2004

A Twist on Packing Analytical Columns for Reversed Phase Liquid Chromatography

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A TWIST ON PACKING ANALYTICAL COLUMNS FOR
REVERSED PHASE LIQUID CHROMATOGRAPHY

By

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A Dissertation submitted to the
Department of Chemistry and Biochemistry
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

Degree Awarded:
Fall Semester, 2004
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ABSTRACT

Liquid chromatography is one of the most widely used analytical techniques for the separation of complex mixtures. Great advances have been made in the last twenty years in column technology, i.e. stationary phases, but few advances have been made in column packing technology. In this study, two new methods for packing analytical reversed-phase liquid chromatography (RPLC) columns are introduced: radially spinning slurry packing and surfactant aided slurry packing. The column performance of columns packed by the traditional slurry packing procedure, the radially spinning slurry packing procedure, and the surfactant aided slurry packing procedure was evaluated and compared. It was determined that the different slurry and pushing solvents utilized during the different packing methods had little effect on column performance. The packed beds of columns packed by the traditional, radially spinning, and surfactant aided slurry packing procedures were investigated by generating a van Deemter plot for columns packed by each method. These van Deemter plots indicated that columns packed by the radially spinning slurry packing technique and the surfactant aided slurry packing technique had different bed structures than those of the control columns packed by the traditional slurry packing procedure. The columns packed by the radially spinning slurry packing procedure process had better column-to-column reproducibility in %RSD of retention time and the capacity factor than columns packed by the traditional slurry packing procedure and the surfactant aided slurry packing procedure. Due to the difficulty of packing columns by the radially spinning technique, the use of a surfactant modifier when packing RPLC columns according to the traditional slurry packing procedure would be much more appealing to column manufacturers.
CHAPTER 1

INTRODUCTION AND BACKGROUND

1.1 Introduction

Chromatography has long been used for both analytical separation and large-scale purification. It was invented and used for the first time by Tswett, a Russian botanist, in the early 1900’s to separate plant pigments on a column of calcium carbonate [1]. There is not another technique that provides scientists with such a capability to separate, purify, identify, and quantify complex mixtures. Following Tswett’s invention of chromatography, a number of advances were made in the field of separations that led to gas chromatography, paper chromatography, thin-layer chromatography, and modern liquid chromatography [2].

The term Liquid Chromatography (LC) can be used to describe any chromatographic technique that uses a liquid as the moving phase. In the case of modern LC, also referred to as High Pressure Liquid Chromatography or High Performance Liquid Chromatography (HPLC), the liquid is forced through a stationary phase that is contained within a column. The term “modern” refers to the instrumentation developed that makes this possible. Improvements in instrument design allow the liquids used in LC to be forced through the stationary phase at higher pressures and with greater reproducibility than previously possible [2].

Liquid chromatography can be broken down into two subsets, normal phase liquid chromatography and reversed-phase liquid chromatography (RPLC), the latter being the more popular mode of HPLC. Two conditions must be met in order to operate in the reversed mode of HPLC. The first condition being that the separation media (stationary phase) employed must be non-polar. The separation media generally consists of a hydrocarbon chain (C-18 or C-8) chemically bonded to a silica substrate. The non-polar stationary phase is then packed into a stainless steel column, which comes in a variety of lengths and diameters. The second condition required to operate in the reversed mode is
that the liquid (mobile phase) that transports the solutes through the stationary phase must be polar. The mobile phases used in RPLC are typically water mixed with a known fraction of an organic modifier such as methanol, acetonitrile, or tetrahydrofuran.

The stationary phases used to pack RPLC columns have definitely improved and evolved over the past 20 years; however, there has not been any advancement during this time in the technology used to pack RPLC columns with these stationary phases. With the advancements in available stationary phases, the shortcomings of current column packing technologies have become more evident.

1.2 Different methods for packing RPLC columns

There are three procedures used to pack reversed-phase liquid chromatography (RPLC) columns, but no one method is superior for packing all types of stationary phases [3]. The best packing procedure is usually chosen based upon characteristics of the packing material and the applications intended for the column being packed. Axial compression [4-9] and radial compression [10-15] are used for the preparation of wide bore columns for preparative applications. Radial compression has also been used to prepare analytical columns. The most commonly used packing technique for packing analytical columns is slurry packing, but slurry packing is also used to prepare some columns for preparative scale applications [16]. Each of these techniques involves the application of a certain level of mechanical stress to the particles during the packing process. However, this mechanical stress is not conveyed homogeneously throughout the bed during the packing process; it varies depending upon the axial and radial position. This leads to heterogeneity within the packed beds of conventional RPLC columns of both analytical and preparative scales [4].
1.2.1 Axial Compression

This packing technique requires the use of a column that can be thought of as a huge syringe, the barrel of which contains the packing material, and the piston of which is moved by a hydraulic jack. The instrumental set-up for axial compression is shown in figure 1.1. A mechanical stress up to 100kg/cm² can be applied with the jack [4-9]. A dilute slurry of the packing material is placed in the column barrel, and the barrel is closed with a frit and a bolted flange. The jack is then used to move the piston upward to compress the slurry. Excess liquid can exit through the frit at the end of the column opposite the piston. As the liquid is forced out of the column, a consolidated bed builds up progressively [4].

Figure 1.1 Schematic of an instrument for axial compression [6].
1.2.2 Radial Compression

With this technique a plastic cartridge that is closed at both ends by a frit and a stream distributor is filled with the packing material and placed inside a steel cylinder. The design of both the plastic cartridge and steel cylinder allow for the formation of a leak-proof seal at both ends of the column. Radial compression of the bed is accomplished by forcing a hydraulic fluid, under pressure, between the plastic cartridge and the steel cylinder [10-15]. As shown by figure 1.2, the plastic cartridge takes on the shape of the packed bed when the column is under pressure from the hydraulic fluid used to compress the stationary phase bed.

Adapted from http://www.laboratorytalk.com/books/chem/chrom/rs_11/rs_11_34.html

Figure 1.2 Diagram of a radial compression column.
1.2.3 Slurry packing

Slurry packing involves the use of high pressure, up to 800 atms, to push a dilute slurry of stationary phase through a stainless steel LC column that is closed at the outlet with a frit and end fitting to prevent the stationary phase from being extruded from the column. When preparing the slurry, it is important to use a solvent that is good at wetting and dispersing the stationary phase [16]. This is necessary to prevent aggregation of the stationary phase particles during the packing process and to prevent the particles from settling out of the slurry solvent prior to the packing process. A pushing solvent is then pumped through the column at a high flow rate and pressure to force bed consolidation. Bed consolidation takes between 15 to 30 minutes depending upon the length of the column being packed.

The particles in the slurry are initially carried rapidly down the column by the pushing solvent and collide with the rising surface of the bed. Then, the fast-moving stream of solvent forces the particles further into the column consolidating the bed. A

Figure 1.3: Slurry packer set-up.
typical slurry packing set-up is shown in figure 1.3. This set-up consists of a solvent reservoir, pump, pre-column assembly, and analytical RPLC column. The solvent reservoir houses the pushing solvents used during the packing process. The pump, a Haskel double action air driven fluid pump in this case, delivers the required pressure for bed consolidation of the stationary phase in the column. The pre-column assembly serves as a reservoir for the stationary phase slurry prior to pressurizing the system.

