation exchange resin. The separation involves untypical peak formation depending, for example, on the pH and the cation composition. The retention mechanism was found to be hydrogen bonding between glycinebetaine and the resin.

In the experimental part, all four resin types – strong acid cation, strong base anion, weak acid cation and weak base anion exchange resins – were used. In addition, adsorption equilibria data of seven monosaccharides and sucrose were measured with the resins in sodium and sulfate forms because such data have been lacking. It was found out that the isotherms of all sugars were linear under industrial conditions.

A systematic method for conceptual process design and sequencing of chromatographic separation steps were developed. Heuristics were drawn from the current industrial practices also for the selection of a suitable ion exchange resin for the separation of a sugar from a biomass hydrolysate.

**Keywords** chromatography, fucose, galactose, arabinose, mannose, glycinebetaine, conceptual process design, ion exchange resin, adsorption isotherm

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**Tiivistelmä**

Hiilihydraatit koostuvat erilaisista sokereista, joista glukoosi on yleisin. Jotkut monosakkarideista ovat arvokkaita yhdisteitä, joita käytetään lääke- ja elintarviketeollisuudessa. Niitä voidaan erotella biomassahydrolysaateista esimerkiksi kromatografisin menetelmin.

Tässä väitöskirjassa tutkittiin arvokkaiden komponenttien kromatografista erottelua teollisessa mittakaavassa käyttäen ioninvaihtohartseja. Erityisen tutkimuksen kohteena oli harvinaisten sokerien erottelu biomassahydrolysaateista. Fukoosille kehitettiin uusi kromatografinen erotusprosessi, jonka lähtöaineena oli esiprosessoitu sulfiittijäteliemi. Keskeistä prosessissa ovat kolme kromatografista erotusta erityyppisillä ioninvaihtohartseilla. Galaktoosin kromatografista erotusta testattiin kolmella eri bioperäisellä hydrolysaatilla: laktoosi-, arabikumi- ja hemiselluloosahydrolysaatilla. Kokeilla osoitettiin, että myös galaktoosia voidaan erotella kromatografisesti kompleksista hiilihydraattimatriisista. Lisäksi arabinoosille kehitettiin talteenottoprosessi sitruspektiinin jäteliemestä, ja mannoosille puuselluloosa-hydrolysaatista.

Monosakkaridien lisäksi glysiinibetaiinin kromatografista erotusta tutkittiin vetymuotoisella heikolla kationinvaihtajalla. Erotuksessa piikin muodostuminen riippuu esimerkiksi pH:sta ja syötön kationikoostumuksesta. Retentiomekanismia tutkittiin tarkemmin käyttäen apuna samankaltaisia molekyylejä, ja mekanismin huomattiin johtuvan vetysilloista glysiinibetaiinin ja hartsin välillä.

Kokeellisissa töissä käytettiin kaikkia neljää ioninvaihtohartsityyppiä: vahvaa ja heikkoa kationinvaihtajaa sekä vahvaa ja heikkoa anioninvaihtajaa. Lisäksi adsorptiotasapainot mitattiin seitsemälle monosakkaridille ja sakkaroosille kaikilla hartsityypeillä natrium- ja sulfaattimuodoissa teollisissa olosuhteissa, koska sellaisia mittaustietoja ei ole ollut aiemmin käytettävissä. Jakautumiskertoimien havaittiin saavan vakioarvon koko tutkitulla alueella.

Väitöskirjassa kehitettiin myös systemaattinen menetelmä kromatografisten erotusten järjestämiseen. Nykyisistä teollisista käytännöistä kehitettiin heuristiikkoja, joiden avulla myös sopiva ioninvaihtohartsi voidaan valita tietyn sokerin erottamiseen hydrolysaatista.

**Avainsanat** kromatografia, fukoosi, galaktoosi, arabinoosi, mannoosi, glysiinibetaiini, konseptuaalinen prosessisuunnittelu, ioninvaihtohartsi, adsorptio isotermi

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# Preface

The work in this thesis has been carried out in two independent research institutes during the period 2002 - 2010. All the experimental work was done at the R&D center of Danisco Sweeteners in Kantvik, in the group of Chromatographic Separation. The other place of research was the Aalto University, Plant Design research unit.

I wish to express my gratitude to Professor Markku Hurme for his constructive feedback. I owe my sincere thanks to Mr. Heikki Heikkilä for his willingness to share his practical experience and theoretical knowledge about chromatography. Special thanks to Katja Häkkä for her enthusiasm and interest in rare sugars. Other co-authors, Juho Jumppanen and Hannu Paananen, are also acknowledged for their contribution.

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Finally, and most importantly, I want to thank Tuomo, Inka and Lauri for bringing joy and meaning to my life. I also want to express my deepest thanks to my parents and my sister for their love, encouragement and support at all turns of my life. Each and every one of my friends has a place in my heart, although they are not listed here.

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Pia Saari Espoo, 5th May 2011

# List of Publications

I Saari, P., Häkkä, K., Heikkilä, H., Jumppanen, J., Hurme, M. A Novel Chromatographic Production Scale Separation Process for L-Fucose, *J. Liq. Chromatogr. Related Technol.* **32** (2009) 2050–2064.

II Saari, P., Häkkä, K., Jumppanen, J., Heikkilä, H., Hurme, M. Study on Industrial Scale Chromatographic Separation Methods of Galactose from Biomass Hydrolysates, *Chem. Eng. Technol.* **33** (2010) 137–144.

III Saari, P., Paananen, H., Hurme, M., Study on the Retention of Glycinebetaine on a Weak Acid Cation Exchange Resin, *J. Liq. Chromatogr. Related Technol.* **34** (2011) 622–633.

IV Saari, P., Heikkilä, H., Hurme, M., Adsorption Equilibria of Arabinose, Fructose, Galactose, Glucose, Mannose, Rhamnose, Sucrose, and Xylose on Ion-Exchange Resins, *J. Chem. Eng. Data* **55** (9) (2010) 3462–3467.

V Saari, P., Hurme, M., Process Design Principles in the Chromatographic Separation of Sugars from Biomass Hydrolysates, *Chem. Eng. Technol.* (2011) **34** 282-288.

# Author's Contribution

I The author participated in planning the experiments. Test trials were performed together with the pilot plant personnel. The author processed the results and wrote the manuscript.

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III The author planned the experiments. Test trials were performed together with the pilot plant personnel. The author processed the results and wrote the manuscript.

IV The author planned and carried out the experiments. The author processed the results and wrote the manuscript.

V The author participated in planning the experiments. Test trials were performed together with the pilot plant personnel. The author processed the results, developed the method and wrote the manuscript.

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# Notation

### Nomenclature

| | |
|---|---|
| α | separation factor |
| c | concentration |
| ΔC | the rise in sugar purity |
| $K$ | distribution coefficient |
| $k$ | retention factor |
| m | mass |
| $q$ | solid-phase concentration of a saccharide |
| $R^2$ | coefficient of determination |
| $t$ | (elution) time |
| $V$ | (elution) volume |

### Abbreviations

| | |
|---|---|
| BV | bed volume |
| GB | glycinebetaine |
| IEX | ion exchange |
| DM | dry matter (equals DS) |
| DS | dissolved solids |
| DVB | divinylbenzene |
| HPLC | high performance liquid chromatography |
| MAX | methyl-α-D-xylopyranoside |
| NA | not available |
| PS-DVB | poly(styrene-co-divinylbenzene) |
| RI | refractive index |
| SAC | strong acid cation exchange resin |
| SBA | strong base anion exchange resin |
| SMB | simulated moving bed |
| SSL | spent sulfite liquor |
| SSMB | sequential simulated moving bed |
| TMB | true moving bed |
| WAC | weak acid cation exchange resin |
| WBA | weak base anion exchange resin |

# 1 Introduction

The current economy is mostly based on unrenewable raw material and energy sources, especially crude oil. There are debates about the exact year of peak oil production but it is generally believed to occur before 2025 (Cheng 2010). Therefore, the need to produce energy and materials from renewable resources has become evident over the past decades. It is estimated that the annual production of biomass is 170 billion tons (Röper 2002). This growth would make it possible to move over to a bio-based economy from the present hydrocarbon economy.

Utilizing the biomass, which, from the chemical point of view, is far more diverse than crude oil, is, however, challenging. The concept of biorefineries has evolved to cover the various processes needed to convert the biomass into fuels, energy and materials. The ultimate idea in biorefineries is that various processes are used to convert biomass into one or more low-volume, high-value chemical products together with a low-value, high-volume liquid transportation fuel, while generating electricity and process heat for its own use and/or export. (Kamm et al. 2006)

The biocatalytic route for the production of bulk and fine chemicals from renewable resources is fermentation. Lignocellulosic biomass contains various sugar monomers such as xylose, mannose, glucose, fructose and galactose but also arabinose and rhamnose, which are released under hydrolysis. Economic fermentation of all monosaccharides in biomass is not likely in the near future, however. For example, wild-type *S. cerevisiae* strains ferment glucose, mannose, galactose and fructose into ethanol but other potentially fermentable substrates, such as xylose, arabinose and rhamnose, are a major challenge in metabolic engineering (van Maris et al. 2006). Therefore, many high-value chemicals, e.g. relatively rare sugars in nature, in the lignocellulosic biomass remain in the hydrolysate, ready to be separated.

Chromatographic separation of sugars using ion exchange resins offer a manufacturing process where the resin is used as a selective medium to separate dissolved compounds from others. However, major part of all written literature

about chromatography is about various analytical measurement techniques. Ion exchange (IEX), on the other hand, is a well-established unit operation used widely in the process industry, especially in water treatment. Large-scale chromatographic separation of sugars using ion exchange resins is a niche which lies in the middle ground of IEX and chromatography having several special characteristics.

The aim of this thesis is to find new or improved approaches to the chromatographic separation of valuable compounds from biomass hydrolysates or industrial side streams. Valuable compounds in this context are mainly monosaccharides, especially L-fucose (Paper I), D-galactose (Paper II) but also D-mannose and L-arabinose (in Paper V).

In Paper I, a novel chromatographic separation process for L-fucose from wood-based hydrolysate with several separation steps is presented. The process is the first viable method for the recovery of L-fucose from a low starting purity. Paper II enlarges on different chromatographic methods to separate D-galactose from various sources: gum arabic hydrolysate, lactose hydrolysate and pre-processed spent sulfite liquor.

Glycinebetaine (Paper III) is another type of valuable compound already chromatographically separated from molasses. It was noticed earlier that glycinebetaine is retained strongly on a weak acid cation (WAC) exchange resin in hydrogen form, unlike in sodium form. Paper III is a study of the retention mechanism of GB on WAC exchange resin in hydrogen form. The acidic WAC exchanger may be a suitable and more straightforward method for the recovery of GB from vinasse.

All the chromatographic experiments in this thesis are carried out with ion exchange (IEX) resins. In literature, strong acid cation (SAC) exchange resin is the most studied resin type, and consequently, adsorption equilibrium data of monosaccharides are basically non-existing with the other types of resins. However, in this thesis, other types of ion exchangers – weak acid cation (WAC), strong base anion (SBA) and weak base anion (WBA) exchange resins – are also

examined. Therefore, distribution coefficients of the most common monosaccharides – arabinose, fructose, glucose, galactose, mannose and xylose – and the well-known disaccharide, sucrose, were measured for all the four resin types (Paper IV). Since the measurements for all possible types of cations and anions at different concentrations and temperatures would be a vast job, the variables were restricted to the main ion forms, sodium and sulfate, in industrial conditions regarding the temperature and the sugar concentration.

Separation processes need to be designed for new products or raw materials. Therefore, another topic in this thesis is the conceptual process design of chromatographic separation processes. The subject is studied in Paper V which presents some heuristics for the conceptual process design of chromatographic separation of sugars from biomass hydrolysates.

Figure 1 illustrates the research areas of this thesis. Chromatographic separation using ion exchange resins is the core of this study, where monosaccharides (sugars) are strongly involved with glycinebetaine also in focus. Process design aspects are also studied but taking into account the valuable products. Therefore, conceptual process design and chromatographic separation were not studied in general.

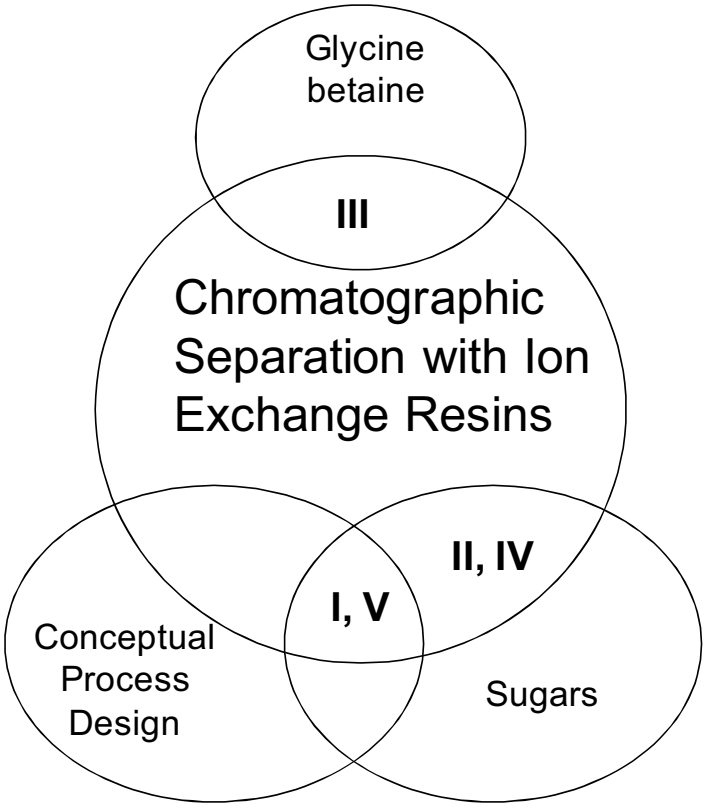


Figure 1. Areas of research of this thesis presented in Papers I-V.

