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*Lappeenranta University of Technology*

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**INFLUENCE OF STATIONARY PHASE AND ELUENT PROPERTIES  
ON CHROMATOGRAPHIC SEPARATION OF CARBOHYDRATES**

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## ABSTRACT

Jari Tiihonen

### **Influence of Stationary Phase and Eluent Properties on Chromatographic Separation of Carbohydrates**

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In this thesis, the sorption and elastic properties of the cation-exchange resins were studied to explain the liquid chromatographic separation of carbohydrates.  $\text{Na}^+$ ,  $\text{Ca}^{2+}$  and  $\text{La}^{3+}$  form strong poly(styrene-co-divinylbenzene) (SCE) as well as  $\text{Na}^+$  and  $\text{Ca}^{2+}$  form weak acrylic (WCE) cation-exchange resins at different cross-link densities were treated within this work. The focus was on the effects of water-alcohol mixtures, mostly aqueous ethanol, and that of the carbohydrates. The carbohydrates examined were rhamnose, xylose, glucose, fructose, arabinose, sucrose, xylitol and sorbitol. In addition to linear chromatographic conditions, non-linear conditions more typical for industrial applications were studied. Both experimental and modeling aspects were covered.

The aqueous alcohol sorption on the cation-exchangers were experimentally determined and theoretically calculated. The sorption model includes elastic parameters, which were obtained from sorption data combined with elasticity measurements. As hydrophilic materials cation-exchangers are water selective and shrink when an organic solvent is added. At a certain deswelling degree the elastic resins go through glass transition and become as glass-like material. The increasing cross-link level and the valence of the counterion decrease the sorption of solvent components in the water-rich solutions. The cross-linkage or the counterions have less effect on the water selectivity than the resin type or the used alcohol. The amount of water sorbed is higher in the WCE resin and, moreover, the WCE resin is more water selective than the corresponding SCE resin. The increased aliphatic part of lower alcohols tend to increase the water selectivity, i.e. the resins are more water selective in 2-propanol than in ethanol solutions.

Both the sorption behavior of carbohydrates and the sorption differences between carbohydrates are considerably affected by the eluent composition and the resin characteristics. The carbohydrate sorption was experimentally examined and modeled. In all cases, sorption and moreover the separation of carbohydrates are dominated by three phenomena: partition, ligand exchange and size exclusion. The sorption of hydrophilic carbohydrates increases when alcohol is added into the eluent or when carbohydrate is able to form coordination complexes with the counterions, especially with multivalent counterions. Decreasing polarity of the eluent enhances the complex stability. Size exclusion effect is more prominent when the resin becomes tighter or carbohydrate size increases. On the other hand, the elution volumes between different sized carbohydrates decreases with the decreasing polarity of the eluent.

The chromatographic separation of carbohydrates was modeled, using rhamnose and xylose as target molecules. The thermodynamic sorption model was successfully implemented in the rate-based column model. The experimental chromatographic data were fitted by using only one adjustable parameter. In addition to the fitted data also simulated data were generated and utilized in explaining the effect of the eluent composition and of the resin characteristics on the carbohydrate separation.

Keywords: Liquid chromatography, Ion-exchange resin, Elasticity, Aqueous alcohol, Sugar, Sugar alcohol, Modeling

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## PREFACE

This thesis was carried out at Lappeenranta University of Technology in the Laboratory of Industrial Chemistry between years 1997 and 2002. The thesis was a part of two research projects “Chromatographic Separation for Food Industry (CHROMFOOD)” and “Use of Subcritical Water as Extractant and Chromatographic Eluent (EXTRACHROM)”. The projects were a part of the National Technology Agency (TEKES) technology programmes “Innovation in Foods 1997–2000” and “Innovation in Foods 2001–2004”.

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Lappeenranta, November 2002

*Jari Tiihonen*



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Scientific publications I-VI





## LIST OF PUBLICATIONS

This thesis is based mainly on the following six publications, which are referred to by the Roman numerals in the text.

- I Tiihonen, J.; Laatikainen, M.; Markkanen, I.; Paatero, E., Sorption of Neutral Components in Ion-Exchange Resins. 1. Effect of Cross-link Density and Counterion on Selective Sorption of Water–Ethanol Mixtures in Sulfonated PS–DVB Resins, *Industrial & Engineering Chemistry Research*, 38, 1999, 4832–4842.
- II Tiihonen, J.; Laatikainen, M.; Markkanen, I.; Paatero, E.; Jumppanen, J., Sorption of Neutral Components in Ion-Exchange Resins. 2. Sorption of *D*-Xylose in Sulfonated PS–DVB Resins from Water–Ethanol Mixtures, *Industrial & Engineering Chemistry Research*, 38, 1999, 4843–4849.
- III Tiihonen, J.; Markkanen, I.; Laatikainen, M.; Paatero, E., Elasticity of Ion-Exchange Resin Beads in Solvent Mixtures, *Journal of Applied Polymer Science*, 82, 2001, 1256–1264.
- IV Tiihonen, J.; Markkanen, I.; Kärki, A.; Äänismaa, P.; Laatikainen, M.; Paatero, E., Modelling the Sorption of Water-Ethanol Mixtures in Cross-linked Ionic and Neutral Polymers, *Chemical Engineering Science*, 57, 2002, 1885–1897.
- V Tiihonen, J.; Markkanen, I.; Paatero, E., Complex Stability of Sugars and Sugar Alcohols with Na<sup>+</sup>, Ca<sup>2+</sup> and La<sup>3+</sup> in Chromatographic Separations using PS–DVB Resins and Aqueous Organic Eluents, *Chemical Engineering Communications*, 189, 2002, 995–1008.
- VI Tiihonen, J.; Sainio, T.; Kärki, A.; Paatero, E., Co-Eluent Effect in Partition Chromatography. Rhamnose–Xylose Separation with Strong and Weak Cation Exchangers in Aqueous Ethanol, *Journal of Chromatography A*, 982, 2002, 69–84.

Jari Tiihonen's contribution in the appended publications:

- I Made the experimental plan. Supervised and participated in the experimental work. Participated in the interpretation of the results and preparation of the manuscript together with co-authors.
- II Made the experimental plan. Supervised the experimental work. Participated in the interpretation of the results and preparation of the manuscript together with co-authors.
- III Made the experimental plan. Supervised and participated in the experimental work. Interpreted the results and wrote the manuscript with a help from co-authors.
- IV Made the experimental plan. Supervised the experimental work. Interpreted the results and wrote the manuscript with a help from co-authors.
- V Made the experimental plan. Supervised and participated in the experimental work. Interpreted the results and wrote the manuscript.
- VI Made the experimental plan. Supervised and participated in the experimental work. Interpreted the results and wrote the manuscript except the column model and calculation part, which was written by the first co-author.



## NOMENCLATURE

### Symbols

$G$	shear modulus, Pa
$K$	distribution coefficient
$K_{el}$	elastic parameter, Pa
$K_{ow}$	octanol–water partition coefficient
$N$	number of datapoints
$q$	swelling degree
$R^2$	coefficient of determination
$\alpha$	separation factor
$\Delta E_{ij}$	interaction energy parameter, J/mol
$\Delta\alpha$	difference in separation factor
$\varepsilon$	dielectric constant

### Acronyms

AAD	absolute average deviation
DVB	divinylbenzene
NA	not available
NRF	non-random factor
PS-DVB	poly(styrene-co-divinylbenzene)
SCE	strong cation-exchange resin, also abbreviated by SAC
UNIQUAC	universal quasi-chemical theory
WCE	weak cation-exchange resin, also abbreviated by WAC

### Definitions

#### *Materials and properties*

**Anion-exchanger.** Ion-exchanger with anions as counterions. The term anion-exchange resin may be used in the case of solid organic polymers. [1]

**Capacity.** The number of counterions relative to the number of exchange sites per unit weight of material. *Theoretical specific capacity:* milliequivalent of ionic groups per unit weight of dry cation-exchanger in the  $H^+$  form. *Volume capacity:* milliequivalent of ionic groups per true volume of swollen ion-exchanger. *Bed volume capacity:* milliequivalent of ionic groups per bed volume determined under specified conditions. [2]

**Cation-exchanger.** Ion-exchanger with cations as counterions. The term cation-exchange resin may be used in the case of solid organic polymers. [1]

**Counterion.** In an ion-exchanger, the mobile exchangeable ions. [1]

**Cross-linkage.** Permanent chemical bonds between polymer chains. Make polymer insoluble in all nondegrading liquids. [2]

**Elasticity.** Large deformability with complete recoverability (rubber elasticity). [3]

**Fixed ions.** In an ion-exchanger, the non-exchangeable ions that have a charge opposite to that of the counterions (functional group). [2]

**Gel-type.** Polymer matrix that do not contain permanent pores. Porosity exists in the swollen state (microporous). [2]

**Glass transition.** Change of the material from glassy to elastic state or vice versa.

**Ion-exchanger.** A solid or liquid, inorganic or organic substance containing ions exchangeable with others of the same charge, present in a solution in which the ion-exchanger is considered to be insoluble. [1]

**Macroporous.** Polymer matrix that contains permanent pores (macroreticular). [2]

**Modulus.** The ratio between the applied stress and the corresponding deformation. [4]

**Resin matrix.** The molecular network of an ion-exchange resin that carries the ionic groups. [2]

**Resin swelling.** Volume change due to the liquid sorption into the resin.

**Shear modulus.** A measure of stiffness (rigidity) of the material. [4]

### *Chromatography*

**Bed porosity.** The void volume between stationary phase particles in a packed column.

**Column chromatography.** A separation technique in which the stationary bed is within a tube. The particles of the solid stationary phase or the support coated with a liquid stationary phase fill the whole inside volume of the tube (packed column). [1]

**Eluent.** Mobile phase which percolates through or along the stationary bed, in a definite direction (liquid phase in the liquid chromatography). [1]

**Ligand exchange chromatography.** Separation is based mainly on the difference in ability of the components to form coordination complexes with the central metal ions (counterion in the cation-exchange resin) [V].

**Linear chromatography.** The equilibrium isotherms are linear beginning at the origin. The peak shapes and the retention times (volumes) are independent of the sample composition and amount. The peak height is proportional to the amount of each component in the injected sample. [5]

**Non-linear chromatography.** The equilibrium isotherms are non-linear and their shapes depend on the concentration of all components in the mixture. The maximum of the peaks, peak shapes and the retention times (volumes) depend on the sample composition and amount. [5]

**Partition chromatography.** Separation is based mainly on differences between the solubilities of the components in the mobile and stationary phases (liquid chromatography). [1]

**Polarity.** Uneven distribution of electron density.

**Resolution.** The separation of two peaks in terms of their average peak width at base. [1]

**Retention volume.** Peak elution volume. The volume of mobile phase entering the column between the start of the elution and the emergence of the peak maximum. [1]

**Separation factor.** The relative retention value calculated for two peaks. By definition, the value of the separation factor is always greater than unity. [1]

**Size exclusion chromatography.** Separation is based mainly on differences in molecular size. [1]

**Stationary phase.** Stagnant phase (bed) in the chromatographic column (solid phase in the liquid-solid chromatography). [1]

## **1 INTRODUCTION**

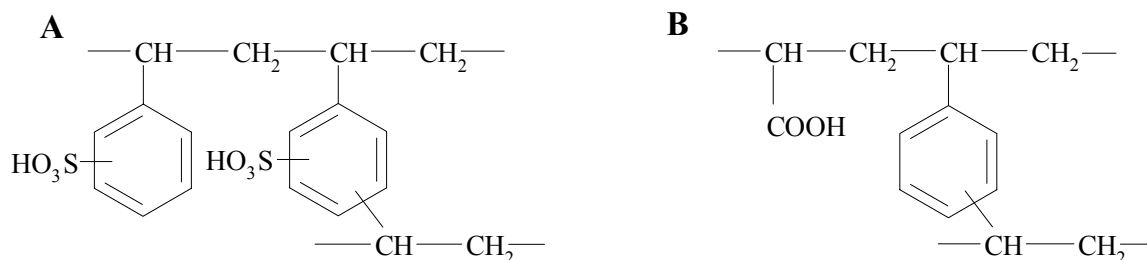
This thesis focuses on cation-exchange resins and their use as stationary phase in liquid chromatographic separation of carbohydrates in aqueous co-solvent eluents. The research is here extended beyond the conventional usage of the cation-exchange resins in the industrial carbohydrate separation, i.e. the separation with the strong cation-exchange (SCE) resins with water eluent. Thus, the effect of new characteristics, the chemical structure of the cation-exchange resin and the counterion valence, in the aqueous alcohol eluents are introduced.

