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ANALYSIS OF OLIGOMERS, FORMED DURING EMULSION POLYMERIZATION PROCESSES, USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A lot of kinetic-mechanistic aspects of the emulsion polymerization process are still unexplained. Particularly at the initiation of the latex particles oligomers play an important role as is explained in chapter one. To get more insight in the role of oligomers it is necessary to collect qualitative and quantitative information about these compounds. In chapter one also an overview of the possible separation methods for these oligomers is given.

In many cases a charged initiator is used in emulsion polymerization processes, for instance persulfate. Therefore, charged oligomers will be formed with one or two sulfate groups. Subsequently these sulfate groups can hydrolyse to alcohol functions. Therefore the separation procedures to be developed for oligomers must be able to separate charged and uncharged oligomers as well. The use of so called mixed mode (reversed phase/anion exchange) stationary phases for these separations has been investigated by using model compounds for the oligomers mentioned above. As described in chapter two a number of columns showed promising results with regard to the separation of oligomers.

Because these analytical separations have to be scaled-up to preparative HPLC to allow further research on the structure of the isolated compounds, the performance of a preparative column system, the Annular Expansion (A/E) system was investigated as is described in chapter three. In this system the column is, after packing, axial as well as radial compressed. The column quality and the reproducibility of the packing procedure were investigated. The efficiency of the column was good for the first couple of packing times reusing the same packing material and reduced plate heights of 2-3 were obtained. But after that the column quality gradually decreased.

In chapter four the preparative separation of, uncharged, styrene oligomers (n = 1 - 6) is described. With the A/E column system, described in chapter three, in theory it was possible to separate up to 5 ml of the original styrene oligomers sample per run.

Before the water phase of the emulsion polymerization product can be analyzed, the, very small, latex particles have to be removed and for preparative separations the water phase has to be concentrated. Therefore three types of filters have been investigated for the removal of these latex particles as described in chapter five. They were able to remove the latex particles but adsorption on the filters of uncharged oligomers was also observed. Further the applicability of solid phase extraction (SPE), freeze drying and evaporation as
concentration techniques was investigated. Using reversed phase SPE it proved to be possible to concentrate the uncharged oligomers. However, for the charged oligomers the recovery was lower. When freeze drying and evaporation were used as concentration techniques irreproducible and incomplete recoveries of the oligomers was observed.
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CHAPTER ONE: EMULSION POLYMERIZATION THEORY: INFLUENCE OF Oligomeric Radicals

1.1 SUMMARY

In this chapter first the basic principles of the emulsion polymerization process are described. Further the role the oligomer radicals formed in the water phase play in this process is discussed. At last methods to analyze these oligomers are presented.

1.2 INTRODUCTION

In emulsion polymerization a recipe is used in which the main components are monomer(s), dispersing medium (usually water), a, usually water soluble, initiator and emulsifier, usually present in concentrations above the critical micelle concentration (CMC) to generate micelles. Although the emulsion polymerization process is applied widely in industry, a lot of the kinetic-mechanistic aspects are still unexplained. The quality of the latex obtained depends upon small variations in the polymerization parameters as well as the skill of the operator[1]. In this chapter first the basic principles of emulsion polymerization will be discussed.

Since in general the monomers are nearly insoluble in water there will be hardly any polymerization in the water phase. Besides, the total surface area of the micelles is much larger than the total surface area of the monomer droplets present in the reaction mixture (about 1000 times) so the chance of entering of an radical from the water phase is much larger for the latex particles. As a result the polymerization will proceed almost exclusively in the monomer swollen latex particles[2].

The emulsion polymerization process can be defined in three distinct intervals[3]. During interval I polymer and monomer containing latex particles are formed by the nucleation process which will be discussed in 1.3. During interval II the newly formed latex particles grow, at the expense of the monomer droplets. The monomer concentration in the water phase is constant during this interval because there is a constant transportation of the
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monomer from the monomer droplets to the water phase and therefore the polymerization rate will also be constant. In the ideal case no new particles are formed during this stage. Interval II ends when all monomer droplets have disappeared so in interval III the two remaining phases are the water phase and the monomer swollen latex particles. In this interval the monomer concentration in the latex particles will decrease because there is no constant supply of monomer from the monomer droplets which causes a decrease in the rate of polymerization.

1.3 PARTICLE NUCLEATION

The complex process of particle nucleation is still not well understood. A widely used theory[3] proposes the micelles as locus of nucleation: The radicals generated in the aqueous phase enter the monomer swollen micelles and initiate polymerization, the otherwise rather dynamic micelles will be stabilized and grow to form monomer swollen polymer latex particles. However, in many cases it was observed that a polymer latex was formed using emulsifier concentrations below the CMC or even when no emulsifier was used. For these systems other nucleation models were proposed: A homogeneous nucleation model[4] was developed in which the radicals react in the water phase with solubilized monomer to form growing oligomeric species. These oligomers will form particles when the critical water solubility length is reached. Other authors[5] refined this model by proposing that first colloidal unstable precursor particles are formed, as a result of the previous described growth of oligomers in the water phase, which will coagulate with each other and with mature particles to form growing polymer latex particles. In practice the micellar and the homogeneous nucleation will occur concurrently.

1.4 PARTICLE GROWTH

After and also during the formation of the stable latex particles polymerization will take place inside these latex particles for reasons which were already described before. The
overall polymerization rate equation for emulsion polymerization processes is as follows[2]:

\[ r_p = \frac{k_p C_M \bar{n} N_p}{N_{av}} \]

where \( r_p \) is the polymerization rate per unit volume, \( k_p \) the propagation rate constant, \( C_M \) the monomer concentration in the particles, \( N_p \) the number of particles per unit volume, \( N_{av} \) Avogadro's number and \( \bar{n} \) the average number of radicals per particle. Smith and Ewart[6] described a model for the determination of the average number of radicals per particle. The formation of a particle with \( n \) radicals can be described as follows:

\[
\frac{dN_n}{dt} = R_{entry} + R_{desorption} + R_{termination}
\]

where:

\[
R_{entry} = (\rho/N_p)(N_{n-1} - N_n)
\]
\[
R_{desorption} = k_d([n+1]N_{n+1} - nN_n)
\]
\[
R_{termination} = c([n+2][n+1]N_{n+2} - n[n-1]N_n)
\]

where \( N_i \) is the number of particles with \( i \) radicals, \( \rho \) the second order rate coefficient of entry of radicals, \( k_d \) the rate coefficient of desorption of the radicals from the particles, \( c \) the rate coefficient for bimolecular termination of radicals in the particles and \( v \) the volume of a monomer swollen latex particle. Combining gives:

\[
\frac{dN_n}{dt} = \frac{\rho}{N_p} (N_{n-1} - N_n) + k_d ([n+1]N_{n+1} - n N_n) + \frac{c}{v} ([n+2][n+1]N_{n+2} - n[n-1]N_n) \tag{1.1}
\]

Smith and Ewart distinguished three cases for the average number of radicals per particle.

Case 1: \( \bar{n} \ll 0.5 \)

In this situation the desorption is a lot faster than the adsorption of the radicals by the latex particles. As a result the number of radicals in the latex particle will be at most be one and the average number will be far smaller than one.

Case 2: \( \bar{n} = 0.5 \)

In this situation there will be negligible desorption of radicals and instantaneous termination.
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will take place when a second radical enters the latex particle. Because the time interval between the entries of radicals varies in a random fashion and \( n = 1 \) when a (first) radical enters the latex particle and \( n = 0 \) when a second one enters the particle, the average \( n \), \( \bar{n} \), will be 0.5.

Case 3: \( \bar{n} \gg 0.5 \)

In this situation the termination in the latex particle is no longer instantaneous when a second radical enters the particle and the desorption of the radicals is negligible. In this case more than one radical can propagate at the same time in a particle.

A simple equation for \( \bar{n} \) can be derived from equation (1.1) for case 1 and case 2 using the so called zero-one model. In this model it is assumed that there will never be more than one radical in a latex particle so only particles with 0 or 1 radicals are considered, \( N_0 + N_1 = N \). In that case the following equation for \( \bar{n} \) can be derived:

\[
\bar{n} = \frac{N_1}{N} = \frac{\rho}{2\rho + k_d}
\]

A more general solution for equation (1.1) was derived by Stockmayer[7] and later modified by O'Toole[8].

\[
\bar{n} = \frac{a}{4} \frac{I_m(a)}{I_{m-1}(a)}
\]

where \( I_m \) and \( I_{m-1} \) are Bessel functions of the first kind, \( m \) a measure for the rate of desorption over the rate of bimolecular termination and \( a \) a measure for the rate of entry over the rate of bimolecular termination.

1.5 RADICAL ENTRY

The oligomer radicals formed in the water phase do not only play a role in the emulsion polymerization process during particle nucleation as is described above but also during the entry of the (oligomer) radical in the latex particle and in this way influence the
particle growth. Gilbert and Napper[9] distinguished the following steps during the entry of a radical in the latex particle:

1. a. Decomposition of the initiator \( I \) and b. formation of the oligomer radical \( R^* \) that can enter the latex particle.

\[
\begin{align*}
a) & \quad \text{Decomposition of the initiator:} \\
& \quad l_2 \rightarrow 2 I^* \\
\text{b) & \quad \text{Formation of the oligomer radical:} \\
& \quad l^* +zM \rightarrow M_z^* (=R^*)
\end{align*}
\]

2. Entry of this oligomer radical into the latex particle \( P \).

\[
k_e \\
R^* + P \rightarrow \text{entry}
\]

3. Bimolecular termination between the radicals in the water phase.

\[
k_t \\
2R^* \rightarrow \text{inert products}
\]

4. Desorption of a radical from the particle.

\[
k_{d^r} \\
P \rightarrow E^*
\]

5. Reentry of desorbed radicals \( E^* \) into the latex particle.

\[
k_r \\
E^* + P \rightarrow \text{entry}
\]

6. Hetero termination in the water phase.

\[
k_{ht} \\
E^* + R^* \rightarrow \text{inert products}
\]

The overall entry rate can be written as:
Emulsion polymerization theory: influence of oligomeric radicals

\[ \rho = k_e [R^\bullet] + k_r [E^\bullet] \]

or simplified:

\[ \rho = k_e [R^\bullet] \]

where \([R^\bullet]\) is the radical concentration in the water phase, \([E^\bullet]\) the concentration of the desorbed radicals in the water phase, \(k_e\) the first order rate coefficient of entry, \(k_r\) the rate coefficient of entry of already desorbed radicals.

Several models are described in the literature for the calculation of the entry rate such as:

a. Collision model[10]


c. Surfactant displacement model[12]

d. Colloidal entry model[5]

e. Propagational model[13]

The principles of these models will be described.

a. Collision model[10]

In this model the author suggests that the entry rate can be given by the collision frequency between an oligomer radical with a latex particle. In this model the size of the oligomer radical is neglected with regard to the size of the latex particle. \(k_e\) can be described by:

\[ k_e = \sqrt{\frac{8\pi k T}{m}} r_s^2 \]

where \(k\) is Boltzman's constant, \(T\) the absolute temperature, \(m\) the mass of the radical and \(r_s\) the radius of the latex particle.


In this model it has been suggested that the rate controlling step for entry is the diffusion of the free radical to the particle surface. The equation for \(k_e\) becomes:
\[ k_e = \frac{kT r_s}{3\eta r_{ol}} \]

where \( \eta \) the viscosity of the water phase and \( r_{ol} \) the radius of the oligomer radical.

c. Surfactant displacement model[12]

In this model it is suggested that the rate determining step for entry is the displacement of a surfactant molecule from the particle surface by an oligomer radical.

d. Colloidal entry model[5]

In 1.3 one of the models proposed for the particle nucleation was the coagulation of precursor particles to latex particles. In this entry model the same idea is applied to the entry of oligomer radicals. It is assumed that the entering radical species is a precursor particle, formed by the oligomers in the water phase. \( k_e \) can be calculated by:

\[ k_e = \frac{kT(r_s + r_{pp})^2}{3\eta W r_{pp}} \]

where \( r_{pp} \) the radius of the precursor particle and \( W \) the stability ratio for a precursor-latex particle interaction which can be calculated using the DLVO theory.

e. Propagational model[13]

In this model the length of the oligomer radical when entering the latex particle is taken in consideration. It is assumed that irreversible free-radical capture by the latex particles is negligible if the aqueous phase radical is below a critical degree of polymerization \( z \) and that irreversible free radical capture by the latex particles is instantaneous for oligomeric free radicals of a degree of polymerization \( z \). The rate determining step for the entry of radicals is the growth of the radicals in the water phase to that particular degree of polymerization. Asua and de la Cal[14] developed a relation for the \( k_e \) using this model:
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where \( \sigma \) the chance that an oligomer radical propagates in the water phase, \( \sigma_0 \) the chance that an initiator radical propagates in the water phase, \([M]_w\) the monomer concentration in the water phase and \( N_T \) the number of latex particles in the water phase.

To verify these entry models several authors[13-15] calculated the entry rates predicted by these models and compared them with experimental values. Comparing these results the last model, the propagational, gave the best approximation[14].

1.6 ANALYSIS OF OLIGOMERS

Using this kinetic-mechanistic propagational model it is important to know the degree of polymerization \( z \) at which the oligomer radicals are irreversible captured by the latex particle. Maxwell et al.[13] gave \( z \)-values for some polymers by fitting the model with the experimental values but to verify the model it will be necessary to determine them. This \( z \) can not be measured directly because the lifetime of the radicals is too short. Information about \( z \) can be obtained from the termination products of the oligomer radicals in the water phase formed during steps 3 and 6 in the process of entry as is described above. In addition, from the analysis of these oligomers information can be obtained about the kind of termination that takes place in the water phase and the amount of termination. When termination takes place due to combination of two radicals the following termination products will be formed[2]:

\[ I - (M)_n - I \]

where \( M \) is the monomer and \( n \) the number of monomers.

When termination takes place due to disproportionation the following products will be formed[2]:
When termination takes place due to chain transfer these termination products will be formed[2]:

\[ \text{I - (M)}_{n-1} - \text{C} = \text{C} \] and \[ \text{I - (M)}_{n-1} - \text{C} - \text{C} \]

Especially when \( z \) is large the amount of termination products formed can be very large. But even when \( z \) is not large, as is the case for butadiene where \( z = 2 \) or \( 3 \), the amount of compounds that can be formed will be considerable, especially with butadiene since it has the possibility of 1,4 and of 1,2 addition to the growing chain.

The initiator most widely used in emulsion polymerization is the persulfate ion[1]. This decomposes to give sulfate ion radicals, these radicals will propagate in the water phase giving oligomeric radicals until they become surface active and irreversible enter the latex particles as is described above. The termination products formed from these oligomer radicals in the water phase will therefore be charged oligomers. Subsequently, the sulfate end groups of these oligomers can hydrolyse to alcohol functions[16-21]. Moreover, it is known that by the dissociation of persulfate \( \text{O} - \text{OH} \) radicals are generated which can also initiate the polymerization and thereby forming uncharged oligomers[22]. Due to these reactions the amount of compounds that have to be analyzed is increased by the addition of uncharged oligomers and charged oligomers containing an alcohol function. The structures of these additional formed oligomers are:

\[ \text{I - (M)}_n - \text{OH}, \text{HO} - \text{(M)}_n - \text{OH} \] and \( \text{(M)}_n - \text{OH} \)

So to obtain information about \( z \) the water phase has to be analyzed but due to the amount of compounds that can be formed this is complicated.

The separation of the charged oligomers of butadiene and styrene formed during emulsion polymerization was obtained using isotachophoresis (ITP)[23-25]. In this technique the species are separated according to their molecular weight, geometry and charge[26]. It was possible to separate charged oligomers differing in chain length and the concentration
of these compounds could be determined. Identification of the oligomers was performed using model compounds. Because the model compounds are not exactly the same as the oligomers, for instance for unsaturated and possible branched butadiene oligomers saturated and linear alkyl sulfonic acids were used as model compounds, only the length and the charge of the oligomers could be determined and not their structure[25]. In ITP also not enough material can be separated to obtain information about the structure of the compounds after the separation. Another drawback of this technique is that no information can be obtained about the uncharged oligomers.

High performance liquid chromatography (HPLC) is a technique which can easily be scaled up to preparative liquid chromatography allowing the isolated unknown compounds to be characterized by other methods as nuclear magnetic resonance (NMR), mass spectroscopy (MS) and infrared (IR). Using reversed phase HPLC it is possible to separate uncharged oligomers, see for instance references[27-33]. The most difficult part using HPLC is the separation of the charged oligomers. In this case reversed phase HPLC can not be used because the sulfate group in these molecules makes that the charged oligomers are not retained on the column[34,35]. Also anion exchange HPLC can not be used because in this technique the separation mechanism is based on the difference in the charged group of the solutes[36]. In these charged oligomers the charged group is in all the cases the same, namely a sulfate group. When looking in the literature similar compounds, anionic surfactants, are easily separated using ion pair reversed phase HPLC (see for instance references [37-43]). A drawback of this technique is that an ion pair is added to the eluent, for instance tetra methyl ammonium bromide, which can not be removed easily after the separation. Removal of the eluent is, in our case, necessary because information about the structure of the oligomers has to be obtained. In this project the use of mixed mode (reversed phase/anion exchange) stationary phases is investigated for the separation of the oligomers. In these kind of stationary phases two retention mechanisms are present, reversed phase and anion exchange. By using these mixed mode stationary phases the uncharged oligomers can be separated by the reversed phase mechanism and the charged oligomers can be separated by a combination of the reversed phase and the anion exchange mechanism. These stationary phases allow the use of a combination of a specific buffer and an organic modifier (for instance methanol, acetonitrile or tetrahydrofuran) as the eluent which can be removed easily.
after the separation. More information about mixed mode stationary phases will be given in chapter two.