# 2 Valuable Compounds in Biomass Hydrolysates and Industrial Side Streams

Terrestrial biomass consists of a wide variety of different compounds. It is estimated that 75 % of the annually renewable biomass are carbohydrates, 20 % is lignin, and only 5 % are other natural compounds (Röper 2002). The variety of carbohydrates in nature is great and the number of theoretical possibilities is almost limitless.

In terms of quantity, wood is the most important renewable natural resource (Nimz et al. 2005). Generally, lignocellulosic biomass consists of three main polymers; cellulose, hemicellulose and lignin, of which the first two are composed of sugar units. Cellulose is a linear polysaccharide consisting of easily fermentable glucose subunits, whereas hemicelluloses are a group of branched hetero-polysaccharides consisting mainly of pentose and hexose sugars. In wood, the amount of cellulose is between 40 and 50 % of dry wood, hemicelluloses vary between 25 and 35 % of dry wood.

In the following, the valuable monosaccharides – fucose, galactose, mannose, arabinose, rhamnose and xylose – are presented briefly. Chromatographic separation methods of these sugars from biomass hydrolysates are discussed further later in this thesis. In addition, glycinebetaine is introduced, because it is a valuable compound and it is also recovered by chromatographic techniques from the side streams of sugar beet processing.

## 2.1 Monosaccharides

Monosaccharides are the simplest carbohydrate units, which are polyhydroxy aldehydes or polyhydroxy ketones, or their derivates. Furthermore, they are carbohydrates which cannot be broken down by hydrolysis (BeMiller 2010). Classification of common monosaccharides according to chemical structures is presented in Figure 2.

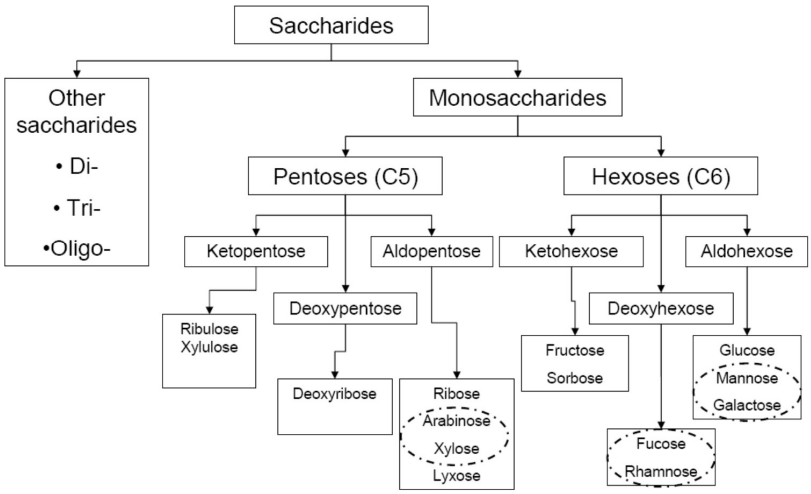


Figure 2. Classification of common saccharides. The monosaccharides of interest in this thesis are circled.

Typical sugar monomers in biomass hydrolysates include C6 sugars: glucose, galactose, mannose, but also C5 sugars: xylose and arabinose. In addition, there are deoxy sugars such as rhamnose and fucose. Most of the naturally occurring sugars are in D-form but exceptions are fucose, rhamnose and arabinose which generally occur in L-form (Figure 3).

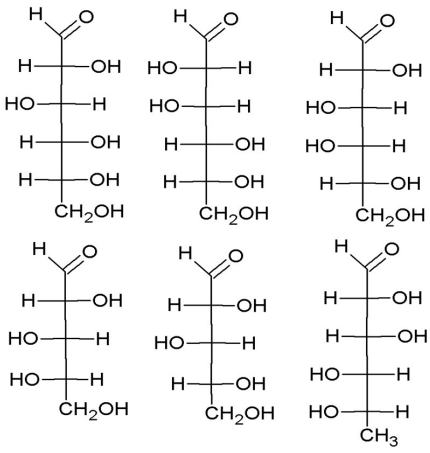

Figure 3. Chemical structure of the most common monosaccharides in biomass: D-glucose, D-mannose, D-galactose, L-arabinose, D-xylose and L-rhamnose.

**Fucose**

L-fucose (6-deoxy-L-galactose) is a methyl pentose sugar which belongs to the family of deoxy sugars. Interest in L-fucose and L-fucose containing oligosaccharides has increased in the medical field as more information of the role of fucose in biological functions has been published. For example, recent studies have indicated that L-fucose and L-fucose rich oligo- and polysaccharides could be used as anti-ageing aids (Robert et al. 2005, Peterszegi et al. 2003, Robert et al. 2003), and that they inhibit immuno-allergic reactions on skin (Hasegawa et al. 1980). In addition, L-fucose has been shown to facilitate long-term memory formation (Matthies et al. 2000). The role of L-fucose in disorders like diabetes, cancer and inflammatory diseases is under research.

Occurrence of fucose and various synthesis methods are covered in Paper I. Raw materials for fucose with natural origin often have low fucose purity, and therefore recovery methods are needed. The aim of Paper I was to develop a chromatographic separation sequence which would separate fucose with sufficient purity from an industrial side stream (spent sulfite liquor origin). The starting purity of fucose was approximately 7 % on dissolved solids (DS), and the target purity > 80 % on DS.

**Galactose**

D-Galactose is an aldohexose, also called brain sugar. Not only the liver but also the brain has the capability to take up and metabolize galactose. Galactose plays a crucial role in many biological processes such as cell–cell adhesion and recognition (Varki 1993). It is specifically characteristic of the blood group substance B (Lloyd, Kabat 1968). Cancer patients who receive pre-, intra-, and post-operative infusion of galactose experience fewer metastases than those who receive no galactose (Kosik et al. 1997). Galactose is also used in sport drinks to improve performance (King et al. 2006).

Galactose is easily produced by hydrolysis of lactose by β-galactosidase. Plant-based galactose can be recovered from hemicellulose hydrolysates but the matrix is more complex compared to lactose hydrolysate. Paper II deals with chromatographic separation of galactose from various biomass hydrolysates.

**Mannose**

D-mannose is an aldohexose which is not well metabolized in humans (Alton et al. 1998). A study suggests that mannose is effective in dislodging the *E. coli* bacteria from the bladder wall (Toyota et al. 1989). It is also used in intravenous inflammation conditions and as raw material in mannitol production.

In nature, mannose occurs in hemicellulose, especially in softwoods in glucomannan and galactoglucomannans (Thomson 2000). Therefore, recovery of mannose from wood-based liquors is an interesting option. A recovery process of mannose from wood-based liquors by a chromatographic method has been patented (Heikkilä et al. 2006). An example of the chromatographic separation of mannose from wood pulp hydrolysate is reported in a case study in Paper V.

**Arabinose**

L-Arabinose is named after gum arabic which it was first isolated from. Arabinose is found in nature as a component of biopolymers such as hemicellulose and pectin.

Arabinose is used as a culture medium and as a natural sweetener for food and pharmaceutical products. It strongly inhibits the absorption of sucrose from the small intestine. The addition of a small amount of L-arabinose (from 2 to 3 %) with sucrose reduces the digestion of sucrose with about 60 % (Susumu 1999). It also acts as the starting material in the synthesis of L-ribose (Jumppanen et al. 2000).

Chromatographic separation of arabinose is discussed in Paper II (gum arabic hydrolysate) and Paper V (case study II, citrus pectin waste stream).

**Rhamnose**

L-Rhamnose is a deoxy sugar and is mainly used in a synthesis to produce fruity aromas, resembling those of strawberry and raspberry (van der Schaft 2007).

**Xylose**

D-Xylose is produced from wood or straw-based xylans. Reduction of xylose produces xylitol which is widely used as a sugar substitute (Olinger & Pepper 2001). Xylitol and its production has been the subject of many studies, and therefore it is not discussed any further in this thesis.

## 2.2 Glycinebetaine

Glycinebetaine ($(CH_3)_3N^+CH_2CO_2H$) is an important osmoprotectant, synthesized by many plants in response to abiotic stresses. It is a naturally occurring compound, found particularly in sugar beet (*Beta vulgaris*). Glycinebetaine (GB), also known as trimethyl glycine or simply betaine, accumulates in molasses, up to 8 % on DS, and can be recovered as a by-product to sucrose (Heikkilä et al. 1992). GB also ends up in vinasse, if the sucrose in molasses is fermented into other chemical(s). Paper III presents a novel method for the recovery of GB from acidic streams, such as vinasse.

GB is the fully methylated version of glycine. Since nitrogen is fully methylated, no proton is available for transferring to its $-COO^-$ end. Therefore, GB is a zwitterion or cation depending on the pH. GB has $pK_a$ of 1.83 (Lide 1996).

GB is used as an ingredient in food and feed applications. GB and its salts have commercial uses also in pharmaceuticals, dental products, and cosmetics (Danisco 2010). GB is also used in the fermentation industry to alleviate the osmotic stress that high substrate concentrations can cause (Kawahara et al. 1990, Farwick et al. 1995).

## 2.3 Biomass Hydrolysates and Industrial Side Streams

The most abundant and feasible source for lignocellulosic biomass is a local matter. This may be, for example, agricultural residues (corn stover, sugar cane, bagasse), energy crops (switch grass), or a side stream from a pulping plant (cooking liquor). Hemicelluloses may also be extracted before traditional kraft-

pulping and collected as a pre-hydrolysate (Mao et al. 2008). Side streams from pulping processes (e.g. sulfite cooking liquors, pre-hydrolysates) are of interest, because they are easily available in large volumes and may offer synergies with the pulping process, and consequently raw material at a relatively low price.

Hydrolysis of lignocellulosic material is carried out enzymatically with cellulases and hemicellulases, or using an acid, most often dilute sulfuric acid. The hydrolysis conditions have great effect on the composition of hydrolysate, if a chemical is used. For example, wood cellulose is more resistant to dilute acid hydrolysis than is hemicellulose. (Lee et al. 1999) Thus, the milder the condition, the larger is the proportion of hemicelluloses in the hydrolysate. Similarly, the degree of depolymerization in the gum depends on the severity of the hydrolysis conditions. The chemical structure of gum arabic has been widely studied and is known to be a highly branched polysaccharide consisting of galactose backbone with linked branches of arabinose and rhamnose, which terminate in glucuronic acid (Stephen 1995). If hydrolysis is carried out under harsh conditions, galactose is also present in the hydrolysate (Heikkilä et al. 2005). Therefore, partial concentrations of sugars in a hydrolysate can vary notably, even if the raw material is the same.

### 2.3.1 Composition of Lignocellulosic Biomass Hydrolysates

Hydrolysates of the agricultural residues (such as bagasse, corn stover and wheat straw) are rich in xylose and glucose (Table 1). In softwood hydrolysates, mannose is one of the main sugar components in addition to xylose, galactose and glucose. Hardwood and softwood differ notably in their hemicellulose compositions, especially in xylose, galactose and mannose contents (Thomson 2000). There are also rare sugars in wood hydrolysates, such as deoxy sugars rhamnose and fucose but their amount is minor. However, their relative concentration increases as the major sugars (glucose, xylose) are converted biochemically or removed by separation techniques.

Table 1. Sugar of common lignocellulosic feed stocks. Adapted from (van Maris et al. 2006, Mao et al. 2008, Helle et al. 2004, Casebier et al. 1972).

| | **Bagasse** | **Corn stover** | **Wheat straw** | **Hardwood (mixture) pre-hydrolysate** | **Softwood (pine) pre-hydrolysate** | **Spent sulfite liquor (Mg)** |
|---|---|---|---|---|---|---|
| Total sugars, % on dry substance | 66 % | 60 % | 57 % | 29 % | 38 % | 15 % |
| Sugar, % on total sugars | | | | | | |
| Arabinose | 3 % | 4 % | 4 % | 2 % | 4 % | - |
| Glucose | 61 % | 60 % | 59 % | 9 % | 17 % | 12 % |
| Galactose | 1 % | 2 % | 1 % | 7 % | 21 % | 7 % |
| Mannose | 1 % | 1 % | 1 % | 4 % | 37 % | 24 % |
| Xylose | 34 % | 33 % | 35 % | 77 % | 21 % | 57 % |

### 2.3.2 Feed Solutions in the Experiments

Several real biomass hydrolysates were experimentally treated in this thesis. The solutions which were chromatographically separated in Papers I, II, and V are presented in Table 2. They are all industrially available streams which contain a mixture of monosaccharides.

Table 2. Feed solution composition in the experiments. (SSL = spent sulfite liquor)

| **Paper number** | **I** | **II** | **II** | **II** | **V** | **V** | **III** |
|---|---|---|---|---|---|---|---|
| Origin of raw material | Pre-treated SSL | Lactose hydrolysate | Gum arabic hydrolysate | Pre-treated SSL | Citrus pectin residual stream | Pulp hydrolysate | Vinasse |
| % on total dissolved solids | | | | | | | |
| Arabinose | 3 | - | 34 | - | 11 | 8 | - |
| Fructose | - | - | - | - | 4 | 4 | - |
| Glucose | - | 45 | - | - | 10 | 26 | 1 |
| Galactose | - | 42 | 9 | 23 | - | 3 | - |
| Fucose | 7 | - | - | - | - | - | - |
| Mannose | - | - | - | 20 | - | 18 | - |
| Rhamnose | 14 | - | 3 | - | - | - | - |
| Xylose | - | - | - | 40 | - | 39 | - |
| Betaine | NA | NA | NA | NA | NA | NA | 20 |

The hydrolysis of lactose was performed enzymatically, whereas gum arabic was hydrolyzed with sulfuric acid and neutralized with calcium hydroxide (Paper II).