1.3 Descriptors of column performance

Once a column has been packed, it is necessary to evaluate its performance. There are several parameters that can be used to evaluate the performance of a given column. The column efficiency (N) or the number of theoretical plates is one such descriptor. There are several methods for calculating N, the Foley-Dorsey equation [17,18]; equation 1.1, will be used in this study. This equation assumes a modified gaussian peak and has been shown by Berthod [19] to be one of the most accurate methods for calculating the column efficiency. The retention time \( t_R \) is the time that it takes for the solute of interest to come off the column, and \( W_{0.1} \) is the width of the peak of interest at ten percent of its height. The asymmetry factor \( b/a \) is used to describe the shape of the peak of interest, and it is also used in equation 1.1. This ratio must be greater than or equal to unity for equation 1.1 to be accurate; therefore, if the peak is fronting the ratio must be inverted. Figure 1.4 illustrates how to calculate the asymmetry factor. The asymmetry factor is measured by drawing a line down through the peak maximum and measuring the width of the peak on each side of the line at 10% of the peak’s height. As expected, a column that produces peaks with an asymmetry factor approaching unity will have better column efficiencies than columns that produce peaks with large asymmetry factors.

\[
N = \frac{41.7 \left( \frac{t_R}{W_{0.1}} \right)^2}{1.25 + \frac{b}{a}}
\]

Equation 1.1
Another descriptor that can be used to evaluate column performance is the height equivalent to a theoretical plate (HETP or H). This value can be determined with equation 1.2, where L is the column length and N is the column efficiency.

Equation 1.2: \[ H = \frac{L}{N} \]

Therefore, H is a measure of the column efficiency per unit length, and small H values indicate a more efficient or better column.

The reduced height equivalent to a theoretical plate (h), also referred to as the reduced plate height, is useful when describing column quality. It can be found using equation 1.3, where H is the HETP and d.p. is the diameter of the stationary phase particles. The reduced plate height for a good RPLC column will range from 2 to 5. This is a unitless number, and a smaller value indicates better column quality.
1.4 Heterogeneity found within the packed beds of current RPLC columns

The packed beds of columns packed by the previously mentioned techniques all suffer from some degree of heterogeneity. This has been confirmed by a number of researchers. Knox and Parcher [20] were the first to describe a heterogeneous region in the packed beds of chromatography columns. They found this region to exist close to the column wall and referred to it as the “Extent of Wall Region”. It was proposed that this region was approximately 3 particle diameters thick. This region of heterogeneity was found to cause an increase in the reduced height equivalent to a theoretical plate and an overall loss of efficiency resulting in lower resolution.

Knox and Parcher [20] proposed operating in the infinite diameter mode to prevent solutes from reaching the wall region. They suggested that if the column diameter were sufficiently large compared to the column length and particle diameter, solute molecules injected centrally onto the top of the column would not reach the walls while traveling the length of the column. For a given column to behave as if it were of infinite diameter, the column diameter \(d_c\) should exceed about four standard deviations of the radial dispersion \(\sigma_r\), \(d_c \geq 4\sigma\) where \(\sigma_r\) is the radial dispersion. Although, this theory has been proven to be valid [21-23], it is not possible to operate in this mode with valve injection [24].

With that being the case, chromatographers have chosen to ignore the wall effect attributed to the extent of wall region. The wall effect has been investigated by several groups including Knox et al. [25] and Eon [26]. Both studied the wall effect using dry packed stainless steel HPLC columns. Knox et al. determined that the extent of wall region was 30 particle diameters thick instead of the 3 particle diameters thickness originally thought to exist. Both groups found the mobile phase velocity to be greater close to the wall than in the central region of the column (figure 1.5). And, they found that the reduced axial plate height increased significantly close to the column wall. Eon also studied radially compressed columns and found that both the dry packed and radially compressed columns suffered from some degree of heterogeneity. However, the radially compressed column did not suffer as greatly from the wall effect as did the dry packed
Figure 1.5: The dependence of axial reduced plate height (a) and peak maximum velocity (b) upon radial position [25].

column [26]. Baur et al. [27] investigated the wall effect using slurry packed HPLC columns. They found the mobile phase velocity to be less in the region close to the column wall and greater in the center, figure 1.6. This is a result of the stationary phase in a slurry packed column being more dense close to the column wall than it is in a dry packed column [4]. Baur et al. [27] used a microvoltammetric electrode that could be placed, with a micropositioner,
The findings of Knox et al. [25], Eon [26], and Baur et al. [27] were confirmed by those of Farkas et al. [24]; however, Farkas et al. found the extent of wall region to be 30 to 50 particle diameters thick as opposed to only about 30 particle diameters thick as proposed by Knox et al. Knox et al. felt that in order to overcome the wall effect, chromatographers must operate under conditions where a large amount of the column’s potential performance is wasted [25].
In a later study, Farkas et al. [28] again confirmed the presence of a non-uniform region of packing along the column wall. The column efficiency calculated from peak width at half height for peaks of pyrromethane recorded at differential radial locations with on column fluorescence detection is plotted in figure 1.7. The column efficiency, as indicated by the plot, was much lower at and close to the column wall than it is in the central region of the packed bed. Again, this led to a reduction in the overall resolution. The heterogeneity of the stationary phase close to the column wall was photographed by Yun and Guiochon [29]. They packed HPLC columns with alternating layers of virgin Zorbax C\textsubscript{18} and blue colored Zorbax. From the photographs, figure 1.8, it was evident that there was friction along the column wall during consolidation. The observed friction led to a region of heterogeneity close to and at the column wall.
Figure 1.8: Extruded column beds from columns packed with alternating layers of
virgin Zorbax C$_{18}$ and blue colored Zorbax [29].

This extent of wall region theory is further supported by a recent NMR study by
Harding and Baumann [30]. NMR Imaging, Pulsed Gradient Spin Echo (PGSE) NMR,
and Dynamic NMR were used to measure and characterize flow through
chromatographic columns. There was an obvious region of lower than average velocity
about 150 µm wide near the column wall. They also observed that the low velocity
region near the column wall became more apparent as the packing density was increased.

Guiochon et al. have studied the “Wall Effect” extensively. They found that
current columns are heterogeneous, both axially and radially [31-33] and that the density
of the bed of the column decreases towards the top of the column [31]. Heterogeneity of
the bed in the axial direction has only a minimal effect on column performance; however,
the presence of heterogeneity in the radial direction has a much greater effect [33,34].

There are two main factors that attribute to variations in the radial packing
density: the geometry of the packing in the wall region and the friction between the wall
and the particles and also between the particles themselves. This packing variation leads to two different wall regions. One is next to the wall of the column due to the increase in the void fraction near the wall since the stationary phase particles cannot penetrate the wall surface. Because of this, the mobile phase velocity in this region is greater than the mobile phase velocity in the center of the column. The second wall region is located near the column wall and is packed more densely than the first resulting in a lower mobile phase velocity in this region than that observed in the center of the column [31].

This evidence confirms that the beds of conventional packed columns used in LC are not homogeneous. Knox et al. [25], Eon [26], Baur et al. [27], and Farkas et al. [24,28] have demonstrated that the local velocity of the mobile phase and the local HETP vary across the column. Therefore, the local column efficiency varies across the column as well [4]. With the presence of radial heterogeneity in all conventional HPLC columns [35], it is clear that improvements in column packing could lead to an increase in overall column performance.