### **1.1 Structure and properties of cation-exchangers**

The cation-exchange resins belong to the group of solid, organic ion-exchangers. The organic ion-exchangers are considered to be the most important ion-exchanger type. In addition to small scale analytical use, ion-exchange resins are also widely applied in industrial applications.

The ion-exchange resins are either cation-exchange or anion-exchange resins, depending on the charge of the exchangeable ion i.e. the counterion. In accordance with this, the cation-exchangers possess negatively charged functional groups having acidic nature and the anion-exchangers positive groups having basic nature. The ion-exchange resins function as strong or weak acids, bases, or salts depending on the functional group and counterion. In addition to the monofunctional ion-exchangers, polyfunctional and amphoteric ion-exchangers exist. In the ion-exchange resins the structure of the polymer matrix carrying the functional groups is a three-dimensional network, where the polymer chains are cross-linked to each other with a suitable cross-linking agent. The resins are normally manufactured as beads with a diameter ranging from a few micrometers to over one millimeter. In this thesis the mean particle sizes from 0.3 mm to 0.4 mm, typical in industrial scale chromatographic separations, and the mean particle size 0.1 mm have been used. The matrix can be gel-type (microporous) or macroporous depending on the manufacturing process. The gel-type ion-exchange resins are regarded as porous only in the swollen state whereas the macroporous resins have permanent pores [6,7].

The exact molecular structure of the resins depends on the manufacturing process and monomers used [7,8]. This research focuses on the most common gel-type cation-exchange resins; the sulfonated poly(styrene-co-divinylbenzene) (PS-DVB) resins and the poly(acrylic acid) resins cross-linked with divinylbenzene [I–VI]. The sulfonated PS-DVB resin and the acrylic resin are the strong cation-exchange resin and the weak cation-exchange resin respectively (Fig. 1).



**Figure 1.** Structure of (A) the sulfonated strong cation-exchange (SCE) resin and (B) the acrylic weak cation-exchange (WCE) resin in the acidic form.

The SCE resins are normally manufactured by copolymerizing styrene with divinylbenzene as a cross-linking agent. After polymerization, the swollen polymer matrix, made to swell with a suitable hydrocarbon based swelling agent, is sulfonated with sulfuric acid [2]. The cross-link density of the gel-type SCE resin is typically 12 wt% DVB or less in the monomer mixture. In this thesis the cross-link density of the SCE resins varied from 4 wt% to 8 wt% [I–VI]. The theoretical capacity (every benzene ring monosulfonated) of the SCE resin is around 5.3 mequiv/g (H<sup>+</sup> form) of the dry resin depending slightly on the cross-link density. Normally, the SCE resins are considered monosulfonated.

Usually the WCE resins are manufactured from methacrylic acid, acrylic acid or esters of those [9]. In making the WCE resin from an ester, the acrylic ester and divinylbenzene (cross-linking agent) are polymerized, after which the polymer is hydrolyzed. In Papers [III], [IV] and [VI] the cross-link density of the WCE resins was between 3 wt% and 8 wt%. The capacity of totally hydrolyzed resin is around 13 mequiv/g (H<sup>+</sup> form) of the dry resin and it depends on the cross-link density. In practice, all ester groups are not hydrolyzed and the capacity remains below theoretical value.

The SCE and WCE resins are mechanically and chemically stable materials. The mechanical stability can be tested with physical attrition, repetitive swelling and drying procedures, mechanical stress and osmotic stability during repetitive ion exchange procedures [10]. The chemical stability is normally understood as stability of the functional groups and polymer

chains against oxidation. The resins are insoluble in all common solvents, but can degrade with strongly oxidizing agents if exposed long enough to oxidation [10]. Normally, the cation-exchange resins are stable at least up to 393 K [11]. At higher temperatures they start to defunctionalize and above 523 K the polymer matrix starts to degrade [9,10]. In this thesis [I–VI] the resins have been studied at 298 K.

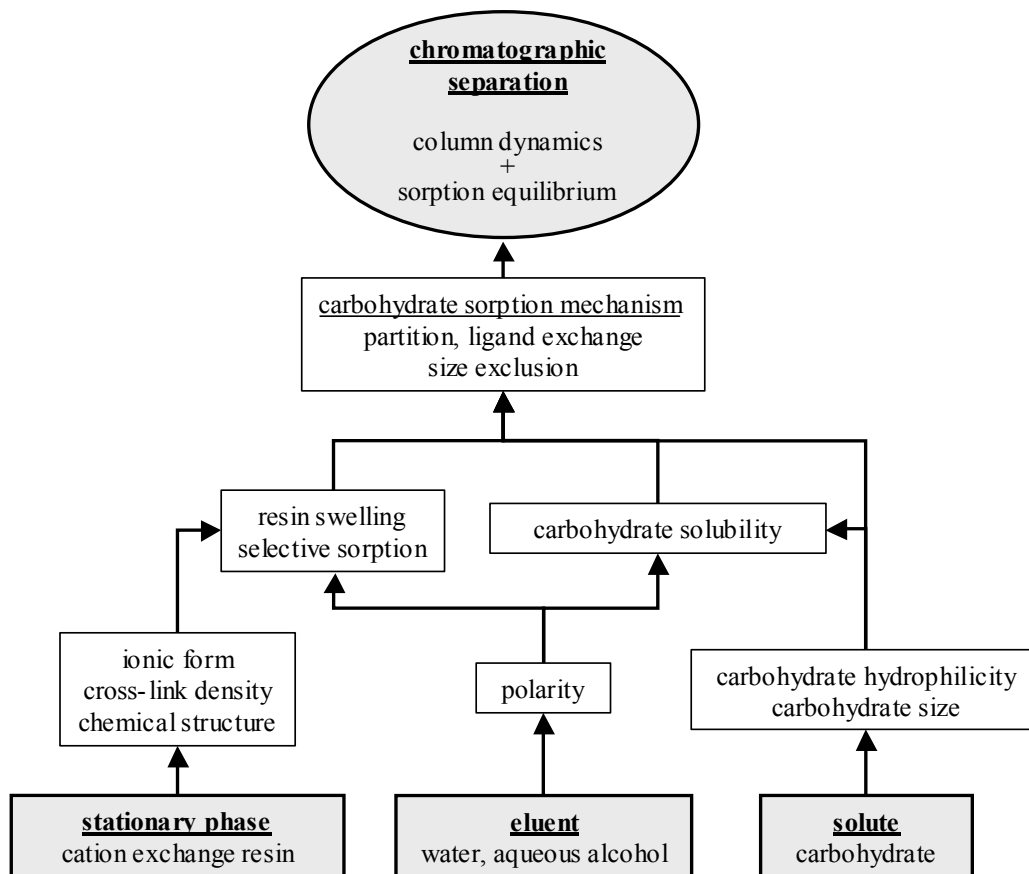
In addition to the chromatographic separation, which is the topic of this thesis, the properties of the resins are utilized in a number of applications. The methods can be classified as ion exchange, where the ionic form of the resin changes, and as methods, where no ion exchange in the resin occurs. Typical ion exchange applications include substitution of undesirable for desirable ions, purification from ionic impurities, separation of ionic from ionic or non-ionic components, concentration of ionic components and neutralization. Methods, where the resins do not act as ion-exchangers, include ion exclusion, ion retardation, ligand exchange, non-ionic adsorption, drying, catalysis, etc. Examples of different application fields of ion-exchangers include water treatment, hydrometallurgy, food and dairy industry, medical industry, biochemistry and analytical chemistry [2].

## **1.2 Purpose and scope of work**

The main emphasis in this thesis is to study the interactions between the stationary phase (gel-type cation-exchanger), the eluent (water or aqueous alcohol) and the solutes (carbohydrates) during the chromatographic separation (Fig. 2). The problem is approached by experimental and theoretical sorption isotherm, mechanical (resin elasticity) and chromatographic studies. The previous studies of binary water–alcohol sorption found in the literature (see the cited articles in Papers [I,IV] in the Appendix) have been focused on either monovalent cations or concentrated alcohol solutions and, exclusively, the SCE resins. The data in those articles are evaluated by the authors only in qualitative manner. Furthermore, the carbohydrate separations in aqueous co-solvent eluents found in the literature (see the cited articles in Papers [II,V,VI] in the Appendix) are limited to the analytical scale and only to SCE resins carrying monovalent counterions and concentrated ethanol eluents. Thus, in this thesis in addition to low feed concentrations high feed concentrations at moderate alcohol concentrations are studied.

The variables summarized in Fig. 2 are taken into account for discovering the fundamental dependencies between the stationary phase properties and the carbohydrate separation in aqueous alcohol eluents. The binary solvent (water–ethanol) sorption into the strong cation-exchange [I,IV] and the weak cation-exchange (WCE) [IV] resins of different cross-link densities and ionic forms are evaluated. The aqueous co-eluent sorption behavior are treated in detail in the Section 2.1.3.

During the sorption process the elasticity of the cation-exchangers changes. The change can be rather drastic depending on the degree of resin swelling and has to be taken separately into account, when sorption and chromatographic behavior of the resins are studied. The experimental elastic behavior at different sorption equilibrium states has been investigated in Paper [III] and the results are summarized in the Section 2.1.2. The experimental shear modulus data have also been utilized in evaluation of the sorption model parameters [IV].



**Figure 2.** Stationary phase, eluent and solute properties affecting the chromatographic separation of carbohydrates.



In this thesis the swollen resins are considered as homogeneous phases and the effect of cross-links are incorporated only through elasticity of the resins [I,II,IV,VI]. The cross-links make the resin insoluble and a cross-link density dependent swelling pressure affects the swelling equilibrium. The swelling pressure affects the sorbed molecules through the exclusion phenomenon, which increases with the increased size of the molecule. However, the ionic interactions are not separately treated in the sorption models.

Despite of the above simplifications, the sorption data can be satisfactorily connected with the proposed thermodynamic sorption models [I,II,IV,VI]. In the models the chemical potentials of the liquid and resin phases are equal at the equilibrium. The equilibrium conditions are presented with two contributions. The mixing term included all other interactions except for the elastic effects. The modeling focuses on fitting the sorption of rhamnose and xylose in aqueous ethanol as a model case in variant combinations of those. Different ionic forms and cross-linkages of both the SCE and WCE resins are treated.

The carbohydrate sorption studies in aqueous alcohols inevitably show that the selective sorption of the aqueous co-solvent eluent strongly affects the distribution of carbohydrates between the external liquid phase and the liquid inside the resin [II,VI]. Furthermore, the stationary phase properties such as the chemical structure (resin type), cross-link density and ionic form give their contributions on the distribution coefficient of carbohydrates as summarized in the Section 2.2.

The outcome of the sorption and elastic studies are utilized in the chromatographic experiments as summarized in the Section 2.3. The chromatographic separation process includes in addition to the sorption equilibrium data the dynamic parameters such as flow rate, mass transfer kinetics and dispersion. The thermodynamic sorption model has been implemented in the dynamic column model used in the chromatographic calculation procedure in Paper [VI]. In addition to rhamnose and xylose, chromatographic separation data of several other carbohydrates are evaluated in the Section 2.3.

## 2 RESULTS

### 2.1 Eluent–stationary phase interactions

The behavior of the SCE resins in the aqueous organic and pure organic solvents has been studied extensively (as reviewed in [I] and [IV]). The WCE resins have received much less attention [III,IV,12]. Most studies have focused on concentrated organic solutions and monovalent counterions (as reviewed in [I] and [IV]). Thus, it is interesting to see the behavior of the resins also in less concentrated solutions and in multivalent cation forms. First, a short summary about the behavior of the cation-exchange resins in water and in organic solvents will be given based mainly on literature data. Next, the elastic properties of the resins are summarized, followed by a more detailed survey on the behavior in the aqueous alcohol solutions. The elasticity has been studied in Papers [I], [III] and [IV]. The discussion on sorption of aqueous ethanol solutions in the resins is extracted from Papers [I] and [IV]. Previously unpublished data on aqueous 2-propanol sorption both in the SCE and in the WCE resins and aqueous ethanol sorption in the WCE Ca<sup>2+</sup> resin have been added.