Pretreated for spent sulfite liquor (SSL), in this context, means a solution after the recovery of sugars, such as xylose and/or rhamnose. SSL is considered a very low value by-product. Prior to chromatographic separations, suspended solids were removed from the hydrolysates by filtration using diatomaceous earth as filter aid; body feed and pre-coat.

In addition, recovery of GB from acidic solutions was studied (Paper III). Generally, GB is separated from molasses which is a by-product of sucrose production (Heikkilä et al. 1992). However, the sugar reform in the EU (European Commission 2003) has changed the profitability of sucrose production drastically within the last few years. At the same time, the demand for bio-ethanol has increased due to changes in oil prices and political decisions related to climate change. Therefore, instead of recovering sucrose, beet based syrups or molasses are fermented into alcohol, organic acid or some other organic compound. Vinasse, a residual substance left after the recovery of the product, is the by-product in which GB ends up. Major components in sugar beet alcohol vinasse are GB (> 15 % of the dry matter), glycerol (> 9 % of the dry matter), and many inorganic compounds such as potash ($K_2O$ > 7 % of the dry matter) (Decloux et al. 2002). Vinasse is different from molasses in many aspects: it has a higher GB content, poorer filterability, lower pH, etc. Therefore, the recovery process for GB is also more complicated from vinasse than from molasses. Filtration process becomes heavier, alkali consumption increases, and more chromatographic separations are needed, for example.

## 2.4 Correlation between the Sugar Concentration in Biomass and the Sugar Price

Since monomeric sugars are not freely occurring compounds, they are commonly produced from hydrolysates containing these. Alternatively, they are synthesized chemically, enzymatically or microbiologically.

Generally, the concentration of a component in the feed stock has a major effect on the price of the product and the same correlation was found with sugars. The concentrations of sugars in the starting material vs. market prices were studied.

Based on the results, Figure 4, which presents the purity of a sugar in the raw material (% on dissolved solids) vs. market price ($/kg), was drawn. The data points in Figure 4 range between sucrose, with the highest sugar concentration on DS and lowest price, and fucose which had the lowest concentration and highest price of the sugars studied.

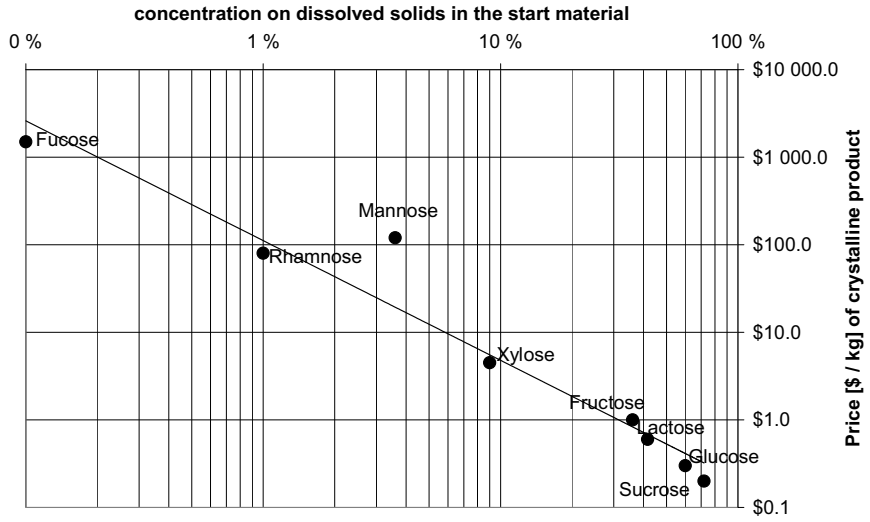


Figure 4. Correlation of sugar concentration in the starting material vs. price.

Sucrose content (% on DS) in Figure 4 was calculated assuming that the starting material for sucrose was sugar beet, having a sucrose content of 18 % and 25 % total dissolved solids (DS). Wheat grain, which was estimated to contain 60 % glucose on DS in the form of starch, is the cheapest raw material for glucose. Milk, which contains 5 g/l lactose, and approximately 12 g/l DS, was the raw material for lactose. Fructose is produced from sucrose; therefore, the raw material concentration for fructose is half the sucrose concentration in sugar beet. Concentrations of xylose, mannose, rhamnose, and fucose as % on DS are estimated concentrations in spent sulfite liquor (Helle et al. 2004, Heikkilä 1985). The bulk price data were collected from several sources, and are only indicative (Kamm et al. 2006, Saari 2007, Anonymous 2010).

The concentration of a natural (not synthetically manufactured) sugar in the raw material correlates with the price of crystalline sugar on a logarithmic scale. The majority of the sugars are bulk sugars with a relatively low price (< 10 $/kg). Mannose deviates from the correlation trend line for an unknown reason, whereas the other sugar purities in the starting solution vs. the price of the crystalline product correlate well.

# 3 Ion Exchangers in the Chromatographic Separation of Carbohydrates and Glycinebetaine

## 3.1 Structure and Properties of Ion Exchangers

Synthetic organic ion exchange resins became available already in the late 1930's. Styrenic resins dominate the ion exchange market but acrylic resins have increased their importance (Dardel, Arden 2008). The resins have four primary types of functionality; strong acid cation (SAC), weak acid cation (WAC), strong base anion (SBA) and weak base anion (WBA). Chemical structures of all the functionally different resin types are presented in Figure 5.

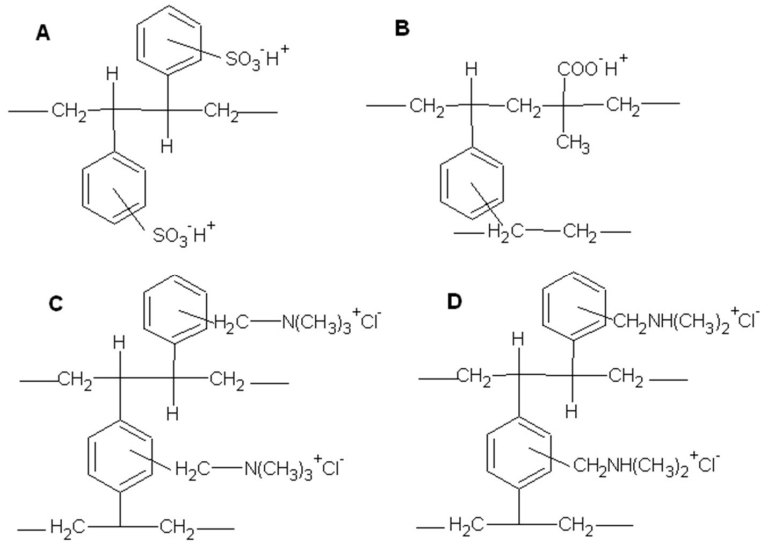


Figure 5. Chemical structures of (A) strong acid cation, (B) weak acid cation, (C) strong base anion, and (D) weak base anion exchanger types.

All the resin types used in the thesis are presented in Table 3. All resins were manufactured by Finex Ltd (Kotka, Finland).

Table 3. The ion exchange resins types used in the experiments of this thesis.

| | Paper number | | | | |
|---|---|---|---|---|---|
| **Exchanger type** | **I** | **II** | **III** | **IV** | **V** |
| SAC | X | X | | X | X |
| SBA | X | X | | X | X |
| WAC | X | | X | X | |
| WBA | | | | X | |

### 3.1.1 Cation Exchange Resins

Strong acid cation (SAC) exchange resins have a styrene or acrylic skeleton. Most often the resin is a sulphonated polystyrene-divinylbenzene (PS-DVB) resin, like in this thesis (Papers I, II, IV, V). Other alkenylaromatic polymer resins like those based on monomers of alkyl-substituted styrene or mixtures thereof can also be applied. The resin may also be cross-linked with other suitable aromatic cross-linking monomers, such as divinyltoluene, divinylxylene, divinylnaphtalene, divinylbenzene, or with aliphatic cross-linking monomers such as isoprene, ethylene glycol diacrylate, ethylene glycol dimethacrylate, N,N'-methylene bisacrylamide or mixtures thereof. (Heikkilä et al. 2005) SAC resins are durable and less expensive compared to other resin types.

Weak acid cation (WAC) exchange resins are most commonly acrylic or methacrylic exchange resins having carboxylic functional groups. However, the resin may also be, for example, a styrene resin, and the functional groups may be other than a carboxylic group, e.g. another weak acid. Such an acrylic resin is preferably derived from methyl acrylate, ethyl acrylate, butyl acrylate, methylmethacrylate or acrylonitrile or acrylic acids or mixtures thereof. The resin may be cross-linked with a cross-linking agent, e.g. DVB, or with the other cross-linking agents mentioned above. WAC exchangers in this study (Papers III and IV) are acrylic acid-DVB gel type resins.

### 3.1.2 Anion Exchange Resins

Strong base anion (SBA) exchange resins have a styrene or acrylic skeleton. Resins may be cross-linked with DVB or other alkenylaromatic polymer resins like those based on monomers like alkyl-substituted styrene or mixtures thereof. The resin may also be cross-linked with other suitable aromatic cross-linking monomers, the same as mentioned above.

Adding functional groups to SBA resins involves two steps: chloromethylation followed by amination. The resulting functional group is a quaternary ammonium group. There are two chemicals that are generally used for the amination step, each producing a different type of SBA resin with different chemical properties. Type I SBA resins are aminated with trimethylamine. Type II SBA resins, developed after the SBA Type I, are aminated with dimethyl-ethanolamine. Type I SBA exchanger with styrene-DVB skeleton functionalized with trimethylamine was used in this study (Papers I, II, IV, V).

WBA exchange resins are the newest type of resins, still lacking large scale industrial applications. The WBA resin used in this thesis (Paper IV) was methacrylate-DVB exchanger having dimethylamino-propylamine as the functional group.

## 3.2 Retention Mechanism in the Chromatographic Separation with Ion Exchange Resins

The retention of a component on the resin is generally a sum of various retention mechanisms. The mechanisms of sugars are most studied for the SAC exchange resin in alkali or alkaline earth metal forms. Mainly, they include ion exclusion, size exclusion and ligand exchange (Caruel et al. 1991). Separation phenomena of the other resin types taking place on chromatographic exchange resins are rather unknown but may also be a combination of known phenomena: steric exclusion, ion exclusion, hydrophobic-hydrophilic interactions, electrostatic attraction-repulsion and ligand exchange.

### 3.2.1 Ligand Exchange

Ligand exchange relates to a cation exchange resin which is loaded with a complexable metal ion. Separation of the components of mixtures occurs by virtue of the differences in the stabilities of the various metal-ligand complexes formed. The complexation between sugars and metal ions coordinated to (especially strong) cation resins is a major mechanism with some ion forms (such as calcium) in the separation of sugar mixtures (Goulding 1975, Walton 1985). The selectivity is determined largely by the nature of the cation employed.

The type of the counter-ion is a crucial parameter affecting the efficiency of chromatographic separation of monosaccharides. Some retention data for selected monosaccharides are available for strong acid cation exchangers in $Ca^{2+}$, $Sr^{2+}$, $Ba^{2+}$, $Pb^{2+}$, $Y^{3+}$, $La^{3+}$, $Pr^{3+}$ ion forms (elution in a column) (Caruel et al. 1991), $Na^{+}$, $K^{+}$ (static method) (Nobre et al. 2009, Gramblicka, Polakovic 2007, Lei et al. 2010).

### 3.2.2 Ion Exclusion

Ion exclusion chromatography is a chromatographic technique used for the separation of ionized and non-electrolyte solutes. No ion exchange occurs and the electrolyte is excluded from the resin on the grounds of the Donnan theory. It states that the electrical potential must remain balanced (Helfferich 1962). Strong and weak electrolytes are separated, the first at the beginning of the elution order and the latter at the end.

With hydrolysates which contain non-electrolytes (sugars) and ionized components (acids, salts), ion exclusion mechanism is of great importance. The characteristic feature of ion exclusion chromatography is the same charge on the dissociated functional groups of the ion exchange resin and on the solute. SAC exchanger in hydrogen form has been used as the stationary phase for the acid-sugar separation (Springfield, Hester 1999, Xie et al. 2005).

On analytical scale, the SAC resin in hydrogen form is also used for the separation of organic acids (Small 1989). A WAC exchange resin in hydrogen form has also been used for the separation of organic acids with acidic eluent on analytical scale (Mori et al. 2006, Tanaka et al. 1999, Helaleh et al. 2003).

### 3.2.3 Size Exclusion

Cross-linkage of the resin is an important factor affecting also the position of the elution profile. The degree of cross-linking governs the extent of swelling of the dry ion exchange resin upon absorbing water. The more weakly cross-linked the resin, the greater is the swelling and the water uptake. The resin capacity is directly proportional to the cross-linkage of the resin.

Cross-linking affects the separation of monosaccharides from oligosaccharides or larger molecules in general. The DVB content can be chosen such that oligosaccharides are excluded but monosaccharides are able to be sorbed by the resin retarding their elution (Vente et al. 2003). Thus, oligomers elute in order of decreasing molecular mass. However, for the monosaccharide separation the DVB content is not as crucial (Adachi et al. 1999). Not surprisingly, industrial chromatographic separations of sugars are carried out with resins of 3 to 8 % by weight cross-linking for resin bed depths of 3 to 6 meters (Hongisto 1977).

### 3.2.4 Hydrogen Bonding (Paper III)

In the chromatographic separation of sugars with cation exchangers, hydrogen bonding is considered a minor effect (Goulding 1975). In other applications it may, however, be an important factor. One is the chromatographic separation of glycinebetaine (GB) with acidic WAC exchanger, which was concluded in Paper III. Namely, when the chromatographic separation of vinasse was studied on an acidic WAC exchanger, it was noted that GB is strongly retained by the resin but the retention time is not a constant in the process of time. Further testing revealed (Julku 2008) that the retention of GB is somehow related to the pH of the solution, to the cation content in the feed and to the resin, but clear understanding of the retention mechanism was not found.