1.5 Research goals

The goal of this work is the development of new column packing technologies. The development of these new methods for packing RPLC columns will be based on the current slurry packing procedure used for packing RPLC columns. The slurry packing procedure was chosen for modification since it is the most commonly used procedure for packing analytical scale RPLC columns. A portion of this work will look at the overall column performance of columns packed by a modified slurry packing procedure that utilizes centripetal force as an aid during the slurry packing process and compare those results to those from columns packed by the traditional slurry packing procedure. Another portion of this work will focus on the benefits of adding surfactants to the slurry solvent(s) and pushing solvent(s) used during the slurry packing procedure.
1.5.1 Centripetal force as a tool for packing RPLC columns

The use of centripetal force while slurry packing to create a more homogeneous bed during consolidation is the driving force in this study. Centripetal force was utilized by Fermier and Colon [36] in the packing of capillary columns for use in Capillary Electrochromatography (CEC). The use of long capillaries in CEC is advantageous since CEC is not pressure limited technique. Just as in RPLC, the packing of long capillary columns for use in CEC is very tedious and time consuming. The combination of the small inner diameters and long lengths of capillaries used in CEC make column packing by the traditional pressure procedure very difficult. Fermier and Colon used centripetal force in the longitudinal direction in place of pressure driven flow. The development of this method not only reduced the amount of time required to pack a capillary column, but it also allowed for the packing of up to eight capillary columns simultaneously. The results of studies using these centripetally packed capillary columns were very similar to those of traditionally packed capillary columns.

In order to use centripetal force as a tool during the slurry packing procedure, the column must be spun during the packing process. This spinning should create a radial centripetal force while pressure driven flow creates an axial force. A large part of this work will be the design and manufacturing of a high-speed coupling for column rotation in house. It will allow for the RPLC column being packed to be spun at variable speeds during the slurry packing procedure. During this new packing procedure there will be two forces acting upon the stationary phase particles. One force will push the particles downward toward the outlet of the column, just as in the traditional slurry packing method. The other will force the particles toward the column wall during the packing process. This should result in the formation of a more homogeneous packed bed within the column.

1.5.2 The use of surfactants for packing RPLC columns

The term surfactant is used to refer to surface-active agents. These surface-active agents are some of the most versatile compounds in the world today. Surfactants are
typically organic compounds that contain both a hydrophobic group and a hydrophilic group making them semi-soluble in both organic and aqueous solvents. Surfactants have an amphipathic structure, meaning they have a polar head group and a non-polar tail. So, if a surfactant is present in the proper concentration it has the ability to greatly reduce or possibly eliminate the interfacial free energy between two immiscible phases. The interfacial free energy, or surface tension, is the minimum amount of work needed to create an interface between two immiscible phases. Surface tension can also be described as the amount of work required to transport a molecule from the interior of the phase to the surface of that phase [37,38].

![Surfactant Behavior](http://www.ilpi.com/genchem/demo/tension/)

Figure 1.9: Surfactant behavior at a two-phase interface

Figure 1.9 indicates how surfactant molecules organize at the interface of two immiscible phases. The polar head group inserts itself into the aqueous phase, and the non-polar tail is in the non-polar phase. This organization that occurs at the interface of the two immiscible phases is what leads to the reduction observed in the surface tension between the two phases [37].

Another term used when working with surfactants is wettability or wetting. The process of displacing one liquid from a surface by another is referred to as wetting, and wetting is very similar to surface tension [37]. As stated previously, the solvent used to prepare the slurry of stationary phase to be used in the slurry packing procedure should be good at wetting and dispersing the stationary phase [16]. The stationary phase
particles in the slurry must not aggregate, and the particles must be held in the suspension long enough to be packed into the column without any sedimentation-sizing occurring. It is important to have fluid velocities as high as possible during the packing process. This allows for uniform and dense compactation into the developing bed [2]. In previous studies, solvents (slurry and pushing) have been chosen so as to give a balanced-density between the solvent and stationary phase and to have low viscosity to allow for high flow rate velocities. To our knowledge there have been no investigations of the effects of surface tension. Surfactants allow the manipulation of surface tension with minimal effects on density and viscosity. The addition of surfactant to the solvent used to prepare the slurry and to the pushing solvents used during the packing process could lead to better wetting and dispersion of the stationary phase. Better wetting and dispersion of the stationary phase could ultimately lead to the formation of a more homogeneous bed of stationary phase within the column.
2.1 Radially spinning slurry packing

This method of packing is based upon the traditional downward flow slurry packing procedure with one significant difference; the column will be radially spinning during the packing process. The radial spinning should create a centripetal force that will act upon the stationary phase particles and push them toward the column wall. In order to spin the column radially during the slurry packing process, a coupling that allows the column being packed to be spun radially without the loss of pressure required for stationary phase bed consolidation was designed and manufactured in-house, figure 2.1. There were a number of things that were considered during the design phase of the coupling. The material used to form the housing had to be strong enough to withstand the pressure used during the packing process, the bearings had to be able to function at the pressure and rotation rate used during the packing process, and the seals had to be able to maintain pressure while spinning radially and be resistant to the solvents used during the packing process.

Since the housing had to withstand pressures as high as 6000 psi during the packing process, it had to be made of a material that could withstand these pressures. The housing (main, upper, and lower) was machined from 304 stainless steel, and the main body of the housing is shown in figure 2.2. The housing was sufficiently thick, 1.271 inches, to prevent any changes in shape from occurring due to the pressure that would be pressing outward on the walls of the coupling. The rotor, figure 2.3, was
Figure 2.1: Exploded cross sectional view of the coupling. All dimensions are in inches.
Figure 2.2: Main housing body.

also machined from 304 stainless steel so that it too could withstand the pressure as well as any friction created from spinning during the packing process. The rotor is the piece of the coupling that spins during the radially spinning packing process. The column inlet is connected to the bottom of the rotor with a Swagelock union, and the column outlet is connected to an electric motor so that it can be used as a drive shaft to spin the rotor. The stationary phase slurry passes through the center of the rotor as it is forced into the column being packed.
Figure 2.3: Schematic of the rotor from the coupling. All dimensions are in inches.
Figure 2.4: Schematic of the seal capture assembly from the coupling. All dimensions are in inches, and TYP indicates each segment has the same length and each pin has the same width.
The seal capture assembly, figure 2.4, was machined from the same stainless steel used for the housing and rotor for durability purposes. The seal capture assembly is made of three separate segments that are connected to each other and to the rotor with dowel pins, figure 2.5, so that the assembly will spin with the rotor during the radially spinning slurry packing process. Both ends of the dowel pins are rounded to make insertion and removal easier, and the dowel pins are case hardened for better durability. The dowel pins used in the seal capture assembly have a diameter of 1/8 inch and a length of 1/2 inch. The three segments of the seal capture assembly, figure 2.4, capture the o-rings and secure them in place when the coupling is assembled and tightened down capturing the o-rings in a fashion that helps to maintain their shape and create a better seal between the rotor and the inlet shaft of the upper housing. The seals that are captured by the assembly spin with the rotor while creating a seal between the assembly and the inlet shaft of the upper housing cap, figure 2.1.