#### 2.1.1 Pure solvent sorption

Both the SCE resins and the WCE resins are hydrophilic polyelectrolytes. The WCE resins have stronger affinity for water than the SCE resins [IV]. The swelling degree depends on the material properties of the resins, the cross-link density and the ionic form and the nature of the swelling solvent. The swelling of the salt form resins is invariably strongest in water [I,IV,6]. The swelling in organic solvents and aqueous organic solvents is dealt later in this thesis. The swelling of the SCE resins and the WCE resins in water with different cross-linkages and ionic forms are shown in Table 1 [III,IV,9]. The water uptake decreases with increasing cross-linkage and ionic valence as can be seen from Table 1 and, for example, from Refs [13] and [14]. Moreover, a clear difference in water sorption is seen between the SCE resins and the WCE resins. In the case of the SCE resins, the acidic form resin sorbs more water than the univalent salt form resin, whereas for the WCE resins the order is reversed. This phenomenon demonstrates clearly the acid strength difference between the strong resins and the weak resins: the functional groups are more dissociated in the SCE resins than in the WCE resins [2]. Water in the resins is usually considered to exist in

different states. Part of the water is bound around the ionic moieties (hydration water) and part of the water is considered as hygroscopic water (free water). The amount of bound water depends on the ionic form and cross-linkage of the resins [I,IV,14].

The affinity of the SCE and WCE resins toward water is very strong, as discussed above. Lower polarity of the solvents makes resins swell less. For instance, the SCE Na<sup>+</sup> resins swell 3 fold more in water than in methanol, when the methanol sorption values in Refs. [15] and [16] are compared to the water sorption values in Table 1. According to Paper [I], the ethanol content of the SCE Na<sup>+</sup> resin in pure ethanol is smaller than in pure methanol. Furthermore, according to Komata and Gohara [17] sorption of organic solvents in the SCE Na<sup>+</sup> resin decreases with the sequence: methanol, ethanol, 2-propanol, dioxane and hexane. These values are in accordance to the corresponding decreasing dielectric constant values,  $\epsilon$ , [18]. It should be also noted that there is a clear difference in swelling between the resin types. The WCE resins swell less than the SCE resins in lower polarity solvents such as in alcohol. Although no data for pure organic solvents are available, this can be deduced from the level of alcohol sorption attained in concentrated aqueous solutions. These data are presented in Paper [IV] and the discussion below. It should be noted, however, that the resins become in the low polarity environments, such as in ethanol, glass-like materials. This thesis focuses on the higher polarity environments, where the resins are gel-like materials. The glassy and gelly nature of the resins are discussed in more detail in the next Section.

**Table 1.** Swelling and elasticity of the strong cation-exchange (SCE) and the weak cation-exchange (WCE) resins in water

resin name	resin type	ionic form	cross-linkage, wt% of DVB	water content, wt%	$G$ in water, MPa	resin name	resin type	ionic form	cross-linkage, wt% of DVB	water content, wt%	$G$ in water, MPa	
CS16G	SCE X8 <sup>a</sup>	H <sup>+</sup>	8	52	27	CS08G	SCE X4 <sup>a</sup>	La <sup>3+</sup>	4	48	2.8	
	SCE X8 <sup>a</sup>	Na <sup>+</sup>	8	46	21		IRC86	WCE NA <sup>a</sup>	H <sup>+</sup>	NA	50	2.5
	SCE X8 <sup>a</sup>	Ca <sup>2+</sup>	8	44	29			WCE NA <sup>a</sup>	Na <sup>+</sup>	NA	67	8.9
	SCE X8 <sup>a</sup>	La <sup>3+</sup>	8	40	37			WCE NA <sup>a</sup>	(CH <sub>3</sub> ) <sub>4</sub> N <sup>+</sup>	NA	64	17
CS11G	SCE X5.5 <sup>a</sup>	Na <sup>+</sup>	5.5	56	9.6	CA16G		WCE X8 <sup>b</sup>	Na <sup>+</sup>	8	64	14
	SCE X5.5 <sup>a</sup>	(CH <sub>3</sub> ) <sub>4</sub> N <sup>+</sup>	5.5	50	14	CA12G	WCE X6 <sup>c</sup>	Na <sup>+</sup>	6	70	7.9	
	SCE X5.5 <sup>a</sup>	Ca <sup>2+</sup>	5.5	50	11	WCE X6 <sup>b</sup>	Ca <sup>2+</sup>	6	48	NA		
	SCE X5.5 <sup>a</sup>	La <sup>3+</sup>	5.5	44	8.2	CA08G	WCE X4 <sup>c</sup>	Na <sup>+</sup>	4	76	4.1	
CS08G	SCE X4 <sup>a</sup>	Na <sup>+</sup>	4	65	3.8	CA06G	WCE X3 <sup>d</sup>	H <sup>+</sup>	3	64	NA	
	SCE X4 <sup>a</sup>	Ca <sup>2+</sup>	4	57	3.3	WCE X3 <sup>c</sup>	Na <sup>+</sup>	3	81	2.5		

<sup>a</sup>Paper [III]. <sup>b</sup>New data for this summary. <sup>c</sup>Paper [IV]. <sup>d</sup>Ref. [9].

### 2.1.2 Elasticity of the swollen resins

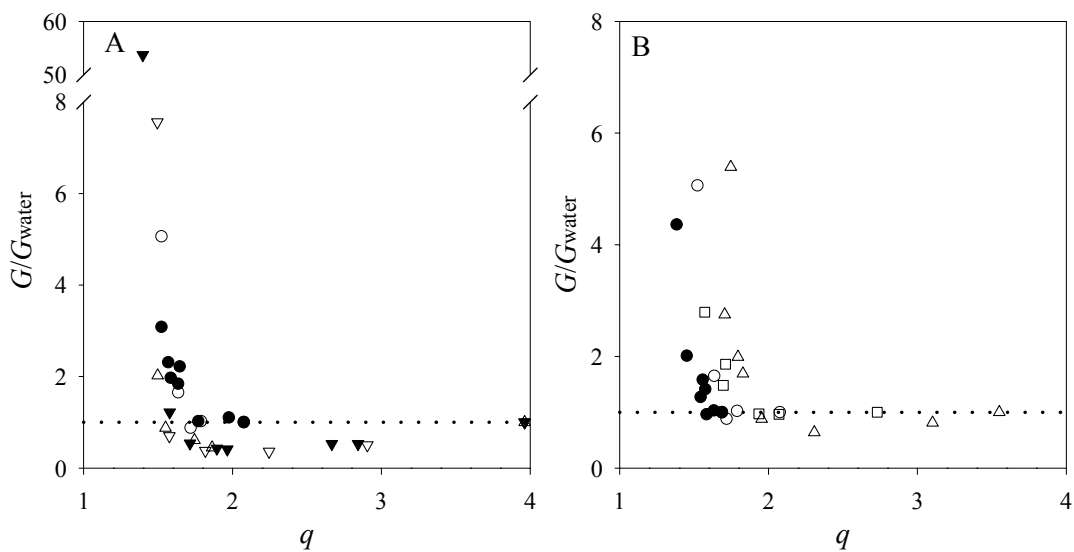
The cation-exchange resins are elastic materials and their elasticity depends strongly on the swelling degree. The elastic behavior of the cation-exchange resins in water and in aqueous alcohol solutions is treated here based on Papers [I], [III] and [IV]. A special instrument was constructed for this research project to measure the shear modulus of the resins. The elasticity was obtained by pressing a single bead, immersed in an appropriate solution, between two parallel plates. The force applied and corresponding degree of deformation were recorded. Substantial number of points were measured for every bead. The shear modulus was calculated from the recorded data by applying the Hertz equation [III]. The experimental elasticity data were utilized on the validation of fitted elastic parameters in the sorption models, mentioned in the Introduction. The same trend between experimental data and calculated elastic parameters were observed [I,IV]. In addition to parameter validation the experimental elastic data were directly utilized in the parameter estimation. In other words, two different elastic equations were applied [IV]. The difference of the elastic equations are treated in more detail at the end of this Section.

The resins become in the water swollen state less elastic with increasing cross-linkage, which corresponds to shorter polymer chain length between cross-links. This is in accordance with the classical theory of rubber elasticity referred in Paper [III]. The shear moduli of the resins in water are summarized in Table 1. At higher cross-link densities elasticity decreases with the increasing valence of the equal sized counterions ( $\text{Na}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{La}^{3+}$ ). On the other hand, at lower cross-link densities the differences in the moduli vanish. Two explanations are offered in Paper [III]. ‘The ionic cross-links’ and the stronger hydrated multivalent counterions restrict the movement of the chains in the denser cross-linked resins.

The aqueous alcohol solvent composition has a strong impact on the elasticity of the cation-exchange resins. The swelling degree of the resins decreases with the decreasing polarity of the solution. The elasticity of the resins changes with the swelling state of the resins as can be seen from Fig. 3 [III]. In Fig. 3, the shear modulus at different swelling degrees in relation to the modulus in fully swollen state (in water) is illustrated. Two phenomena are seen. Firstly, at a resin specific swelling degree the shear modulus of the resin starts rapidly to increase, i.e. the elasticity decreases. The point where the elasticity starts to decrease indicates the change in the form of the resin and it is called as glass transition point. At higher swelling degrees the resins are gel-like materials and at lower swelling degrees they

change to glass-like materials. The change from gel-like to glass-like materials means also that the basic assumptions of the thermodynamic sorption models fail [I,IV].

Secondly, depending on the resin the shear modulus decreases to a minimum when moving from a fully swollen resin to a less swollen state, until aforementioned rapid upturn of the modulus takes place. The decrement of the modulus is most distinct with the WCE Na<sup>+</sup> resins and the lower cross-linked SCE Na<sup>+</sup> resin (Fig. 3). This phenomenon indicates that the polymer chains are strained at the high swelling degrees decreasing the elasticity and that shrinking of the resins reduces the tension.



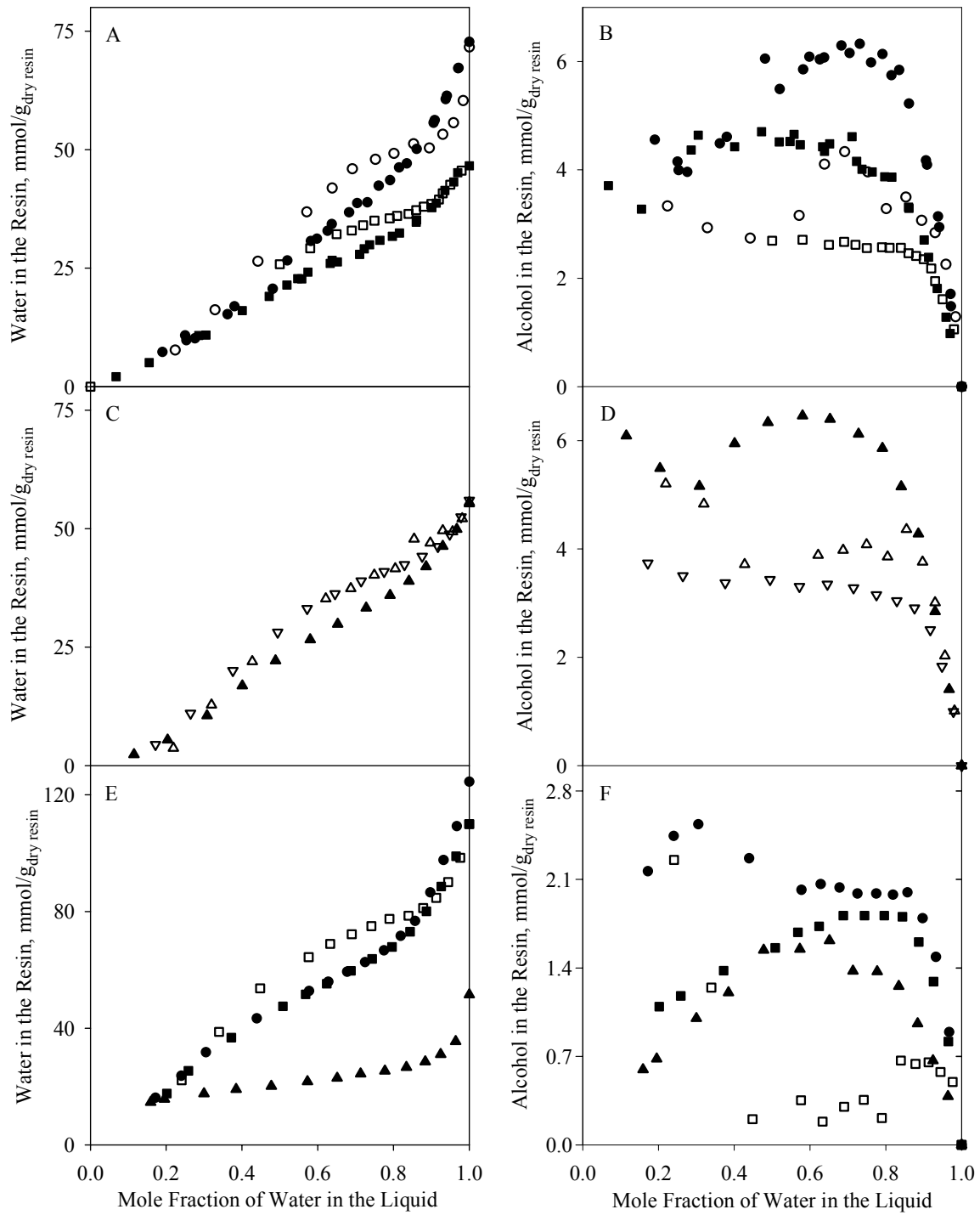
**Figure 3.** Relative shear modulus of the ion-exchange resins at different swelling degrees,  $q$ , of the resins in water or in binary water–alcohol solutions [III]. (A) SCE Na<sup>+</sup> X8 resin: ○) ethanol; ●) 2-propanol. Weak Na<sup>+</sup> cation-exchanger: △) water; ▽) ethanol; ▼) 2-propanol. (B) Strong cation-exchanger: ○) Na<sup>+</sup>, X8, ethanol; ●) La<sup>3+</sup>, X8, ethanol; □) Na<sup>+</sup>, X5.5, ethanol; △) Na<sup>+</sup>, X4, ethanol.