In Paper III, the behavior of pure GB on WAC exchange resins in hydrogen, monovalent, and intermediate ionic forms (25, 50, 75 %) was studied in a batch column to reveal the retention mechanism of GB. Sodium was chosen as the counter ion, although it could as well have been potassium, as these are the most common cations in vinasse. Aqueous eluent at neutral and acidic pH were used in the tests because the charge of GB is different at neutral and at low pHs. Elutions of three other components – glycine, choline chloride and glycerol (see Figure 6) – were also studied under the same conditions.

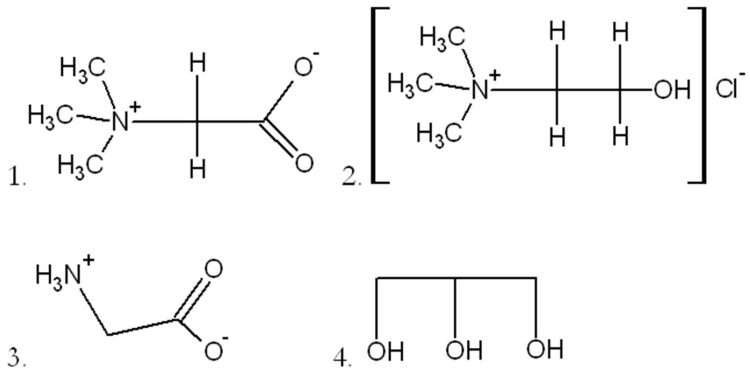


Figure 6. Chemical structures of 1) trimethyl glycine alias glycinebetaine 2) choline chloride 3) glycine 4) glycerol. (Paper III)

Choline chloride was chosen for the experiments, because it has a similar structure as GB but is a permanent cation. As a salt, it also models the behavior of inorganic matter in vinasse. Glycerol, one of the major components in vinasse, would show if the hydroxyl groups (-OH) have to do with the retention mechanism. Glycine, on the other hand, is a zwitterion like GB but is a smaller molecule than GB.

Elution of GB on a sodium/hydrogen form WAC resin (100% - 75 % - 50 % - 25 % - 0 %) is presented in Figure 7. The more the resin is in sodium form, the faster GB elutes. The elution volume (peak maximum) of GB in sodium form is 52 % bed volume whereas in hydrogen form approximately 128 % of bed volume.

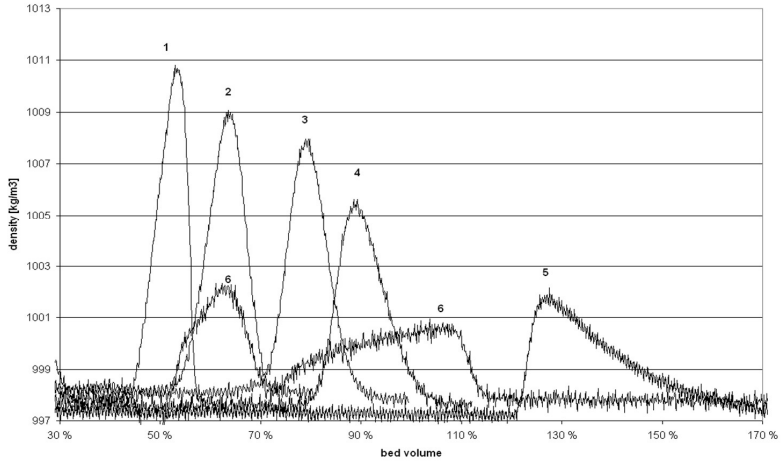


Figure 7. Elution of glycinebetaine on sodium/hydrogen form WAC resin. Numbers refer to test conditions: 1) 100 % Na, 2) 75 % Na, 3) 50 % Na, 4) 25 % Na, 5) 100 % H and no pH adjustment in eluent, 6) 100 % H and pH 1 (eluent). (Paper III)

It was concluded that the retention mechanism of GB to hydrogen form WAC resins depends on two separate phenomena. The first is the ion form of the resin and the second is the pH of the feed solution and/or eluent. The ion form of the resin is strongly related to the cation composition in the feed solution, however. If the feed solution and eluent are cation-free, ion exchange does occur. However, vinasses generally have a heavy salt loading. WAC exchangers easily convert into salt form, if the operating pH is not clearly below the $pK_a$ value of the resin. It is also a well-known fact that WAC-resins have a high selectivity for divalent cations.

An earlier study (da Costa, Leite 2001) using Raman spectroscopy revealed that in aqueous solution of zwitterionic betaine, there is a mixture of monomer and hydrogen-bonded dimer molecules (see Figure 8). On the other hand, WAC exchanger in acidic form offers a carboxyl group (-COOH) to form a hydrogen bond with. Therefore, when GB is a zwitterion, the retention is based on hydrogen bonding between the molecule and the resin.

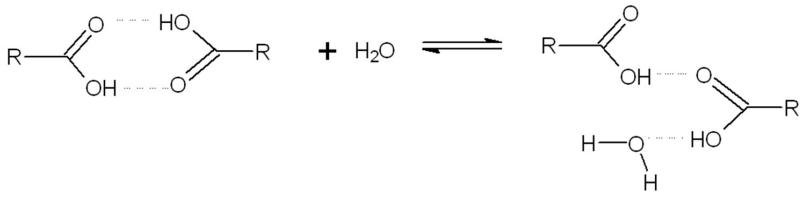


Figure 8. The equilibrium between the dimer + water ↔ hydrated monomers of carboxylic acids (da Costa, Leite 2001).

The other factor is the pH of the feed solution and eluent, which affects the net charge of GB. The lower the pH, the stronger the cation GB becomes. Therefore, the elution behavior of GB with 100 % hydrogen form WAC exchanger is different at a neutral pH and at a very low pH (see Figure 7).

At a neutral pH, the GB peak is asymmetric. At a very low pH (betaine hydrochloride and sulfuric acid in the feed solution), the peak form is asymmetric but it also forms two peaks. The first is assumed to be a salt because a rise in conductivity can be detected simultaneously, and the latter peak is GB (no conductivity).

Thus, the retention mechanism of GB on hydrogen form WAC exchanger is based mainly on hydrogen bonding between the zwitterionic molecule and the resin. At a very low pH (ca. 1), a positive charge of GB, however, explains the faster elution on hydrogen form WAC exchange resin. When GB is a zwitterion, the retention is based on hydrogen bonding between the molecule and the resin.

### 3.2.5 Other Retention Phenomena (Paper I)

There are still other phenomena than ion exclusion, size exclusion, ligand exchange or hydrogen bonding, which affect the retention of components on ion exchange resins. This chapter presents some individual phenomena which have been utilized in this thesis.

**Bisulfite Addition to SBA Exchange Resin**

The use of strong base anion (SBA) exchange resin in bisulfite form was used for sugar separation on analytical scale as early as in the 1950's. It was discovered that some sugars, like glucose and fructose, having carbonyl groups can effectively be separated from each other (Samuelson 1953). In the 1960's, it was reported that ketoses and aldoses can effectively be separated from each other (Lindberg, Slessor 1967). The addition of bisulfite ion makes carbonyl groups undergo a nucleophilic addition reaction with bisulfite (see Figure 9) (Igawa et al. 1990, Kuzmanovic et al. 2003). The reaction of bisulfite with carbonyl groups explains the good separation between aldehydes and ketoses on SBA exchange resin in bisulfite form. This property was utilized in Paper I, as one chromatographic separation step, in the separation of L-fucose from other monosaccharides.

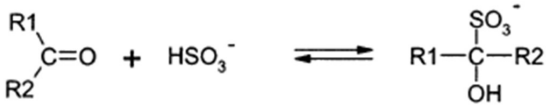


Figure 9. Bisulfite addition – a characteristic reaction of carbonyl compounds

Despite the excellent selectivity, the bisulfite form SBA exchanger offers, the use of resin is inconvenient. Retention is so strong that large volumes of eluent are needed to elute the components. In addition, the ion form inevitably converts into sulfate form due to oxidation that takes place on the resin.

**Hydrophobic-Hydrophilic Interactions**

According to Goulding (1975), hydrophobic interactions play a minor role in the adsorption process of polyols and sugars on strong cation exchangers. However, the resin matrix of weak acid cation (WAC) resins is inherently more hydrophilic than in PS-DVB structure due to the smaller amount of benzene groups which are hydrophobic. For example, common aldoses and ketoses differ by a hydroxyl group from deoxy sugars, which makes a difference in hydrophobicity. Therefore, it is assumed that hydrophobic-hydrophilic interactions may affect the separation of components on the resin surface of the WAC exchanger.

The phenomenon has not been scientifically verified by other methods but experiments support the theory. More hydrophobic saccharides, such as deoxy, methyl and anhydrosugars, are not retained on the resin as long as more hydrophilic saccharides. Rhamnose, for example, elutes earlier than other monosaccharides on the WAC exchange resin in sodium form (Kärki et al. 2002). This was utilized in the separation of rhamnose from other monosaccharides in Paper I.

## 3.3 Physical Variables

The physical variables, such as particle size and size distribution, affect the hydrodynamics in the chromatographic separation column.

The average resin bead size in sugar separations is typically from 200 to 450 μm. The smaller the beads are the faster is the overall diffusion into and out of the resin beads. On the other hand, the pressure drop in the column should not become excessive.

The bead size distribution should preferably be as narrow as possible. Small beads will elute the solute more rapidly and the larger ones less rapidly, which causes additional spreading or tailing of the elution profile. The narrow bead size distribution makes also a "linear" pressure drop in the bed, which is essential for a successful plug flow. Ideally, the beads should be of the same size, which they, in practice, are not.

## 3.4 Adsorption Isotherms (Paper IV)

The design of an industrial-scale chromatographic separation system begins with the selection of a suitable adsorbent material. The adsorption equilibria of the compounds in the feed stream are of essential information in the selection process. The adsorption equilibrium data of sugars have been published mainly for SAC exchangers (Caruel et al. 1991, Nobre et al. 2009, Gramblicka, Polakovic 2007, Lei et al. 2010, Vente et al. 2003). However, adsorption isotherms of saccharides

with the other three main types, namely weak acid cation (WAC), strong base anion (SBA) and weak base anion (WBA) ion exchange resins are scarce.

Paper IV presents the adsorption isotherms for arabinose, fructose, galactose, glucose, mannose, rhamnose, sucrose and xylose on the four ion exchanger types (SAC, SBA, WBA and WAC) in typical ion forms ($Na^+$ and $SO_4^{2-}$). A static method (batch method) was used in the determination of the distribution coefficients. The concentration of individual sugars was varied from 0 to 350 g/l, the temperature was 65 °C and the duration of the test was 8 hours. The sugar concentration of the solution was determined according to refractive index, and the solid-phase concentration of a saccharide, q, at equilibrium was calculated as follows:

$$q = \frac{V(c_{initial} - c_{final})}{m_{dryresin}} \quad (1)$$

where V is the volume of the added sugar solution, $c_{initial}$ the initial concentration of the sugar solution, $c_{final}$ the concentration of the sugar solution at equilibrium and $m_{dry\ resin}$ the mass of the dry resin in the system. All experimental data were fitted with the linear parameter, distribution coefficient, *K*:

$$q = K \cdot c \quad (2)$$

Possible decomposition of sugars was analyzed by HPLC measurements. All the sugars were stable with the SBA exchange resin. No decomposition was detected either with the SAC resin except for sucrose, of which up to 10 % decomposed into glucose and fructose. Besides, the acidic conditions with the WBA exchange resin (pH 3) hydrolyzed sucrose, and therefore, the distribution value could not be determined. The alkaline operating conditions of the WAC exchanger (pH 9) were too challenging for many saccharides. Sucrose, rhamnose and mannose were quite resistant: less than 5 % decomposed during the test. Distribution factors could not be determined for the other sugars, since from 10 to 30 % were isomerized (such as glucose into fructose or vice versa) or decomposed into components other than common sugars. Therefore, the use of the WAC exchanger is restricted.

All the equilibrium data were described with linear isotherms over the concentration range. An example of the adsorption isotherms is presented in Figure 10, where adsorption isotherms of mannose are depicted on the four resin types.

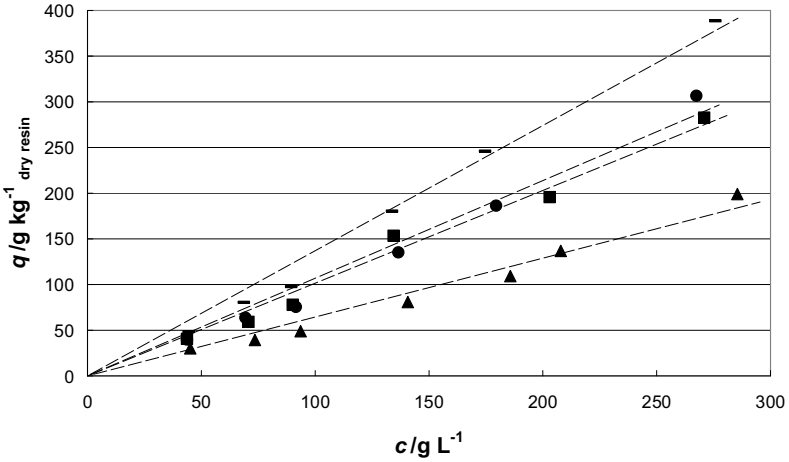


Figure 10. Single-component adsorption isotherms of **mannose** on SAC ($Na^+$) / SBA ($SO_4^{2-}$) / WAC ($Na^+$) / WBA ($SO_4^{2-}$) exchange resins. ■ SBA; ▲ WBA; ▬ WAC; ● SAC. Symbols represent experimental data and lines best fits of Eq 2. (Paper IV)

The WBA exchange resin showed high selectivity for xylose and arabinose. The SBA exchange resin demonstrated good selectivity for xylose and rhamnose, which implies that these could be separated from biomass hydrolysates with the SBA exchange resin.