Several different types of bearings were used in the coupling as shown in figure 2.1. Two steel and stainless steel ball bearings, figure 2.6, were chosen because they were capable of spinning at the rotational rate desired. The large lower ball bearing (part # 6206) could withstand being spun up to 14,000 RPM, and the small upper ball bearing (part # 6002) could withstand an even higher spinning rate of 30,000 RPM. The other type of bearings used in the coupling are thrust bearings, figure 2.7. The upper thrust bearing is a steel needle-roller bearing that had a 7/8” shaft, 1/8” race, and a 31 thousandths race. The lower thrust bearing (part #
8110895R04&5) is a roller bearing. The difference between a needle-roller thrust bearing and a roller thrust bearing is the diameter of the rollers that are placed in the cage assembly. These thrust bearings have a large contact area between the roller and
raceway, can be used for medium to heavy duty tasks, have a low profile design for limited spaces, and have cage assemblies and washers made of hardened steel. This type of bearing was used to aid with any deflection that might occur in the steel and stainless steel ball bearings due to the upward force placed on the rotor from the pressure used to pack the column during the radially spinning slurry packing process. Bearing deflection is the elastic deformation that can occur between the ball bearings and the raceways because of external loads.

It is very difficult to find a seal that will perform well for this type of application. None of the available seals have all of the specifications required to function properly under the conditions of this application. A number of manufacturers make seals that are capable of sealing under the high pressure used during the packing process or while spinning during the packing process, but there is not a seal available that is capable of both. The shape of the two surfaces where the seal is needed is cylindrical; therefore, this application required the use of an o-ring seal, figure 2.8.

![Diagrams of the shape and cross sectional view of an o-ring style seal.](image)

Figure 2.8: Diagrams of the shape and cross sectional view of an o-ring style seal.

The decision on what type of material the seal should be made from was difficult. The material for the seals had to be compatible with the solvents used during the packing process and able to withstand the friction created from the radial spinning during the packing process. Therefore, it was necessary to try seals made from several different materials. Initially, Teflon o-rings were used in the coupling because Teflon is resistant to the solvents used during the packing process, but these
seals did not perform well. The stationary phase slurry acted like a fine sand paper during the packing process and created a flat surface on the Teflon o-rings causing the seals to fail. This failure allowed the stationary phase slurry and pushing solvent to escape into the chamber of the main housing body and reach the upper bearings. When this happened the coupling had to be disassembled so the seals could be changed and the bearings could be thoroughly cleaned. Also, the stationary phase was contaminated with bearing lubricant and could not be recovered. The second type of o-ring used was made from Viton (fluorocarbon), and this material offers a wide spectrum of solvent compatibility. The Viton o-rings performed well and did not fail as often as the Teflon o-rings. Another style of Viton o-rings, Quattro seal (figure 2.9), was used in conjunction with the style of o-ring shown in figure 2.8 to form a seal between the lower housing and the bottom of the rotor, figure 2.1. The Quattro seal was chosen because it has four sealing surfaces and double the sealing ability of a standard o-ring. This style of seal is symmetrically designed to seal on the inner diameter, outer diameter, top, and bottom. This design reduces the force needed to create a positive seal. Two seals of each design, standard and Quattro, were stacked in an alternating fashion in the lower housing to form a seal with the bottom of the rotor. Number 112 Viton o-rings were used in the seal capture assembly (figure 2.4), and number 114 Viton o-rings were used in the lower housing.

High tensile strength grade 8 bolts were used to assemble the coupling. Swagelock fittings were used to mate the column inlet to the bottom of the rotor and to attach the plumbing from the pump used to provide the pressure needed during the
packing process to the inlet of the upper housing. This hardware was selected for its strength and durability. The coupling was mounted to the frame of a SorVall superspeed Automatic Refrigerated centrifuge model RC2-B. The centrifuge was stripped down to the frame, and the motor and coupling were then mounted to the frame. The electric motor from the centrifuge was used to spin the column and rotor during the radially spinning slurry packing process. The column being packed was used as a drive shaft during the packing process to connect the electric motor to the rotor of the coupling. The speed of the electric motor was controlled with the control panel from the RC2-B centrifuge. The coupling, electric motor, and frame are shown in figure 2.10. A piece of PVC pipe was placed between the coupling and electric motor as a safety precaution to aid in the collection of solvent used during the

Figure 2.10: The coupling, electric motor, and frame.
packing process. A piece of quarter inch tubing runs from the base of the PVC pipe
to a waste container, and the solvent drains out of the pipe through the tubing into the
waste container.

All materials used to manufacture the coupling were obtained from
McMaster-Carr (Atlanta, GA).

2.2 Surfactant aided slurry packing

The physical properties of surfactants make them an attractive slurry and
pushing solvent modifier for use during the traditional downward flow slurry packing
procedure. Surfactants have the ability to greatly reduce the surface tension between
two phases and allow the manipulation of surface tension without significantly
altering the density or viscosity of the solvent. The addition of the surfactant
modifier to the slurry and pushing solvents used in the slurry packing procedure could
lead to better wetting and dispersion of the stationary phase particles during the
packing process which might reduce the heterogeneity found within the packed bed of
the RPLC column.

The surfactant to be used as a modifier must have a critical micelle
centreration (CMC) above the concentration that will be used in this study. The
volume percent of surfactant modifier added to the slurry and pushing solvents
resulting in a maximum in column efficiency will be determined experimentally, and
this percentage of modifier will then be added to the slurry and pushing solvents prior
to the slurry packing process.
CHAPTER 3

EXPERIMENTAL METHODS

3.1 Synthesis of stationary phase

3.1.1 Reagents

Anhydrous methylene chloride was obtained from Aldrich (Milwaukee, WI), and all other organic solvents were purchased from Fisher Scientific (Milwaukee, WI) and were of HPLC grade. Water was de-ionized and passed through a Barnstead (Boston, MA) Nanopure II purification system fitted with a 0.45 µm filter. The anhydrous methylene chloride was used as received from Aldrich. The silane, n-octadecyl-dimethyl-chlorosilane, was purchased from Gelest, Inc. (Morrisville, PA) and used as received. 4-dimethylaminopyridine (4-DMAP; Aldrich, Milwaukee, WI) was also used as received.

AstroSil porous silica particles (5 µm) were obtained from Stellar Phases, Inc. (Yardley, PA). These silica particles have an average pore size of 106 angstroms and an absolute surface area, as measured by BET, of 325 m²/g.

3.1.2 Bonding Reaction

It is essential that the silane bonding reaction be carried out under extremely dry conditions in order to prevent the water-initiated dimerization of the silane reagent; therefore, 50g of the silica was placed in a 1000mL round bottom flask and dried at 170°C under vacuum for a period of 24 hours. The dried silica, 4-DMAP, and silane were placed in a dry box and kept under a dry nitrogen atmosphere at all times. A two-fold excess of silane was added to the flask containing the silica particles, and a four-fold excess of the base (4-DMAP) was added to serve as both an
acid acceptor for the hydrogen chloride produced in the reaction and as a reactive intermediate at the silica-solution interface. Anhydrous methylene chloride was used as the reaction solvent, using a ratio of 10 mL per gram of silica. The mixture was then refluxed at 40°C using an oil bath and magnetic stirrer. The mixture was allowed to reflux for 36 hours. This reaction scheme was adapted from Sentell [39] and is shown below in figure 3.1.