The sorption models, as earlier introduced, consist of two parts, mixing and elastic contributions. The elastic contribution was formulated in two ways: one, which was able to take into account the non-ideal elastic behavior of the resins, i.e. the decrement of the modulus and the other, which was not able to predict the decrement [IV]. The experimental shear modulus data were utilized in parameter estimation, when the equation capable of taken into account the increased elasticity was used. It should be noted, however, that the primary importance of the elastic contribution was to determine the level where the resin swelled in water. Thus, the elastic equation, which was unable to predict the decrement of the modulus, gave equal results in the sorption modeling when compared to the equation capable of predicting the decreasing modulus [IV].

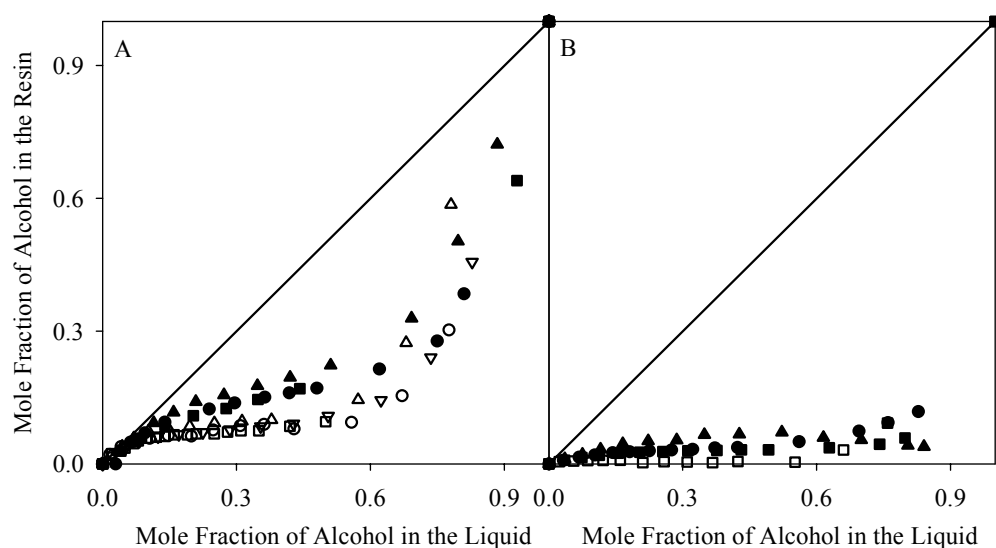
### 2.1.3 Sorption of aqueous organic solvents

The swelling of the resins in the solvent mixtures is affected by the resin characteristics and by the solution properties, i.e. the nature of the added organic solvent and the composition of the solution. The effect of the resin dependent variables (cross-link density, ionic form and resin type) on the sorption behavior of the cation-exchange resins in aqueous ethanol are treated in detail in Papers [I] and [IV] and a summary is given later in this Section. But first, the effects of the solvent nature (ethanol vs. 2-propanol) and of the solvent composition are considered here. The solvent sorption rarely takes place with the same mole ratio as is the mole ratio of the solvent components outside of the solid (resins). Thus, the solvent mixture sorption is primarily selective. The strongest swelling in water of the salt form cation-exchange resins suggest at least some water selectivity in sorption of aqueous organic solvent mixtures. Selective sorption of water in water–alcohol solutions is obvious and clearly demonstrated in Papers [I] and [IV] and in Figs 4 and 5. The data in Figs 4 and 5 have been obtained by the methods presented in Papers [I] and [IV].

If the swelling in pure solvent has any correlation with the water selectivity in aqueous alcohol solutions, higher selectivity should be observed for alcohols having longer aliphatic chains. This rule should apply, at least for the lower alcohols. In fact, the stronger selectivity toward water in 2-propanol solutions than in ethanol solutions can be ensured from Figs 4 and 5 and Refs [19] and [20]. The increased selectivity can be attributed to lower polarity and higher molar volume of 2-propanol. These conclusions are also supported by the fact that according to data in literature [19,20] the water selectivity is higher in aqueous 2-propanol than in aqueous ethanol. Though the selectivity was not so clear between concentrated 2-butanol and 2-propanol solutions used in Ref. [19]. On the other hand, the data in Fig. 5 suggest that the selectivity difference between 2-propanol and ethanol solutions vanishes at high alcohol mole fractions. The selectivity difference disappears at the glass transition region (external water mole fractions 0.3–0.5).



**Figure 4.** The water–alcohol sorption in the cation-exchange resins at different chemical structures, ionic forms and cross-link densities of the resins. (A–D) SCE resins: ○ Na<sup>+</sup> X5.5, □ Na<sup>+</sup> X8, △ Ca<sup>2+</sup> X5.5, ▽ (CH<sub>3</sub>)<sub>4</sub>N<sup>+</sup> X5.5. (E,F) WCE resins: ○ Na<sup>+</sup> X6, □ Na<sup>+</sup> IRC86 (unknown cross-link density), △ Ca<sup>2+</sup> X6. Closed symbols refer to the ethanol solutions and open to the 2-propanol solutions. Data are from Papers [I] and [IV]. The 2-propanol data are original for this summary.



**Figure 5.** The solvent selectivity of the cation-exchange resins in water–alcohol solutions at different chemical structures, ionic forms and cross-link densities of the resins. (A) SCE resins:  $\circ$ )  $\text{Na}^+$  X5.5,  $\square$ )  $\text{Na}^+$  X8,  $\Delta$ )  $\text{Ca}^{2+}$  X5.5,  $\nabla$ )  $(\text{CH}_3)_4\text{N}^+$  X5.5. (B) WCE resins:  $\circ$ )  $\text{Na}^+$  X6,  $\square$ )  $\text{Na}^+$  IRC86 (unknown cross-link density),  $\Delta$ )  $\text{Ca}^{2+}$  X6. Closed symbols refer to the ethanol solutions and open to the 2-propanol solutions. Data are from Paper [I]. The 2-propanol and the WCE data are original for this summary.

The differences in selectivity reflect the differences in the sorption isotherms. The shape of the water isotherm in water–2-propanol mixtures differs from that of water–ethanol mixtures. A clear change in the isotherm shape irrespective of the resin type or ionic form is observed between water mole fractions 0.3 and 0.9 in Fig. 4. Between these limits water sorption in aqueous 2-propanol exceeds sorption in aqueous ethanol. The excess water sorption in the aqueous 2-propanol resin originates in the difference in interactions between water–2-propanol and water–ethanol solutions [21]. The limits can be clearly connected to the alcohol sorption isotherms. Below the external water mole fraction 0.9 the 2-propanol isotherm deviates from the ethanol isotherm. Thus, 2-propanol is more strongly excluded than ethanol because of its greater molar volume and weaker polarity. Larger molecules are more strongly affected by the swelling pressure of the resin and decreased polarity decreases affinity toward the hydrophilic resin. At the glass transition region the water isotherms and the alcohol isotherms start to approach each other, again. In the case of the WCE resin, 2-propanol sorption seems to exceed ethanol sorption at high polymer contents in Fig. 4D. However, there are too few points at that area to be certain that the last points are just not scattered due to very limited sorption of 2-propanol leading to relatively large experimental error in concentrated 2-propanol.

The resin characteristics have some evident impacts on the swelling degree and the isotherm shapes of the resins. Increasing cross-linkage hardens the resins [I–IV], which is clearly seen



an increase in the elastic modulus and decreasing water sorption at water rich solutions. Moreover, the sorption properties of the SCE and the WCE resins have a clear difference. In both cases the water sorption decreases consistently when moving toward the lower external water mole fractions. On the other hand, the water sorption isotherms of different cross-link densities merge at much lower water mole fraction in the SCE resins than in the WCE resins (Fig. 4) [IV]. For the SCE resins, the merging and transformation from the gel-like to the glass-like state are directly combined whereas the WCE resins have a significant gap between the isotherm merging and glass transition [IV]. This gap is considered as 'an intermediate phase' between the true gel and the glass transition [IV]. The unique sorption characteristics of the resin type are connected to the different ionic interactions inside the resin in Paper [IV]. Stronger association tendency of the WCE resin can be responsible for the early merging of the water isotherms.

The alcohol sorption isotherms significantly differ from the water isotherms, which is expected for the water selective resins. Instead of monotonous increase alcohol sorption goes through a maximum, which is most prominent at low cross-link densities (Fig. 4) [I,IV]. At high cross-link densities the isotherms can also have a local maximum (Fig. 4D, 4F), reach a plateau (Fig. 4F) or even become a monotonously rising curve (Fig. 4B, 4D) due to the hindered sorption of alcohol (and water) by the stiff polymer matrix [I,IV]. At higher cross-link densities (or with more hydrated ions) the amount of free liquid compared to the solvated liquid decreases [7]. Consequently, the sorption of alcohol can be a result of two competing sorption mechanisms. At high water contents alcohol is sorbed by dissolution in the adsorbed water, whereas at low water contents alcohol begins to displace the more strongly bound water from the hydration shell. From this point of view, in stiff resins, where even the pure water sorption is very limited, high hydrated liquid and free liquid ratios lead to a local sorption maximum or even monotonous sorption of alcohol. However, the selectivity at different cross-link densities follows the same trend irrespective of cross-link density, though a small increase in selectivity can be observed with increasing cross-linkage from Fig. 4 in Paper [I] or by redimensioning the results reported in Fig. 3 of Paper [IV].

It is reasonable to assume that also the counterion valence has some impact on the aqueous alcohol sorption. Therefore, sorption studies were made by using  $\text{Na}^+$ ,  $\text{Ca}^{2+}$  and  $\text{La}^{3+}$  counterions, which all have approximately equal ionic radii. The increased counterion valence decreases water sorption at high external water mole fractions as is proved to be case in Papers [I] and [IV] as well as in Fig. 4. The water sorption isotherms of the SCE resins at

different ionic forms merge at the glass transition region. According to the Fig. 4 also in the WCE resins the water sorption difference between the  $\text{Na}^+$  resin and the  $\text{Ca}^{2+}$  resin vanishes at the glass transition region. The decreased water sorption in the multivalent resins can be linked to the following. Multivalent ions, though more strongly hydrated than monovalent ions, have lower number in the resin decreasing the free water sorption into the resin and leading to the less swollen resin [I]. Moreover, the possible 'ionic cross-links' in the case of the multivalent ions can decrease the swelling degree of the resins [I,IV]. The difference in water uptake of the  $\text{Na}^+$  and  $\text{Ca}^{2+}$  resins is much larger in the WCE resin than in the SCE resin (Fig. 4 and Paper [IV]). On the other hand, the isotherm shape and the sorbed amount of alcohol remains the same level irrespective of ionic valence (Fig. 4 and Paper [I]). The decreased water sorption and unchanged alcohol sorption leads to decreased water selectivity with increased ionic valence.