The retention mechanism of sugars on SAC exchange resin was speculated to be related to a partitioning which takes place both in the bound non-freezable pore-water and in the freezable pore-water. Besides ordinary free water, there are two types of bound water in the narrow pores of the resin, which are freezable and unfreezable water, tightly bound to the polymer backbone. (Vaňková et al. 2010) This model would explain better the linear isotherm than the previously suggested weak complexes and the mutarotation equilibrium (α and β forms) of sugars (Nobre et al. 2009). There is a finite surface area for adsorption, and the saturation

effect should be observed, if the retention mechanism is related to interactions on the resin surface.

## 3.5 Separation Factor vs. Rise in Sugar Purity (Paper V)

Chromatographic separations may be characterized by a separation factor (α) which gives an indication of the difficulty of the separation. The separation factor is calculated as presented in Equation 1:

$$\alpha = \frac{k_2}{k_1} = \frac{V_2 - V_0}{V_1 - V_0} = \frac{t_2 - t_0}{t_1 - t_0} \qquad (3)$$

where k is the retention factor, V (or t) is the retention volume (or time) of a component 1 or 2, and $V_0$ (or $t_0$) is the void fraction, i.e. elution volume (or time) of a non-retained component.

The separation factor (α) correlates with the rise in sugar purity (ΔC) over a separation step as presented in Figure 11. These separation factors were measured on an industrial scale for separations with the sugar content in feed > 90 % on DS (Papers I-II, Heikkilä et al. 2006, Jumppanen et al. 2000, Heikkilä et al. 2005, Heikkilä et al. 2005, Strube et al. 1998). The separation factors were calculated from chromatograms, assuming that the void volume is 33 % of the bed volume, if not stated otherwise. For sugar mixtures, the pair consists of the product sugar and the major impurity sugar. The separations were carried out batch wise, except for the fructose-glucose separation which was a simulated moving bed (SMB) example.

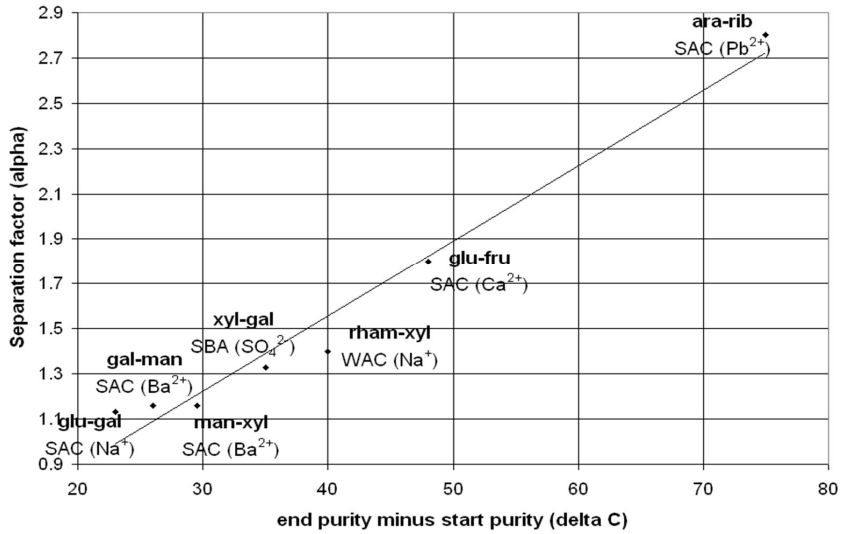


Figure 11. The separation factor (α) vs. the rise in purity (ΔC) in sugar separations (sugars > 90 % on dissolved solids). Fuc, rha, glu, gal, man, xyl, ara, and rib refer to fucose, rhamnose, glucose, galactose, mannose, xylose, arabinose and ribose respectively. SAC, SBA, and WAC refer to strong acid cation, strong base anion and weak acid cation respectively. (Paper V)

Figure 11 shows that the general level of separation factors in some sugar separations is relatively low, even < 1.2, which is considered a very difficult separation in chromatography (Bidlingmeyer 1987). The correlation between the rise in purity and the separation factor is strong. For example, if a higher than 40 % on dissolved solids (DS) rise in purity is targeted over one separation step, the separation factor of the product sugar with the main impurity sugar should be higher than 1.5. The sugar yield in the individual separations varied between 70 % and 95 % in literature. With a constant yield, the correlation between the rise in purity and separation factor would have been even better. The complexity of the feed solution (i.e. number of sugars), not shown in the figure, also affects the result.

# 4 Current Industrial Chromatographic Processes

## 4.1 Operation Modes

### 4.1.1 Batch Chromatography

In preparative chromatography, the application of classical batch elution is the prevailing technology (Guichon et al. 1994). All experimental chromatographic work in this thesis (Papers I-III, V) was also carried out in pilot scale batch columns, with the inner diameter (i.d.) between 9.3 cm and 1.0 m. The schematic construction of the columns is presented in Figure 12.

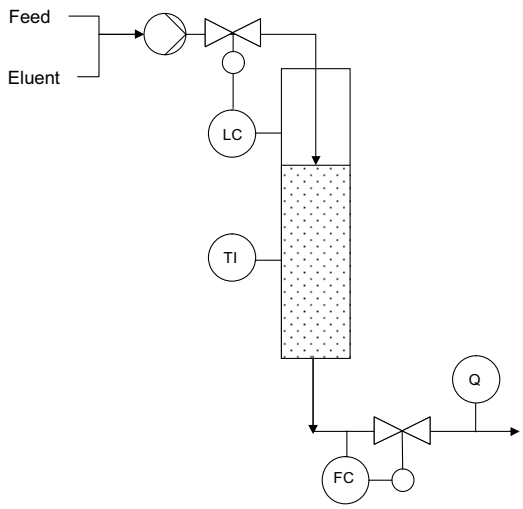


Figure 12. Experimental set-up in the chromatographic tests.

The resin bed height varied between 1.6 m and 5.3 m, depending on the column i.d. The column had a thermostated water jacket which kept the temperature at the target temperature in the column. The elution water was degassed by boiling the water before the tests.

### 4.1.2 Continuous Chromatography

Some limitations of batch chromatography, such as productivity, recovery and solvent economy, can be overcome with continuous chromatography. Broughton and Gerhold from Universal Oil Products introduced the concept of simulated moving bed (SMB), as a practical implementation of the true moving bed (TMB) process in the 1960's (Broughton, Gerhold 1961). The TMB is a hypothetical process which assumes that there is a real counter-current motion between the fluid phase and the solid phase (Guichon et al. 1994).

Nowadays, among the continuous techniques, the most important ones are the simulated moving bed (SMB) and the sequential simulated moving bed (SSMB) (Karlsson 2001). In the continuous SMB process, all fluids (feed, eluent and outtake products) typically flow continuously. In the sequential SMB, the flows are sequential but the concentration gradients move continuously in the columns. The main advantage of the sequential process is that more than two components can be separated with high purity and yield.

The classical four-zone SMB principle is described in Figure 13. The principal idea is to simulate a true moving bed where counter-current movement between a solid and a liquid is achieved. The simulation is achieved by switching periodically the injection and the withdrawal points. This enhances the separation and alleviates the problems associated with batch chromatography.

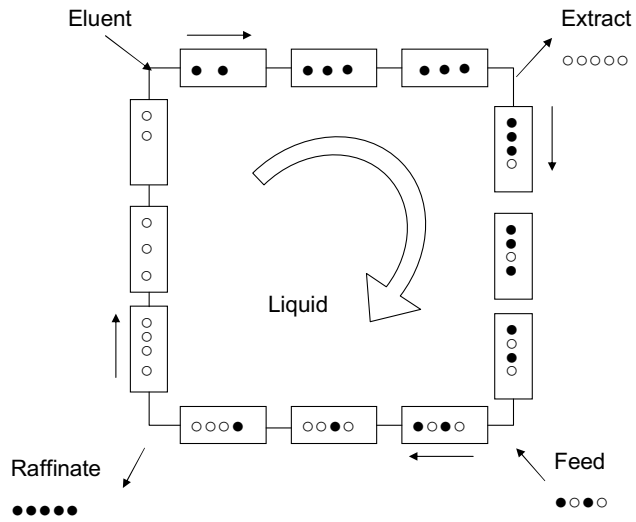


Figure 13. Principle of simulated moving bed.

Research in the SMB technology has been very active during the past decade. There are several publications covering recent developments in SMB chromatography (Seidel-Morgenstern et al. 2008, Gomes et al. 2006, Xie et al. 2001). Seidel-Morgenstern et al. (2008) describe new variants of SMB chromatography which include, for example, modulation of the feed concentration and flow rates. The pharmaceutical industry and difficult enantioseparations using chiral stationary phases have become a strong driving force (Rajendran et al. 2009).

In modeling, an SMB unit can be considered as a single chromatography column with several inlet and outlet ports along the column. Because continuous chromatography is basically a method to intensify the process economy, it has not been widely covered in this thesis. Generally, SMB is considered a more efficient technology but a comparison of batch elution and continuous SMB chromatography by Strube et al. (1998) shows that this does not always apply. It was stated that it is not possible to generally predict what kind of process will provide the greater productivity, batch or SMB.

## 4.2 Optimization in Chromatography

Separation processes account for 40 - 70 % of both capital and operating costs in industry (Rousseau 1987). Pharmaceutical products, food and fine chemicals are subject to tight specifications for product purities, and therefore efficient processes for the high-purity separation of sensitive products are needed. This necessitates optimization which is an important topic also in large-scale chromatography.

The total separation cost is the ultimate objective function which must be optimized in any separation process. Typical objective functions over a chromatographic step are productivity, eluent consumption, yield and purity, as they are clearly defined functions (Schmidt-Traub 2005). The parameters affecting these are flow rate, resin bed length, feed size (volume and concentration) and the cut points. It has been shown that the minimum separation costs for neither batch nor SMB units are located at the maximum productivity (Jupke et al. 2002). The most economical separation process is a compromise between the eluent consumption and the productivity.

Cut strategies in batch chromatography of binary components can be divided into two approaches (Figure 14): I) three fractions, or II) two fractions. The cut strategy I is a better method in optimizing the chromatographic separation process. This method was also used in Papers I, II and V, but with the exception that a recycle fraction was used.

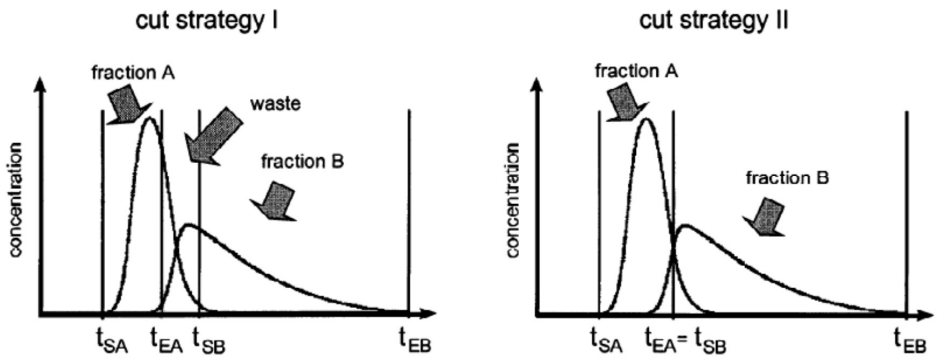


Figure 14. Cut strategies for batch optimization (Jupke et al. 2002).

The fraction between the product and waste fraction, called a recycle fraction, can be used to dilute the original concentrated feed solution or directed to the column before or after the main pulse. In some cases, the recycle fraction is re-processed separately but because this increases operating costs, direct pre/post-feed is preferred. In all cases, the volume of the recycle fraction should not exceed a limit which is determined by the process (need for dilution, broadening of the profile, evaporation capacity, etc.). In addition, the composition of the recycle fraction should be similar to the feed solution to avoid weakening of the separation. It has been experimentally tested that the volume of the pre/post-feed should not exceed 2 % BV, and should preferably be 1 % BV.

Another important factor concerning the optimization of a separation process is the integration of other unit operations or processes. Common unit operations related to the chromatographic separation are filtration, pH adjustment and concentration by e.g. evaporation. Examples of the pre- or post-unit processes are softening, IEX, fermentation, membrane separation and/or crystallization.

Crystallization and chromatography, despite their close interaction, are in practice often carried out separately in many industries. Chromatography is followed by crystallization to recover products in solid form. The coupling of chromatographic separation and crystallization aims to improve the overall separation efficiency of the process by the synergistic effects of chromatography and crystallization. For example, chromatographic separation alone may be an expensive alternative,

while using lower cost crystallization methods is limited to the existence of thermodynamic boundaries. Lim et al. (1995) and Lorenz et al. (2001) have studied the separation of enantiomers by the hybrid technique. They report that an important task is to identify optimal crossover purification between the chromatography and crystallization operations. Fung et al. (2005) have proposed a systematic procedure for synthesizing an optimal chromatography-crystallization separation process. More recently, hybrid processes have also been presented for the recovery of a natural drug ingredient (Qu et al. 2010a, Qu et al. 2010b).

## 4.3 Applications

Essentially, IEX resins are used for two types of chromatographic separation of compounds on a large scale. The first is the chromatographic separation of hydrolysates, or vegetable-derived industrial by-products of the food and fermenting industries, where the target is to separate a sugar or a group of sugars form other compounds (such as salts, inorganic material and larger molecules). The other type is the chromatographic separation of essential sugar mixtures, such as presented in Figure 11.