![Bonding Reaction Scheme](image)

Figure 3.1: Bonding reaction scheme for reversed-phase packing material.

Once the reaction time was complete, the bonded phase product was washed with three 150 mL portions of each solvent using the rinse sequence methylene chloride, methanol, methanol-water (50/50, v/v), methanol, and diethyl ether. After the ether was allowed to evaporate from the product, the derivatized silica was dried under vacuum at 125°C for 16-24 hours to ensure that any adsorbed organic residue from the synthetic procedure was removed. A small sample of product was sent to QTI (Whitehouse, NJ) for elemental analysis. The analysis determined that there was 20.01% carbon on the surface of the silica particles. This correlated to a bonding density of approximately 3.46 µmol/m². Equation 3.1 was used to calculate the bonding density of the stationary phase; $\alpha$ is the bonding density in µmol/m², $n_c$ is the number of carbon atoms, $S$ is the silica surface area, $M$ is the molecular weight of the ligand, and $L$ is the molecular weight of the leaving group. Initial experiments were performed using stationary phase prepared by Karyn Usher, a group member. The

\[
\alpha = \frac{\%C \times 10^6}{12.01 \ln_c S \left[ 100 - \left( \frac{\%C}{12.01 \ln_c} \right) (M - L) \right]}
\]
starting materials were identical to those described above, and the same bonding procedure was followed. The bonding density for this stationary phase is not known.

3.2 The downward flow slurry packing procedure

3.2.1 Design of downward flow slurry packing system

The packer set-up for packing RPLC analytical columns by this method consisted of a regulator, air driven fluid pump, solvent reservoir, pre-column assembly, and chromatography column. The regulator was connected to a compressed air tank and set to provide a constant pressure of 50 psi to the double action air driven fluid pump. This pump is capable of delivering and maintaining a pressure of 6000 psi. This pressure is adequate for packing analytical RPLC columns. The solvent reservoir was attached to the top of the pump, and the desired pushing solvent was placed in this reservoir prior to and during the packing process. The pre-column assembly was removed from the pump prior to packing, and the column to be packed was attached to the end of the pre-column assembly. This column had a porous metal frit at the outlet held in place with a compression fitting. With the column attached to the pre-column assembly, the pre-column assembly was then connected to the pump. The pre-column assembly has a volume of approximately 45 mL. It is important to fill the pre-column assembly completely with the slurry mixture to prevent any air from disrupting the formation of the packed bed during the packing process.
3.2.2 Procedure for cleaning the slurry packing system and column hardware

Before an empty column could be packed, it was necessary to clean the slurry packing system. Methanol (200 mL) was added to the solvent reservoir, and the system was pressurized to force the methanol through the pump and the pre-column assembly. This was done without the empty column attached to the pre-column assembly. It was also necessary to clean the column blank to be packed as well as the frits and end fittings that would be used to prevent the stationary phase from being extruded from the column. The column blank was placed in a 100 mL graduated cylinder, and methanol was added to the cylinder until just above the column blank. The column blank was then sonicated for five to ten minutes. Then, the methanol was removed and the process was repeated once more. The end fittings and frits were cleaned in the same fashion as the column blank. The column, end fittings, and frits were allowed to dry and were then visually inspected for residual silica. If any residual silica was present, the cleaning process was repeated.
3.2.3 Selection of slurry and pushing solvents

There are several important criteria that must be met when choosing slurry and pushing solvents when packing columns by the slurry packing procedure and the stationary phase is less than 20 \( \mu m \) in diameter. First, the stationary phase particles in the slurry must not aggregate, and the particles must be held in the suspension long enough to be packed into the column without any sedimentation-sizing occurring. It is important to have fluid velocities as high as possible during the packing process. This will allow uniform and dense compactation into the developing bed. The solvent used to make the slurry must not damage the stationary phase and must be easily removed from the packing material. The particles must be strong enough to withstand the high pressures used during the packing process [2].

Since the stationary phase particles used in this study are less than 10 \( \mu m \) in diameter, it was not necessary to use the balanced-density slurry packing technique [2]. Therefore, the slurry and pushing solvents that were used in the procedure developed by C.A. Doyle were used in this study [40]. Chloroform was chosen as the slurrying solvent because it provided necessary wetting and dispersion of the stationary phase particles. It also has a relatively high density (1.5 g/mL) [2], and this helps to keep the stationary phase particles in suspension. The first of three pushing solvents was a 50/50 (v/v) mixture of chloroform and methanol. Both of these solvents have the same viscosity, 0.6 cP, [2], and methanol has a density of 0.8 g/mL, which is about half that of chloroform [2]. Therefore, the mixture of chloroform and methanol should have suitable velocity for use in the slurry packing procedure. Pure methanol was chosen as the second pushing solvent because the velocity of the pushing solvent would not be changed during the packing process since the viscosity of the pushing solvent was not changed. Also, the methanol should remove most of the chloroform from the stationary phase as it is forced through the column. A 50/50 (v/v) mixture of methanol and water was chosen as the final pushing solvent. The primary reason for using this mixture was to prepare the column for use in RPLC.

In order to ensure consistency across the scope of this study, three columns were packed with a slurry solvent that was a 50/50 (v/v) mixture of chloroform and
methanol and a pushing solvent that was a 40/60 (v/v) mixture of methanol and water. This slurry and pushing solvent composition was chosen to make sure that any differences observed in the performance of columns packed by each of the packing methods used in this study could indeed be attributed to the differences between the packing techniques and not the different solvents used during the packing process.

3.2.4 The downward flow slurry packing process

The pre-column assembly was removed from the slurry packer system, and the pump was primed with approximately 100 mL of the first pushing solvent, 50/50 chloroform and methanol. This was done to remove any air that might have been in the air driven fluid pump. An additional 200 mL of the chloroform and methanol mixture was added to the solvent reservoir. The preparation of the stationary phase slurry was the next step in the packing process. Approximately 1.2 grams of 5 µm silica particles that were previously derivatized using n-octadecyldimethylchlorosilane was weighed out using a top loading analytical balance into a 100 mL beaker. A 10-15 percent excess of stationary phase was used when preparing the slurry to ensure the column to be packed would be completely filled. 45 mL of chloroform was then added to the beaker containing the stationary phase. The slurry was sonicated for five minutes to suspend the particles in the solvent and to remove unwanted gases. The slurry mixture was immediately added to the pre-column assembly, and the pre-column assembly was attached to the pump. The system was pressurized, and the pump began to force the slurry into the column blank. Once the level of the 50/50 mixture of chloroform and methanol was very low in the solvent reservoir, the next pushing solvent (100 mL of methanol) was added to the reservoir. When the level of this solvent was low, the last pushing solvent was added. The last pushing solvent was 200 mL of a 50/50 (v/v) mixture of methanol and water. The column was held under constant pressure for 30 minutes. Afterwards, the pressure was allowed to bleed off the system, and the pre-column assembly and column were removed from the pump. The column was removed from the pre-column assembly, and the top of the stationary phase bed was leveled off with
a spatula. The column was then closed with another frit and end fitting. This end of
the column will be used as the column inlet during chromatographic evaluation.
Another column blank with a frit and end fitting was attached to the pre-column
assembly, and the pre-column assembly and column were placed on the pump to
catch the excess stationary phase.