In conclusion, the experimental data discussed above unambiguously demonstrate that the SCE resin sorbs less water and more alcohol than the WCE resin at the same cross-linkage and at the same ionic form. The markedly higher water selectivity of the WCE resin can be utilized in the carbohydrate separation as will be shown later in this thesis.

Different thermodynamic models were tested in order to correlate the data of water–ethanol sorption to the cation-exchangers [I,IV]. The models were also extended to more complex mixtures, i.e. water–ethanol–sugars sorption [II,VI]. Sugar sorption is summarized in the next Section. The equilibrium conditions in the models consisted of two contributions, mixing and elastic, as explained in the Introduction. The modified UNIQUAC based model was suitable to predict the water–ethanol sorption behavior in the SCE resins [I,IV], but it was essentially inaccurate to predict the sorption in the WCE resins [IV]. In contrast to these findings, a model based on the Non-Random Factor (NRF) equation was derived and it was successfully applied to water–ethanol sorption both in the SCE resins and in the WCE resins [IV]. Thus, the NRF model was also applied in the water–ethanol–rhamnose–xylose–resin systems in Paper [VI]. The same elastic contribution was applicable to all sorption models and, in all cases, the random mixing was given by athermal Flory–Huggins equation. However, the non-random interactions were the mixing equation dependent as shown for UNIQUAC and for NRF in Equation 3 of Papers [I] and [IV], respectively.

It should be noted, that in this thesis (Papers [I,II,IV,VI]) successful fitting was obtained without separating the ionic interactions from the rest of the mixing effects. However, in the

case of linear polymers, which were structurally similar as the resins studied here, it was necessary to include the effect of the free counterions in order to explain satisfactorily the ternary liquid equilibrium data (water–alcohol–polymer) [21]. On the other hand, the localized sorption of solvent molecules in the model NRF in Papers [IV] and [VI] was taken into account by introducing two different binding sites in the resin. The stronger interactions formally correspond the hydration of the ionic moieties in the resins.

## 2.2 Sorption of carbohydrates in aqueous alcohol eluents

Sorption of carbohydrates from aqueous organic solvents in the cation-exchange resins, especially in the SCE resins, from aqueous organic solvents has been studied to some extent (as reviewed in [II] and [VI]). Common features in these previous studies have been the use of low solute concentrations and often concentrated organic solvent solutions as is reviewed in Papers [II] and [VI]. Furthermore, those studies have focused on the monovalent counterions as pointed out in Paper [II]. In this thesis, emphasis is on the less concentrated aqueous ethanol solutions and on a wider sugar concentration range than in the previous studies (as reviewed in [II] and [VI]).

Next, sorption of monosaccharides (rhamnose and xylose) in the SCE resins and in the WCE resins is summarized and the discussion is based mainly on Papers [II] and [VI]. New experimental data, original in this summary, on rhamnose–xylose sorption have been added (Figs 6 and 7). Calculated results in Figs 6 and 7 are based on the parameters presented in Table 2 and in Paper [VI]. Table 2 contains previously unpublished resin–liquid component NRF parameters for the sorption data of the SCE La<sup>3+</sup> X8 resin. The experimental and calculated results have been obtained by the methods and procedures presented in Papers [I], [II] and [IV].

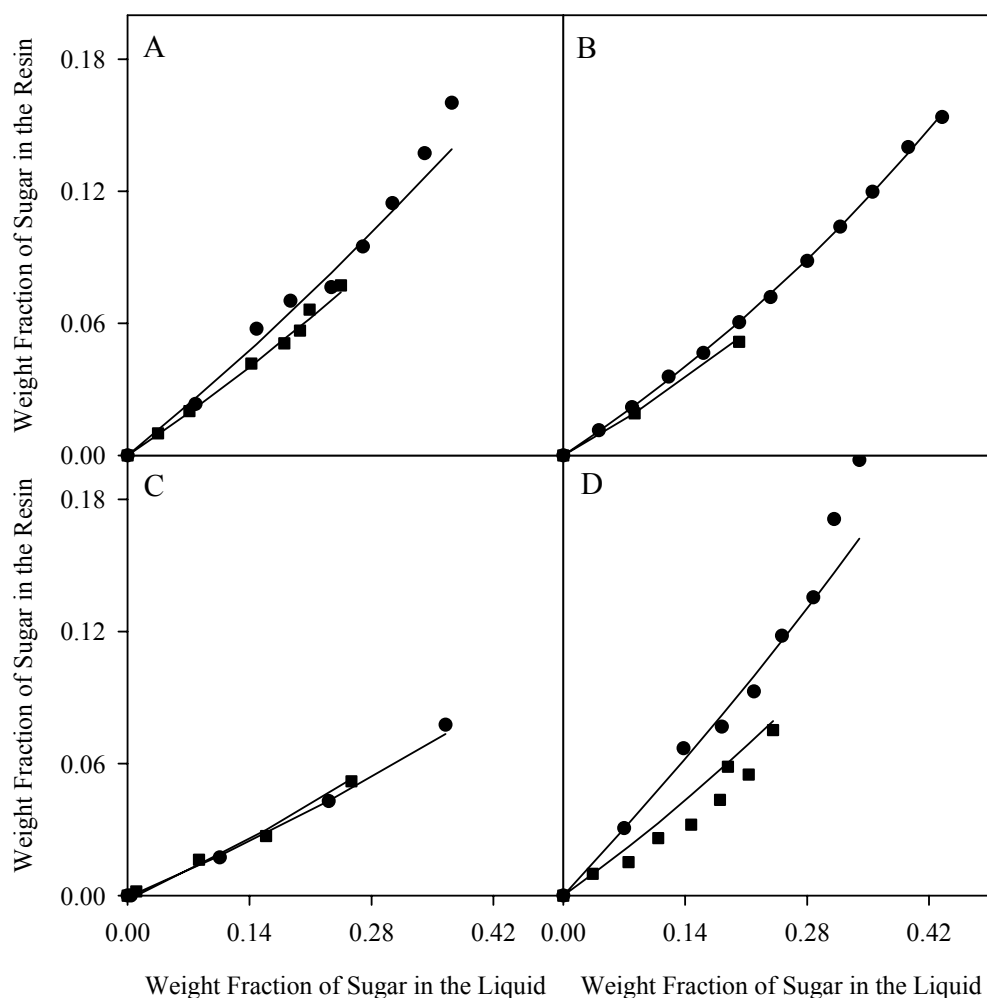
**Table 2.** Interaction,  $\Delta E_i$ , and elastic parameters,  $K_{el}$ , as well as coefficient of determination values,  $R^2$ , absolute average deviation values, AAD, and number of datapoints,  $N$ , of the NRF isotherm model used in Paper [VI]. These values are original for this summary

resin	$\Delta E_{ij}$ , J/mol								$K_{el}$ , MPa
	water		ethanol		rhamnose		xylose		
	site $a^a$	site $b^a$	site $a^a$	site $b^a$	site $a^a$	site $b^a$	site $a^a$	site $b^a$	
SCE La <sup>3+</sup> X8	650	-1400	86	-124	718	-2000	316	-1200	17
	$R^2$ , %	AAD, %	$R^2$ , %	AAD, %	$R^2$ , %	AAD, %	$R^2$ , %	AAD, %	$N$
SCE La <sup>3+</sup> X8	85.01	4.70	97.00	7.86	90.36	11.5	-	-	22
SCE La <sup>3+</sup> X8	84.76	4.86	91.99	10.9	-	-	98.56	8.23	21

<sup>a</sup> Coordination numbers used in NRF model: site  $a$  is 7 and site  $b$  is 3 [IV,VI].

### 2.2.1 Sorption in water

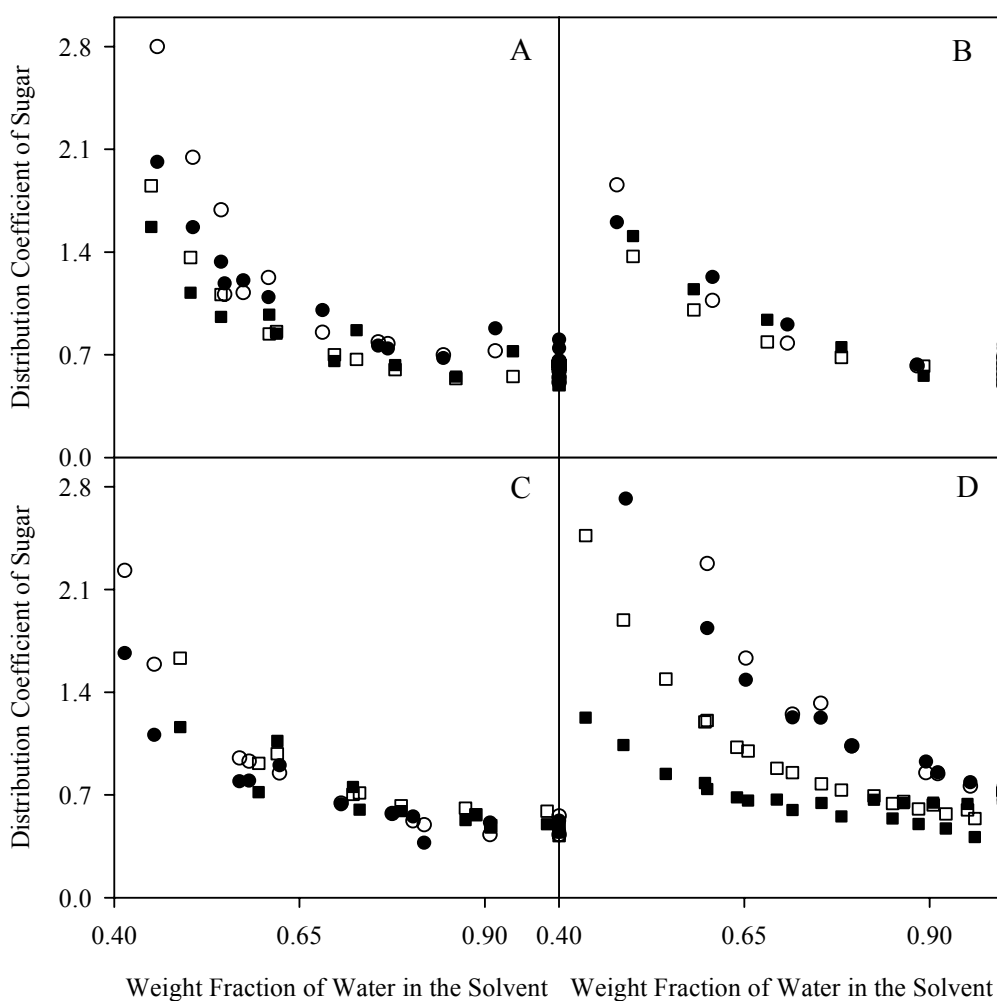
Before moving on the solvent mixtures, sugar sorption in water is briefly discussed. Thus, the effect of the cross-link density and the ionic form on the sugar sorption isotherm in water is shown in Fig. 6 and in Fig. 3 of Paper [II]. The increased cross-link density and ionic valence decreases sugar sorption in the resins as shown from the Figures above. The explanation lies on the decreased water sorption at the higher cross-link density and higher counterion valence resins (Table 1). Thus, free water content of the resins also decreases and sugar sorption diminishes [II]. In the case of rhamnose, a weak complex formation with multivalent cations may occur reducing the sorption difference in the  $\text{Ca}^{2+}$  and  $\text{La}^{3+}$  form resins when compared to the  $\text{Na}^+$  form [V,VI]. The effect of the resin type on sugar sorption from water is treated in the next Section.



**Figure 6.** Rhamnose (square) and xylose (circle) sorption in the cation-exchange resins in water at different chemical structures and ionic forms of the resins (part of data have been presented in Paper [II]). (A) SCE  $\text{Na}^+$  X5.5, (B) SCE  $\text{Ca}^{2+}$  X8, (C) SCE  $\text{La}^{3+}$  X8 and (D) WCE  $\text{Na}^+$  X6. Symbols represent experimental datapoints and lines have been calculated with the NRF model used in Paper [VI].