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CHAPTER TWO: ANALYSIS OF OLIGOMERS USING MIXED MODE STATIONARY PHASES

2.1 SUMMARY

The possibility to separate charged and uncharged oligomers formed during emulsion polymerization processes by high performance liquid chromatography (HPLC) using mixed mode (reversed phase/anion exchange) stationary phases was investigated. To examine this possibility, several columns were evaluated on their reversed phase, anion exchange and mixed mode properties using model compounds. The OmniPac PAX-500, the PRP-X100 and the mixed phase PLRP-S/PLSAX columns showed promising results for the separation of real samples.

2.2 INTRODUCTION

A lot of kinetic-mechanistic aspects of the emulsion polymerization process are still unexplained. Particularly at the initiation of the latex particles oligomers play an important role. One of the questions concerns the length of the oligomer radicals when they enter the latex particles[1]. Besides that, it is also important to know what kind of termination takes place in the water phase. In many emulsion polymerization processes a charged initiator, for instance persulfate, is used. Consequently, charged oligomers will be formed having one or two sulfate groups. Subsequently these sulfate groups may hydrolyse to alcohol functions.

To get more insight in the role of oligomers in emulsion polymerization processes it is necessary to collect qualitative and quantitative information about these compounds. Preferably the separation procedures to be developed for oligomers should be able to separate charged and uncharged oligomers as well in one analysis procedure. Also, in this study these (analytical) separations had to be scaled-up to preparative liquid chromatography to allow further research on the structure of the isolated compounds. This latter consideration puts specific demands on the composition of the applied eluents with respect to the removal of it after having finished the preparative separation.
While especially charged oligomers are difficult to separate either by reversed phase or ion exchange chromatography in this investigation the use of so called mixed mode stationary phases for the separations of charged and neutral oligomers have been investigated. In mixed mode, stationary phases with two different retention mechanisms on one stationary phase are intentionally prepared by the manufacturer. Here the columns selected for this study showed reversed phase and anion exchange properties by\(^2\): 

\(i.\) interaction of the apolar part of the sample molecules to the apolar backbone of the stationary phase and 

\(ii.\) anion exchange interactions of the ionic part of the sample molecules with the cationic groups on the support. In this paper the term mixed mode reversed phase/anion exchange will further be indicated as mixed mode.

In the literature a number of examples of mixed mode stationary phases have been reported. Some early examples of mixed mode columns are soft-gel anion exchange resins like DEAE-sephadex or \(-\)cellulose\(^3,4\). A disadvantage of these columns is the low pressure allowed across these columns. Another example is the RPC-5 column, this type of column consists of a nonporous spherical polymer support (polychlorotrifluoroethylene, Plaskon 2300) which is coated with trioctylmethyl ammonium chloride\(^3-5\). General drawbacks of these columns are bleeding of the ammonium groups from the column, and the nonporous support which is not of a constant quality.

Several authors prepared mixed mode columns based on silica gel supports which already showed one of the two retention mechanisms. Two methods were used by McLaughlin et al. to prepare these mixed mode stationary phases\(^6-9\). The first method was by modifying an anion exchange matrix with alkyl chains. A commercially available aminopropylsilyl bonded-phase silica (APS-hypersil) was reacted with several organic acids that contained hydrophobic sites as well as sites for ionic interactions\(^6-8\). Other authors also used this first method to prepare mixed mode silica gel supports by reacting an anion exchange silica support with octadecyltrichlorosilane\(^10\). The second method was to modify a reversed phase matrix by attaching anionic groups to the support. For this purpose ODS-hypersil was coated with trioctylmethyl ammonium chloride\(^8,9\).

Other authors used bare silica gels to prepare mixed mode stationary phases. Crowther et al.\(^11,12\) prepared mixed mode stationary phases containing C-8 groups and quaternary ammonium groups by a two-step procedure. Kopaciewicz et al.\(^13\) coated silica
with polyethyleneimmine, after crosslinking with diepoxides the coating was derivatized with monoepoxides to control the hydrophobicity of the coating. El Rassi et al.[14] prepared mixed mode phases with strong anion exchange/weak hydrophobic and weak anion exchange/weak hydrophobic properties on silica supports by initially attaching dimethylpropylsilane groups to the surface and subsequently binding polar moieties to the carbon chains.

The above described columns were mainly used for the separation of (oligo)nucleotides, RNA, DNA and peptides.

Another group of columns show, beside their main retention activity, also some secondary selectivity not intentionally introduced by the manufacturer, e.g. low capacity anion exchange columns show in many cases also some reversed phase properties. The Hamilton PRP-X100 is an example of these kinds of columns. Basically it is a low capacity anion exchange column however it also shows reversed phase properties because the sample solutes may undergo interactions with its hydrophobic matrix of polystyrene crosslinked with divinylbenzene. These reversed phase properties were demonstrated by several groups[15-19].

Mixing a reversed phase with an anion exchange stationary phase (mixed phase) or by connecting an anion exchange and a reversed phase column in series are other possibilities to achieve mixed mode properties. Comparing a mixed mode reversed phase/cation exchange with a mixed phase (reversed phase/cation exchange) and with a cation exchange- and a reversed phase column in series, Issaq et al.[20] observed the best results for the mixed mode column using antidepressants as the model compounds. The performance of the mixed phase column was less compared to that of the mixed mode column but the retention times were considerably shorter compared to the two columns in series. Crowther et al.[12] also observed that the mixed phase approach showed inferior results compared to mixed mode stationary phases.

A number of commercial mixed mode columns have been coming available over the last few years. Examples are the ABx columns of J.T. Baker[21,22] and the mixed mode RP C-4, C-8, C-18/Anion columns of Alltech[23,24]. These columns contain silica based stationary phases. Another example is the OmniPac PAX-500 of Dionex which is a polymer based mixed mode column. This column has a macroporous core of ethylvinylbenzene
Analysis of oligomers using mixed mode stationary phases

crosslinked with divinylbenzene showing reversed phase properties. To this macroporous core a latex with quaternary ammonium groups is attached giving the stationary phase its anion exchange properties[25-27].

In this research a number of columns were investigated on their mixed mode, in this case reversed phase/anion exchange, properties and their suitability to separate charged and neutral oligomers. Mainly polymer based columns were investigated because of the relatively high pH of the eluent used in this study. The columns were investigated on their hydrophobic, anion exchange and their mixed mode properties respectively by using three different test mixtures consisting of i. alkylbenzenes, alkylpyridines and alkylhydroxybenzoates; ii. a number of anorganic anions and iii. a number of alkyl sulfonic acids and styrene sulfates. Finally the results obtained on the investigated columns will be discussed.

2.3 EXPERIMENTAL

Instrumentation

The HPLC system consisted of a Bischoff HPLC pump (Bischoff, Leonberg, Germany), a Metrohm 690 Ion Chromatograph (Metrohm, Herisau, Switzerland), a Bischoff Lambda 1000 UV detector and two recorders (Kipp&Zonen, Delft, The Netherlands).

Columns

A number of columns were investigated on their hydrophobic, anionic and mixed mode properties. The OmniPac PAX-500 (250 x 4 mm ID) column was provided by Dionex, Breda, the Netherlands. Two specially prepared mixed phase columns were a gift of Polymer Labs (Shropshire, United Kingdom); the columns (150 x 4.6 mm ID) contained a mixture of 50% of PLRP-S, 8 μm, 1000Å particles and 50% of PLSAX, 8 μm, 1000Å particles as the stationary phase. The columns were slurry packed. Column 1 (PL1) was packed as reversed phase PLRP-S columns are usually packed, using acetonitrile/water (7/1 w/w) as the packing solvent. Column 2 (PL2) was packed as ion exchange PLSAX columns are
usually packed, using a buffer as the solvent. Also a number of low capacity anion exchange columns were tested: A Hamilton PRP-X100 (125 x 4 mm ID), (Hamilton, Nevada, USA); a HEMA-S 1000 Q-L, 7 and 10 μm, and a HEMA-BIO 1000 NH₂ 7 μm (150 x 3 mm ID) (Tessek, Prague, Czech Republic). The HEMA columns were a gift from Tessek.

In addition, also two non-polymer based columns were investigated, a Zorbax Bioseries Oligo (80 x 6.2 mm ID, 5 μm, 150Å) from Rockland Technologies Inc., Newport, USA, and an Aluspher RP-Select B (5 μm, 100Å, 119 x 4.6 mm ID) from Merck, Darmstadt, Germany. The Zorbax oligo is a silica based support of which the silica has a zirconia-treated surface which is stable up to a pH of 8.5, and was a gift from Rockland, Nuenen, the Netherlands. The Aluspher RP-Select B is an alumina based material which can be used in a pH range of 2-12 and was borrowed from Merck, Amsterdam, the Netherlands.

Chemicals

Methanol and acetonitrile, HPLC grade, were purchased from Merck. Tetrahydrofuran was purchased from Westburg, Leusden, the Netherlands. Water was purified using a Milli Q Water Purification System (Millipore Corporation, Milford, Massachusetts, USA). The aqueous buffers used consisted of solutions of ammonium carbonate (Merck), pH ~9, while this type of buffer can readily be removed after preparative scale separations. Before use, the eluents were filtered and degassed ultrasonically.

Benzene, toluene, ethylbenzene, propylbenzene, butylbenzene and p-styrene sulfate (styrene sulfate 1) were from Aldrich, Steinheim, Germany; methyl p-hydroxybenzoate and propyl p-hydroxybenzoate were from Sigma, St. Louis, USA; butane sulfonic acid was from Kodak Eastman, Rochester NY, USA; pentane sulfonic acid and octane sulfonic acid were from FSA Laboratorium Supplies, Loughborough, United Kingdom; dodecane sulfonic acid, sodium fluoride, sodium chloride, sodium nitrite, sodium nitrate, sodium sulfite and sodium sulfate (p.a.) were from Merck; o-pentylpyridine, o-hexylpyridine and 2-phenylethyl sulfate (styrene sulfate 2) were synthesised in our department.

Methods

The columns were tested in three modes: i. reversed phase, ii. anion exchange and
iii. mixed mode.

The reversed phase properties of the columns were tested using mixtures of an organic modifier (methanol, acetonitrile or tetrahydrofuran) and water as the eluents. As test compounds the alkylbenzenes, the alkylhydroxybenzoates and the alkylpyridines were used. The compounds were detected by UV detection at 254 nm. The column performance and the capacity factors of the compounds were measured at several eluent compositions. The hold up time of the column, $t_0$, was determined by injection of methanol.

The anion exchange properties of the columns were tested using aqueous solutions of ammonium carbonate as the eluents. For the Polymer Labs and the OmniPac columns 1% of organic modifier was added to the eluent to ensure a sufficient wetting of the columns. The anorganic anions were used as the test compounds. The compounds were detected using conductivity detection. The capacity factors of the compounds were measured for a range of ammonium carbonate concentrations. The $t_0$ was determined by injection of water.

The mixed mode properties of the columns were tested using mixtures of an organic modifier and an aqueous solution of a specific concentration of ammonium carbonate as the eluents. As test compounds the alkyl sulfonic acids and the styrene sulfates were used. The alkyl sulfonic acids were used as model compounds for butadiene oligomers and the styrene sulfates as model compounds for styrene oligomers. The detection of the alkyl sulfonic acids was performed by conductivity detection, while the styrene sulfates were detected by UV detection at 254 nm. The capacity factors of the test compounds were measured for concentrations of 1 to 90% of the organic modifiers in aqueous ammonium carbonate solutions of two concentrations, $5 \times 10^{-3}$ and $10^{-2}$ M. The column performance under mixed mode conditions was measured for the styrene sulfates. The $t_0$ was determined by injection of water.

Except for the HEMA columns the flow used in the above described experiments was 1.0 ml/min. In case of the HEMA columns a flow of 0.5 ml/min was used.

2.4 RESULTS AND DISCUSSION

Because only the PRP-X100, the OmniPac PAX-500 and the Polymer Labs columns
(PL1 and PL2) showed initially promising results for the separation of charged and uncharged oligomers, the results of the other columns used in this work will not be discussed in detail. The HEMA columns showed only few reversed phase properties and it was impossible to obtain baseline separation for the alkylbenzenes. The plate numbers for the alkylbenzenes were \(-2000-3000\) pt/m, assuming Gaussian peak shapes, and considerable fronting of the peaks occurred. It was possible to achieve some separation between the alkyl sulfonic acids but the peaks were very broad and the reproducibility was bad. Also the column performance decreased rapidly after only a few weeks of use, probably due to the fact that these columns were not suitable for the applied eluents[28]. With the Zorbax Oligo column it was only possible to obtain separation between the alkylbenzenes at very low organic modifier concentrations due to the low retention times of these compounds. This column showed almost no separation of the inorganic ions. Using this column in reversed phase and anion exchange mode these results could be expected because it consists of a normal silica based stationary phase. In the mixed mode test of this column the alkyl sulfonic acids and the styrene sulfates also showed low capacity factors so it was not possible to achieve a separation between these compounds. Finally the Aluspher Select-B also showed only low capacity factors for the alkyl sulfonic acids and styrene sulfates and therefore also this column was not suitable for these separations.

**Reversed phase mode:** To test the columns in the reversed phase mode first useful performance criteria were defined using the retention times and the resolution of the alkylbenzenes as the parameters. The criteria were to obtain a resolution as high as possible but larger than at least one with a retention time below 25 minutes for butylbenzene. In some cases, if it was not possible to obtain a resolution higher than one with these restrictions, a longer retention time was accepted.

The plate number \(N\) was calculated from:

\[
N = 5.54 \times \left( \frac{t_R}{w_h} \right)^2
\]

Where \(t_R\) the retention time and \(w_h\) the peak width at half height.

The asymmetry factor, \(asf\), was calculated at 10% of the peak height.
Analysis of oligomers using mixed mode stationary phases

The resolution $R_s$ was calculated using the following formula:

$$R_s = \frac{t_{R2} - t_{R1}}{(w_{h1} + w_{h2}) / 1.18}$$

Where $t_{R1}$ and $t_{R2}$ the retention time and $w_{h1}$, $w_{h2}$ the peak width at half height for respectively the compounds 1 and 2.

Under these conditions, the plate number ($N$), the resolution ($R_s$) and the asymmetry factor ($asf$) of the test compounds for the OmniPac, the PRP-X100 and the Polymer Labs (PL1 and PL2) columns were measured and are respectively given in the figures 2.1, 2.2 and 2.3 for the three different organic modifiers. The highest plate numbers were observed using acetonitrile as the organic modifier (figure 2.1). The plate numbers for methanol were very low, especially for the Polymer Labs columns. For the PL1 column it was impossible to obtain a resolution of one under these conditions. It is known from literature that polymeric based stationary phases show a poor performance when methanol is used as the organic modifier[29]. The plate numbers were also low for all columns using tetrahydrofuran as the organic modifier this in contrast with earlier published results[29]. No results are provided in this study for the OmniPac column using tetrahydrofuran because these columns are not compatible with eluent concentrations containing more than 10% tetrahydrofuran. Also the best resolution (figure 2.2) of the test compounds was observed using acetonitrile as the organic modifier. Better results in this respect were obtained for methanol compared with tetrahydrofuran. In almost all cases peak tailing on the investigated columns was observed, $asf > 1$ (figure 2.3). Comparing the two Polymer Labs columns, PL2 showed higher plate numbers but the $asf$ was also higher.

As mentioned the reversed phase performance of the columns was determined for the alkylbenzenes. With the alkylhydroxybenzoates and the alkylpyridines as the test compounds the behaviour of the columns for other types of compounds was investigated under the same conditions. As an example the retention times of the alkylbenzenes, the hydroxybenzoates and the pyridines for methanol as the organic modifier in the eluent are given in table 2.1 under the conditions used in the figures 2.1-2.3. The retention times on the Polymer Labs and the OmniPac columns were for both the hydroxybenzoates and the pyridines about the same as for benzene, as could be expected considering the polarity of the compounds. However, the
Figure 2.1: Plate numbers $N$ for benzene (ben), toluene (tol), ethylbenzene (eb), propylbenzene (pb), butylbenzene (bb), methyl p-hydroxybenzoate (mh), propyl p-hydroxybenzoate (ph), $o$-pentylpyridine (pp) and $o$-hexylpyridine (hp) in the reversed phase mode.

Eluents:  
- a. Methanol/water PL1, PL2: 80/20; PRP-X100: 90/10 and OmniPac PAX-500: 95/5 v/v  
- b. Acetonitrile/water PL1, PL2: 45/55; PRP-X100: 73/27; OmniPac PAX-500: 77/23 v/v  
- c. Tetrahydrofuran/water PL1, PL2: 30/70; PRP-X100: 38/62 v/v.
Analysis of oligomers using mixed mode stationary phases

Figure 2.2: Resolution $R_f$ between the solute pairs benzene-toluene (ben-tol), toluene-ethylbenzene (t-eb), ethylbenzene-propylbenzene (eb-pb), propylbenzene-butylbenzene (pb-bb), $p$-hydroxybenzoic acid methylster-$p$-hydroxybenzoic acid propylster (mh-ph) and a-pentylpyridine-o-hexylpyridine (pp-hp) in the reversed phase mode

Eluent: 
- a. Methanol/water
- b. Acetonitrile/water
- c. Tetrahydrofuran/water

Columns: PL1, PL2, PRP-X100 and OmniPac PAX-500. For experimental conditions see figure 2.1.
Figure 2.3: Asymmetry factors for benzene, toluene, ethylbenzene, propylbenzene, butylbenzene, $p$-hydroxybenzoic acid methylester, $p$-hydroxybenzoic acid propylester, $o$-pentylypyridine and $o$-hexylpyridine in the reversed phase mode.