### 4.3.1 Hydrolysates and By-Products

Acidic hydrolysates contain a variety of toxic inhibitory by-products for fermentation (acetic acid, furfural, etc.), and efficient separation of these compounds is of great importance, when high yield fermentation is pursued. Chromatographic separation of all sugars as a group using IEX resin from a lignocellulosic hydrolysate has been studied as a promising method to improve the fermentation yield (Springfield, Hester 1999, Xie et al. 2005).

Examples of chromatographic separations of hydrolysates on an industrial scale are presented in Table 4. These include, for example, sucrose recovery from molasses, betaine recovery from molasses or vinasse, lactose removal from milk, and xylose recovery from spent sulfite liquor. In all presented processes, a SAC exchange resin in monovalent or divalent form is used, and the separation is mainly based on ion exclusion and size exclusion. SAC exchangers have the

longest history and their chemical stability and durability are best known of the other IEX types.

Table 4. Industrial scale, single-step, chromatographic processes utilizing size exclusion and/or ion exclusion chromatography. Purity is expressed as % on dissolved solids (DS).

| **Product** | **Starting material** | **Resin type** | **Starting purity % on DS** | **Target purity % on DS** | **Ref.** |
|---|---|---|---|---|---|
| Sucrose | Molasses | SAC ($Na^+/K^+$) | 40 - 60 | ~ 90 | (Heikkilä et al. 1992, Paananen, Kuisma 2000) |
| Betaine | Molasses/ Vinasse | SAC ($Na^+/K^+$) | 3 - 15 | 50 - 60 | (Heikkilä et al. 1992, Decloux et al. 2002, Paananen, Kuisma 2000) |
| Xylose | Spent sulfite liquor | SAC ($Mg^{2+}$) SAC ($Ca^{2+}$) | ~15 | 60 - 70 | (Heikkilä 1985) |
| Arabinose | Gum arabic hydrolysate | SAC ($Ca^{2+}$) SAC ($Na^+$) | 34 | 85 | (Heikkilä et al. 2005) |
| Lactose free milk | Milk | SAC ($Ca^{2+}/K^+$/ $Mg^{2+}$/ $Na^+$) | ~ 30 | ~ 0 | (Harju 1989) |

### 4.3.2 Sugar Mixtures

The other type of chromatographic separations are essentially saccharide-saccharide separations, where other compounds than sugars are in minority.

*Single-step.* Chromatographic separation of fructose from glucose-fructose mixture is performed industrially with a calcium form SAC exchanger from approximately 42 % on DS to 90 % on DS and in simulated moving bed (SMB) mode (Strube et al. 1998, Dow 2010). A relatively high separation factor of fructose/glucose (around 1.8) makes it possible to achieve this high rise in purity, not compromising the yield. In Paper II, galactose was separated from a lactose hydrolysate with a SAC exchanger in sodium form from 42 % on DS to 65 % on DS. It was stated, however, that a calcium form resin would have been a better choice selectivity-wise.

Fermentation products or conversion mixtures of sugars are also separated chromatographically. Dendene et al. (1995) carried out lactulose purification from lactose and galactose with a SAC resin in potassium form (Dendene et al. 1995). In the manufacturing process of tagatose, chromatographic separation can be used to recycle lactose for hydrolysis and galactose for isomerization (Bertelsen et al. 2006). The separation of dextran and fructose from a fermentation broth was also carried out with an SMB mode (Coelho et al. 2002). Chromatographic separation of psicose from a reaction mixture containing fructose was recently proposed to be carried out with calcium form SAC exchange resin in SMB mode (Long et al. 2009)

*Multi-step.* Separation of a monosaccharide from a solution with a low starting purity usually requires several chromatographic separation steps with various resin types. Examples of multi-step separation sequences of sugar-sugar separations are presented in Figure 15. These are chromatographic separation sequences for fucose (Paper I), mannose (Heikkilä et al. 2006), rhamnose (Heikkilä et al. 2002) and galactose (Heikkilä et al. 2005), originally from spent sulfite liquor side streams.

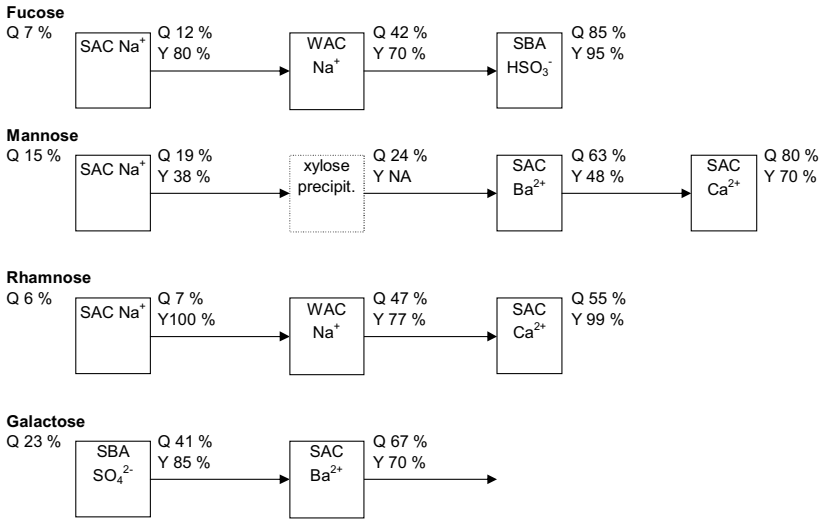


Figure 15. Industrial scale chromatographic processes with multiple chromatographic steps. SAC = strong acid cation, WAC = weak acid cation, SBA = strong base anion, Q = purity of the product sugar as % on dissolved solids, Y = yield of the product sugar.

The final purities for sugars of the processes vary from 55 % on DS (rhamnose) to 80 % on DS (mannose). It can be seen in Figure 15 that also other IEX resins types than SAC exchangers are used to increase the purity of the product sugar. For example, a WAC exchanger in sodium form is used in the fucose and rhamnose separation processes. Ion form may be a heavy metal ($Ba^{2+}$), like in the mannose process, although this requires an IEX process for the effluent. SBA resins can also be used (fucose and galactose processes), although they have lower stability compared to SAC exchangers. The WAC exchanger in sodium form has been used in the fucose and rhamnose separations, because it separates rhamnose better than other IEX resins. Its disadvantage is the operating pH that needs to be alkaline, which causes some sugars to degrade. In addition to chromatographic steps, other unit operations are also needed. These may include, for example, filtration of suspended solids and final purification by crystallization, ion exchange, etc. These are not presented in Figure 15.

# 5 Novel Chromatographic Separation Processes

In this thesis, several new chromatographic separation processes were developed. In Paper I, a novel chromatographic separation sequence for L-fucose is proposed. Paper II discusses chromatographic separation of D-galactose from biomass hydrolysates. In Paper III, a workable method for chromatographic separation of glycinebetaine (GB) is presented, although the focus is on understanding the retention mechanism of GB on the resin.

## 5.1 Chromatographic Separation Process for L-Fucose (Paper I)

As explained in Chapter 2.1, production of fucose from biomass-based hydrolysates has been lacking recovery methods. Production of fucose by synthetic means also leads to multi-step processes with a relatively low L-fucose yield due to the low conversions and several steps, as discussed in Paper II.

The origin of the raw material used in this study (Paper I) was spent sulfite liquor presented in Table 2. The raw material was a fucose-enriched fraction after xylose and rhamnose recovery from SSL by chromatographic methods. The composition of the liquor was analyzed by HPLC to contain 47.2 % on dry matter (DM) various sugars (fucose, rhamnose, xylose, arabinose, xylulose) and sugar-like compounds such as methyl-α-D-xylopyranoside (MAX). The rest was unidentified compounds consisting of inorganic and organic components, like other carbohydrates. The fucose content was ~ 7 % on DM and the target purity > 80 % on DM.

### Strong Acid Cation Exchange Resin in Sodium Form

First, the raw material was subjected to a chromatographic separation step where the column filling material was SAC exchange resin in sodium form. This resin was chosen because it is generally used to remove ionic and larger compounds (ion and size exclusion). Rhamnose, arabinose and xylose are the first identified sugars which start to elute after the conductivity peak (Figure 3 in Paper I).

Fucose, xylulose and MAX are completely overlapping and they are not separated from each other. At the end of the profile, unknown compounds are again abundant. The fucose content of ca. 11 % on DM can be achieved, on an average, in the product fraction. The fucose yield is ca. 59 % without recycling. Main impurities in the fucose fraction are still rhamnose and MAX.

### Weak Acid Cation Exchange Resin in Sodium Form

The collected fucose fraction was subjected to the second chromatographic separation with the WAC exchange resin in sodium form. According to the earlier experiments, the resin separates compounds also according to their hydrophobic-hydrophilic properties. This was confirmed in these separations, as MAX elutes as the first component, and is followed by deoxy sugars, fucose, and rhamnose (Figure 16). The first concentration peak consists mostly of MAX, fucose and rhamnose, and the second of arabinose, xylose, galactose, mannose, fructose and glucose.

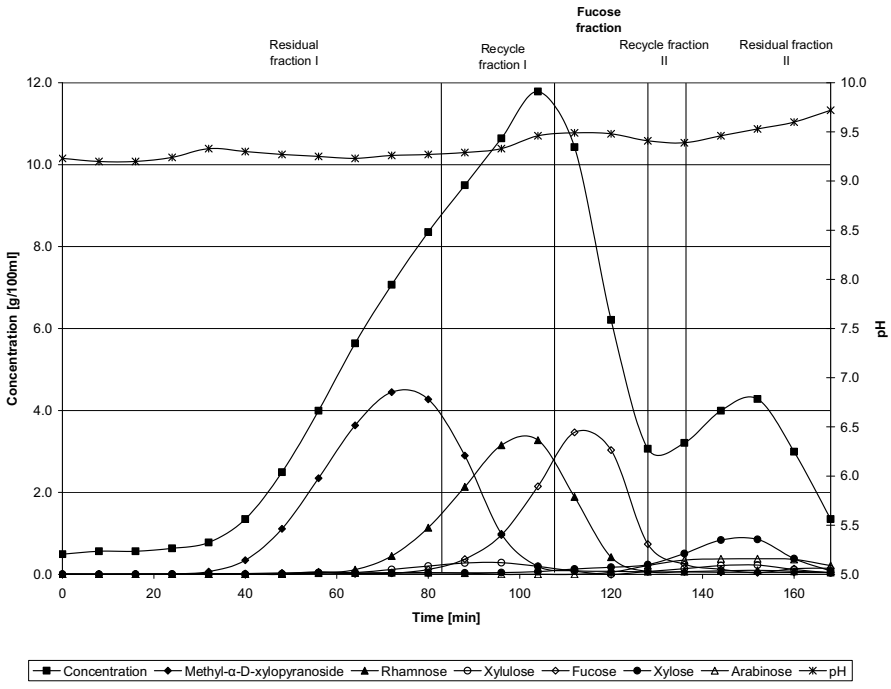


Figure 16. Chromatographic separation profile: WAC exchange resin in sodium form. (Paper I)

The fucose content in the feed solution to the second separation was ca. 11 % on DM, and the fucose content ca. 38 % on DM is achieved, on an average, in the product fraction. The fucose yield over this step is ca. 50 % without recycling. A WAC exchanger in sodium form degrades many sugars, because its operating pH should be above 7 to avoid ion form conversion. In paper IV it was measured that from 10 % to 40 % of a sugar may be degraded under alkaline conditions and at an elevated temperature.

### Strong Base Anion Exchange Resin in Bisulfite Form

As nearly half of the DM in the product fraction was still unknowns, a SBA exchange resin in bisulfite form was tested to separate the compounds based on carbonyl groups. This was the right choice because the separation is excellent between the unidentified compounds and the deoxy sugars. Rhamnose elutes after fucose and that decreases the fucose content at the end of the profile (Figure 5 in Paper I). The fucose purity of 83 % on DM in the product fraction can be reached with 95 % yield without recycling.

Despite the excellent selectivity of the SBA exchange resin in bisulfite form, it is a challenging ion form to work with for many reasons. It is not a stable ion form, as oxidation takes place on the resin continuously. This causes change in the ion form (to sulfate) which weakens the separation drastically. Therefore, a pre-column was designed to remove most of the oxidizing agents and elongating the lifetime of the main bisulfite column. Regeneration of the columns (with sodium disulfite) is more complicated than general regeneration procedures. In addition, due to extremely great retardation of all compounds on the resin, a very large feed size and a faster flow velocity are needed.

The process was concluded to have three chromatographic separation steps as presented in Figure 15 and in more detail in Figure 17. The process was piloted till crystalline product and found to be a technically viable method, however, not fully optimized.