Dr. Kate Rimmer packed a set of control columns at NIST, Gaithersburg, MD,
by the traditional downward flow slurry packing procedure using isopropanol as both
the slurry and pushing solvent. These columns were packed with the same column
hardware and stationary phase used to pack columns in-house. These columns were
used for comparison throughout the study.

3.2.5 The downward flow slurry packing procedure with a surfactant modifier

A series of columns were packed by the downward flow slurry packing
procedure using 2-butoxyethanol (Aldrich, Milwaukee, WI) as a slurry and pushing
solvent modifier. 2-butoxyethanol was chosen for use in this study because its use as
a mobile phase modifier in RPLC has already been investigated and was found to not
form micelles under any conditions [39]. These columns were packed using a
mixture of chloroform, methanol, and 2-butoxyethanol as the slurry solvent and a
mixture of methanol, water, and 2-butoxyethanol as the pushing solvent. The first
column was packed using a slurry solvent with the composition of 49/49/2 percent by
volume of chloroform, methanol, and 2-butoxyethanol respectively and a 39/59/2
percent by volume mixture of methanol, water, and 2-butoxyethanol respectively as
the pushing solvent. Other columns were packed by increasing the percentage of 2-
butoxyethanol from 4 to 10% in increments of two percent and adjusting the volume
of the other solvents accordingly.
3.3 Radial spinning slurry packing procedure

3.3.1 Design of the radially spinning slurry packing system

The packer set-up for packing RPLC columns by this method consisted of a regulator, air driven fluid pump, solvent reservoir, pre-column assembly, high speed coupling, chromatographic column, and electric motor. The regulator was connected to a compressed air tank and set to provide a constant pressure of 50 psi to the double action air driven fluid pump. The solvent reservoir was attached to the top of the pump and the pushing solvent to be used was placed in the reservoir prior to the packing process. The pre-column assembly was attached to the top of the high-speed coupling (Figure 3.3), and stainless steel tubing was used to connect the pump to the pre-column assembly. The chromatographic column was attached to the bottom of
the coupling, and a fitting that contained a porous metal frit and small spacer to hold the frit in place was attached to the bottom of the column. This fitting was then connected to the electric motor.

### 3.3.2 Procedure for cleaning the radial spinning slurry packing system

The cleaning procedure used for this packing system was the same as that used to clean the downward flow slurry packing system with one exception, the high speed coupling had to be cleaned prior to packing a column. The coupling was taken apart, and each piece was cleaned individually with methanol and rinsed with water. The bearings used in the coupling were re-oiled, and the seals were replaced. The coupling was then reassembled and placed back into the packing system.

### 3.3.3 Selection of slurry and pushing solvents

The selection of a suitable slurry solvent was more challenging for this method of packing. Chloroform quickly deteriorated the Viton o-ring seals that were used in the high-speed coupling, and pure methanol did not sufficiently suspend the stationary phase for packing. Therefore, a 50/50 (v/v) mixture of chloroform and methanol was used as the slurry solvent. This solvent mixture held the stationary phase in suspension and did not deteriorate the seals in the coupling as quickly as pure chloroform.

The seals in the coupling also caused difficulties in selecting suitable pushing solvents. Again, chloroform was not suitable nor was methanol. These solvents in the volume required for the packing process caused too much damage to the o-ring seals. Due to this problem a 40/60 (v/v) mixture of methanol and water was chosen as the pushing solvent to be used for this procedure. This mixture did not damage the seals as much as the pure organic solvents and prepared the column for use in RPLC.
3.3.4 The radial spinning slurry packing procedure

The tubing connecting the pump to the top of the pre-column assembly was removed from the pre-column assembly, and the pump was primed with approximately 100mL of the 40/60 (v/v) methanol and water mixture. An additional 500mL of this mixture was placed in the solvent reservoir. The slurry was prepared using approximately 1.2g of the stationary phase previously described and 45mL of the 50/50 (v/v) chloroform/methanol mixture. The slurry was sonicated for several minutes to remove unwanted gases and placed in the pre-column assembly. The electric motor was engaged, and the column was spun at 6000 rev/min; this speed was chosen arbitrarily. Once the column was up to speed, the packing system was pressurized. The column was held under constant pressure for 30 minutes. Then, the pressure to the pump was turned off, and the pressure was allowed to bleed off from the system. The column was removed from the packing system and an end fitting and frit assembly was placed on the inlet of the column. The fitting used to mate the column to the electric motor was removed, and an end fitting and frit assembly was placed on the outlet of the column. The excess stationary phase was forced out of the high speed coupling by pressurizing the system and collected in a small beaker.

3.4 Collection of chromatographic data

3.4.1 Determination of test solutes

The test solutes used in this study were as follows: thiourea, benzene, toluene, and ethyl benzene. The thiourea and ethyl benzene were purchased from Aldrich (Milwaukee, WI), and the benzene and toluene were purchased from Fisher Scientific (Fair Lawn, NJ). These solutes were chosen for use in the test mixture for several reasons. Neutral solutes were desired to prevent any secondary equilibria from playing a role in the separation, and these solutes also generally exhibit good peak shape and provide good detector response.
3.4.2 Preparation of mobile phase and test mixture

Initial studies were performed using a mobile phase consisting of 40% acetonitrile (Fisher Scientific, Fair Lawn, NJ) and 60% water by volume. The mobile phase composition was adjusted to 30% acetonitrile and 70% water by volume for later studies to increase retention and reduce extra column affects. The test mixture was prepared using the same solvent composition that was to be used as the mobile phase for each set of experiments. This was done to prevent any shifts in retention times as well as to prevent peak splitting.

3.4.3 Determination of column efficiencies and reduced plate height values

Each column was allowed to equilibrate for 30 minutes to ensure that all the pushing solvents from the packing process were flushed out of the column. The test mixture was then injected onto the column. All injections were made in triplicate to check for repeatability. The Turbochrom software (version 3.3 or 4.1, Perkin Elmer, San Jose, CA) used for data collection was capable of calculating the number of theoretical plates (N) by several methods. The Foley-Dorsey equation was selected for use in this study. The plates calculated by the software were checked periodically by manually calculating the asymmetry factor (B/A) and using an excel spreadsheet to calculate the number of theoretical plates. If the asymmetry factor was less than 1, the ratio was inverted for use in the Foley-Dorsey equation. The column efficiencies were then used to calculate the reduced height equivalent to a theoretical plate (h). An average and standard deviation of both N and h was determined for each column as well as for all columns packed by a particular method.
3.4.4 van Deemter analysis

The column efficiencies at flow rates from 0.2 to 5.0 mL/min in increments of 0.2 mL/min were determined for a column packed by each of the packing techniques described as well as a control column. The mobile phase used was composed of 50% acetonitrile and 50% water by volume. The columns were thermostatted at 30°C. Benzene was used as the test solute, and thiourea was used as the void time marker. The Foley-Dorsey equation was used to calculate the number of theoretical plates, and the plates were then used to determine the height equivalent to a theoretical plate (H). Excel was used in conjunction with the add-on program Scientific Data Analysis Software (Prentice Hall, Upper Saddle River, NJ) to perform regression based upon the Van Deemter equation. The values obtained were plotted against the flow velocities (cm/s) to generate van Deemter curves.