Eluent:  
- a. Methanol/water
- b. Acetonitrile/water
- c. Tetrahydrofuran/water

Columns: PL1, PL2, PRP-X100 and OmniPac PAX-500. For experimental conditions see figure 2.1.
Analysis of oligomers using mixed mode stationary phases

Retention times on the PRP-X100 column were for the alkylhydroxybenzoates considerably larger than for the pyridines and were in the same range as butylbenzene suggesting a strong specific interaction of these esters to the PRP-X100 column.

On the PL1 column the propylester eluted before the methylester (table 2.1). For all the investigated columns it was observed that when the amount of organic modifier in the eluent was increased at a certain organic modifier concentration the propylester eluted before the methylester.

On these polymeric columns the hydroxybenzoates and the pyridines showed about the same performance compared with the alkylbenzenes, as can be seen in figures 2.1, 2.2 and 2.3. The columns did not show, in comparison with silica based reversed phase columns, a worse performance for basic compounds. This is because of the lack of interfering interactions of the basic pyridines with the free silanol groups present on silica surfaces[30]. The hydrophobicity of the columns increases in the range PL1, PL2, PRP-X100 and OmniPac PAX-500 taking into account the percentage organic modifier necessary to elute the alkylbenzenes from a column.

TABLE 2.1
RETENTION TIMES OF THE ALKYLBENZENES, THE HYDROXYBENZOATES AND THE PYRIDINES
Eluent: methanol/water: PRP-X100: 90/10 v/v; PL1,PL2: 80/20 v/v; and OmniPac PAX-500: 95/5 v/v

<table>
<thead>
<tr>
<th>Compound</th>
<th>PRP-X100 tₚ (min)</th>
<th>PL1 tₚ (min)</th>
<th>PL2 tₚ (min)</th>
<th>OmniPac PAX-500 tₚ (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>benzene</td>
<td>5.02</td>
<td>4.29</td>
<td>5.58</td>
<td>7.67</td>
</tr>
<tr>
<td>toluene</td>
<td>7.29</td>
<td>6.53</td>
<td>8.98</td>
<td>10.42</td>
</tr>
<tr>
<td>ethylbenzene</td>
<td>9.59</td>
<td>9.46</td>
<td>13.51</td>
<td>13.06</td>
</tr>
<tr>
<td>propylbenzene</td>
<td>13.59</td>
<td>14.35</td>
<td>20.82</td>
<td>16.94</td>
</tr>
<tr>
<td>butylbenzene</td>
<td>20.89</td>
<td>23.92</td>
<td>34.69</td>
<td>23.34</td>
</tr>
<tr>
<td>methylester</td>
<td>21.20</td>
<td>5.78</td>
<td>3.31</td>
<td>6.00</td>
</tr>
<tr>
<td>propylester</td>
<td>27.35</td>
<td>5.62</td>
<td>3.99</td>
<td>6.19</td>
</tr>
<tr>
<td>pentylpyridine</td>
<td>4.96</td>
<td>4.25</td>
<td>5.59</td>
<td>7.88</td>
</tr>
<tr>
<td>hexylpyridine</td>
<td>7.11</td>
<td>6.20</td>
<td>8.48</td>
<td>9.92</td>
</tr>
</tbody>
</table>

The following formula applies for the capacity factor as a function of the concentration organic modifier in the eluent for reversed phase stationary phases:
\[ \log k' = a - m \cdot x \]

Where \( x \) the concentration of the organic modifier in the eluent, \( k' \) the capacity factor, \( a \) and \( m \) are constants. Bowers et al.[29] showed that this formula also applies using polystyrene based reversed phase columns. This equation also proved to be valid for the data measured on the PL1, PL2, PRP-X100 and the OmniPac PAX-500 columns for all the three organic modifiers used, as is shown in figure 2.4.

Also generally for reversed phase columns the logarithm of the capacity factors of homologous series are linearly dependent on the length of the alkyl chain[31]. Also this relationship proved to be valid for the columns used in this study using alkylbenzenes as the homologous series (figure 2.5).

From the data it can be concluded that the PRP-X100, the OmniPac PAX-500 and both the Polymer Labs columns showed typical reversed phase behaviour when tested in the reversed phase mode.

**Ion exchange mode:** Equation (2.1) holds for ions eluting from an ion exchange column[32].

\[ \log k' = \frac{f}{b} \log C - \frac{f}{b} \log [E^-] + \text{constant} \quad (2.1) \]

Where \( C \) the resin capacity, \([E^-]\) the ion concentration in the eluent, \( f \) the charge of the solute ion and \( b \) the charge of the eluent ion.

This relation proved also to be valid for the investigated columns (figure 2.6). This result could be expected for the PRP-X100 column because it was developed specifically for anion exchange separations. For the Polymer Labs columns this means that the reversed phase part of the stationary phases do not influence the anion exchange behaviour of these columns. The anion exchange behaviour of the OmniPac PAX-500 was already shown in literature[25]. The slopes and the correlation coefficients (\( R \)-values) of the lines in figure 2.6, calculated by using linear regression, are presented in table 2.II. The low value for \( R \) in case of the fluoride ion for the OmniPac column is caused by the low retention times, so
Figure 2.4: Logarithm of the capacity factor $k'$ versus the percentage organic modifier in the eluent. Compounds: toluene and butylbenzene. Columns: PL1, PL2 and OmniPac PAX-500.

△ toluene on PL1, ○ toluene on PL2, □ toluene on OmniPac, ● butylbenzene on PL1, ▷ butylbenzene on PL2 and □ butylbenzene on OmniPac
Figure 2.5: Logarithm of the capacity factor $k'$ versus the length of the alkyl chain of a homologous series of alkylbenzenes. Compounds: benzene, toluene, ethylbenzene, propylbenzene and butylbenzene. Columns: PL1, PL2, PRP-X100 and OmniPac PAX-500.

Eluent:  
a. Methanol/water PL1(+), PL2(●): 80/20 v/v; PRP-X100(●): 90/10 and OmniPac PAX-500(●): 95/5 v/v  
b. Acetonitrile/water PL1(+), PL2(●): 45/55 v/v; PRP-X100(●): 73/27 v/v; OmniPac PAX-500(●): 77/23 v/v  
c. Tetrahydrofuran/water PL1(+), PL2(●): 30/70 v/v; PRP-X100(●): 38/62 v/v
Figure 2.6: Logarithm of the capacity factor versus the logarithm of the carbonate concentration in the eluent, the column is in the anion exchange mode. Eluent: ammonium carbonate, for PL1, PL2 and OmniPac PAX-500 1% methanol was added. Compounds: Fluoride(+), chloride(●), nitrite(□), nitrate(▲), sulfite(♦) and sulfate(●). Columns: a. PRP-X100, b. OmniPac PAX-500, c. PL1 and d. PL2.
interaction occurs of the fluoride peak with the system peaks yielding inaccurate results for the retention times. The slope was in all cases $\sim 1$ for the monovalent solute anions. Inspecting equation (2.1) this means that the eluent is in the bicarbonate form, as can be expected at a pH of $\sim 9$. The slope was smaller than -1 but not -2 as should be expected when divalent anions are used as test compounds. This implies that the divalent anions are not retained as divalent anions. To be retained as a divalent anion it must face two adjacent anion exchange sites on a stationary phase that are appropriately spaced[25]. This is apparently not the case.

In table 2.II capacity factors and plate numbers of the columns for nitrate are given. The poorest anion exchange performance was observed for the OmniPac column using these eluent mixtures. However, it should be emphasized that the OmniPac column is specifically designed for hydroxide as the eluent anion[25]. Better results were observed for the Polymer Labs columns, especially when higher buffer concentrations were used. In this case short retention times were observed, indicating that the reversed phase stationary phase has a negative influence on the performance in the anionic mode. The results for the PRP-X100 column were constant over the ionic strength range and were therefore better at lower buffer concentrations and worse at the higher buffer concentrations.

To find out whether there is a difference between the nature of the organic modifier used for wetting the OmniPac and the Polymer Labs columns the plate numbers and the retention times for nitrate were measured for 1% methanol and for 1% acetonitrile added to the eluent, table 2.III. The difference in retention time using methanol or acetonitrile was
small. Somewhat higher plate numbers were observed for the OmniPac column when wetting this column with acetonitrile. The plate numbers for the Polymer Labs columns were higher using 1% methanol instead of 1% acetonitrile, especially for the PL1. This is in contrast with the results observed for the reversed phase behaviour of these columns observed in this study and in the literature[29].

**TABLE 2.11**

**PLATE NUMBERS N AND CAPACITY FACTORS k' OF NITRATE**

Eluent: Ammonium carbonate in water; for the OmniPac PAX-500 and the Polymer Labs columns 1% organic modifier was added to the eluent. Unless otherwise noted methanol was as organic modifier.

<table>
<thead>
<tr>
<th>(NH₄)₂CO₃ concentration [mol/l]</th>
<th>OmniPac N[pt/m] k'</th>
<th>PL1 N[pt/m] k'</th>
<th>PL2 N[pt/m] k'</th>
<th>PRP-X100 N[pt/m]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.10⁻³</td>
<td>2286</td>
<td>-</td>
<td>953</td>
<td>-</td>
</tr>
<tr>
<td>2.5.10⁻³</td>
<td>1352</td>
<td>1347</td>
<td>1168</td>
<td>2172</td>
</tr>
<tr>
<td>3.10⁻³</td>
<td>-</td>
<td>2453</td>
<td>345</td>
<td>-</td>
</tr>
<tr>
<td>5.10⁻³</td>
<td>1566</td>
<td>5612</td>
<td>3845</td>
<td>2178</td>
</tr>
<tr>
<td>7.5.10⁻³</td>
<td>1621</td>
<td>14959</td>
<td>7447</td>
<td>2166</td>
</tr>
<tr>
<td>7.5.10⁻³ (1% ACN)</td>
<td>1818</td>
<td>10218</td>
<td>7239</td>
<td>2.89</td>
</tr>
</tbody>
</table>

**Mixed mode:** As indicated above in reversed phase chromatography the logarithm of the capacity factors of the test compounds versus the percentage organic modifier in the eluent provides a straight line and in anion exchange chromatography the logarithm of the capacity factor is linearly dependent on the logarithm of the anion concentration in the eluent. These linear dependencies were not observed for the PL2 column when used under mixed mode conditions, figure 2.7a and b. Also the other columns exhibited typical mixed mode behaviour as is shown in figure 2.7c, d and e. At lower organic modifier concentrations the sulfonic acids and the styrene sulfates exhibit 'reversed phase' properties: the capacity factor decreases with increasing organic modifier concentration. Here the limiting factor for elution is the organic modifier concentration. At higher concentrations organic modifier, the capacity factor increases rapidly for all components. In this case the anion concentration in the eluent is the limiting factor for elution. In appendix 2.1 a complete overview is presented of the retention behaviour of the model compounds for all the three organic modifiers, methanol, acetonitrile and tetrahydrofuran, under mixed mode conditions.
Figure 2.7: Influence of the concentrations of the organic modifier and ammonium carbonate in the eluent under mixed mode conditions.

a. PL2, percentage organic modifier versus the logarithm of the capacity factor; organic modifier: acetonitrile, concentration (NH₄)₂CO₃ in water: 10⁻² M

b. PL2, logarithm of the concentration (NH₄)₂CO₃ versus the logarithm of the capacity factor; organic modifier: acetonitrile, concentration (NH₄)₂CO₃ in water: 10⁻² M

c. PL1, percentage organic modifier versus the logarithm of the capacity factor; organic modifier: acetonitrile, concentration (NH₄)₂CO₃ in water: 10⁻² M

d. OmniPac PAX-500, percentage organic modifier versus the logarithm of the capacity factor; organic modifier: acetonitrile, concentration (NH₄)₂CO₃ in water: 10⁻² M

e. PRP-X100, percentage organic modifier versus the logarithm of the capacity factor; organic modifier: methanol, concentration (NH₄)₂CO₃ in water: 10⁻² M

+ butane sulfonic acid, △ pentane sulfonic acid, ○ octane sulfonic acid, □ dodecane sulfonic acid, ◆ styrene sulfate 1, ● styrene sulfate 2 and ■ fluoride
Analysis of oligomers using mixed mode stationary phases

The same dependency of the retention mechanism with the organic modifier concentration was also observed by for instance Grego et al.[33]. They observed for polypeptide hormones on a reversed phase column at low concentrations of the organic modifier reversed phase behaviour of the column while at higher concentrations the ionogenic polypeptide hormones showed polar interactions with the column.

The fluoride ion also deviates from the usual ion exchange behaviour using a combination of buffer and an organic modifier as the eluent, as is shown in figure 2.7b. This is caused by the fact that the organic modifier present in the eluent will induce a decreased hydration of the solute anion which results in an increase in the retention time[34]. The change of the capacity factors of the alkyl sulfonic acids as a function of the shorter alkyl chains with the concentration of the organic modifier shows in some cases similar behaviour as the fluoride ion. In these cases the alkyl chain is probably too short to have a substantial interaction with the hydrophobic backbone of the stationary phase.

The separation factor $\alpha$ for the solute pairs butane/pentane sulfonic acid and butane/octane sulfonic acid using methanol or acetonitrile as the organic modifier in two aqueous ammonium carbonate solutions of concentrations of respectively $5 \times 10^{-3}$ and $10^{-2}$ M as the eluents is presented in figures 2.8 and 2.9. In all cases the separation factor was higher for methanol compared to acetonitrile. Also $\alpha$ was higher using a higher anion concentration in the eluent. This is another proof for the mixed mode behaviour of the columns, no difference in $\alpha$ would be obtained when only anion exchange as the retention mode was active. This can be explained as follows. Combining equation (2.1) and equation (2.2) for $\alpha$, with $k_1'$ and $k_2'$ as the capacity factors for component 1 and 2 respectively:

$$\alpha = \frac{k_1'}{k_2'} \quad (2.2)$$

gives:
Figure 2.8: Separation factor $\alpha$ as function of the organic modifier concentration in the eluent. Ammonium carbonate concentration in water was $5 \times 10^{-3}$ M. Columns: PRP-X100 (+), PL1 (+), PL2 (x) and OmniPac PAX-500 (●).

a. Methanol, $\alpha$ butane sulfonic acid-pentane sulfonic acid  
b. Methanol, $\alpha$ butane sulfonic acid-octane sulfonic acid  
c. Acetonitrile, $\alpha$ butane sulfonic acid-pentane sulfonic acid  
d. Acetonitrile, $\alpha$ butane sulfonic acid-octane sulfonic acid.
Figure 2.9: Separation factor $\alpha$ as function of the organic modifier concentration in the eluent. Ammonium carbonate concentration in water was $10^{-2}$ M. Columns: PRP-X100 (●), PL1 (+), PL2 (▲) and OmniPac PAX-500 (○).

a. Methanol, $\alpha$ butane sulfonic acid-pentane sulfonic acid
b. Methanol, $\alpha$ butane sulfonic acid-octane sulfonic acid
c. Acetonitrile, $\alpha$ butane sulfonic acid-pentane sulfonic acid
d. Acetonitrile, $\alpha$ butane sulfonic acid-octane sulfonic acid.
Where $K_1$ and $K_2$ the constants for the compounds 1 and 2 respectively. From the resulting equation (2.3) it is obvious that $\alpha$ is independent of the eluent ion concentration. Also no difference in $\alpha$ would be observed when only reversed phase behaviour would take place. Because no difference in the capacity factors between the two ion concentrations in the eluent would be observed when the same concentration organic modifier was used. The increase in $\alpha$ could be explained with a combination of these two retention modes. Considering the solute pair butane and octane sulfonic acid the octane sulfonic acid will show more reversed phase behaviour and less anion exchange behaviour. The influence of increasing the anion concentration in the eluent will decrease the capacity factor less compared to the butane sulfonic acid which shows less reversed phase and more anion exchange behaviour. Besides the higher $\alpha$-values another advantage of higher anion concentrations in the eluent are the lower retention times of the test compounds.