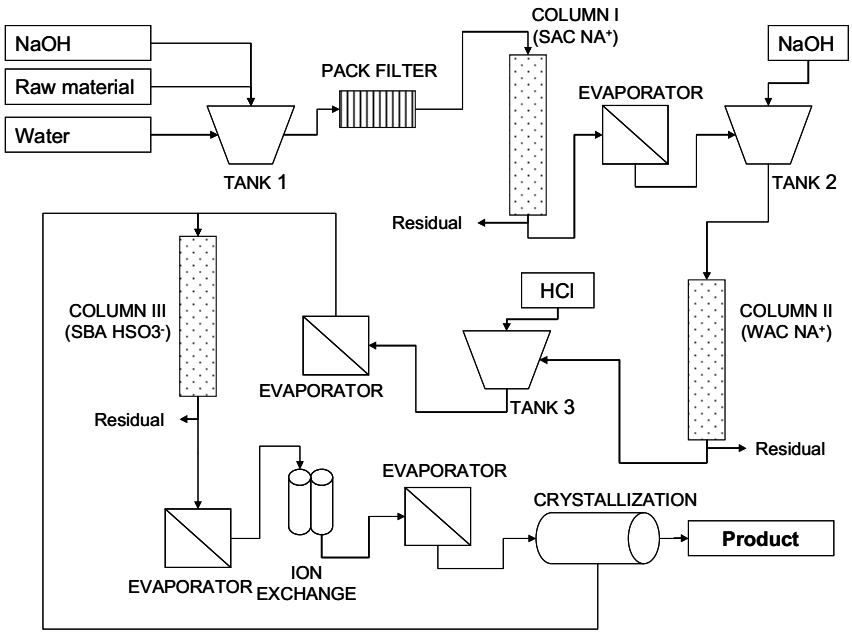


Figure 17. Diagram of the L-fucose production process. (Paper I)

## 5.2 Chromatographic Purification of D-Galactose (Paper II)

Purification methods for galactose from various sources by chromatography were studied in Paper II. Three possible sources for D-galactose - gum arabic hydrolysate, lactose hydrolysate and SLL based side stream - were experimentally compared. The compositions of the feed solutions are presented in Table 2.

Chromatographic separation of galactose in various ion forms of the SAC and SBA exchange resins has been reported in the scientific literature and patents as presented in Paper II, Table 1. A closer look at the references reveals that the separation factors between galactose and other sugars (xylose, mannose, glucose, arabinose and tagatose) can vary notably. For example, with the calcium form SAC exchanger, α ($^{arabinose}/_{galactose}$) is 1.28 whereas α ($^{galactose}/_{xylose}$) is only 1.06 (Caruel et al. 1991). Therefore, the most suitable ion exchange resin for the separation of galactose depends on impurities (sugars) in the solution. This aspect or the selection of the right kind of ion exchanger type for a separation task is discussed further in Paper V.

## Lactose Hydrolysate

In this study, a monovalent ion form SAC resin was tested for glucose-galactose-lactose separation because this has not been done earlier on a large scale. It was demonstrated, that although the calcium form SAC exchanger has higher selectivity for galactose-glucose, sodium form SAC exchanger can also be used in the chromatographic purification of galactose (Figure 18). The separation factor of the galactose-glucose pair in this system is 1.13 whereas for calcium form it is 1.24. Glucose elutes after lactose and is partially overlapping with galactose which elutes as the last compound.

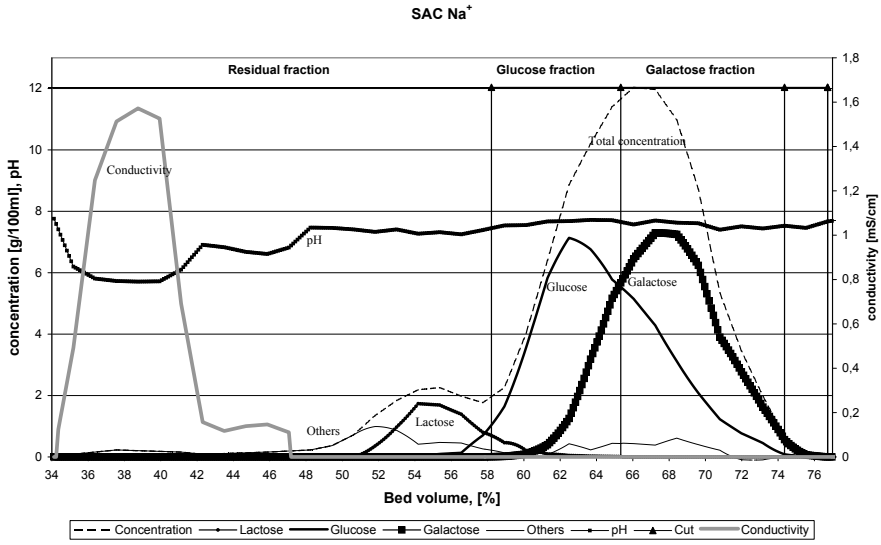


Figure 18. Lactose hydrolysate separation with sodium form strong acid cation exchange resin. Feed volume 12 l, concentration 28 g/100 ml, column i.d. 0.23 m, resin bed height 5.3 m, flow rate 30 l/h, temperature 60 °C. (Paper II)

The glucose fraction may suit the crystallization as such because the glucose content is as high as 71 % on DS. The glucose yield without recycling is 62 %. In the product fraction, the galactose content is 65 % on DS, which is high enough for crystallization with this impurity profile. The galactose yield without recycling is 78 %.

### Gum Arabic Hydrolysate

The gum arabic hydrolysate was chromatographically separated with a calcium form SAC exchange resin. This resin type was selected, as there is a relatively large amount of ionic compounds and larger molecules in the hydrolysate (54 % on DS other than monosaccharides). As the neutralization was done by $Ca(OH)_2$, calcium was a natural choice for the counter ion.

Figure 3 in Paper II shows that the elution begins with unknown components (other than sugars) which may be unhydrolyzed starting material, inorganic material, and/or degradation products from the hydrolysis. The first eluting monosaccharide is galactose which is exceedingly overlapping with rhamnose whose retention time is very close to galactose. They are both hexoses (C6 sugars), whereas the last sugar, arabinose, is a pentose (C5 sugar). Galactose can be recovered as a fairly pure fraction (44 % on DS) with a relatively good yield (69 %). Arabinose would naturally be the main product in this kind of process because it could be recovered with 85 % yield and 94 % on DS purity. The yield could be further increased by recycling methods.

### Hemicellulose Hydrolysate

The third case, separation of galactose from hemicellulose hydrolysate, was carried out with a SBA exchange resin in sulfate form. Mannose, galactose and rhamnose, which are all hexoses, elute with a very similar retention time leaving xylose, the only pentose, as the last eluting component (Figure 19). Therefore, the resin type suits the xylose recovery best. As galactose elutes almost simultaneously with other monosaccharides except for xylose, the galactose purity does not increase very much, from 23 % on DS to 45 % on DS with 53 % yield with no recycling. The galactose purity in the galactose fraction is probably too low for a successful crystallization. Therefore, further purification should be carried out.

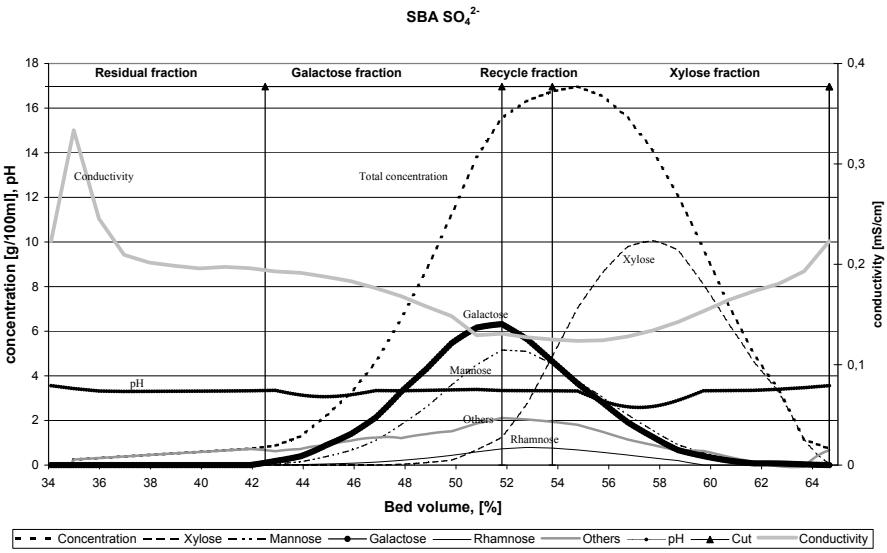


Figure 19. Chromatographic separation of hemicellulose hydrolysate with sulfate form strong base anion exchange resin. Feed volume 15 l, concentration 30 g/100ml, column i.d. 0.23 m, resin bed height 5.3 m, flow rate 30 l/h, temperature 60 °C. (Paper II)

Concerning all three presented chromatographic galactose separations, the intensification of chromatography using for example SMB techniques and/or recycling of eluent water would allow a better feasibility, if separations were carried out as industrial processes.

## 5.3 Glycinebetaine from Vinasse (Paper III)

In Paper III, the retention mechanism of glycinebetaine (GB) on a WAC exchange resin in hydrogen form was studied. It was concluded that hydrogen bonding is the main mechanism causing a higher retention of betaine than other components on hydrogen form WAC exchange resin (Chapter 3.2.4).

The ability of the WAC exchanger in hydrogen form to separate glycinebetaine from other compounds was utilized applying it to the chromatographic separation of vinasse. The feed volume in the chromatographic separation test was 13.5 % bed volume (BV). The GB content in the feed was 19.7 % on DS, glycerol 2.8 %

on DS, sucrose 0.8 % on DS and glucose 1.0 % on DS. The dissolved solids content in the feed solution was 35 % and with this DS the conductivity was 26 mS/cm and pH 3.1. More than 50 feeds were done prior to the fraction series collection to reach the equilibrium ion form of the resin. Samples for HPLC analyses were collected at 5-min intervals at the outlet of the column (see Figure 20). Separations of vinasse were done at pH 3.1. Furthermore, this pH was low enough to maintain the original ion form (hydrogen) of the resin despite the cations in vinasse.

Betaine elutes as the last component, basically alone, making its recovery at high purity relatively easy (peak GB content 89 % on DS). Therefore, it offers an alternative separation method for the recovery of GB from vinasse. This type of resin and ion form allows chromatographic separation at an acidic pH, unlike a WAC exchanger in alkali or alkaline earth metal form. In addition, the new method enables the recovery of GB from vinasse with only one chromatographic separation step, instead of current multi-step chromatographic separations (Kärki et al. 2002).

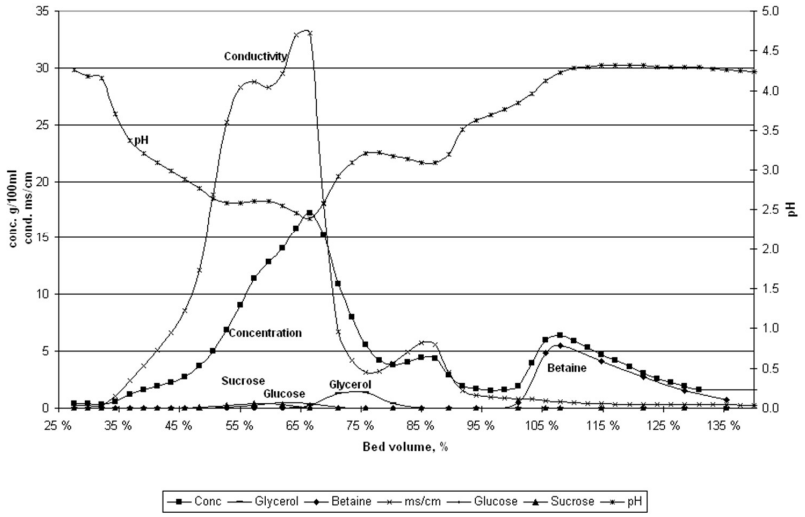


Figure 20. Chromatographic separation of vinasse with weak acid cation exchange resin in hydrogen form. (Paper III)

Productivity could be raised by a larger feed size but optimization of possible chromatographic separation process was not done at this stage. The feed volume was, however, already relatively large compared to common values in the industry. However, tailing, caused by the effects related to the characteristic behavior of GB and –COOH group, makes the achievement of high yield and high productivity for GB challenging.

From the resin property point of view, a methacrylic acid resin may be a better alternative for a WAC exchange resin in hydrogen form used for the separation of vinasse. This is because the apparent dissociation constant, $pK_a$, of the methacrylic acid is higher than that of the acrylic acid. The characteristics of some commercial methacrylic- and acrylic-based WAC exchange resins were reported by Riveros (2004). Apparent $pK_a$ values were measured to be within a range of 4.9 – 5.5. The amount of cross-linking also has an effect on the properties of carboxylic polymers (Fisher & Kunin 1956) but the effect is equal, if the cross-linking degree is the same. Using methacrylic acid as the raw material in the resin would allow a lower operating pH with less chemicals and would, thus, help to maintain the right ion form (hydrogen) in the resin.

# 6 Synthesis of a Chromatographic Separation Sequence (Paper V)

The separation of a monosaccharide from a solution with a low starting purity usually requires several chromatographic separation steps with various IEX resins. In addition, other auxiliary unit operations are needed. General separation process design principles and more detailed rules-of-thumb have been presented for distillation (Liu 1987) and bio-separations (Garciá et al. 1999) but process synthesis guidelines for the sequencing of chromatographic steps have not been proposed earlier.

Paper V discusses the topic and presents systematics for the synthesis of a chromatographic separation sequence for the separation of sugars on an industrial scale with IEX resins. The current industrial practice of chromatographic separation sequences was studied to find out systematics and heuristics for the sequencing (Heikkilä et al. 2006, Jumppanen et al. 2000, Heikkilä et al. 2005, Strube et al. 1998, Heikkilä et al. 2002, Heikkilä et al. 2002, Papers I and II). The systematics is divided into general heuristics and more detailed engineering guidelines for the sequencing of chromatographic separation of sugars.

## 6.1 General Heuristics

Some simple heuristics can be drawn from the chromatographic separation sequences presented in the referred literature. These are categorized into two subclasses in Table 5: method (M) and feed composition (C) related heuristics as presented in Paper V.