3.4.5 Pyconometry Study

Pyconometry was used to determine the void volume of columns packed by each of the techniques previously described. Chloroform and Acetonitrile (Fisher Scientific, Fair Lawn, NJ) were chosen as the solvents for this study. There is a significant difference between their densities: acetonitrile has a density of 0.7138 g/mL and chloroform has a density of 1.484 g/mL, which makes them appealing solvents for a study of this type. The column was equilibrated with chloroform for 45 minutes, and the mass of the column was measured. Then, the column was equilibrated with acetonitrile for 45 minutes, and the mass of the column was measured. The void volume was then calculated using equation 3.2.

Equation 3.2: \[ V_0 = \left( \frac{W_x - W_y}{P_x - P_y} \right) \]
3.5 Instrumentation

The column hardware used in this study was obtained from Sci-Con (Winter Park, FL). Parker ¼ inch end fitting assemblies were used in conjunction with 100 mm by 4.6 mm stainless steel column blanks. Stainless steel and peek 0.25 x 4.6 mm frit assemblies were used to prevent the stationary phase from being extruded from the column. Comparison tests for columns packed by different slurry packing techniques were performed on a Spectra-Physics SP8800 ternary pump (San Jose, CA) or a Shimadzu LC-10AD liquid chromatography pump (Kyoto, Japan). Van Deemter and pyconometry experiments were carried out using the Shimadzu LC-10AD LC pump. For all experiments, the columns were water-jacketed, and the temperature was regulated at 30°C with a Fisher Isotemp refrigerated circulator model 1016S (Pittsburgh, PA) circulating a mixture of ethylene glycol and water. Solutes were injected using a Valco (Houston, TX) six-port injector fitted with a 20 µL sample loop. An ABI Analytical Spectroflow 757 variable-wavelength detector (Ramsey, NJ) with the wavelength set at 204 nm was used with both systems. A PE Nelson (Cupertino, CA) series 900 interface was used to relay the detector signal to the computer system. Turbochrom version 3.3 or 4.1 software Perkin Elmer, San Jose, CA, was used for data collection and manipulation.
CHAPTER 4

RESULTS AND DISCUSSION

4.1 Evaluating overall column performance

Column efficiency (N) and the reduced height equivalent to a theoretical plate (h), as previously mentioned, are typically used to describe the overall performance of an LC column. Several studies were performed to determine column efficiencies and reduced plate heights for columns packed by the traditional downward flow slurry packing procedure, the radially spinning slurry packing procedure, and the traditional downward flow slurry packing procedure with a surfactant modifier present in both the slurry and pushing solvents.

4.1.1 Initial column performance study

The initial study was performed using three columns packed by the traditional downward flow slurry packing procedure and three columns packed by the radially spinning slurry packing procedure. These columns were packed with stationary phase prepared by a fellow group member, and the bonding density of the C18 ligand is not known. The results from the column efficiency tests are shown in table 4.1. The columns packed by the traditional downward flow slurry packing method performed poorly. There was an approximate thousand plate increase in the average column efficiency observed for the columns packed by the radially spinning slurry packing procedure. It is evident that there were some extra-column effects present in this study. The column efficiency increased as the retention time of the solute increased. This is normally not the case, typically column efficiency for a well-retained solute, toluene in this case, will suffer due to the band broadening that occurs from the solute being on the column so long. Also, note that the standard deviations for the column
Table 4.1: Average column efficiencies with standard deviations for three columns each packed by traditional and radially spinning slurry packing procedures. Mobile phase was 40/60 by volume acetonitrile/water.

<table>
<thead>
<tr>
<th>Solute</th>
<th>Traditional N +/- SD</th>
<th>Radially Spun N +/- SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetophenone</td>
<td>2700 +/- 900</td>
<td>3700 +/- 800</td>
</tr>
<tr>
<td>Benzene</td>
<td>3300 +/- 1000</td>
<td>4200 +/- 1100</td>
</tr>
<tr>
<td>Toluene</td>
<td>3400 +/- 1200</td>
<td>4500 +/- 1300</td>
</tr>
</tbody>
</table>

Efficiencies for columns packed by both methods are basically the same. The reduced plate height values determined for these columns, figure 4.1, also indicate that the columns packed by the radially spinning slurry packing technique are of better quality than those packed by the traditional slurry packing procedure.

Figure 4.1: Average reduced plate height (h) values for three columns each packed by traditional and radially spinning slurry packing procedures.
4.1.2 Column performance study with control group

Columns packed by the traditional slurry packing procedure and the radially spinning slurry packing technique were compared to columns packed by an outside source, Dr. Kate Rimmer currently at NIST, according to the traditional slurry packing procedure. The columns packed by the outside source were intended to serve as a control group of columns. The performance of these columns was tested by Dr. Rimmer using standard reference 869a, and the average column efficiency for the solute 1,2:3,4:5,6:7,8-tetrabenzonaphthalene was found to be 5100 with a standard deviation of 700. The mobile phase strength was decreased for this study in an attempt to reduce the extra-column effects observed in the previous study. Also, the solute acetophenone was replaced with ethyl benzene. The results of this study are shown below in table 4.2. The columns packed in-house by the traditional slurry technique exhibited the best overall column efficiencies in this study. The column efficiencies for the columns packed by the radially spinning slurry packing method fell between those of the traditional columns and the control columns. The control

<table>
<thead>
<tr>
<th>Solute</th>
<th>Traditional</th>
<th>Control</th>
<th>Radially Spun</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene</td>
<td>5800 +/- 500</td>
<td>4400 +/- 200</td>
<td>4600 +/- 200</td>
</tr>
<tr>
<td>Toluene</td>
<td>6100 +/- 500</td>
<td>5100 +/- 100</td>
<td>5500 +/- 300</td>
</tr>
<tr>
<td>Ethyl benzene</td>
<td>5100 +/- 400</td>
<td>4200 +/- 100</td>
<td>5100 +/- 200</td>
</tr>
</tbody>
</table>

Table 4.2: Average column efficiencies with standard deviations for three columns each packed by traditional and radially spinning slurry packing procedures. Mobile phase was 30/70 by volume acetonitrile/water.
columns, which were packed by the traditional slurry packing procedure, exhibited the poorest performance. The different solvents used during each of the packing procedures may have caused some of the differences observed in the column efficiencies of these columns. One reason the efficiencies observed in this study is higher than those observed in the previous study is the difference in the mobile phase composition used in each study. The mobile phase strength was decreased in this study to reduce extra-column effects. The reduction of the extra-column effects resulted in an increase in the observed column efficiencies for this study. The reduced plate height values, figure 4.2, for these columns also indicate that the columns packed by the traditional slurry packing procedure are of higher quality than the radially spun columns and the control columns.

![Reduced Plate Height Values](image)

**Figure 4.2:** Average reduced plate height (h) values for three columns each packed by traditional and radially spinning slurry packing procedures.
4.1.3 Column performance study when the same slurry and pushing solvents are used during the packing process

Another efficiency study was performed in which the slurry and pushing solvents were the same throughout the packing procedures to determine if the differences in the observed efficiencies from previous studies might be due to the different solvents that were utilized during the different packing procedures. The columns packed by the traditional slurry packing procedure still exhibited better column efficiencies than those of the columns packed by the radially spinning slurry packing technique, table 4.3. The results obtained with the columns packed by the traditional slurry packing procedure in this study are very similar to those obtained in the previous study where the same batch of stationary phase was used to pack the columns. The differences observed in the column efficiencies for columns packed by both techniques are not due to the different slurry and pushing solvents used in prior studies.