Next the efficiency of the columns for styrene sulfate 1 and 2 was investigated. The plate numbers and the asymmetry factors for the styrene sulfates are presented in table 2.IV for the OmniPac PAX-500 and the PL2 columns using methanol and acetonitrile as the organic modifier in the eluent. A higher plate number was observed in almost all cases for styrene sulfate 1 compared to styrene sulfate 2 which is probably caused by the differences of the location of the sulfate group on the styrene molecule. The $\alpha_{sf}$ was, for high or low concentrations of an organic modifier, also better for styrene sulfate 1. For styrene sulfate 2 high asymmetry factors were observed using low organic modifier concentrations while at high organic modifier concentrations asymmetry factors below one were observed for this compound. No significant difference in performance was observed between methanol and acetonitrile when used as the organic modifier in the eluent, this in contrast with the reversed phase and the anion exchange mode. The retention times between the two styrene sulfates differed in most cases not more than 10%, figure 2.7. The plate numbers for the alkyl sulfonic acids were not measured because of the interference of system peaks which occurs using a conductivity detector.
Analysis of oligomers using mixed mode stationary phases

TABLE 2.IV
PLATE NUMBERS AND ASYMMETRY FACTORS OF STYRENE SULFATE 1 AND 2 ON THE OMNIPAC PAX-500 AND THE PL2 COLUMNS

Eluent: mixture methanol or acetonitrile as the organic modifier and an aqueous ammonium carbonate solution; the ammonium carbonate concentration in aqueous buffer was $10^{-2}$ M

<table>
<thead>
<tr>
<th>percentage organic modifier [%]</th>
<th>solute</th>
<th>OmniPac</th>
<th></th>
<th>PL2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Methanol</td>
<td>asf</td>
<td>Acetonitrile</td>
<td>asf</td>
</tr>
<tr>
<td>10</td>
<td>styrene sulfate 1</td>
<td>-</td>
<td>-</td>
<td>16824</td>
<td>1.38</td>
</tr>
<tr>
<td></td>
<td>styrene sulfate 2</td>
<td>-</td>
<td>-</td>
<td>1873</td>
<td>10.70</td>
</tr>
<tr>
<td>30</td>
<td>styrene sulfate 1</td>
<td>13348</td>
<td>3.19</td>
<td>16144</td>
<td>1.93</td>
</tr>
<tr>
<td></td>
<td>styrene sulfate 2</td>
<td>1621</td>
<td>6.25</td>
<td>3999</td>
<td>5.02</td>
</tr>
<tr>
<td>50</td>
<td>styrene sulfate 1</td>
<td>2728</td>
<td>2.50</td>
<td>10066</td>
<td>2.43</td>
</tr>
<tr>
<td></td>
<td>styrene sulfate 2</td>
<td>2270</td>
<td>5.40</td>
<td>4608</td>
<td>0.56</td>
</tr>
<tr>
<td>70</td>
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<td>16538</td>
<td>2.11</td>
<td>10181</td>
<td>0.89</td>
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<tr>
<td></td>
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<td>3478</td>
<td>3.26</td>
<td>960</td>
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<tr>
<td>90</td>
<td>styrene sulfate 1</td>
<td>13345</td>
<td>1.16</td>
<td>18584</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>styrene sulfate 2</td>
<td>998</td>
<td>3.63</td>
<td>246</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>percentage organic modifier [%]</th>
<th>solute</th>
<th>Methanol</th>
<th>asf</th>
<th>Acetonitrile</th>
<th>asf</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>styrene sulfate 1</td>
<td>8964</td>
<td>3.16</td>
<td>8466</td>
<td>1.76</td>
</tr>
<tr>
<td></td>
<td>styrene sulfate 2</td>
<td>1043</td>
<td>9.45</td>
<td>1887</td>
<td>3.73</td>
</tr>
<tr>
<td>30</td>
<td>styrene sulfate 1</td>
<td>8316</td>
<td>1.25</td>
<td>8609</td>
<td>1.71</td>
</tr>
<tr>
<td></td>
<td>styrene sulfate 2</td>
<td>6328</td>
<td>2.20</td>
<td>3927</td>
<td>1.01</td>
</tr>
<tr>
<td>50</td>
<td>styrene sulfate 1</td>
<td>7211</td>
<td>1.31</td>
<td>8455</td>
<td>1.64</td>
</tr>
<tr>
<td></td>
<td>styrene sulfate 2</td>
<td>6461</td>
<td>1.39</td>
<td>1190</td>
<td>0.28</td>
</tr>
<tr>
<td>70</td>
<td>styrene sulfate 1</td>
<td>6943</td>
<td>1.29</td>
<td>14333</td>
<td>1.22</td>
</tr>
<tr>
<td></td>
<td>styrene sulfate 2</td>
<td>7605</td>
<td>-</td>
<td>349</td>
<td>0.24</td>
</tr>
<tr>
<td>90</td>
<td>styrene sulfate 1</td>
<td>7588</td>
<td>1.18</td>
<td>21288</td>
<td>0.76</td>
</tr>
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<td>styrene sulfate 2</td>
<td>984</td>
<td>3.26</td>
<td>455</td>
<td>0.18</td>
</tr>
</tbody>
</table>

In conclusion, all the columns were able to separate butane from octane sulfonic acid, a separation factor higher than one could be obtained using low concentrations of an organic modifier, figure 2.8 and 2.9. Therefore with the investigated columns it should be possible to separate the sulfate monomer from the dimer of butadiene.

For the PRP-X100 column the retention times for dodecane sulfonic acid and the styrene sulfates were for all the eluent mixtures used very high, figure 2.7e. For butadiene
It is known that oligomers at least up to \( n = 4 \), of both monosulfates and disulfates, are formed \([1,35,36]\). For styrene sulfate oligomers up to \( n = 2 \) or 3 are formed \([1,37]\). This implies that with the PRP-X100 columns the separation of these oligomers would be very time consuming. One way to decrease the retention times of these compounds is to increase the ammonium carbonate concentration in the eluent. There are two problems concerning this solution. With the increase of the ammonium carbonate concentration conductivity detection becomes difficult as the conductivity of the eluent may exceed the range of the conductivity detector. Second, using a higher ammonium carbonate concentration yielded a more instable base line due to a larger decrease in conductivity by the decomposition of the eluent. This also caused a decrease in the reproducibility of the retention times.

The Polymer Labs columns showed reasonable retention times for the alkyl sulfonic acids and consequently are more useful for this separation. However the selectivity between the butane and the pentane sulfonic acid is low, \( \alpha = 1 \), figure 2.8 and 2.9, so probably it will be a problem to separate isomers formed during the polymerization. In this study the mixed phase approach, the Polymer Labs columns, showed comparable results to the mixed mode approach. The difference in retention times between the PRP-X100 and the Polymer Labs columns could be explained considering the resin capacity of the columns. The resin capacity of the PRP-X100 is \( \sim 290 \mu\text{eq/ml}[25] \). The resin capacity of PLSAX is \( >200\mu\text{eq/ml}[38] \), resulting in a resin capacity of \( >100\mu\text{eq/ml} \) for the Polymer Labs columns. From equation (2.1) it is clear that increasing the resin capacity of a column increases the retention times of alkyl sulfonic acids as was also shown by Pietrzyk et al.\([39]\).

For the OmniPac column it is possible to separate butane from pentane sulfonic acid and to obtain reasonable retention times for the larger alkyl sulfonic acids, figure 2.7d. Here a disadvantage is that to perform the separation of the smaller and the larger alkyl sulfonic acids in one analysis it is necessary to use a gradient.

### 2.5 CONCLUSIONS

A number of columns were investigated with respect to their ability to separate
Analysis of oligomers using mixed mode stationary phases

charged and uncharged oligomers formed during emulsion polymerization processes. The PRP-X100, the OmniPac and the Polymer Labs columns showed promising results with respect to the possibility to separate charged and uncharged oligomers of butadiene and styrene. The columns showed typical reversed phase, anion exchange or mixed mode behaviour when respectively tested under reversed phase, anion exchange or mixed mode conditions. As expected, the reversed phase quality of the columns was not comparable to silica based reversed phase materials. Under mixed mode conditions it was shown that manipulation of the eluent, i.e. the ionic strength and the nature and the concentration of the organic modifier therein, provides good possibilities to separate charged oligomers. It was possible to obtain a separation factor large enough to enable the separation between charged oligomers. The other investigated columns, the Tessek HEMA, the Aluspher select B and the Zorbax Bioseries Oligo, were less useful for the separations of oligomers used under the conditions applied in this study.

2.6 REFERENCES


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APPENDIX 2.1: MIXED MODE EXPERIMENTS

2.1.1 INTRODUCTION

In chapter two some examples of mixed mode behaviour of the PRP-X100, PL1, PL2 and the OmniPac PAX-500 were presented in figure 2.7 using model compounds. In this appendix an overview of the mixed mode behaviour of these columns is presented using the three organic modifiers (methanol, acetonitrile and tetrahydrofuran) in the eluent. The organic modifier was mixed with aqueous ammonium carbonate solutions in a ratio of 1/99 to 90/10 v/v. Using these percentages organic modifier, the retention times of the model compounds were measured at two concentrations of ammonium carbonate in the aqueous phase: $5 \times 10^{-3}$ and $10^{-2}$ M.

2.1.2 MIXED MODE RESULTS

In figures 2.1.1 to 2.1.4 the mixed mode results for respectively the PL1, PL2, PRP-X100 and the OmniPac PAX-500 are presented. The experimental conditions were:

- a. organic modifier: methanol; concentration ammonium carbonate in the aqueous phase: $5 \times 10^{-3}$ M
- b. organic modifier: methanol; concentration ammonium carbonate in the aqueous phase: $10^{-2}$ M
- c. organic modifier: acetonitrile; concentration ammonium carbonate in the aqueous phase: $5 \times 10^{-3}$ M
- d. organic modifier: acetonitrile; concentration ammonium carbonate in the aqueous phase: $10^{-2}$ M
- e. organic modifier: tetrahydrofuran; concentration ammonium carbonate in the aqueous phase: $5 \times 10^{-3}$ M
- f. organic modifier: tetrahydrofuran; concentration ammonium carbonate in the aqueous phase: $10^{-2}$ M

+ butane sulfonic acid, a pentane sulfonic acid, O octane sulfonic acid, □ dodecane sulfonic acid, * styrene sulfonate 1, ● styrene sulfonate 2 and ▲ fluoride
Figure 2.1.1: Influence of the concentration and the kind of organic modifier (methanol, acetonitrile and tetrahydrofuran) and the ammonium carbonate concentration in the eluent under mixed mode conditions for the PL1 column.
Figure 2.1.2: Influence of the concentration and the kind of organic modifier (methanol, acetonitrile and tetrahydrofuran) and the ammonium carbonate concentration in the eluent under mixed mode conditions for the PL2 column.
Figure 2.1.3: Influence of the concentration and the kind of organic modifier (methanol, acetonitrile and tetrahydrofuran) and the ammonium carbonate concentration in the eluent under mixed mode conditions for the PRP-X100 column. No peaks were observed under the conditions used in c, e and f.
Figure 2.1.4: Influence of the concentration and the kind of organic modifier (methanol, acetonitrile and tetrahydrofuran) and the ammonium carbonate concentration in the eluent under mixed mode conditions for the OmniPac PAX-500 column. No experiments were performed using tetrahydrofuran as the organic modifier because this column was not suitable for eluents with more than 10% tetrahydrofuran.
CHAPTER THREE: EVALUATION OF THE PERFORMANCE OF THE ANNULAR EXPANSION SYSTEM FOR PREPARATIVE LIQUID CHROMATOGRAPHY

3.1 SUMMARY

Because it is necessary in a later stage to scale up the separation of the oligomers, a preparative column system, the Annular Expansion (A/E) system, was evaluated. The column system showed good efficiencies, reduced plate heights of 2-3 were observed, and the capacity factors were reproducible for several repacking steps. Using the 'true' plate heights it was shown that the conclusions that can be drawn from the theoretical plate heights were misleading.

3.2 INTRODUCTION

One of the major problems encountered in the operation of preparative high performance liquid chromatography (HPLC) is the lack of the long term stability of the applied columns. The background of this problem is not fully understood yet nor clearly related to any factor of the packing process. The phenomena involved in the preparation and maintaining of preparative HPLC columns are very complex and only very few systematic studies have been undertaken to investigate them. During the operation of a preparative column a zone of low density packing appears to be formed at the column inlet resulting in the formation of a void at the beginning of the column[1]. This causes considerable back mixing of the sample resulting in a strong drop in the column performance. Especially the large inner diameter analogues of conventional analytical packed columns may suffer of this problem. Moreover, this often results in a considerable waste of packing material since this seldom will be reused.

Compression technology has been developed to overcome this problem. This technique includes the compression of the chromatographic bed in the column to prevent and to repair void volumes in it. Some manufacturers only use bed compression during the packing of the column while others also compress the bed during the chromatographic
Evaluation of the performance of the A/E system for preparative liquid chromatography process. This latter approach will also remove the voids formed during the use of the column[2]. Compression can be accomplished either radially or axially or by a combination of both. An example of a column system in which radial compression is applied during the chromatographic process are the radial compression module (RCM) cartridges from Waters Millipore[3]. Axial compression during the chromatographic process is for instance applied in the preparative column systems from Prochrom and Rainin[1,2]. A general disadvantage of applying the compression technique is the possibility of crushing the particles which may lead to permeability and performance problems. Of course this strongly depends on the hardness of the applied packing material.

A combination of both radial and axial compression of the chromatographic bed during the packing and the chromatographic process is applied in the user-packable Annular Expansion (A/E) system, which was investigated in this study. In the A/E system hydraulic action drives a piston and a tapered shaft. As the piston moves upward, it axially compresses the packing; as the tapered shaft moves into the column, it radially compresses the surrounding packing toward the column wall. Up to now only few literature references are available concerning the performance and the reproducibility of the packing procedure of A/E columns. Lawing et al.[4] used an improved method to pack A/E columns by adding surfactants to the packing slurry. Improved reproducibility and performance of the columns were observed. In this study the reproducibility of a specific packing procedure of an A/E column system was investigated. Moreover, the possibilities of the reuse of packing materials was studied and the column performance was compared to columns prepared with fresh packing materials.

Also in this study the influence of the equation used for the calculation of the plate height was investigated. In most studies the theoretical plate height, \( H \), based on Gaussian shaped peaks, is used in analytical HPLC and in preparative HPLC as well[5-9]. The theoretical plate height can be determined by using equation (3.1):

\[
H = \frac{L}{N}
\]  

(3.1)

where \( L \) is the length of the column and \( N \) the number of theoretical plates which can be calculated as from:
where $t_R$ is the retention time of the solute and $w_h$ is the peak width at half height.

Experimentally Gaussian peak shapes are rarely observed due to various intra and extra column sources of asymmetry. This may lead to an overestimation of the plate count of more than 100%[10]. Better methods to describe the distribution properties of a peak, without making assumptions about its exact mathematical description are for instance the statistical moments method and the method developed by Foley and Dorsey which takes into account the peak asymmetry[11]. Using this last method the 'true' plate height, $H_{\text{sys}}$, can be determined using equation (3.3)

$$H_{\text{sys}} = \frac{L}{N_{\text{sys}}} \tag{3.3}$$

and equation (3.4)

$$N_{\text{sys}} = \frac{41.7 \times \left(\frac{t_R}{w_0.1}\right)^2}{(1.25 + \text{asf})} \tag{3.4}$$

where $N_{\text{sys}}$ the 'true' plate number, $w_{0.1}$ the peak width at 10% of the peak height and asf the asymmetry factor calculated at 10% of the peak height. Equation (3.4) calculates the number of plates accurately to within $\pm 1.5\%$ for $1.00 \leq \text{asf} \leq 2.76$[11] and is therefore one of the most accurate methods for the determination of the number of plates[12]. To make a better comparison possible between the efficiency of an analytical column and a preparative column reduced plate heights can be used. The reduced plate height can be calculated as follows.

$$h_x = \frac{H_x}{d_p}$$

where $H_x$ is the theoretical or the 'true' plate height, $h_x$ is the reduced theoretical or 'true' plate height and $d_p$ is the particle diameter of the packing material.

In this study the column efficiencies were determined by assuming Gaussian peak
shapes as well as by taking into account the asymmetry of the peaks by using the Foley/Dorsey method. The difference in the conclusions that can be drawn from the two different reduced plate heights, $h$ and $h_{sys}$, will be discussed.

3.3 EXPERIMENTAL

Instrumentation

The preparative HPLC experiments were performed on a Waters Delta Prep 4000 equipped with a Waters 486 Tunable Adsorbance detector (Millipore Corporation, Milford, Massachusetts, USA) and a recorder (Kipp&Zonen).

Columns

The investigated preparative column system was the Annular Expansion system, 250 x 50 mm ID (Septech, Merck, Darmstadt, Germany) borrowed from Merck, Amsterdam, the Netherlands. LiChrospher RP-18, 15 μm (Merck), was used as the packing material.

Chemicals

Methanol, HPLC grade, and aceton, p.a., were from Merck. Toluene, propylbenzene and butylbenzene were from Aldrich, Steinheim, Germany. Uracil was from Fluka AG, Buch SG Switzerland. Water was purified using a Milli Q Water Purification system (Millipore Corporation). Before use, the eluents were filtered and degassed by purging with helium.

Methods

The packing procedure for the A/E column was as follows. A slurry, containing 250 g of the packing material (LiChrospher RP-18) and aceton, of a total volume of 750 ml was prepared. The mixture was placed in an ultrasonic bath for 30 minutes and after that shaken vigorously to obtain a good wetting and dispersion of the particles. Next the slurry was poured into the column body with a reservoir attached to it on top (figure 3.1a). The aceton was removed using a running water aspirator connected with the column inlet. When all the aceton had been aspirated the vacuum was disconnected. The excess of the packing was
removed and the frit and the outlet cap were placed on top of the packing (figure 3.1b). After closing, the column was compressed using a hydraulic pump (Dayton, Chicago, USA). The column was compressed at a rate of about one stroke of the handle per 10 seconds until a pressure of 1100 psi was obtained. After that the safety stop was tightened by hand. After 10 minutes the column was recompressed to 1100 psi and this was repeated after having pumped one column volume of eluent through the column. During the first day no measurements were performed and the bed was given time to settle. After that, each day the column was used it was recompressed to 1100 psi prior to use.

The column performance and the reproducibility of the packing method was tested using 80/20 v/v methanol/water as the eluent. Toluene, propylbenzene and butylbenzene were applied as the test compounds and detected using UV detection at 254 nm. The flow through the column was in between 10-90 ml/min. The hold up time of the column, $t_0$, was calculated by injection of uracil.
3.4 RESULTS AND DISCUSSION

The quality of the column was judged on the minimum reduced theoretical and minimum reduced 'true' plate height, respectively $h^{\text{min}}$ and $h_{\text{sys}}^{\text{min}}$, and the magnitude and on the reproducibility of the capacity factors of the test compounds. The minimum reduced theoretical and the 'true' plate height of the column were determined graphically by plotting the reduced plate height versus the eluent velocity[13]. At least 12 measurements were performed to draw the reduced plate height vs eluent velocity graph, which was reported to be more than sufficient for the determination of the minimum reduced plate height[9].