Table 5. Method (M) and composition (C) heuristics for the chromatographic separation of hydrolysates.

| | |
|---|---|
| M1 | Favor a SAC exchanger. |
| M2 | Use only water as eluent. |
| M3 | Avoid high pH. |
| M4 | Keep the solvent or eluent consumption as low as possible. |
| M5 | Integrate chromatography with other unit operations. |
| M6 | Aim at a high yield over every process step. |
| C1 | Try to accept the same ion form for the IEX resin as that of the feed solution. |
| C2 | Separate the most plentiful component first. |
| C3 | For salt-sugar separations choose an IEX resin with higher cross-linking. |

The first heuristic method (M1) suggests favoring SAC exchangers. This is because the SAC exchangers are the most common and durable type of resins. In ion- and size-exclusion they are the working horses.

Using only water as eluent (M2) refers to the fact that other solvents may cause emissions to air and water. In addition, they require an ATEX (ATmosphéres EXplosives) status and complicate the recovery process.

Reasoning behind avoiding the high pH (M3) is that many sugars decompose under alkaline conditions. Therefore, WAC exchangers should only be used in selected situations.

It is important to diminish the need for evaporation (M4) because recycling of the eluent causes energy costs. In practice, this means recycling techniques and using as large a feed as possible (concentration and volume). The eluent/feed –ratio should be optimized to be as low as possible.

Integrating chromatography with other operations (M5) helps to achieve a better product yield and to reach lower production costs. These pre- and final treatments may be, for example, evaporation, IEX, crystallization, fermentation and/or

membrane separation. Integration of chromatography and crystallization was discussed in Chapter 4.2.

One should always aim at a high yield over every process step (M6) because the total recovery becomes very low, if several low-yield steps are involved (Figure 21). For example, with 3 steps and 70 % step yield, the total recovery of a component is only 34 % (see Figure 21).

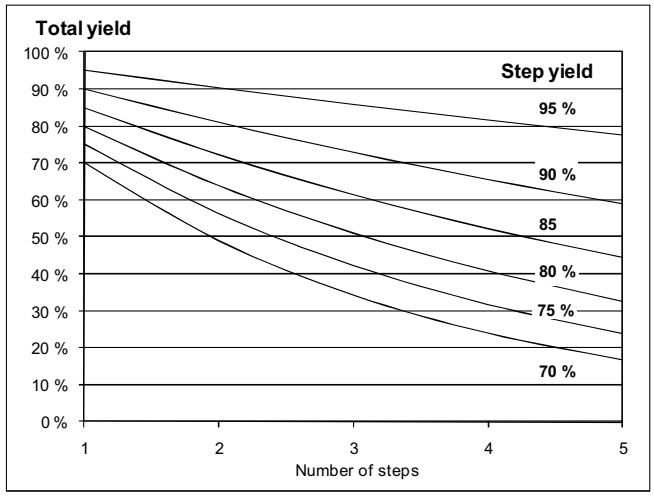


Figure 21. Total yield of a product as a function of step yield (70 – 95 %) over the number of unit operations.

Composition heuristics relate to the composition in the feed solution. Operating with IEX resins means that the ion composition in the feed solution must be taken into account. Trying to accept the same ion form for the IEX resin as in the feed solution (C1) allows lower operating costs, if this heuristic can be followed. Otherwise, an expensive ion removal prior to the chromatographic separation is needed. A frequent regeneration of the IEX resin causes a load on the water treatment.

The second feed composition related heuristic is to separate the most plentiful component first (C2), whether it is a group of non-sugars or an individual sugar. The separation load depends on the material to be processed and this affects the separation cost.

An IEX resin with higher cross-linking should be used for salt-sugar separations (C3) because resins typically suffer from repeated shrinking and swelling cycles. Tougher IEX resins can withstand osmotic stress better.

## 6.2 Sequencing

The selection of the right type of IEX resin for the separation is of great importance. Not only functionality but also cross-linking and counter-ion type are essential parameters to be considered. Based on industrial practices, guidelines for the sequencing and the selection of a feasible IEX resin for the sugar separation are given in Figure 22 as a block diagram.

A basic assumption for chromatography is that the solution can be filtered to be clear. Otherwise, other separation techniques must be used. The left side of Figure 22 (route 1) illustrates the questions for hydrolysates with the majority of non-sugars; salts, larger molecules than sugars, organic acids, inorganic material, etc. Practically, all these types of separation are carried out with SAC exchangers with a varying degree of cross-linking. The cross-linking degree depends on the main separating phenomenon (major impurity); ion or size exclusion. The counter-ion type is selected taking into account the ion composition of the hydrolysate.

The right-hand side (route 2) in Figure 22 depicts the sequencing of the separation steps of sugar mixtures. Typically, these mixtures have already gone through a chromatographic or membrane separation, which has removed the majority of other components than sugars. The selection of the right resin type focuses on the selection of the highest separation factor between the product and the major impurity. The separations are typically done by ‘Separate the most plentiful component first’ (C2) principle as the target purity is reached. This target purity is decided based on the requirements of the final separation process (e.g. crystallization).

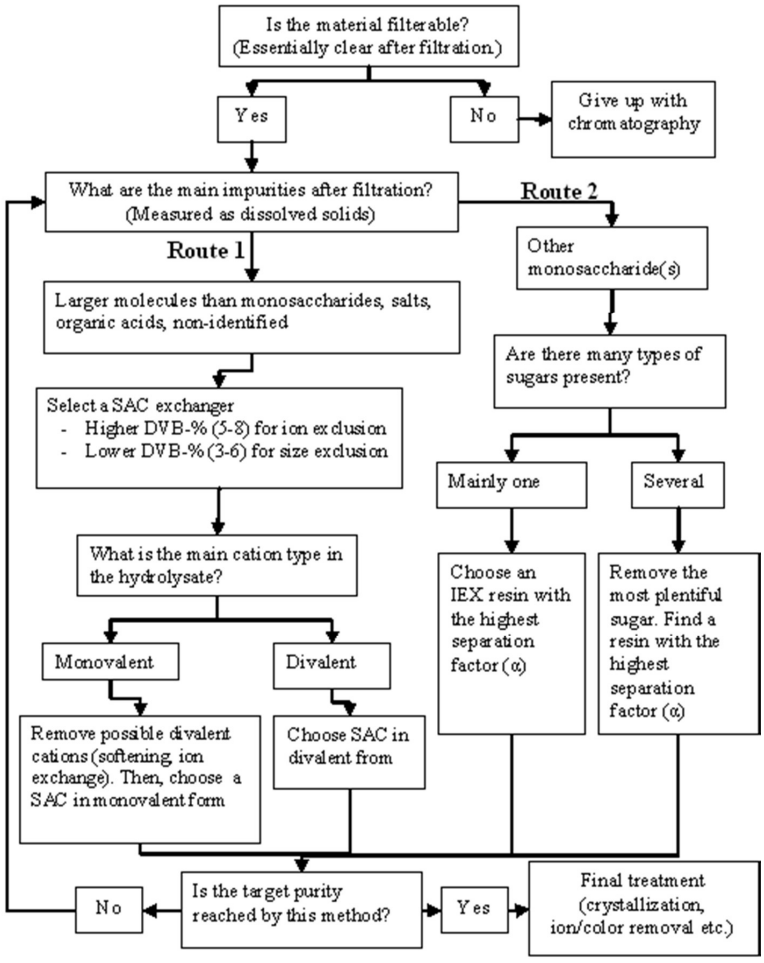


Figure 22. Flow diagram illustrating the process synthesis of chromatographic separation of sugars from biomass hydrolysates (or plant extracts) with ion exchange resins. (Paper V)

## 6.3 Case Studies

The viability of the developed method was verified with two case studies presented in Paper V. These were a chromatographic separation process for mannose from a wood pulp hydrolysate and a recovery process for arabinose from citrus pectin liquid residual. The sugar compositions of the feed solutions are presented in Table 6. The target purity for a sugar was set to 65 % on DS, and the step yield to 70 % without recycling (heuristic M6).

Table 6. Sugar composition of the filtered feed solutions in the case studies as % on dissolved solids

| | Arabinose | Glucose | Galactose + rhamnose | Mannose | Xylose | Fructose | Non-sugars |
|---|---|---|---|---|---|---|---|
| Lignocellulose hydrolysate | 8 | 26 | 3 | 18 | 39 | - | 7 |
| Citrus pectin plant liquid residue | 11 | 10 | - | - | - | 4 | 75 |

In the first case study, an advantageous separation sequence for mannose from lignocellulosic hydrolysate was concluded to be started by removal of xylose with SBA ($SO_4^{2-}$) (route 2 in Figure 22 and heuristics C1 and C2). The next step, removal of glucose, would be carried out with SAC ($Ba^{2+}$) but ion removal in-between is needed to avoid the formation of $BaSO_4$. The process is presented in Figure 23. It was confirmed experimentally in Paper V and found to be technically viable.

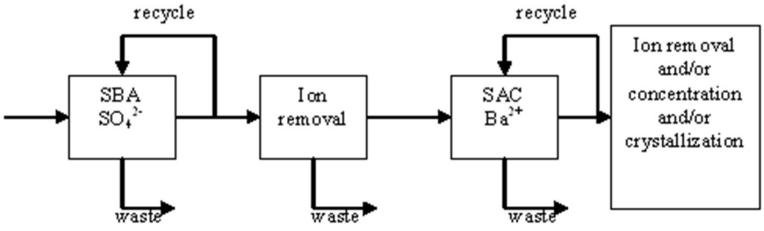


Figure 23. A potential separation sequence for the mannose recovery from lignocellulose hydrolysate.

In the second case study, the separation of arabinose from citrus pectin liquid residual which contained 75 % on DS non-sugars, was reasoned to begin with a SAC exchange resin (route 1 in Figure 22 and heuristics M1). The same ion form as of the solution, which was mainly sodium, was chosen as suggested in heuristic C1. Experimental verification for the choice was done in a pilot scale column. The target purity could be reached with 74 % yield without recycling.

# 7 Conclusions and Future Work

The recent interest in biorefining has been a strong driver for the development of industrial scale separation technology capable of fractionating complex solutions. One of the aims of this thesis was to find new or improved approaches to the large scale liquid chromatographic separation of valuable components from biomass hydrolysates or industrial side streams. This objective was reached: several new chromatographic processes were introduced for monosaccharides, such as L-fucose, D-galactose, L-arabinose and D-mannose, from biomass-based solutions (Papers I – III and V).

One of the novel chromatographic processes is the recovery process for L-fucose (Paper I). The raw materials for fucose with natural origin often have low fucose purity and recovery methods from sugar mixtures have been lacking. The presented method is the first manufacturing process based mainly on chromatographic separation from a natural raw material. The method may also help in the process design of fucose recovery from other types of raw materials, such as hydrolysates of fucose containing polymers (exopolysaccharides, fucoidan). However, there is still room for improvements in the process. For example, an alternative for the bisulfite form SBA resin is desired, because the chemical stability of the ion form is poor due to oxidization.

In literature, articles related to chromatographic separation of individual sugars have, so far, mainly discussed the sugar separations of synthetic solutions but have ignored other advantages the liquid chromatography offers. These are for example the possibility to simultaneously recycle unhydrolyzed material (by size exclusion) and to separate ionic compounds from the products (by ion exclusion). These advantages of chromatographic separation were demonstrated well in the recovery of galactose from lactose hydrolysate, gum arabic hydrolysate and hemicellulose hydrolysate which was a SSL side stream (Paper II). Chromatographic separation of galactose and glucose from lactose hydrolysate was carried out with sodium form SAC exchange resin having as low separation factor as ~ 1.1. In fact, chromatographic separations of many monosaccharides are carried out successfully with very low separation factors on a large scale. This is

encouraging information for the chromatographic separation process development because a low separation factor may be sufficient to reach the target purity. In this thesis it is illustrated that the purity of the sugar in the starting solution is the price determining factor cost wise.

A novel chromatographic process was also developed for the recovery of glycinebetaine (GB) from vinasse using a WAC exchange resin in hydrogen form (Paper III). The main purpose of the work was to elucidate the retention mechanism of GB on the resin. The retention was found to be based on hydrogen bonding between the resin and the zwitterionic GB. Understanding the mechanism is of great importance in the further development and optimization of the GB separation process. The novel process enables an acidic operating pH unlike the current chromatographic method. However, the relation between 1) the pH in the feed and eluent, 2) the cation composition in the feed and 3) the retention behavior of GB should still be better understood and quantified.

Adsorption isotherms of eight sugars were measured for SAC, SBA, WAC and WBA resins in sodium and sulfate forms (Paper IV). The equilibrium data for the sugars were described with linear isotherms over the concentration range between 0 and 350 g/l. Such data can be applied by other researchers in the development of chromatographic separation processes for various carbohydrate containing biomass hydrolysates. The data also help in the selection of a feasible stationary phase for a sugar separation.

During the determination of adsorption isotherms also potential decomposition of sugars was followed by HPLC. This was very relevant because it was found out that under some conditions, considerable amount of sugars may be either isomerized (e.g. glucose into fructose or vice versa) or decomposed. Therefore, the possibility of decomposition of a sugar should be taken into account, if adsorption isotherms of sugars are determined by refractive index method. For more accurate decomposition figures, further studies on the topic should be conducted.

No systematic methods for sequencing multistage chromatographic processes have been presented in the academic literature. Paper V discusses conceptual process synthesis principles and presents systematics for the synthesis of a chromatographic separation sequence for the separation of sugars on an industrial scale with IEX resins. Now there is a method for the selection of a feasible IEX resin for the separation of a sugar from a solution. The information can also be used in estimating how many chromatographic steps are needed to purify a sugar from a solution, if the retention data is available. The recommendations lead to a technically viable separation process, which was also demonstrated by two examples, but not necessarily to an economically feasible process configuration.

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# Errata

- In Paper I the eluent composition in HPLC analyses was 79 % acetonitrile (HPLC grade) in which 50 % $H_3PO_4$ (Fisher) 6mL/L was used (instead of 1.6 mL/L).

- In Paper II: Table 6, the mannose yield in recycle fraction should be 24 % (instead of 2 %).

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