## 2.2.2 Sorption in aqueous alcohol solvents

As pointed out in Paper [II], the primary sorption data in Papers [II] (Figs 1–2) and [VI] (Fig. 1) are an inconvenient way of analysing sugar sorption in the water–ethanol solutions. A better way of illustrating the alcohol effect on sugar sorption is to use distribution coefficients,  $K$  (Fig. 7 and Paper [II] (Figs 5 and 6)). Distribution coefficients in Fig. 7 are calculated from the ratio of sugar weight fractions in the solution inside the resin and in the external solution. In paper [II], mole fractions were used.



**Figure 7.** The distribution of rhamnose (square) and xylose (circle) between the external liquid and the liquid inside the cation-exchange resins in aqueous ethanol solutions at different chemical structures and ionic forms of the resins (part of data have been presented in Paper [II]). (A) SCE Na<sup>+</sup> X8, (B) SCE Ca<sup>2+</sup> X8, (C) SCE La<sup>3+</sup> X8 and (D) WCE Na<sup>+</sup> X4. Filled symbols represent experimental datapoints and open symbols have been calculated with the NRF model used in Paper [VI].

First of all, the apparently scattered distribution coefficients at the water weight fraction 1.0 in Fig. 7 simply reflect the slightly concave shape of sugar isotherms in water (Fig. 6 and Fig. 3 of Paper [II]). In the aqueous ethanol solutions the general trend observed in the distribution coefficients is the same irrespective of the resin: increased ethanol concentration increases the  $K$ . As hydrophilic components, sugars have a much better solubility in water than in ethanol. As shown earlier, in the water selective resin the ethanol content in the external liquid is substantially higher than in the liquid inside of the resin, which enhances sugar sorption into the resin [II].

In relatively dilute aqueous ethanol solutions, sugar sorption follows the trend observed in water, i.e. increased cross-link density decreases sugar sorption. At lower water contents the higher water selectivity of the higher cross-linkage resins (Section 2.1.3 and Paper [I]) overcomes the effect of increased free water content of the less cross-linked resins and sugar sorption is favored in the more densely cross-linked resins [II]. Sugar sorption tends to decrease with increasing counterion valence in the case of non-complexing or very weakly complexing sugars [II]. The explanation lies again on the relatively smaller free water content of the multivalent ions [II]. Strongly complexing sugars will turn the situation over especially with increased ethanol content of the resin [V].

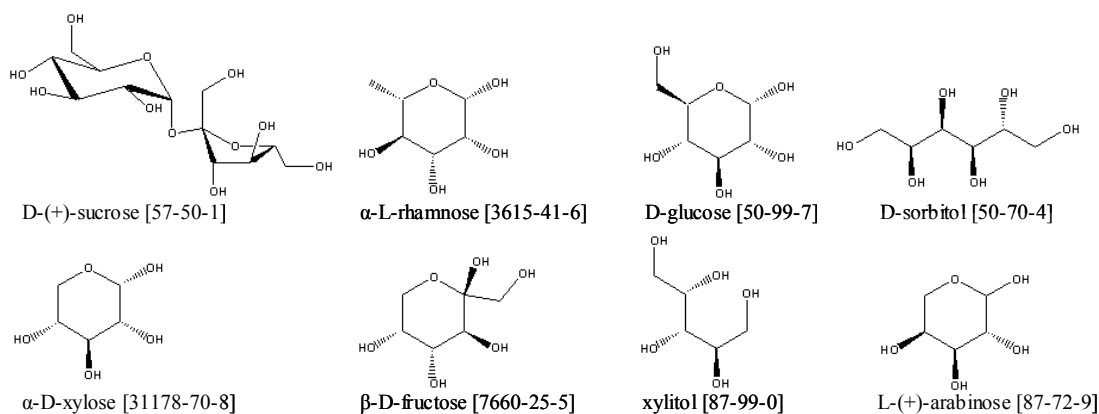
The WCE  $\text{Na}^+$  resin sorbs more xylose and less rhamnose from water, when compared with the corresponding SCE resin (Fig. 6). Moreover, sugar sorption increases faster in the WCE resins than in the SCE resins with increasing ethanol concentrations, which stems from the higher water selectivity of the WCE resins (Fig. 5 and Fig. 7). It should be noted that for the rhamnose sorption in the WCE  $\text{Na}^+$  resin (Fig. 7D) the effect of the sugar concentration on  $K$  was also tested. As a consequence, two different trends of the  $K$  of rhamnose in the WCE  $\text{Na}^+$  at high water contents can be found. Upper points are for concentrated sugar solutions and lower points for dilute sugar solutions. The difference in  $K$  between the concentrated and the dilute solution values illustrates the concave isotherm shape in less concentrated ethanol solutions and this is analogous to the isotherm shape in water. As can be seen from Fig. 4 of Paper [II], the concave shape of the isotherm gradually vanishes when going to lower external water concentrations. The isotherm shape transforms from concave to linear (Fig. 4 of Paper [II]), when moving to more concentrated ethanol solutions, and therefore the data points representing different sugar concentrations gradually overlap in Fig. 7D. On the other hand, the lower sugar solubility diminishes the sugar concentration differences and decreases the  $K$  value differences at lower water fractions in Fig. 7D

The calculated isotherms in Fig. 6 correlate the experimental data points reasonably well, but the curves tend to be systematically less concave than observed experimentally. In the case of xylose sorption, deviations are observed only at high sugar concentrations in the SCE Na<sup>+</sup> and in the WCE Na<sup>+</sup> resins. In rhamnose sorption by the WCE Na<sup>+</sup> resin the calculated values are somewhat higher than the measured ones. Moreover, the calculated and the experimental *K* values in Fig. 7 agree satisfactorily down to the external water weight fraction of 0.6. Below this value, the calculated *K* values start to exceed increasingly especially in the SCE Na<sup>+</sup> and La<sup>3+</sup> resins. In the WCE resin the gap between the measured and the calculated values start somewhat earlier. It should be noted that the calculated isotherms are a compromise to fit the sorption data and the chromatographic data in Paper [VI] as accurately as possible.

### **2.3 Chromatographic separation of carbohydrates with aqueous organic eluents**

Water is the most commonly used eluent for the liquid chromatographic separation of carbohydrates by cation-exchange resins. Addition of an organic co-solvent has been limited to analytical scale separations. In the case of carbohydrates, the most common co-solvent used has been ethanol, which is an acceptable solvent also for food industry. Analytical scale separations in solvent mixtures have been carried out with a number of carbohydrates ranging from monosaccharides to oligosaccharides, mainly in concentrated ethanol ([22], [23] and Refs in [VI]). In this thesis, more emphasis is put on less concentrated alcohol solutions. Furthermore, the effect of the resin type and of the ionic form are treated. Separations in larger than strictly in analytical scale are also included.

Carbohydrate, especially rhamnose–xylose, separation is discussed on the basis of Papers [V] and [VI]. Some new data, original in this summary, on separation of several carbohydrates in water and in aqueous 2-propanol or in ethanol are also added. Section 2.3.1 gives an insight into the effect of the properties of the cation-exchanger on carbohydrate separation. Rhamnose and xylose are used as model components. In the Section 2.3.2 the effect of eluent composition on carbohydrate separation in the cation-exchange resins is illustrated. In addition to rhamnose and xylose, several other carbohydrates were tested (Tables 4–5 and Figs 8–10). The molecular structure of the carbohydrates are shown in Fig. 8.



**Figure 8.** Carbohydrates studied. The CAS numbers are given in brackets.

### 2.3.1 Cation-exchanger properties

Cross-link density, ionic form and resin type affect the separation efficiency in the cation-exchange resins. According to Paper [VI], increased cross-linkage improves separation in water and in aqueous ethanol down to a certain ethanol concentration. This is not exactly in accordance with the sorption characteristics of the resin. Increased cross-linkage decreases the sorbed amount of the sugar down to a certain ethanol concentration [II]. On the other hand, the capacity of the resin in the column increases when the swelling of the resin decreases due to the increased cross-linkage (Table 1). The increased capacity in the case of ligand exchange chromatography affects the separation [24]. Moreover, adding alcohol to the eluent also favors increasing cross-linkage, because higher cross-linkage increases water selectivity (Fig. 5 and Paper [I]) and improves separation of hydrophilic carbohydrates. Naturally, the separation can also get worse if the increased retention is in favor of the more rapidly eluted component. The effect of the eluent composition is discussed in more detail in the next Section. However, the kinetic problems in the column (peak tailing) limit the separation benefit due to the higher cross-link density [VI]. Thus, the low cross-link density resins can operate efficiently at more concentrated organic solutions than the high cross-link density resins because of higher liquid contents (Fig. 4, Papers [II] and [VI]). This is especially true with the SCE resins. Another limiting property at higher cross-link densities is the size of the solute. The maximum size of the component capable of sorbing in the resin decreases with increasing cross-linkage as well as with decreasing swelling degree.



The effect of the ionic form on the separation efficiency of the carbohydrates is twofold i.e. either ligand exchange or partition dominates. The separation by ligand exchange is straightforward. The higher ionic valence enhances separation, when complex forming carbohydrates are separated from each other or from noncomplexing carbohydrates [V]. However, the correct elution order has to be taken into account. Separation enhances, if the elution volume of the stronger complexing solute is bigger than of the weaker complexing solute also in the noncomplexing environment. In the opposite case, separation improves only if the elution order changes in the complexing environment and if the elution volume difference is greater than in the noncomplexing environment. An example of the combined effect of ligand exchange and partition is given in the next Section.

Noncomplexing carbohydrates or very weakly complexing counterions (monovalent cations) make the situation different. Walton [25] has concluded that in the case of monovalent cations, the retention volume of the hydrophilic carbohydrates decreases in the order  $\text{Li}^+ < \text{Na}^+ < \text{K}^+$ , which corresponds to the order of increasing free water content of the resin. The case for monovalent cations seems to be the same as for noncomplexing carbohydrates with multivalent cations mentioned in Section 2.2 and in Paper [II].

Separation between rhamnose and xylose is significantly enhanced even in water, when the WCE  $\text{Na}^+$  resin is used instead of the SCE resin as the stationary phase [VI]. The efficiency difference is also seen in the  $K$  values in Fig. 7. The stronger water affinity of the WCE than that of the SCE resin enhances the elution difference between different hydrophilicity carbohydrates.

### 2.3.2 Eluent composition

The eluent composition affects the elution volume difference of the carbohydrates (Fig. 8) and occasionally even changes the elution order as can be seen from Figs 9–10. Fig. 9 shows the chromatographic data of the carbohydrates in water and in aqueous 2-propanol for the SCE  $\text{Na}^+$  and the WCE  $\text{Na}^+$  resins. The corresponding data for the SCE  $\text{La}^{3+}$  resin in aqueous ethanol is shown in Fig. 10. It should be noted that in Fig. 9 the chromatograms between the different resins are not directly comparable because of the large difference in the particle diameter (Table 3). The negative peak (system peak, considered in more detail in Paper [VI]) due to alcohol sorption properties is omitted in Figs 9 and 10. The negative peak affects only

the shape of the first eluting components (sucrose and rhamnose in Fig. 9 and sucrose, rhamnose and glucose in Fig. 10).