The A/E column was packed six times reusing the same packing material. The results of these experiments 1.1-1.6 are summarized in table 3.1. The column was also packed with a fresh amount of the same packing material to investigate the difference in performance and the reproducibility using fresh and used packing material. The results of these latter experiments 2.1 and 2.2 are also presented in table 3.1. The column performance was good, minimum reduced plate heights of 2-3, for $h$ as $h_{\text{sys}}$ as well, were observed. However, after several times of repacking the column with the same material the column performance gradually decreased. At the same time we observed an increased pressure drop across the column.

Comparing the results observed for the theoretical, $h$, and the 'true', $h_{\text{sys}}$, reduced plate height values it appears that when the theoretical plate height was used as a measure for the column efficiency the conclusion would be that the column performance was good for the first four packing steps while using the 'true' reduced plate height it can be concluded that the performance decreased after only two packing steps due to large differences in $\text{asf}$ that were observed during the different experiments.

The decrease in efficiency was probably due to crushing of the silica particles due to the repacking and recompressing of the column. This was confirmed by the observation that by repacking the column with used material, small particles, 'fines', passed through the frits. This was in contrast with the results observed by Lawing et al.[4] who reported no noticeable creation of 'fines' when using several packing materials. In principle the crushing of the particles can be reduced by recompressing the column after packing at a lower pressure[14]. To investigate whether the decrease of the compressing pressure influences the column performance the plate heights were measured as a function of the compression pressure. In
TABLE 3.1
THE CAPACITY FACTORS, $k'$, MINIMUM REDUCED PLATE HEIGHTS, $h$ AND $h_{sys}$, AND ASYMMETRY FACTORS, $asf$, FOR ALKYLBENZENES DETERMINED ON THE ANNULAR EXPANSION COLUMN
Eluent: methanol/water 80/20, experiments 1.1-1.6: repacking with the same material (LiChrospher RP-18 15 μm);
2.1-2.2: repacking with a new batch of the same material

<table>
<thead>
<tr>
<th>time</th>
<th>1.1</th>
<th>1.2</th>
<th>1.3</th>
<th>1.4</th>
<th>1.5</th>
<th>1.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k'$ toluene</td>
<td>1.81 ± 0.02</td>
<td>1.80 ± 0.044</td>
<td>1.79 ± 0.007</td>
<td>1.81 ± 0.0092</td>
<td>1.80 ± 0.02</td>
<td>1.80 ± 0.014</td>
</tr>
<tr>
<td>propy Benzene</td>
<td>3.69 ± 0.055</td>
<td>3.67 ± 0.032</td>
<td>3.62 ± 0.028</td>
<td>3.69 ± 0.026</td>
<td>3.67 ± 0.050</td>
<td>3.66 ± 0.030</td>
</tr>
<tr>
<td>buty Benzene</td>
<td>5.51 ± 0.089</td>
<td>5.48 ± 0.045</td>
<td>5.39 ± 0.063</td>
<td>5.52 ± 0.041</td>
<td>5.47 ± 0.081</td>
<td>5.45 ± 0.053</td>
</tr>
<tr>
<td>asymmetry factor</td>
<td>1.30 ± 0.089</td>
<td>0.92 ± 0.044</td>
<td>0.58 ± 0.053</td>
<td>0.69 ± 0.050</td>
<td>1.06 ± 0.23</td>
<td>0.80 ± 0.038</td>
</tr>
<tr>
<td>$h_{min}$ toluene</td>
<td>2.7 (20 ml/min)</td>
<td>1.8 (30 ml/min)</td>
<td>2.5 (30 ml/min)</td>
<td>2.6 (30 ml/min)</td>
<td>4.0 (30 ml/min)</td>
<td>4.1 (50 ml/min)</td>
</tr>
<tr>
<td>propy Benzene</td>
<td>2.9</td>
<td>2.0</td>
<td>2.6</td>
<td>2.6</td>
<td>4.1</td>
<td>4.1</td>
</tr>
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<td>2.9</td>
<td>2.0</td>
<td>2.7</td>
<td>2.7</td>
<td>4.2</td>
<td>4.2</td>
</tr>
<tr>
<td>$h_{sys}$ min toluene</td>
<td>3.1 (30 ml/min)</td>
<td>1.9 (30 ml/min)</td>
<td>7.1 (30 ml/min)</td>
<td>4.4 (30 ml/min)</td>
<td>4.8 (30 ml/min)</td>
<td>8.1 (50 ml/min)</td>
</tr>
<tr>
<td>propy Benzene</td>
<td>2.9 (20 ml/min)</td>
<td>2.0</td>
<td>6.9</td>
<td>4.1</td>
<td>4.6</td>
<td>8.1</td>
</tr>
<tr>
<td>buty Benzene</td>
<td>2.9 (30 ml/min)</td>
<td>2.0</td>
<td>8.6</td>
<td>4.2</td>
<td>4.7</td>
<td>8.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>time</th>
<th>2.1</th>
<th>2.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k'$ toluene</td>
<td>1.76 ± 0.019</td>
<td>1.82 ± 0.016</td>
</tr>
<tr>
<td>propy Benzene</td>
<td>3.57 ± 0.059</td>
<td>3.73 ± 0.036</td>
</tr>
<tr>
<td>buty Benzene</td>
<td>5.32 ± 0.099</td>
<td>5.58 ± 0.061</td>
</tr>
<tr>
<td>asymmetry factor</td>
<td>1.93 ± 0.103</td>
<td>1.42 ± 0.097</td>
</tr>
<tr>
<td>$h_{min}$ toluene</td>
<td>8.9 (70 ml/min)</td>
<td>3.2 (20 ml/min)</td>
</tr>
<tr>
<td>propy Benzene</td>
<td>9.0</td>
<td>3.3</td>
</tr>
<tr>
<td>buty Benzene</td>
<td>9.0</td>
<td>3.3</td>
</tr>
<tr>
<td>$h_{sys}$ min toluene</td>
<td>12.1 (70 ml/min)</td>
<td>3.8 (30 ml/min)</td>
</tr>
<tr>
<td>propy Benzene</td>
<td>12.7</td>
<td>3.8</td>
</tr>
<tr>
<td>buty Benzene</td>
<td>12.4</td>
<td>3.9</td>
</tr>
</tbody>
</table>

Figure 3.2a the reduced plate heights versus the compressing pressure of the column are presented. As can be seen the plate height increases when the compressing pressure increases but at the same time, as can be observed in figure 3.2b, the $asf$ of the peaks decreases. Therefore as a net result the reduced 'true' plate height, $h_{sys}$, does not change much (figure 3.2c). So reduction of the compression pressure of the A/E column is a way to reduce the
Evaluation of the performance of the A/E system for preparative liquid chromatography

Figure 3.2: a. Reduced theoretical plate height $h$, b. asymmetry factor $asf$ and c. reduced 'true' plate height $h_{32}$ vs compression pressure of the column. Test compounds: toluene (○), propylbenzene (+) and butylbenzene (○). Flow: 50 ml/min. Eluent: Methanol/water 80/20 v/v. Column: Annular Expansion column.
crushing of the silica particles without losing performance. Another way to avoid the loss of column performance by repacking with the same stationary phase is to remove the 'fines' from the packing material after the unpacking of the column.

As shown in table 3.1, the variation in the capacity factors was small for both experiments 1 and 2. In experiment 1.4 the column performance was tested during a couple of weeks as presented in the figures 3.3 and 3.4. During this time the capacity factor of toluene increased from 1.78 to 1.82, calculated by linear regression (figure 3.3). This decrease can be explained by taking into account the decrease of the column volume due to the recompression of the column every day, using the well known equation for the capacity factor.

\[ k' = K \frac{V_s}{V_m} \]

Where \( K \) the partition coefficient, \( V_s \) the volume of the stationary phase and \( V_m \) the volume of the mobile phase. Assuming that \( V_s \) is constant and equals about 33% of the total volume[15] and that \( K \) is constant, it can be calculated that the decrease in \( V_m \) corresponds with a decrease in the column length of about 3 mm. This is in agreement with our visual observation.

![Figure 3.3: Capacity factor \( k' \) vs day of measurement. Test compound: toluene. Flow: 70 ml/min. Eluent: Methanol/water 80/20 v/v. Column: Annular Expansion column.](image)

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Figure 3.4: a. Reduced theoretical plate height $h$, b. asymmetry factor $asf$ and c. reduced 'true' plate height $h_{sys}$ vs day of measurement. Test compound: toluene. Flow: 70 ml/min. Eluent: Methanol/water 80/20 v/v. Column: Annular Expansion column.
Also as carried out in experiment 1.4, in figure 3.4a the column efficiency expressed in terms of the minimum theoretical reduced plate height, $h$, is presented for a number of weeks of testing. The column efficiency decreased only slightly during this time. For the 'true' plate height, $h_{sys}$, no difference in performance during the weeks was observed (figure 3.4c) due to the improvement observed in $asf$ with time (figure 3.4b).

The bad performance observed for experiment 2.1 was probably due to the bad wetting of the silica particles.

3.5 CONCLUSIONS

In this study the Annular Expansion column, a preparative column system, was investigated. It was shown that the performance of the column in terms of reduced plate heights and reproducibilities of $k'$-values yielded satisfying results and were comparable with values to be obtained on analytical columns. Furthermore it came out that the stationary phase could easily be applied a number of times, maintaining the same column performance when theoretical plate heights were used to measure the column efficiency. When the 'true' plate height was used to measure the efficiency it was observed that the efficiency decreased after only a few repacking steps. The decrease in performance was probably due to the crushing of particles of the packing material. This can be reduced by decreasing the compressing pressure and it was shown that this does not have a negative influence on the column efficiency. In conclusion the A/E system showed to be a useful tool to pack home made preparative HPLC columns.

3.6 REFERENCES

3. Waters *Sourcebook of chromatography* 1992
Evaluation of the performance of the A/E system for preparative liquid chromatography

14. A. Reborek, personal communication
15. C.F. Poole and S.K. Poole, Chromatography Today Elsevier Science Publishers Amsterdam 1991, p 373
CHAPTER FOUR: PREPARATIVE SEPARATION OF, NEUTRAL, STYRENE OLIGOMERS

4.1 SUMMARY

In this chapter the preparative separation of, neutral, styrene oligomers is investigated using reversed phase HPLC. The Annular Expansion (A/E) column was shown to have a comparable performance to an analytical column, which could therefore be used as a pilot column for the A/E column. The separation was optimized, investigating several eluent compositions, using the analytical column. For the preparative separation methanol was chosen as the eluent and 0.5 ml styrene oligomers were separated in one run. Styrene oligomers, \( n = 1 \) to \( n = 6 \), were obtained with a purity higher than 95%.

4.2 INTRODUCTION

Oligomers can roughly be defined as compounds consisting of a series of repeat units whose molecular weights are less than \( \sim 10,000 \). This definition is broad enough to describe the molecular weight region in which the small organic molecule character disappears and measurable polymer physical properties become evident. This means that the separation mechanism will not be the same for all oligomers. In this study the main point of interest are the low molecular weight oligomers.

A lot of research has been done on the separation of, neutral, oligomers especially styrene oligomers. Gas chromatography (GC) can only be used for volatile oligomers with few repeat units and the resolution for stereoisomer separation (i.e. the separation of isotactic, syndiotactic and atactic isomers) is lower than in case of high performance liquid chromatography (HPLC)[1], which will be described later on. For supercritical fluid chromatography (SFC) it is reported that up to 42 styrene oligomers could be separated but with long retention times[2]. Size exclusion chromatography (SEC), where the separation takes place according to their Stokes’ radius using a column packing with pores which have a diameter similar to the size of the analyte molecules, shows only a small peak capacity and
Preparative separation of neutral styrene oligomers has a low resolution which decreases with increasing molecular weight and is therefore only feasible up to a pentamer or hexamer[3].

Up till now the best results for the separation of oligomers were obtained using reversed phase or normal phase HPLC. Bui et al.[4] separated styrene oligomers up to n = 20 using a C-18 column and a tetrahydrofuran/water gradient. Kirkland[5] separated styrene oligomers with polymerization degrees up to 11 on C-18 columns using acetonitrile as the eluent. Lewis et al.[1] successfully resolved, on a C-18 column, stereoisomers of low molecular weight styrene oligomers using an acetonitrile/dichloromethane gradient. Further they tested 27 other mobile phase solvents, either pure or mixed, isocratically or with gradient elution, to obtain a maximum resolution of the stereoisomers. In that study the best results were obtained using an acetonitrile/water/dichloromethane gradient. Other examples of eluents used were methanol/water, ethanol/water, nitromethane and propylene carbonate. It appeared that only a few of the solvents tested produced stereoisomer separation. The Snyder solvent selectivity scheme[6] did not accurately predict selectivities for these separations. Solvents from different groups provided isomer separations, while solvents within the same group showed widely different selectivity for these separations. Hansen's solubility model appeared promising for optimizing the mobile phase composition for these separations. In this model the hydrogen bonding forces are plotted vs the combined dispersion forces and polarity forces of the eluent. It appeared that all the solvents that lay inside the solubility circle of styrene did not give any stereoisomer separation while all the tested solvents that lay outside this circle gave at least some stereoisomer separation. Using other alkyl bonded stationary phases (C-1 to C-18) all stationary phases gave stereoisomer separation using the suitable eluents. Using phenyl bonded stationary phases separation of the oligomers but no stereoisomer separation was obtained[7]. Several authors investigated if it was possible to predict the retention times of the oligomers using retention models. Jandera[8] described the retention behaviour of oligomeric series (polystyrene, ethylene glycols and ethoxylated nonylphenols) on reversed phase columns using a modified version of the lipophilic and polar interaction indices model he used for the description of the retention behaviour of homologous series[9]. This model gave, as a first approximation, a linear relationship between the logarithm of the capacity factor (logk') vs the organic modifier concentration in the eluent (φ) and also between logk' vs the number of structural repeat.
units. Using this retention model he could also account for the remarkable behaviour of ethoxylated nonylphenols where the retention decreased with increasing number of repeat oligo ethylene units due to the high polarity of these units. Larmann et al.[10] separated styrene oligomers from \( n = 2-12 \) using isocratic tetrahydrofuran/water mixtures as eluents using four different C-18 packings. A linear relation between \( \log k' \) vs \( \varphi \) was observed for all the used packing materials. This relation was obtained using the linear solvent strength (LSS) model of Snyder[11]. Lai et al.[12] separated, using phenyl bonded stationary phases, isocratically styrene oligomers using tetrahydrofuran/water, tetrahydrofuran/hexane and acetonitrile/water as eluents. Using the Snyder relation between \( \log k' \) and \( \varphi \) and the linear Martin relation between the \( \log k' \) and the degree of polymerization (the number of repetitive units) the retention of the oligomers could be estimated. No stereoisomer separation was observed using these phenyl bonded phases as was already observed by Lewis et al.[7]. Boehm et al.[13] developed a theoretical model for the retention behaviour of flexible, chainlike, homooligomers and polymers. It appeared that this statistical thermodynamic model was in good agreement with the observed experimental behaviour.

When normal phase stationary phases were used, it was also possible to separate uncharged oligomers. Using nitrile bonded phases almost baseline separation of styrene oligomers up to \( n = 10 \) was obtained using iso-octane/dichloromethane as the eluent[14]. Martins' rule could be used to describe the retention behaviour of the compounds. Jandera and Rozkosná[15] used bare silica gel stationary phases to separate styrene oligomers using 1,4 dioxane/n-heptane and tetrahydrofuran/n-heptane mobile phases. In this case also the Martin and Snyder equations could be used to describe the retention mechanism of the oligomers. These equations were used to develop a model for the prediction of the retention times for these oligomers using gradient elution. Mourey et al.[16,17] investigated silica stationary phases to separate styrene oligomers using gradient elution with n-hexane/tetrahydrofuran, n-hexane/ethyl acetate and n-hexane/dichloromethane as eluent mixtures. With n-hexane/dichloromethane stereoisomer separation was observed. This stereoisomer separation could be explained by taking into account the weak aromatic ring localization on silica supports. When a non localizing solvent is used (e.g. hexane/dichloromethane) this interference will not be disturbed and stereoisomer separation will be observed. According to the authors the elution behaviour of the oligomers using silica
stationary phases could not be explained exclusively by solubilisation of the oligomers in the eluent as was described by Lewis et al.\[1\] for reversed phase HPLC. The adsorption mechanism will in this case also play a role. When using a modification of the solvent displacement model of Snyder, Mourey could not accurately explain all aspects of the adsorption behaviour of oligomeric solutes in contrast with the results obtained by the authors described above\[14, 15\].

Summarizing it can be concluded that when reversed phase or normal phase HPLC is used as the separation technique it is possible to separate (styrene) oligomers and in some case even the stereoisomers of these oligomers. The retention behaviour can be described by theoretical and empirical models. The goal of this research was to investigate wether it is possible to separate low molecular weight styrene oligomers on a preparative scale. In this research a C-18 stationary phase was used because this phase should be well suited for this separation. First the separation was optimized, using several eluents, by using an analytical column with the same packing material as was available for the preparative column. Then the sample loadability of the analytical column was tested and afterwards the separation was scaled up to prep-LC. A preparative separation was performed and the purity of the separated oligomers was tested.

4.3 EXPERIMENTAL

Instrumentation

The HPLC experiments were performed on a Waters Delta Prep 4000 equipped with a Waters 486 Tunable Adsorbance detector (Millipore Corporation, Milford, Massachusetts, USA) and a Rheodyne 7010 injector equipped with a 20 µl, 50 µl or a 1 ml loop. The chromatograms were recorded on a Kipp&Zonen recorder or on a D 2500 Chromato-Integrator (Merck Hitachi, Tokyo Japan). The peak areas were determined using the HPLC manager from Merck.

Columns

For the analytical experiments a home made column, slurry packed, was used;
250x4.6mm ID, stationary phase LiChrospher RP-18, 15 μm (Merck, Darmstadt, Germany). For the preparative experiments the Annular Expansion (A/E) System as described in chapter three was used.

**Chemicals**

The TSK polystyrene standard A-300, \( MW = 4.18 \times 10^2 \), \( M_w/M_n = 1.15 \), was from Tosoh Corporation, Tokyo, Japan. This standard contains oligomers with 1-8 units styrene, see figure 4.1[18]. The monostyrene standard (\( MW = 162 \)) was obtained from Waters Millipore. Toluene, propylbenzene and butylbenzene were from Aldrich, Steinheim, Germany. Methanol, acetonitrile and dichloromethane, HPLC grade, were from Merck. Tetrahydrofuran, unstabilized, HPLC grade, was purchased from Westburg, Leusden, the Netherlands. Water was purified using a Milli Q Water Purification System (Millipore). The eluents were filtrated and degassed by purging with helium before use.

*Figure 4.1: Size exclusion chromatogram of the TSK styrene standard A-300 from reference [18]*
Preparative separation of neutral styrene oligomers

Methods

To validate if the performance of the analytical column and the A/E column was comparable the capacity factors $k'$ and the plate numbers $N$ of a number of test compounds (alkylbenzenes) were compared applying an injection volume of 20 $\mu$L. Further, to investigate whether the increase of the injection volume on the A/E column from 20 $\mu$L to 1 ml will cause considerable peak broadening due to the increase of the extra column volume, the plate numbers of the alkylbenzenes using a 20 $\mu$L and a 1 ml injection loop were compared. The optimization of the separation conditions of the styrene oligomers was carried out on the analytical column. For the optimization several eluent compositions and gradients were investigated. The capacity factors $k'$ of the oligomers and the resolution $R_s$ of every pair of successive oligomers were measured. For this optimization 20 $\mu$L of a 0.5%v/v solution of the styrene oligomers in methanol was injected. To investigate the sample loadability of the preparative column the amount of styrene oligomers injected on the analytical column was systematically increased while it was observed if still baseline separation was obtained.

The preparative separation was performed on the A/E column using methanol as the eluent and 0.5 ml of the original styrene oligomers sample was injected. The first six peaks were manually collected separately. The purity was verified by injection of 20 $\mu$L of the separate peaks on the analytical column. To verify if the first peak of this separation was indeed the styrene monomer the $k'$ of this peak was compared with the $k'$ of a monomer standard.

4.4 RESULTS AND DISCUSSION

In table 4.1 the reduced theoretical plate height $h$, the asymmetry factor $asf$ and the capacity factor $k'$ are given for the analytical column using toluene, propylbenzene and butylbenzene as the test compounds. Comparing these results with the results obtained in chapter three e.g. table 3.1 for the A/E column it can be concluded that both the analytical and the preparative column show a comparable performance. The plate heights of the analytical column are in the same range as for the A/E column and the $k'$-values also, although at lower flow the $k'$ increased somewhat for the analytical column.
In table 4.II the reduced theoretical plate heights are given for the A/E column using toluene, propylbenzene and butylbenzene as the test compounds and using a 20 μl and a 1 ml injection loop. Only a small increase in \( h \) was observed using the 1 ml loop, so the peak broadening due to the increase of the extra column volume is negligible under these conditions.

From the results of tables 4.I and 4.II it can be concluded that the analytical column can be used as a pilot column for the preparative separation on the A/E column. Almost no loss of performance will be expected when the separation is scaled up to preparative chromatography.

### TABLE 4.I: REDUCED THEORETICAL PLATE HEIGHTS \( h \), ASYMMETRY FACTORS \( a_s f \) AND CAPACITY FACTORS \( k' \) FOR THE TEST COMPOUNDS ON THE ANALYTICAL C-18 COLUMN

**Eluent:** methanol/water 80/20 v/v; **Test compounds:** toluene, propylbenzene and butylbenzene; The flow is given in the table.

<table>
<thead>
<tr>
<th></th>
<th>( h )</th>
<th>( a_s f )</th>
<th>( k' )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[ml/min]</td>
<td>[ml/min]</td>
<td>[ml/min]</td>
</tr>
<tr>
<td>flow</td>
<td>1.0</td>
<td>0.5</td>
<td>0.3</td>
</tr>
<tr>
<td>toluene</td>
<td>5.24</td>
<td>3.77</td>
<td>3.38</td>
</tr>
<tr>
<td>propylbenzene</td>
<td>4.80</td>
<td>3.47</td>
<td>3.06</td>
</tr>
<tr>
<td>butylbenzene</td>
<td>4.50</td>
<td>3.28</td>
<td>2.98</td>
</tr>
</tbody>
</table>

### TABLE 4.II: REDUCED THEORETICAL PLATE HEIGHT \( h \) FOR THE A/E COLUMN

**Comparison between the 20 μl and the 1.0 ml injection loop.**

**Eluent:** methanol/water 80/20 v/v; **Test compounds:** toluene, propylbenzene and butylbenzene; **Flow:** 1.0 ml/min

<table>
<thead>
<tr>
<th></th>
<th>( h ) injection volume 20 μl</th>
<th>( h ) injection volume 1.0 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>toluene</td>
<td>4.37</td>
<td>4.58</td>
</tr>
<tr>
<td>propylbenzene</td>
<td>4.62</td>
<td>4.66</td>
</tr>
<tr>
<td>butylbenzene</td>
<td>4.73</td>
<td>4.91</td>
</tr>
</tbody>
</table>

In tables 4.III and 4.IV the capacity factors \( k' \) and the resolution \( R_s \) for the styrene oligomers on the analytical column are respectively given using several eluent mixtures and
Preparative separation of neutral styrene oligomers gradients. All the eluent compositions used were able to separate the oligomers and \( R_g \)-values higher than one were obtained (table 4.IV).

**TABLE 4.III: CAPACITY FACTORS \( k' \) FOR THE FIRST FIVE STYRENE OLIGOMERS FOR THE ELUENT COMPOSITIONS USED IN THIS STUDY**

Flow: 1.0 ml/min; Compounds: styrene oligomers, A-300; Analytical C-18 column; Linear gradients were used; Multiple \( k' \)-values point to peak splitting due to stereoisomer separation

<table>
<thead>
<tr>
<th>gradient</th>
<th>( k'_1 )</th>
<th>( k'_2 )</th>
<th>( k'_3 )</th>
<th>( k'_4 )</th>
<th>( k'_5 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. methanol (100%), isocratic</td>
<td>0.65</td>
<td>1.05</td>
<td>1.49</td>
<td>2.06</td>
<td>2.77</td>
</tr>
<tr>
<td>2. methanol (100%) ( \rightarrow ) tetrahydrofuran (100%) in 40 minutes</td>
<td>0.72</td>
<td>1.17</td>
<td>1.65</td>
<td>2.23</td>
<td>2.85</td>
</tr>
<tr>
<td>3. methanol (100%) ( \rightarrow ) acetonitrile/methanol 50/50 v/v in 20 minutes</td>
<td>0.77</td>
<td>1.21</td>
<td>1.73</td>
<td>2.37</td>
<td>3.20</td>
</tr>
<tr>
<td>4. methanol (100%) ( \rightarrow ) methanol/dichloromethane 90/10 v/v in 10 minutes ( \rightarrow ) methanol (100%) in 2 minutes</td>
<td>0.59</td>
<td>0.89</td>
<td>1.24</td>
<td>1.71</td>
<td>2.27</td>
</tr>
<tr>
<td>5. methanol (100%) ( \rightarrow ) methanol/dichloromethane 90/10 v/v in 20 minutes</td>
<td>0.65</td>
<td>1.02</td>
<td>1.47</td>
<td>2.02</td>
<td>2.73</td>
</tr>
<tr>
<td>6. acetonitrile (100%), isocratic</td>
<td>0.86</td>
<td>1.34</td>
<td>1.94</td>
<td>2.77</td>
<td>4.14</td>
</tr>
<tr>
<td>7. acetonitrile (100%) ( \rightarrow ) acetonitrile/tetrahydrofuran 50/50 v/v in 20 minutes</td>
<td>0.97</td>
<td>1.57</td>
<td>2.26</td>
<td>2.98</td>
<td>3.82</td>
</tr>
<tr>
<td>8. acetonitrile (100%) ( \rightarrow ) acetonitrile/methanol 90/10 v/v in 20 minutes</td>
<td>0.99</td>
<td>1.59</td>
<td>2.31</td>
<td>3.27</td>
<td>4.60</td>
</tr>
<tr>
<td>9. acetonitrile (100%) ( \rightarrow ) acetonitrile/dichloromethane 75/25 v/v in 15 minutes</td>
<td>0.95</td>
<td>1.54</td>
<td>2.15</td>
<td>2.91</td>
<td>3.98</td>
</tr>
<tr>
<td>10. acetonitrile (100%) ( \rightarrow ) acetonitrile/dichloromethane 50/50 v/v in 20 minutes</td>
<td>0.99</td>
<td>1.59</td>
<td>2.25</td>
<td>3.02</td>
<td>3.91</td>
</tr>
<tr>
<td>11. acetonitrile/water ( \rightarrow ) acetonitrile (100%) in 15 minutes, hold 5 min ( \rightarrow ) acetonitrile/dichloromethane 50/50 v/v in 30 minutes</td>
<td>4.33</td>
<td>7.90</td>
<td>10.90</td>
<td>13.09</td>
<td>13.23</td>
</tr>
<tr>
<td>12. acetonitrile/methanol 90/10 v/v, isocratic</td>
<td>0.98</td>
<td>1.54</td>
<td>2.22</td>
<td>3.10</td>
<td>4.51</td>
</tr>
</tbody>
</table>
TABLE 4.IV: RESOLUTION $R_s$ BETWEEN THE FIRST FOUR OLIGOMER PAIRS FOR THE ELUENT COMPOSITIONS USED IN THIS STUDY

Flow: 1.0 ml/min; Compounds: styrene oligomers, A-300; Analytical C-18 column; Linear gradients were used

<table>
<thead>
<tr>
<th>gradient</th>
<th>$R_s$(1-2)</th>
<th>$R_s$(2-3)</th>
<th>$R_s$(3-4)</th>
<th>$R_s$(4-5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. methanol (100%), isocratic</td>
<td>2.44</td>
<td>1.94</td>
<td>1.76</td>
<td>1.49</td>
</tr>
<tr>
<td>2. methanol (100%) $\rightarrow$ tetrahydrofuran (100%) in 40 minutes</td>
<td>2.64</td>
<td>2.01</td>
<td>1.85</td>
<td>1.71</td>
</tr>
<tr>
<td>3. methanol (100%) $\rightarrow$ acetonitrile/methanol 50/50 v/v in 20 minutes</td>
<td>2.57</td>
<td>2.15</td>
<td>1.94</td>
<td>1.71</td>
</tr>
<tr>
<td>4. methanol (100%) $\rightarrow$ methanol/dichloromethane 90/10 v/v in 10 minutes $\rightarrow$ methanol (100%) in 2 minutes</td>
<td>1.94</td>
<td>1.62</td>
<td>1.87</td>
<td>1.50</td>
</tr>
<tr>
<td>5. methanol (100%) $\rightarrow$ methanol/dichloromethane 90/10 v/v in 20 minutes</td>
<td>2.35</td>
<td>1.90</td>
<td>1.71</td>
<td>1.54</td>
</tr>
<tr>
<td>6. acetonitrile (100%), isocratic</td>
<td>2.76</td>
<td>2.57</td>
<td>2.77</td>
<td>1.28</td>
</tr>
<tr>
<td>7. acetonitrile (100%) $\rightarrow$ acetonitrile/tetrahydrofuran 50/50 v/v in 20 minutes</td>
<td>3.36</td>
<td>3.20</td>
<td>1.85</td>
<td>1.84</td>
</tr>
<tr>
<td>8. acetonitrile (100%) $\rightarrow$ acetonitrile/methanol 90/10 v/v in 20 minutes</td>
<td>3.36</td>
<td>2.86</td>
<td>2.11</td>
<td>1.18</td>
</tr>
<tr>
<td>9. acetonitrile (100%) $\rightarrow$ acetonitrile/dichloromethane 75/25 v/v in 15 minutes</td>
<td>3.28</td>
<td>2.89</td>
<td>1.91</td>
<td>1.46</td>
</tr>
<tr>
<td>10. acetonitrile (100%) $\rightarrow$ acetonitrile/dichloromethane 50/50 v/v in 20 minutes</td>
<td>3.32</td>
<td>3.07</td>
<td>2.07</td>
<td>1.40</td>
</tr>
<tr>
<td>11. acetonitrile/water $\rightarrow$ acetonitrile (100%) in 15 minutes, hold 5 min $\rightarrow$ acetonitrile/dichloromethane 50/50 v/v in 30 minutes</td>
<td>8.94</td>
<td>7.14</td>
<td>3.33</td>
<td>-</td>
</tr>
<tr>
<td>12. acetonitrile/methanol 90/10 v/v, isocratic</td>
<td>3.16</td>
<td>2.85</td>
<td>1.90</td>
<td>1.42</td>
</tr>
</tbody>
</table>

Generally it can be concluded that the resolution was better when acetonitrile was the main component in the eluent compared to methanol. The capacity factors were also somewhat higher for acetonitrile as the main component in the eluent compared to methanol. When acetonitrile was present in the eluent peak splitting was observed (figure 4.2 and table 4.III), this is caused by the separation of the stereoisomers of the oligomers[1]. When methanol was the main component in the eluent no stereoisomer separation was obtained. Also in the literature it was reported that when acetonitrile was present in the eluent stereoisomer separation was obtained and with methanol only partial stereoisomer separation was
Preparative separation of neutral styrene oligomers obtained[1]. The fact that no stereoisomer separation was observed in this study using methanol as the main solvent in the eluent is probably caused by the loss of resolution due to the rather large particle diameter, 15 μm, of the stationary phase. The highest resolution was obtained when water was added to an acetonitrile based eluent but using this gradient also long retention times were obtained, e.g. 42 minutes for n = 4.

Figure 4.2: Chromatograms of the styrene oligomers TSK A-300 separation on the analytical C-18 column; a. using acetonitrile as the eluent; b. using methanol as the eluent. Flow: 1.0 ml/min
To determine the sample loadability of the A/E column the sample loadability of the analytical column was determined by increasing the amount of oligomers applied on the column. First by increasing the concentration of the oligomers at a constant injection volume of 20 µl and after that by increasing the sample volume of the applied oligomers. In all these cases the criterion was the baseline separation of the oligomers. The sample loadability of the analytical column was investigated for two eluents, 100% methanol and 100% acetonitrile. These two eluents were selected for several reasons: i. It was possible to obtain baseline separation for both the eluents (table 4.IV, eluent compositions 1 and 6) and ii. using a gradient instead of an isocratic eluent at most a small increase in resolution was obtained. The only eluent which gave a considerable better resolution was eluent no. 11, when water was added to the eluent. Not only long retention times were obtained in this case but water is also more difficult to remove after the separation, especially when volatile compounds are present in the collected samples. In table 4.V the concentration and the amount of the styrene sample applied on the analytical column is presented. As is shown in table 4.V and figure 4.3 almost baseline separation was obtained when 50 µl of the original sample of styrene oligomers was injected using 100% acetonitrile as the eluent. The resolution for the higher sample loadabilities could not be determined because the concentration of the oligomers was too high for the UV detector. The sample loadability was lower when 100% methanol was used as the eluent, as could be expected with respect to the resolution obtained in the optimization experiments (table 4.IV, eluent compositions 1 and 6). The separation was not further optimized because the amount of oligomers that could be applied on the preparative column using these conditions was large enough for our purposes.

Figure 4.3: Chromatogram of the styrene oligomers TSK A-300 separation when 50 µl was injected on the analytical C-18 column. Eluent: acetonitrile; Flow: 1.0 ml/min.
**Preparative separation of neutral styrene oligomers**

**TABLE 4.V: SAMPLE LOAD ON THE ANALYTICAL C-18 COLUMN**

Flow: 1.0 ml/min; Compounds: styrene oligomers, A-300

<table>
<thead>
<tr>
<th>eluent</th>
<th>sample concentration in methanol</th>
<th>injection volume</th>
<th>baseline separation</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% methanol</td>
<td>0.5 mg/ml</td>
<td>20 μl</td>
<td>yes</td>
</tr>
<tr>
<td>100% acetonitrile</td>
<td>0.5 mg/ml</td>
<td>20 μl</td>
<td>yes</td>
</tr>
<tr>
<td>100% methanol</td>
<td>0.279 g/ml</td>
<td>20 μl</td>
<td>yes</td>
</tr>
<tr>
<td>100% acetonitrile</td>
<td>0.279 g/ml</td>
<td>20 μl</td>
<td>yes</td>
</tr>
<tr>
<td>100% methanol</td>
<td>pure</td>
<td>20 μl</td>
<td>almost</td>
</tr>
<tr>
<td>100% acetonitrile</td>
<td>pure</td>
<td>20 μl</td>
<td>yes</td>
</tr>
<tr>
<td>100% acetonitrile</td>
<td>pure</td>
<td>50 μl</td>
<td>almost</td>
</tr>
</tbody>
</table>

From the sample loadability of the analytical column the sample loadability for the A/E column can easily be calculated using the following equation:

\[
\text{sample loadability} = \frac{D_p^2 L_p}{D_a^2 L_a}
\]

where \( D_p \) is the diameter of the preparative column, \( D_a \) the diameter of the analytical column, \( L_p \) the length of the preparative column and \( L_a \) the length of the analytical column. This means that theoretically when using acetonitrile as the eluent 5 ml of the undiluted original sample could be applied on the preparative A/E column. For our purposes 0.5 ml sample was sufficient, therefore methanol was used as the eluent because it is less toxic than acetonitrile.