**Table 3.** Conditions in the chromatographic runs shown in Figs 9 and 10

resin	bed height, cm	particle diameter, <sup>a</sup> mm	alcohol in eluent, mol%	bed porosity	sample volume, cm <sup>3</sup>	flow rate, cm <sup>3</sup> /min
SCE Na <sup>+</sup> X5.5	40.4	0.10	0	0.35	0.5	1.0
SCE Na <sup>+</sup> X5.5	37.6	0.10	15 (37wt%, 2-propanol)	0.36	0.5	1.0
WCE Na <sup>+</sup> X6	29.4	0.38	0	0.34	0.5	1.0
WCE Na <sup>+</sup> X6	23.0	0.38	15 (37wt%, 2-propanol)	0.34	0.5	1.0
SCE La <sup>3+</sup> X5.5	26.1	0.10	0	0.38	0.05	0.25
SCE La <sup>3+</sup> X5.5	25.5	0.10	15 (30wt%, ethanol)	0.33	0.05	0.25

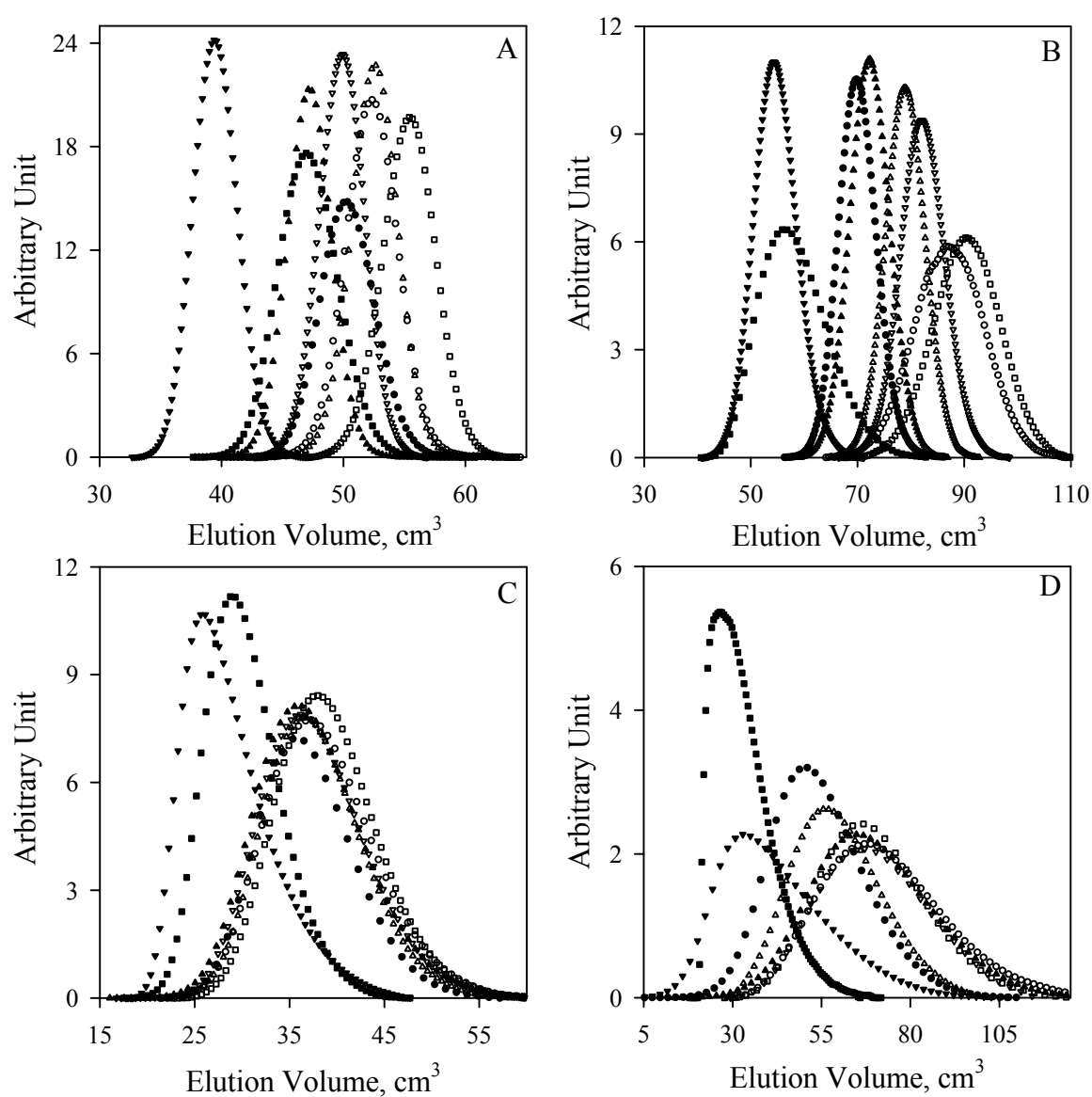
<sup>a</sup> Average diameter in water in the Na<sup>+</sup> form.

In addition to the conditions in the chromatographic runs shown in Table 3 the relevant chromatographic parameters were as follows. The column (diameter 1.6 cm) was thermostated at 298 K. The concentration of the carbohydrates in the feed was 60 g/dm<sup>3</sup> except for glucose and fructose (50 g/dm<sup>3</sup>) in Fig. 10. Carbohydrates were run one component at a time and they were detected with an RI detector. The bed capacities were different for different resins in the chromatographic runs shown in Table 3. The same bed capacity (the amount of dry resin) was used only when the eluent composition was changed and the bed height changed due to the resin deswelling in the less polar environment (Section 2.1.3).

The elution volumes and peak widths are increased for all carbohydrates, when the alcohol content of the mobile phase is increased as shown in Figs 9–10. The resin deswelling decreases the effect but elution volume changes are still seen. The increment is directly related to the increased sorption of the hydrophilic components in the water selective resins. The increased sorption in the more concentrated alcohol solutions was clearly illustrated in Section 2.2.2 and in Paper [II].

The effect of the eluent composition on the separation of carbohydrates is closely related to the relative hydrophilic nature of the carbohydrates. This can be seen from Table 4, where the relative hydrophilicity (octanol–water partition coefficient,  $K_{ow}$ ) and separation factors (Paper [VI]) of the carbohydrates are compared. The absolute  $K_{ow}$  values [26] as well as relevant solubility values of the carbohydrates are presented in Table 5. Smaller  $K_{ow}$  value means a more strongly hydrophilic nature of the component. Hydrophilicity order in Table 4 is read so that first the carbohydrate in the first column is selected and then compared with the other carbohydrates of the same row after the gray box. Furthermore, the change in

separation factor is presented in the left hand side of the gray boxes. Another notable observation is that the carbohydrates in Table 4 are in their elution order in water for the SCE Na<sup>+</sup> resin and the WCE Na<sup>+</sup> resin, respectively. The change in the separation factors in Table 4 indicates that generally the increased hydrophilic nature increases the elution volume as compared with less hydrophilic carbohydrates. Some exceptions found to the aforementioned rule are marked with the letter c in Table 4. Even in the case of these exceptions the water solubility order between the carbohydrates agree with the hydrophilicity rule, although the separation factor changes, in general, do not follow the solubility sequence.



**Figure 9.** The effect of eluent composition on the elution behavior of carbohydrates (A,B) in the SCE Na<sup>+</sup> X5.5 resin and (C,D) in the WCE Na<sup>+</sup> X6 resin. (A,C) water. (B,D) 15 mol% aqueous 2-propanol. Symbols: ▼) sucrose, ■) L-rhamnose, ▲) D-glucose, ●) D-xylose, ▽) D-sorbitol, ○) D-fructose, △) xylitol, □) L-arabinose.

**Table 4.** The effect of the aqueous alcohol eluent on the separation factor,  $\alpha$ , of carbohydrates in relation to water based on data in Fig. 9. Upper right half: hydrophilicity order based on octanol–water partition coefficient,  $K_{ow}$  (smaller  $K_{ow}$  means higher hydrophilicity). Lower left half: difference in separation factor,  $\Delta\alpha$

strong cation-exchange resin (SCE X5.5 Na <sup>+</sup> )									
elution order in water	sucrose	rhamnose	glucose	sorbitol	xylose	fructose	xylitol	arabinose	$K_{ow}$
sucrose		$K_{ow}$	$K_{ow}$	$K_{ow}$	$K_{ow}$	$K_{ow}$	$K_{ow}$	$K_{ow}$	$K_{ow}$
rhamnose	$\Delta\alpha$ -0.6		$>$	$>$	$>$	$=$	$>$	$>$	$K_{ow}$
glucose	$\Delta\alpha$ -0.1	<b>0.5<sup>a</sup></b>		$<^c$	$<$	$<$	$<$	$<$	$K_{ow}$
sorbitol	$\Delta\alpha$ <b>0.1</b>	<b>0.6</b>	<b>0.1</b>		$<$	$<$	$>^c$	$>^c$	$K_{ow}$
xylose	$\Delta\alpha$ -0.4	<b>0.2</b>	-0.1 <sup>b</sup>	<b>0.3<sup>a,b</sup></b>		$<^c$	$>$	$>$	$K_{ow}$
fructose	$\Delta\alpha$ 0.0	<b>0.6</b>	0.1	0.0	<b>0.3</b>		$>$	$>^c$	$K_{ow}$
xylitol	$\Delta\alpha$ -0.3	<b>0.4</b>	-0.1	-0.1 <sup>b</sup>	<b>0.1</b>	<b>0.2<sup>a,b</sup></b>		$>$	$K_{ow}$
arabinose	$\Delta\alpha$ -0.1	<b>0.6</b>	0.0	-0.1	<b>0.2</b>	-0.1	<b>0.1</b>		
	$\Delta\alpha$ $\Delta\alpha$	$\Delta\alpha$ $\Delta\alpha$	$\Delta\alpha$ $\Delta\alpha$	$\Delta\alpha$ $\Delta\alpha$	$\Delta\alpha$ $\Delta\alpha$	$\Delta\alpha$ $\Delta\alpha$	$\Delta\alpha$ $\Delta\alpha$		
weak cation-exchange resin (WCE X6 Na <sup>+</sup> )									
elution order in water	sucrose	rhamnose	xylose	glucose	xylitol	sorbitol	fructose	arabinose	$K_{ow}$
sucrose		$K_{ow}$	$K_{ow}$	$K_{ow}$	$K_{ow}$	$K_{ow}$	$K_{ow}$	$K_{ow}$	$K_{ow}$
rhamnose	$\Delta\alpha$ <b>0.4<sup>b</sup></b>		$>$	$>$	$>$	$>$	$=^c$	$>$	$K_{ow}$
xylose	$\Delta\alpha$ -0.6	<b>0.6</b>		$>$	$>$	$>$	$<^c$	$>$	$K_{ow}$
glucose	$\Delta\alpha$ 0.0	<b>1.4</b>	<b>0.4<sup>a</sup></b>		$<$	$<$	$<$	$<$	$K_{ow}$
xylitol	$\Delta\alpha$ -0.5	<b>0.8</b>	<b>0.1<sup>a</sup></b>	<b>0.2<sup>a,b</sup></b>		$<^c$	$<^c$	$>$	$K_{ow}$
sorbitol	$\Delta\alpha$ -0.1	<b>1.4</b>	<b>0.4<sup>a</sup></b>	0.0 <sup>a</sup>	<b>0.2<sup>a</sup></b>		$<$	$>^c$	$K_{ow}$
fructose	$\Delta\alpha$ 0.0	<b>1.5</b>	<b>0.4</b>	0.0	<b>0.3<sup>a</sup></b>	0.0 <sup>a</sup>		$>$	$K_{ow}$
arabinose	$\Delta\alpha$ -0.2	<b>1.3</b>	<b>0.3</b>	-0.1	<b>0.2</b>	-0.1 <sup>b</sup>	0.0 <sup>a,b</sup>		
	$\Delta\alpha$ $\Delta\alpha$	$\Delta\alpha$ $\Delta\alpha$	$\Delta\alpha$ $\Delta\alpha$	$\Delta\alpha$ $\Delta\alpha$	$\Delta\alpha$ $\Delta\alpha$	$\Delta\alpha$ $\Delta\alpha$	$\Delta\alpha$ $\Delta\alpha$		

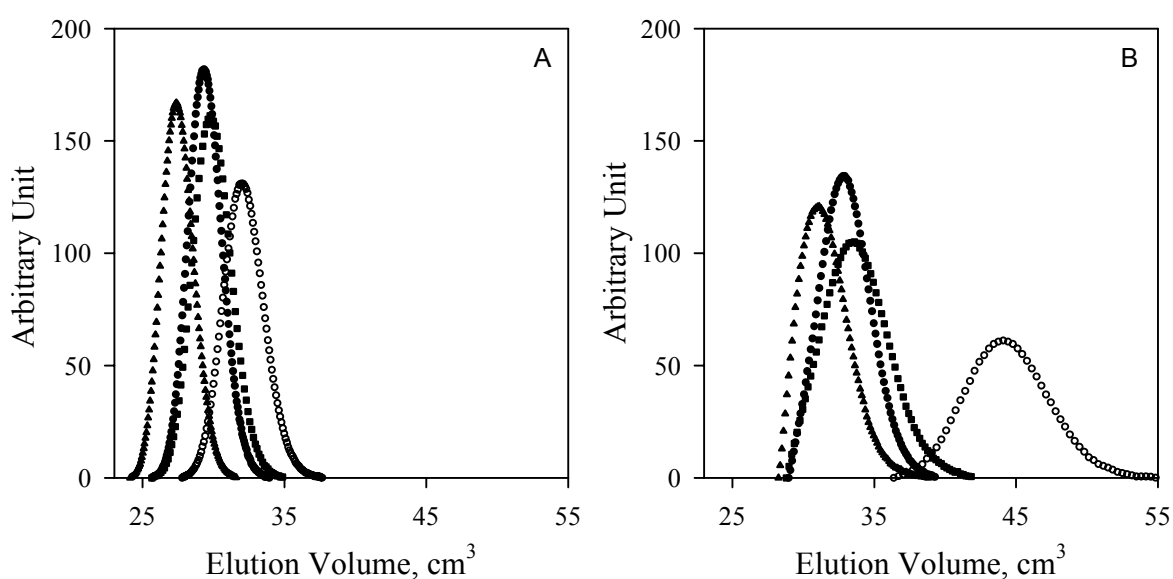
**Criteria,  $K_{ow}$ :**  $<$  increased hydrophilic nature,  $=$  equal hydrophilic nature,  $>$  decreased hydrophilic nature (reading order in Table: column vs row). <sup>a</sup> Separation minimal in water. <sup>b</sup> Elution order changed. <sup>c</sup> Water solubility agrees the effect on the separation factor (see 4<sup>th</sup> paragraph in Section 2.3.2). **Example:** For SCE  $K_{ow}$  order is sucrose  $<$  rhamnose (sucrose more hydrophilic than rhamnose) and  $\alpha$  for sucrose-rhamnose pair decreases 0.6.

The hydrophilicity rule can be overcome by the stronger interactions such as the complexation as mentioned in the previous Section. The complexation is evident in the La<sup>3+</sup> resin as shown in Fig. 10. The separation of fructose, which is considered as a complex forming sugar [V], from other sugars is more efficient in the La<sup>3+</sup> resin than in the Na<sup>+</sup> resin, especially in the aqueous alcohol eluent. It is known that the complex strength increases with decreasing polarity of the solution [V]. The elution order of rhamnose with respect of glucose and xylose changes when the counterion of the resins is changed from Na<sup>+</sup> to La<sup>3+</sup>. Rhamnose elutes later than glucose or xylose in the La<sup>3+</sup> resin. The relative elution volume change between rhamnose and xylose was also observed in Paper [VI] when the Na<sup>+</sup> ion was changed to the Ca<sup>2+</sup> ion. In Paper [VI] it was speculated that the change in the elution volume was an indication of a slight complex forming ability of rhamnose, which increased rhamnose elution volume and made separation negligible. The same phenomenon can be true also in the La<sup>3+</sup> resin. On the other hand, rhamnose, glucose and xylose are considered as very weakly complexing carbohydrates and their complexing ability was found to be

practically equal in Paper [V]. Moreover, the enhanced separation observed for fructose in aqueous alcohol (Fig. 10B) did not take place for rhamnose when the mobile phase composition was changed. Thus, it is probable that some other interactions are also affecting the elution of rhamnose, such as decreasing the hydrophilicity effect due to the decreased free water content in the multivalent cation resins, as noticed in the previous Section and in Paper [II]. In other words, the elution of more hydrophilic glucose and xylose compared to less hydrophilic rhamnose is more rapid in the multivalent resin than in the monovalent resin.

**Table 5.** Octanol–water partition coefficients,  $K_{ow}$ , and solubilities of studied carbohydrates in water, in ethanol and in 2-propanol. Carbohydrates are in the elution order in the SCE Na<sup>+</sup> resin in water (Fig. 9A). Solubility is given as weight percent at 298 K unless otherwise indicated

carbohydrate	$10^3 K_{ow}$ [26]	solubility, wt%			
		water	ethanol (293K, 80%)	ethanol	2-propanol
sucrose	0.20	67.9 [27], 67 [28]	37 g/dm <sup>3</sup> [29]	0 (287K) [29] 0.06 [30]	
rhamnose	28	40 [VI]		10 (293K) [30]	8.4 (310K) [31]
glucose	0.58	49.4 (296K) [27]	45 g/dm <sup>3</sup> [29]	1.6 (296K, 99%) [31] 1.5 g/dm <sup>3</sup> [32] 0.4 (293K)[30]	1.1 (296K, 99%) [31] 1 g/dm <sup>3</sup> [32]
sorbitol	6.3	72 [28]		4.7 (313K, 96 %) [33]	
xylose	10	55 [VI] 550 g/dm <sup>3</sup> [26]	62 g/dm <sup>3</sup> [29]	4 g/dm <sup>3</sup> [32]	1.5 g/dm <sup>3</sup> [32]
fructose	28	80.5 [34] 778 g/dm <sup>3</sup> (293K) [26]	274 g/dm <sup>3</sup> [29]	3.5 (303 K) [35]	
xylitol	2.8	66 [28]		1.9 (293K, 96%) [33] 4.7 (313K, 96%) [33]	
arabinose	1.2	500 g/dm <sup>3</sup> [26]	19 g/dm <sup>3</sup> [29]	1 g/dm <sup>3</sup> [32]	1.5 g/dm <sup>3</sup> [32]



**Figure 10.** The effect of eluent composition on the elution behavior of carbohydrates in the SCE La<sup>3+</sup> X5.5 resin. (A) water. (B) 15 mol% aqueous ethanol. Symbols: ▲ D-glucose, ● D-xylose, ■ L-rhamnose, ○ D-fructose.

Fast elution of sucrose is directly related to the size of the sugar. Sucrose as a disaccharide has a larger molar volume than monosaccharides. It is known that in cation-exchangers the elution volume of oligosaccharides decreases with increasing number of repetitive monosaccharide units in sugar, when water and water-rich alcohol eluents are used. The situation becomes opposite when concentrated alcohol is used, i.e. the elution volume increases with increasing oligosaccharide size [22]. According to Havlicek and Samuelson [22], the observed elution order in water and in water-rich eluents is due to the increased swelling pressure effect with the increased carbohydrate size. In concentrated alcohol eluents, the increasing elution volume correlates with the increasing number of hydroxyl groups [23].

In conclusion, it is clearly shown in Table 4 that separation of several sugars can be enhanced by adding alcohol to the eluent. The best results can be achieved by separating rhamnose from other monosaccharides and polyols studied. Separation of xylose and xylitol from the rest of the studied monosaccharides and polyols is also enhanced, especially in the WCE Na<sup>+</sup> resin. Conversely, separation of sucrose became worse in all cases. This stems from the effect of alcohol addition on the elution behavior of oligosaccharides as stated above [22].

The thermodynamic NRF sorption model introduced in previous Sections was implemented to a rate-based column model as described in Paper [VI]. The column model was successfully utilized for rhamnose–xylose chromatographic data. Quantitative agreement was achieved between experimental and calculated ethanol, rhamnose and xylose elution data in the SCE and the WCE resins at different cross-link densities and ionic forms. The model was able to adequately calculate the chromatographic behavior at high and low carbohydrate concentrations with small and large relative feed volumes. Furthermore, simulated data were used in Paper [VI] to illustrate the effect of the SCE and the WCE resin properties on the elution behavior of rhamnose and xylose in water and aqueous ethanol.

### 3 CONCLUSIONS

In this thesis the properties of the strong and the weak cation-exchange resins in different aqueous organic solvent, mainly in aqueous alcohol, environments and their use on carbohydrate separation in these environments were studied at 298 K. In addition to the linear conditions (small feed concentrations and volume), high feed concentrations and large feed volumes were used in the chromatographic experiments. The sorption properties and the chromatographic performance of the resins were modeled.

The behavior of the cation-exchangers in the aqueous organic environment is complex. This complex behavior was analyzed experimentally and fitted to the thermodynamic sorption models. The hydrophilic nature of the salt form resins leads to a water selective sorption from aqueous solvent mixtures. The properties of the resins dictates the isotherm shape and the uptake of all liquid components. The cross-link density and ionic form of the resin do not only affect the absolute amount of the sorbed components but also the liquid component ratios inside and outside of the resin (distribution coefficient). However, the changes in the distribution coefficients were shown to be relatively small in binary solvent mixtures, such as water–alcohol, irrespective of cross-linkage or counterion. On the other hand, the altering of the cross-link density or of the ionic form can affect drastically the sorption of solutes such as carbohydrates. In addition to the non-specific partition, specific interactions (complex formation) with counterions may strongly affect carbohydrate sorption and a densely cross-linked polymer network can restrict the sorption of larger molecules.

Increasing concentration of an organic co-solvent not only increase carbohydrate sorption but also strengthens the carbohydrate counterion complexes and affects the size exclusion of carbohydrates. The hydrophilic carbohydrates tend to sorb more efficiently when the water selectivity of the resins increases. The complexing effect becomes significant when multivalent counterions and complex forming carbohydrates are incorporated. The addition of co-solvent increases the complex stability. Decreased polarity of the aqueous solvents deswells resin and the exclusion effect in the narrowed resin may strengthen. On the other hand, the increased water selectivity affects the opposite direction by increasing the sorption of larger molecular size carbohydrates.

A fundamental effect on the sorption properties is evident when the resin type, i.e. both the resin matrix and the functional group, is changed. Not only the water isotherm shape changes

but the shape and magnitude of the alcohol isotherm are also altered. The weak acrylic cation-exchanger has stronger water affinity and it is more water selective than the corresponding strong PS-DVB cation-exchanger. Thus, stronger effects on the hydrophilic carbohydrate sorption due to organic solvent addition are feasible. Moreover, the volume change with the solvent composition and with the ionic form are more prominent for the weak resin than for the strong resin.

In addition to the experimental data the sorption phenomena were characterized by forming suitable thermodynamic sorption models as already mentioned. Several models were tested and the most suitable of them was used in calculations of multicomponent sorption in the weak and strong cation-exchangers. The models were formed so that in the liquid phase all interactions were included in the mixing effects and in the resin phase the mixing effects and the elastic effects were taken separately into account.

In all cases, independently on the properties of the resins, the composition and amount of the sorbed liquid inside the resin affect the elastic nature of the resin. The elastic data were utilized in the estimation of elastic parameters in the sorption models. The calculated elastic parameters showed the same trend as the experimental elasticity data. Generally, the elasticity of the swollen resins decreases with the increasing cross-link density and usually also with the increasing counterion valence. At the certain deswelling range, the elastic polymer becomes a glass-like material. However, whereas the rubber-like strong cation-exchanger converts directly to the glass-like material at the range where the water sorption isotherms merge, the weak cation-exchanger has 'an intermediate state' between the isotherm merge point and the glass-transition.

All the aforementioned characteristics of the resins have a direct impact on the chromatographic behavior of the cation-exchangers in the separation of organic solutes (carbohydrates) with the aqueous organic (alcohol) eluents. By adding alcohol to the eluent or by increasing hydrophilicity of the resin, the separation of the carbohydrates with different hydrophilicity can be enhanced. However, there are some restrictions for the enhanced separation. First, the more hydrophilic carbohydrate have to elute at the same time or later than the less hydrophilic carbohydrate in water. Second, if the elution order changes, the absolute elution volume difference between the carbohydrates must increase. Furthermore, the separation can be even further enhanced if the later eluted carbohydrates have special interactions like complex formation with the counterion.



Up to a system specific cross-link level the separation efficiency increases. Above that limiting cross-link density the diffusional hindrance leads to a significant peak tailing and worse separation. At lower cross-linkages the water content of the stationary phase increases leading to the increased component dilution, which is seen as wider peaks and poorer separation efficiency. On the other hand, by adding alcohol to the eluent the less densely cross-linked resins become a more attractive separation media choice due to the higher initial swelling of the resin. Thus, the lower cross-link resins can maintain the effective separation properties up to more concentrated alcohol solutions than the more strongly cross-linked resins. Naturally, the benefit of the stronger swelling disappears when the sorption isotherms merge and the cross-link effect vanishes. From this point of view the strong cation-exchangers have a wider applicable solvent concentration range than the weak cation-exchangers due to the later isotherm merging.

On the other hand, in concentrated organic solvents the solubility of the carbohydrates reduces and mass transfer kinetics become slower making the large scale separations less appealing. Moreover, the increased dilution due to the increased elution volume of carbohydrates in aqueous alcohol solutions diminishes the benefits of better separation in industrial scale applications. Thus, all four factors, the decreased solubility of carbohydrates, slower mass transfer kinetics, enhanced separation and increased sorption in the aqueous alcohol solutions, must be taken into account when developing the economically feasible separation processes.

The separation process was also characterized by forming a suitable column model. The selected multicomponent sorption model was successfully implemented in the column model, which was utilized in the calculation of rhamnase and xylose separation in different eluent compositions and in different stationary phases. The simulated chromatographic data were used to explain the effect of the resin characteristics on the separation efficiency of the carbohydrates.

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## APPENDICES

### Scientific publications I-VI