After the preparative separation the first 6 peaks were collected separately. The purity was tested by analysing the collected fractions of the styrene oligomers on the analytical column (table 4.VI). For all the oligomers a purity higher than 95% was obtained. The peak recognition was investigated by comparing the retention times of the oligomers in the mixture with a monostyrene standard (table 4.VII). It appeared that the first peak of the oligomer mixture is from the monostyrene.
TABLE 4.VI: PURITY OF THE STYRENE OLIGOMERS AFTER THE PREPARATIVE SEPARATION

Determined on the analytical C-18 column: Eluent: methanol; Flow: 1.0 ml/min

<table>
<thead>
<tr>
<th>a</th>
<th>purity [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&gt;95</td>
</tr>
<tr>
<td>2</td>
<td>&gt;95</td>
</tr>
<tr>
<td>3</td>
<td>&gt;98</td>
</tr>
<tr>
<td>4</td>
<td>&gt;98</td>
</tr>
<tr>
<td>5</td>
<td>&gt;97</td>
</tr>
<tr>
<td>6</td>
<td>&gt;97</td>
</tr>
</tbody>
</table>

TABLE 4.VII: PEAK RECOGNITION BY THE RETENTION TIMES OF THE STYRENE OLIGOMERS

Determined on the analytical C-18 column: Eluent: methanol; Flow: 1.0 ml/min

<table>
<thead>
<tr>
<th>PS1 (standard)</th>
<th>t_R [min]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.66</td>
</tr>
<tr>
<td>2</td>
<td>5.76</td>
</tr>
</tbody>
</table>

4.5 CONCLUSIONS

In this study the preparative separation of, neutral, styrene oligomers was investigated. As was reported in the literature, styrene oligomers can easily be separated using reversed phase HPLC. The preparative A/E column showed a comparable performance to an analytical column. Therefore this latter column could be used as a pilot column for the preparative separation. Investigating several eluent compositions on the analytical column it appeared that the improvement by using a gradient instead of an isocratic eluent was small except when water was added to the eluent. Therefore two relatively simple eluents were used in the scaling up procedure from the analytical to the preparative separation, pure methanol and pure acetonitrile. Investigating the sample loadability on the analytical column it appeared that the amount of sample that could be injected onto the A/E column under
Preparative separation of neutral styrene oligomers

baseline separation conditions was large enough for our requirements. After the preparative separation of the styrene oligomers on the A/E column using 100% methanol as the eluent, the purity for all the oligomers investigated was higher than 95%. After comparison of the retention times of the first peaks with a monomer standard the six collected peaks were identified as \( n = 1 \) to \( n = 6 \).

4.6 REFERENCES

3. S. van der Wal LC-GC Intl. 5 (1992) 36, 38-42
5. J.J. Kirkland Chromatographia 8 (1975) 661-668
CHAPTER FIVE: SAMPLE PRETREATMENT FOR EMULSION POLYMERIZATION PRODUCTS

5.1 SUMMARY

Before the water phase of the emulsion polymerization product (latex) can be analyzed, the latex particles have to be removed and in the case the separation is performed on preparative scale the sample has also to be concentrated. In this chapter the removal of the latex particles by ultrafiltration is discussed, special attention is paid to the adsorption of the uncharged oligomers on the filters. It appeared that this adsorption can be very high, up to almost 100% for the higher oligomers. Further, concentration techniques such as solid phase extraction (SPE), freeze drying and evaporation are also discussed. SPE proved to be a valuable technique for the uncharged oligomers but was less suitable for the charged oligomers. Using freeze drying and evaporation as concentration techniques, from which it was expected that they were better suitable for the charged oligomers, the recovery of the charged oligomers was lower than 100%.

5.2 INTRODUCTION

5.2.1 Removal of the latex particles

Before the water phase of the latex can be analyzed the latex particles have to be removed for several reasons. The latex particles can cause clogging of the HPLC column, they can adsorb on the column and elute on arbitrary times. It was also observed that they can lead to a large decrease in column performance[1]. Pijls[2] investigated several sample pretreatment methods for the removal of latex particles from the emulsion prior to isotachophoresis (ITP) analysis. Filtration of the latex through filters with 200 and 50 nm pores showed almost no improvement of the isotachopherograms. With dynamic light scattering it was observed that there were still particles present in the solution with diameters smaller than the pores in the filters that were used. An other method to remove the latex
particles investigated by Pijls was the separation of the water phase from the latex particles by ultracentrifugation. The problem using this technique was that the density of the water phase is larger than the density of the latex particles. Further Pijls tried to remove the latex particles by coagulation with cetyltrimethylammonium bromide (CTAB) or Al\(^{3+}\). It appeared to be impossible to remove all the latex particles because a certain amount of the particles became positively charged by the used coagulant and were thereby stabilized. Another disadvantage of this method was the coagulation of the higher oligomers. The best method to remove the latex particles proved to be ultrafiltration. The filter type used by Pijls was the anisotropic, hydrophillic YMT30 membrane on polycarbonate from Amicon, this filter was developed to remove proteins of a molecular weight above 30,000 from an aqueous solution. After filtration no latex particles could be detected in the filtrate using light scattering. Morrison et al.\[3\] observed that the charged oligomers did not adsorb on these filters. Because they investigated this using ITP no information about the adsorption of uncharged oligomers could be obtained. In this study the adsorption of the uncharged oligomers of butadiene on the Amicon filter was investigated using model compounds instead of the uncharged butadiene oligomers. Also a couple of other filters were tested for the removal of the latex particles from the latex. For these filters also the adsorption of uncharged oligomers was investigated.

5.2.2 Concentration techniques

To obtain information about the structure of the oligomers, for instance by using nuclear magnetic resonance (NMR), it is necessary to have at least 1 mg of a specific oligomer. The concentration of the oligomers in the water phase of the latex is low. The highest concentration of one particular styrene oligomer observed by Morrison et al.\[3\] was 2.10\(^{-3}\) mol/l, the highest concentration for one particular butadiene oligomer was also 2.10\(^{-3}\) mol/l\[4\]. To obtain information about the structure of the oligomers this means that for the oligomer of the highest concentration, assuming a yield of 100%, at least 100 ml of the water phase must be pretreated and separated. Because the other oligomers, that are present in lower concentrations, must be investigated also and because the yield of these separations
will not be 100%, the sample that has to be analyzed must be at least 5-10 times larger than that volume. Injection of such a large sample can, even on the preparative A/E system, cause volume overload which leads to peak broadening resulting in a decrease in resolution. Therefore it is necessary to concentrate the sample before injection. There are a lot of different concentration techniques such as freeze concentration, freeze drying, lyophilization, vacuum distillation, evaporation, reverse osmosis, ultrafiltration, solvent extraction, solid phase extraction, surface adsorption and gas stripping[5]. In this study three techniques were investigated: solid phase extraction (SPE), evaporation and freeze drying. For these techniques it was investigated for which kind of oligomers they could be used and what the recoveries of the oligomers after the concentration step were.

5.3 EXPERIMENTAL

Instrumentation and columns

The HPLC experiments to investigate the adsorption on the filters and the recoveries of the SPE cartridges were performed on chromatographic system I: a Waters Delta Prep 4000 equipped with a Waters Tunable Adsorbance detector (Millipore Corporation, Milford, Massachusetts, USA). The chromatograms were recorded on a D 2500 Chromato- Integrator (Merck Hitachi, Tokyo, Japan). The peak areas were determined using the HPLC manager from Merck Hitachi. The column used was a home made column, slurry packed, 250x4.6mm ID, stationary phase LiChrospher RP-18, 15 μm (Merck, Darmstadt, Germany).

The HPLC experiments to investigate the recoveries of the freeze drying and the evaporation experiments were performed on chromatographic system II: a Bischoff HPLC pump and a Bischoff lambda 1000 UV detector (Bischoff, Leonberg, Germany). The chromatograms were recorded on a D 2500 Chromato- Integrator and the peak areas were determined using the HPLC manager. The column used was the PL2 column from Polymer Labs, Shropshire, United Kingdom and is described in chapter two.

The light scattering measurements were performed on a Malvern 4700 multi angle light scattering system from Malvern Instruments Ltd., Worcestershire, United Kingdom.

For the SPE experiments a Vac-Elut vacuum manifold from Analyticchem
Sample pretreatment for emulsion polymerization products

International, Harbor City, CA, USA, was used. The SPE cartridges used were Bakerbond SPE Octadecyl 3ml from J.T. Baker, Phillipsburg, NJ, USA.

Chemicals

For the removal of the latex particles three types of filters were used. The hydrophilic YMT30 membranes on polycarbonate, 14 mm diameter, from Amicon, Beverly, CA, USA; MF-Millipore membrane filters composed of mixtures of cellulose acetate and cellulose nitrate, pore size 0.05 μm, diameter 13 mm, from Millipore Corporation; Polycarbonate track etching (PCTE) filter membranes, pore size 0.05 μm, diameter 13 mm, from Poretics Corporation, Livermore, CA, USA. The Amicon membranes were placed in a reusable filter holder from the Micropartition system (MPS-1) from Amicon. The Millipore and the Poretics membranes were placed in a metal filter holder with a luer lock (Millipore).

Crotylalcohol was from Fluka, Buch, Switzerland; 2,4-octadien-1-ol was from Johnson Matthey GmbH Alfa Products, Karlsruhe, Germany; cis-7-dodecen-1-ol and cis-11-hexadecen-1-ol were from Aldrich, Steinheim, Germany. Butadiene latex (RDHPLCO01) was obtained from R. Driessens from the group of German, filtrated styrene latex was obtained from the group of A. Eshuis. Milli-Q purified water was used. Methanol, HPLC grade, and ammonium carbonate were from Merck. The eluents were filtered and degassed by purging with helium before use.

Methods

Filtration: The alcohols, which were used as model compounds for the uncharged butadiene oligomers, were dissolved in Milli-Q purified water: crotylalcohol 1.0 μl/ml, octadienol 0.4 μl/ml, dodecenol 0.2 μl/ml and hexadecenol 0.2 μl/ml. The Amicon filters were placed in the reusable filter holder and centrifuged for 30 min by 3000 rpm. The Millipore and the Poretics filters were placed in the metal holder and the solution was pressed through the filter manually using a glass syringe. The amount of alcohols that passed through the filters was determined by comparing the concentration of the alcohols after filtration with the concentration before filtration by measuring the peak area using the chromatographic system I as described above. For the determination of crotylalcohol methanol/water 40/60 v/v was used as the eluent, for octadienol 75/25, for dodecenol 90/10 and for hexadecenol 95/5.
Crotylalcohol and octadecanol were detected by 220 nm, dodecenol and hexadecenol by 210 nm. To investigate whether the amount of oligomeric alcohols adsorbed on the filter decreased in time due to already adsorbed material on the filter the first and the second milliliter of the filtrated solution were collected separately. To investigate also whether the filter holders themselves adsorb the alcohols, the filtration procedures were also performed without the presence of the filters. For the membranes with the 0.05 μm pores the presence of latex particles after filtration was measured using the light scattering equipment.

**Sample concentration using SPE:** The SPE cartridges were placed on the Vac-Elut. The cartridges were preconditioned by sequential washings with 5 ml methanol and 5 ml water under light vacuum (−20 mmHg on the meter). The vacuum was turned off when the water was close to reaching the top of the sorbent bed to prevent the sorbent from running dry. Then the (Amicon) filtrated butadiene latex (RDHPLC001) was passed through the SPE cartridge again under light vacuum. To find out if breakthrough had occurred, the water phase of the latex leaving the cartridge was sampled every 3 ml and investigated with the chromatographic system I, using a mixture of methanol/water 70/30 v/v as the eluent. The adsorbed compounds were eluted using methanol as the desorbent. The yield was also determined chromatographically.

**Sample concentration using freeze drying and evaporation:** Filtrated styrene latex was evaporated in a rotary vapour or freeze dried, the volume before and after was measured to determine the concentration factor. The recoveries after freeze drying and evaporation were determined using the chromatographic system II by comparing the concentration of the compounds before and after the concentration step. A mixture of 30%v/v methanol and 70%v/v aqueous 10−2 M ammonium carbonate solution was used as the eluent. To remove the smallest latex particles, which were still present in the solution, the latex was filtrated on an Amicon filter before injection.
Sample pretreatment for emulsion polymerization products

5.4 RESULTS AND DISCUSSION

5.4.1 Removal of the latex particles

In table 5.1 the percentage of the model compounds for the uncharged oligomers that passed through the filters and also the percentage of the model compounds still present in the solution after adsorbance on the filter holders is presented. It appeared that on the Amicon filter holder a considerable amount of dodecenol adsorbed: 25%. For the other compounds the adsorption was less but for the crotylalcohol and the hexadecenol still 5% was adsorbed on this filter holder. The adsorption on the Millipore holder was less, probably due to the higher polarity of the holder. From the three filters tested in this study the highest amount of alcohols was adsorbed on the Amicon filter; for the higher alcohols, dodecenol and hexadecenol, almost everything was adsorbed. Less adsorption was observed for the other two filters but for the dodecenol still 20-30% was adsorbed. When octadienol was filtered though the Millipore filter an extra peak was formed (figure 5.1), no explanation was found for this phenomenon. To investigate whether the adsorption of the alcohols on the filters would decrease when already some adsorption of the alcohol on the filter had taken place, a second milliliter of the alcohol solutions was filtered also (table 5.1). In most cases the adsorption decreased somewhat or remained the same. The increase in the adsorption of crotylalcohol using the Amicon filter is probably caused by the competition during filtration between crotylalcohol and glycerol which is initially present on the filter. The reason for the increase using the PCTE filter with dodecenol is not known. No general trend was observed for the decrease in adsorption of the alcohols on the filters. Maybe the adsorption of the alcohols from the solution will decrease further when more analyte is filtered, so the filter will become saturated with the alcohol, but this is not a useful method because when a latex is filtered after a few milliliters the filter will be clogged by the latex particles.

Generally it can be concluded that adsorption of the alcohols will occur during filtration. The amount of adsorption of the investigated alcohols on a specific filter depends on the kind of alcohol investigated. This will cause an additional problem by the analysis of the uncharged oligomers because it can not be predicted how much of a particular oligomer will adsorb.
TABLE 5.1: RECOVERY OF THE ALCOHOLS, AS MODEL COMPOUNDS FOR THE UNCHARGED BUTADIENE Oligomers, AFTER ADSORPTION ON THE FILTER HOLDERS AND THE FILTERS

Model compounds: crotylalcohol, 2,4-octadien-1-ol, cis-7-dodecen-1-ol and cis-11-hexadecen-1-ol; Concentration of the alcohols determined on the chromatographic system I

<table>
<thead>
<tr>
<th></th>
<th>Amicon holder [%] ± SD</th>
<th>Millipore holder [%] ± SD</th>
<th>YMT30 filter [%] ± SD</th>
<th>Millipore MF filter [%] ± SD</th>
<th>PCTE filter [%] ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>crot 1st ml</td>
<td>94 ± 1</td>
<td>98 ± 1</td>
<td>91 ± 2</td>
<td>93 ± 2</td>
<td>90 ± 2</td>
</tr>
<tr>
<td>crot 2nd ml</td>
<td>86 ± 2</td>
<td>96 ± 2</td>
<td>94 ± 1</td>
<td>94 ± 1</td>
<td>90 ± 2</td>
</tr>
<tr>
<td>oct 1st ml</td>
<td>99 ± 0.4</td>
<td>100 ± 1</td>
<td>96 ± 1</td>
<td>84 ± 1</td>
<td>86 ± 0.5</td>
</tr>
<tr>
<td>oct 2nd ml</td>
<td>74 ± 6</td>
<td>91 ± 8</td>
<td>12 ± 3</td>
<td>77 ± 8</td>
<td>71 ± 9</td>
</tr>
<tr>
<td>dod 1st ml</td>
<td>95 ± 4</td>
<td>98 ± 3</td>
<td>2 ± 3</td>
<td>99 ± 9</td>
<td>105 ± 15</td>
</tr>
<tr>
<td>dod 2nd ml</td>
<td>95 ± 4</td>
<td>98 ± 3</td>
<td>2 ± 3</td>
<td>99 ± 9</td>
<td>105 ± 15</td>
</tr>
<tr>
<td>hex 1st ml</td>
<td>95 ± 4</td>
<td>98 ± 3</td>
<td>1 ± 1</td>
<td>102 ± 5</td>
<td>106 ± 7</td>
</tr>
<tr>
<td>hex 2nd ml</td>
<td>95 ± 4</td>
<td>98 ± 3</td>
<td>1 ± 1</td>
<td>102 ± 5</td>
<td>106 ± 7</td>
</tr>
</tbody>
</table>

Figure 5.1: Chromatograms of octadienol on the analytical C-18 column before (a) and after (b) filtration on the Millipore filter. Eluent: methanol/water 75/25 v/v; Flow: 1.0 ml/min
Sample pretreatment for emulsion polymerization products

5.4.2 Concentration techniques

SPE: For the SPE experiments a C-18 cartridge was used. This cartridge will mainly adsorb the apolar compounds and is therefore not suitable for the concentration of the charged oligomers. In figure 5.2 an analysis of the, Amicon filtrated, butadiene latex RDHPLC001 is shown on the C-18 column using 70/30 v/v methanol/water as the eluent. The peak 3 at ~6.3 minutes is from uncharged oligomers[1]. The SPE recovery of these latter oligomers was investigated in this study. No breakthrough was observed when 75 ml butadiene latex had passed through the cartridge. In the desorption step the first milliliter methanol eluted 94.3% of the adsorbed uncharged oligomers from the SPE cartridge. In the second milliliter of desorbent 5.2% of the oligomers was determined. In the third milliliter no detectable amount of oligomers was determined. The amount of charged oligomers present in the adsorbate product from the SPE cartridge was also increased with regard to the amount in the original solution. No quantitative results were obtained because the peaks from the charged oligomers eluted near t₀ (figure 5.2 'peaks' 1 and 2). It was tried to obtain quantitative information about these charged oligomers by analysing this product using the PL2 column (on this column the charged compounds showed more retention, see Appendix 5.1) but in this case also interference with the t₀ occurred.

![Figure 5.2: Chromatogram of the separation of the butadiene latex RDHPLC001 on the analytical C-18 column. Eluent: methanol/water 70/30 v/v; Flow: 1.0 ml/min](image-url)
In conclusion, it appeared that the neutral oligomers could be concentrated at least by a factor 75. In theory this would make a preparative separation of the uncharged oligomers possible. Using an anion exchange cartridge might increase the amount of charged oligomers recovered but a number of problems would arise. The charged oligomers must be desorbed using high salt concentrations in the desorbent which can be difficult to remove afterwards. Further the adsorption of the apolar part of the charged oligomers on the stationary phase can obstruct the desorbing of the compounds.

Freeze drying and evaporation: In table 5.11 the recoveries after evaporation and freeze drying are presented. The recovery is in most cases less than 100% and no general trend in the losses can be observed. In peak 1 interference of the $t_0$ may have influence on the results found for this peak. The reason for the loss of the compounds from the solution is not clear. When analysing the sample on the C-18 column no peaks were observed after $t_0$, this means that the compounds were charged oligomers and therefore not volatile. It is not expected that reaction took place during the concentration step because no extreme circumstances were applied. This is supported by the absence of peak shifting and also no new peaks were observed.

### TABLE 5.11: RECOVERY OF THE STYRENE Oligomers AFTER FREEZE DRYING AND EVAPORATION

Concentration determined on the chromatographic system II, for explanation of peaks 1,2,3 and 4 see figure 5.1.5 in Appendix 5.1

<table>
<thead>
<tr>
<th>peak yield</th>
<th>1 [%]</th>
<th>2 [%]</th>
<th>3 [%]</th>
<th>4 [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>evaporation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4x</td>
<td>215</td>
<td>115</td>
<td>50</td>
<td>98</td>
</tr>
<tr>
<td>20x</td>
<td>49</td>
<td>72</td>
<td>42</td>
<td>61</td>
</tr>
<tr>
<td>freeze drying</td>
<td>90</td>
<td>90</td>
<td>40</td>
<td>55</td>
</tr>
</tbody>
</table>

### 5.5 CONCLUSIONS

Although the factors described in this chapter are not yet investigated thoroughly it can be concluded that the sample preparation of the latices is not straightforward. Several factors have to be taken into account. Adsorption of the uncharged oligomers was observed
Sample pretreatment for emulsion polymerization products on the filters used to remove the latex particles, the Amicon YMT30, the MF-Millipore 0.05\(\mu\)m and the PCTE 0.05 \(\mu\)m filter. Although reversed phase SPE proved to be a useful technique to concentrate the uncharged oligomers it could not be used for the concentration of the charged oligomers. The two other methods investigated to concentrate the charged oligomer solution, freeze drying and evaporation, did not give 100% recovery of the compounds.

5.6 REFERENCES

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APPENDIX 5.1: HPLC SEPARATIONS OF REAL LATEX SAMPLES

5.1.1 INTRODUCTION

In chapter two the use of mixed mode columns for the separation of charged and uncharged oligomers is evaluated using model compounds. In this appendix some examples are given of separations of real latex samples. In some cases a comparison is made between these separations and separations performed using isotachophoresis (ITP).

5.1.2 EXPERIMENTAL

Instrumentation and columns

The reversed phase HPLC experiments were performed on a Waters Delta Prep 4000 equipped with a Waters Tunable Adsorbance detector (Millipore Corporation, Milford, Massachusetts, USA). The chromatograms were recorded on a recorder (Kipp&Zonen, Delft, The Netherlands). The column used was a home made column, slurry packed, 250x4.6mm ID, stationary phase LiChrospher RP-18, 15 µm (Merck, Darmstadt, Germany).

The mixed mode experiments were performed on a Bischoff HPLC pump and a Bischoff lambda 1000 UV detector (Bischoff, Leonberg, Germany). The chromatograms were recorded on a D 2500 Chromato-Integrator (Merck Hitachi, Tokyo, Japan) or on a recorder (Kipp&Zonen). The column used was the PL2 column and is described in chapter two.

Chemicals

Methanol, acetonitrile, HPLC grade, and ammonium carbonate were from Merck. Milli-Q purified water was used. The eluents were filtered and degassed, by ultrasonic treatment for the mixed mode experiments and by purging with helium for the reversed phase experiments, prior to use.

Crotylalcohol was from Fluka, Buch, Switzerland and 2,4-octadien-1-ol was from Johnson Matthey GmbH Alfa Products, Karlsruhe, Germany. Butadiene latices were obtained from the group of German. Styrene latices were obtained from the group of German or from...
the group of Eshuis.

Methods

In the examples presented in this appendix the isotachopherograms were taken from literature and compared with HPLC measurements of the same samples.

On the, Amicon filtrated, latex samples two kinds of HPLC analysis were applied. Measurements on a reversed phase column were performed to investigate the presence of uncharged oligomers and measurements on a mixed mode column were performed to investigate the charged oligomers. For the reversed phase experiments methanol/water or acetonitrile/water mixtures were used as the eluents. For the mixed mode experiments mixtures of methanol and an aqueous ammonium carbonate solution were used as the eluents. The compounds were detected using a UV detector at 220 nm.

5.1.3 RESULTS AND DISCUSSION

Reversed phase column

In figure 5.1.1 a chromatogram of an, Amicon filtrated, butadiene sample, RDHPLC001[1] on the C-18 column is presented using methanol/water 50/50 v/v as the eluent. The first group of peaks is probably from the disulfates and the second group probably from the monosulfates. Group three at 22 minutes is probably from uncharged oligomers. These oligomers could not be identified using the retention times of the model compounds crotylalcohol and 2,4-octadien-1-ol (see chapter five), these were, using this eluent, respectively 5.5 and 45 minutes. It is possible that these peaks are from dialcohols. This is the same sample as shown in figure 5.2 where methanol/water 70/30 v/v was used as the eluent, in that case the peaks of group three eluted as one peak. The fact that peak splitting was observed could mean that stereoisomers are present, see chapter four. When this sample was analyzed using a mixture of acetonitrile/water as the eluent no separation of group three is observed. This is in contrast with the results obtained for the uncharged styrene oligomers (chapter four), where the stereoisomer separation was better using acetonitrile instead of methanol in the eluent. In the RDHPLC001 sample a considerable
amount of uncharged oligomers were present. In the other samples that were analyzed, which will be described in more detail by the description of the mixed mode analysis, only a little amount of uncharged oligomers could be detected.

Figure 5.1.1: Chromatogram of the separation of the butadiene latex RDHPLCO01 on the analytical C-18 column. Eluent: methanol/water 50/50 v/v; Flow: 1.0 ml/min
appendix 5.1

Mixed mode column

In figures 5.1.2 and 3 examples of HPLC separations are presented for two, Amicon filtrated, butadiene samples, respectively 880107 and 880614, using the PL2 column are compared with their ITP separations[2]. Generally it can be concluded that when the concentration and the amount of peaks in the HPLC analysis increased the concentration and the amount of compounds identified in the ITP analysis was also larger. This means that it is possible, using mixed mode columns, to obtain retention and (some) separation of the compounds that must be analyzed. It was not possible to make any peak identification by comparing the retention times of the peaks found in the samples with the retention times observed for the model compounds for butadiene oligomers (chapter two). It was also not possible to obtain information about the identity of the peaks by observing the changes in the retention times of the separated compounds by varying the methanol concentration in the eluent and comparing this with the change of the retention time of the model compounds. Since by changing the methanol concentration the peak shape changed in a large amount so no peak identification could be made. The amount of the latex samples that was available for the HPLC separations was not enough to do a separation on a preparative scale, therefore no research could be performed on the structure of the compounds. In figure 5.1.4 an example of the HPLC and ITP analysis[3] of a styrene sample (S113) is shown. In this case also partial separation was obtained. In figure 5.1.5 the HPLC separation of a styrene sample from the group of Eshuis is presented, in this case no ITP analysis was available.
Figure 5.1.2: Chromatogram (a) and isotachopherogram (b)[2] of the separation of the butadiene latex 880107. HPLC conditions: Column: PL2 column; Flow: 1.0 ml/min; Eluent: methanol/aqueous ammonium carbonate solution of 10^{-2} M 20/80 v/v. For the ITP conditions see reference [2]
Figure 5.1.3: Chromatogram (a) and isotachopherogram (b) [2] of the separation of the butadiene latex 880614. HPLC conditions: Column: PL2 column; Flow: 1.0 ml/min; Eluent: methanol/aqueous ammonium carbonate solution of $10^{-2}$ M 10/90 v/v. For the ITP conditions see reference [2]
Figure 5.1.4: Chromatogram (a) and isotachopherogram (b) of the separation of the styrene latex SI13. HPLC conditions: Column: PL2 column; Flow: 1.0 ml/min; Eluent: methanol/aqueous ammonium carbonate solution of 10^2 M 30/70 v/v. For the ITP conditions see reference [3]. a = M(SO_4)_2; b = M_2(SO_4)_2; c = MSO_4 (unsaturated); d = MSO_4; e = M_2SO_4 (unsaturated); f = M_2SO_4; L = CT; S = standard and T = CH_3CH_2COO where M is a monomer of styrene
Figure 5.1.5: Chromatogram of the HPLC separation of the styrene latex from the group of Eshuis on the PL2 column. Eluent: methanol/aqueous ammonium carbonate solution of 10^{-2} M 30/70 v/v; Flow: 1.0 ml/min

5.1.4 CONCLUSIONS

From the analysis of real samples the following can be concluded. In most of the samples almost no uncharged oligomers were observed as was shown in the reversed phase analysis. Only in the case of the RDHPLC001 sample a considerable amount of uncharged oligomers was observed. But this sample was prepared under extreme reaction conditions[1]. From the mixed mode separations it can be concluded that charged oligomers showed more retention on the PL2 column than on the C-18 column, as was expected. It was not possible to obtain baseline separation of the compounds present in the sample. There was not enough sample available to carry out a preparative experiment so it was not possible to obtain in this way information about the structure of the compounds.
5.1.5 REFERENCES

2. C.G.J.M. Pijls *MS Thesis* Eindhoven University of Technology 1989
CHAPTER SIX: FINAL CONCLUSIONS AND RECOMMENDATIONS

In this study several aspects of the qualitative and quantitative analysis of oligomers formed during emulsion polymerization processes were investigated.

First the separation of the charged and uncharged oligomers was investigated using mixed mode (reversed phase/anion exchange) HPLC as described in chapter two. A number of columns were investigated on their mixed mode properties using model compounds. The PRP-X100, the OmniPac PAX-500 and the Polymer Labs mixed phase columns showed promising results with respect to the possibility to separate charged and uncharged oligomers of butadiene and styrene. The eluent mixtures developed for these analysis can be easily removed after the separation. This makes this technique also suitable for preparative separations of these oligomers to allow further research on the structure of these compounds in a later stage in the investigations. As is shown in Appendix 5.1 the separation of real latex samples is not yet optimal using mixed mode columns.

To scale up these separations to preparative HPLC a preparative column system, the Annular Expansion column, was investigated applying a silica based reversed phase stationary phase as described in chapter three. The efficiency of this column was good for a number of packing times, reusing the same packing material. However, after that the column quality gradually decreased. The Annular Expansion column proved to be a useful system to pack home made preparative columns and showed a performance comparable to its analytical analogue. The question is whether or not this preparative system is also suitable for polymer based packings as are used in the mixed mode experiments.

The preparative separation of, neutral, styrene oligomers is described in chapter four using the preparative Annular Expansion column packed with reversed phase material. The separation proved to be simple and in theory 5 ml of the original styrene oligomers mixture could be separated, still obtaining baseline separation.

In chapter five a number of aspects concerning the sample pretreatment for latices are described. To remove the, very small, latex particles a number of filters were tested: the Amicon YMT30, the MF-Millipore with 0.05 μm pores and the PCTE Poretics with 0.05 μm pores. These filters were able to remove the latex particles but adsorption of the target uncharged oligomers was observed at the same time. As concentration techniques for the
dilute oligomer samples solid phase extraction (SPE), freeze drying and solvent evaporation were investigated. Using C-18 SPE cartridges it proved to be possible to concentrate the uncharged oligomers of butadiene at least by a factor 75. As could be expected the recovery of the charged oligomers was lower under these conditions. When freeze drying and evaporation were used as concentration techniques irreproducible and incomplete recoveries of the oligomers were observed.

The quantitative and qualitative separation of oligomers of real samples is at present still far from a routine technique. Further research has to be focused on several subjects, some of them already pointed out above:

1. Optimizing of the separation of real latex samples using mixed mode HPLC and a thorough comparison of these results with the results that can be obtained with isotachophoresis (ITP) analysis.
2. Testing of the preparative Annular Expansion column with polymer based packing materials. According to the manufacturer (Merck) this should be possible.
3. Further research on the sample pretreatment of latices with respect to the adsorption of the oligomers to filter material and also to concentration techniques of oligomer solutions.
4. Scaling up of the analytical separation to preparative HPLC to allow further research on the structure of the compounds.
   a) The separation of the uncharged oligomers formed during the emulsion polymerization process can be performed using the Annular Expansion column with the reversed phase packing material as described in chapter three and four. In this case concentrating of the sample can be performed using C-18 SPE cartridges. A problem that still has to be solved is the loss of compounds by the filtration of the latex sample.
   b) The separation of charged oligomers is at present more difficult. The separation still has to be optimized as stressed in point 1. The preparative separation for polymer packing materials is not yet investigated (point 2). And although no loss of charged compounds was observed during filtration a suitable technique for the concentration
Final conclusions and recommendations

of these compounds still has to be developed (point 3).

5. In a later stage attention can be paid to the possibility to separate the charged and the uncharged oligomers in one run. This can for instance be performed on a mixed mode column using gradient elution. First the uncharged oligomers can be eluted by using the column in the reversed phase mode by using a mixture of an organic modifier and water as the eluent. In this mode the charged oligomers will not be eluted. After that the charged oligomers can be eluted in the mixed mode by using a combination of an organic modifier and an aqueous ammonium carbonate solution as the eluent.
SYMBOLS AND ABBREVIATIONS

SYMBOLS:

\( C_M \) the monomer concentration in the particles
\( D_a \) diameter analytical column
\( D_p \) diameter preparative column
\( H \) (theoretical) plate height
\( H_{sys} \) 'true' plate height
\( I_m, I_{m-1} \) Bessel functions of the first kind
\( K \) partition coefficient
\( L \) length of the column
\( L_a \) length analytical column
\( L_p \) length preparative column
\( N \) (theoretical) plate number
\( N_{av} \) Avogadro's number
\( N_i \) the number of particles with \( i \) radicals
\( N_p \) the number of particles per unit volume
\( N_{sys} \) 'true' plate number
\( N_T \) the number of latex particles in the water phase
\( R \) correlation coefficient
\( R_s \) resolution
\( T \) the absolute temperature
\( V_m \) volume mobile phase
\( V_s \) volume stationary phase
\( W \) the stability ratio for a precursor-latex particle interaction which can be calculated using the DLVO theory
\( a_{sf} \) asymmetry factor
\( b \) charge of the eluent ion
\( c \) the rate coefficient for bimolecular termination of radicals in the particles
\( d_p \) particle diameter
\( f \) charge of the solute ion
symbols and abbreviations

\( h \) (theoretical) reduced plate height
\( h_{sys} \) 'true' reduced plate height
\( k \) Boltzmann's constant
\( k' \) capacity factor
\( k_d \) the rate coefficient of desorption of the radicals from the particles
\( k_e \) the first order rate coefficient of entry
\( k_p \) the propagation rate constant
\( k_r \) the rate coefficient of entry of already desorbed radicals
\( \bar{n} \) the average number of radicals per particle
\( r_p \) the polymerization rate per unit volume
\( r_{pp} \) the radius of the precursor particle
\( r_s \) the radius of the latex particle
\( t_R \) retention time
\( v \) the volume of a monomer swollen latex particle
\( w_{0.1} \) peak width at 10% height
\( w_h \) peak width at half height
\([E\bullet]\) the concentration of the desorbed radicals in the water phase
\([M]_w \) the monomer concentration in the water phase
\([R\bullet]\) the radical concentration in the water phase
\( \alpha \) separation factor
\( \rho \) the second order rate coefficient of entry of radicals
\( \sigma \) the chance that an oligomer radical propagates in the water phase
\( \sigma_0 \) the chance that an initiator radical propagates in the water phase

ABBREVIATIONS:

A/E Annular Expansion
CMC critical micelle concentration
GC gas chromatography
HPLC high performance liquid chromatography
ID inner diameter
symbols and abbreviations

IR infra red
ITP isotachophoresis
MS mass spectroscopy
NMR nuclear magnetic resonance
PCTE polycarbonate track etching
SEC size exclusion chromatography
SFC supercritical fluid chromatography
SPE solid phase extraction