Table 4.3: Average column efficiencies with standard deviations for three columns each packed by traditional and radially spinning slurry packing procedures. Mobile phase was 30/70 by volume acetonitrile/water.

<table>
<thead>
<tr>
<th>Solute</th>
<th>Traditional</th>
<th>Radially Spinning</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene</td>
<td>5200 +/- 200</td>
<td>4600 +/- 200</td>
</tr>
<tr>
<td>Toluene</td>
<td>6200 +/- 200</td>
<td>5500 +/- 300</td>
</tr>
<tr>
<td>Ethyl benzene</td>
<td>5200 +/- 200</td>
<td>5100 +/- 200</td>
</tr>
</tbody>
</table>
4.1.4 How column performance is affected by the use of a surfactant modifier during the packing process

The last column efficiency test that was performed looked at columns that were packed by the traditional slurry packing procedure, the radially spinning slurry packing procedure, and the traditional slurry packing procedure with a surfactant modifier (2-butoxyethanol) present in both the slurry and pushing solvents. All the columns used were prepared using the same slurry and pushing solvents with the exception of the addition of the surfactant modifier in the procedure for packing columns by the traditional procedure with a slurry and pushing solvent modifier. A series of columns were packed where the percentage of surfactant modifier was increased from 2% by volume to 10% by volume in increments of 2%. These columns were then tested for column performance to determine if there was a certain percentage of surfactant modifier that corresponded to a maximum in column efficiency. The results of this study indicate that over the surfactant modifier range covered there is not a clear maximum for each solute in column efficiency, figure 4.3. Therefore, columns were prepared using 10% by volume surfactant modifier in the slurry and pushing solvents used to pack columns by the traditional slurry packing procedure. The apparent surface tensions, table 4.4, of the slurry and pushing solvents with and without the surfactant modifier were measured using a Fisher surface tensiomat model 21. There was not a significant change between the surface tensions of the slurry solvent with or without the surfactant modifier present, but there was a large difference between the surface tensions of the pushing solvent with the surfactant modifier versus the pushing solvent without the surfactant modifier. The column efficiencies exhibited by columns packed using the surfactant modifier indicate that these columns performed slightly better than columns packed by the traditional slurry packing procedure using traditional solvents and columns packed by the radially spinning slurry packing technique using traditional solvents, table 4.5. The column- to-column standard deviations for the columns packed with the surfactant modifier present were less than those of columns packed by either of the
Figure 4.3: Column efficiency as a function of the percent 2-butoxyethanol used during the packing process. Mobile phase was 30/70 by volume acetonitrile/water.

Table 4.4: Average apparent surface tensions for slurry and pushing solvents with and without 2-butoxyethanol present. Composition is by volume percent.

<table>
<thead>
<tr>
<th>Solvent composition</th>
<th>Avg. apparent surface tension (dynes/cm)</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>50/50 chloroform-methanol</td>
<td>21.13</td>
<td>1.11</td>
</tr>
<tr>
<td>45/45/10 chloroform-methanol-surfactant</td>
<td>23.13</td>
<td>0.322</td>
</tr>
<tr>
<td>40/60 methanol-water</td>
<td>39.80</td>
<td>0.346</td>
</tr>
<tr>
<td>35/55/10 methanol-water-surfactant</td>
<td>31.73</td>
<td>0.252</td>
</tr>
</tbody>
</table>
Table 4.5: Average column efficiencies with standard deviations for three columns each packed by traditional and radially spinning slurry packing procedures. Mobile phase was 30/70 by volume acetonitrile/water.

<table>
<thead>
<tr>
<th>Solute</th>
<th>Traditional N +/- SD</th>
<th>Radially Spinning N +/- SD</th>
<th>Surfactant Aided N +/- SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene</td>
<td>5200 +/- 200</td>
<td>4600 +/- 200</td>
<td>5900 +/- 100</td>
</tr>
<tr>
<td>Toluene</td>
<td>6200 +/- 200</td>
<td>5500 +/- 300</td>
<td>6500 +/- 100</td>
</tr>
<tr>
<td>Ethyl benzene</td>
<td>5200 +/- 200</td>
<td>5100 +/- 200</td>
<td>5300 +/- 100</td>
</tr>
</tbody>
</table>

Figure 4.4: Average reduced plate height (h) values for three columns each packed by traditional and radially spinning slurry packing procedures.
other methods. It is possible, with the surfactant modifier present in the slurry and pushing solvents, that the slurry and pushing solvents were better able to wet and disperse the stationary phase, which led to a reduction in the particle-particle friction and particle-wall friction during the packing process. The result of which was a more homogeneous packed bed within the column. The reduced plate height values indicate that the columns packed with the surfactant modifier present were of better quality than those packed without the surfactant modifier present, figure 4.4.

4.2 van Deemter Analysis

There are three different regions that comprise a van Deemter plot (figure 4.5), and each of these regions corresponds to a different band broadening process during the chromatographic run. These events are eddy diffusion (a term), longitudinal diffusion (b term), and resistance to mass transfer (c term). The results

![Figure 4.5: Typical van Deemter plot.](image)
of the van Deemter study for columns packed by each of the different packing methods examined are shown graphically in figure 4.6. It appears that there is an improvement in plate height in the region that is governed by eddy diffusion for both the radially spin packed columns and the columns packed by the traditional slurry packing procedure (RDS) and traditional slurry packing procedure with 2-butoxyethanol present as a modifier (Surf). Mobile phase was 50/50 by volume acetonitrile/water with benzene as the test solute.
packing procedure using a surfactant modifier in the slurry and pushing solvents. The improvement observed in the plate height translates to about a 1000 plate increase for the columns packed using the radially spinning slurry packing method and the surfactant modifier. Eddy diffusion arises from the different paths that the mobile phase follows between different particles within the column as it traverses down the column. As a result, solute molecules also take different paths through the packed bed within the column, figure 4.7. The mobile phase moves faster in wide paths and

![Diagram of eddy diffusion within a packed bed](image)

Figure 4.7: Diagram depicting eddy diffusion within a packed bed [2].

slower in narrow paths; therefore, the narrow band of solute molecules injected onto the top of the column broadens as it travels down the column [2]. A possible explanation for the differences observed in the eddy diffusion region of the van Deemter plots between the control column (packed by the traditional slurry packing procedure) and the columns packed by the other two methods is a more uniform spacing between the stationary phase particles within the packed bed of the columns.
A more uniform spacing between the particles would lead to a more homogeneous mobile velocity across the column and allow solute molecules to traverse down the column at the same speed. Thus, less broadening of the solute band occurs leading to better peak shape and column efficiency.

There is also a notable difference between the slope of the Van Deemter plots in the region associated with the C term (resistance to mass transfer) for the columns packed using a surfactant modifier in the slurry and pushing solvents and those packed by the other two methods. This is also clearly seen in the values obtained for the C term from regression on the experimental data, table 4.6. The C term value for the columns packed using the surfactant modifier is 30% less than that observed for the control column. Knox stated in a recent publication “little can be done to improve mass transfer within the particles of packing, but much can be done to improve the structure of the chromatographic bed” [40]. The same packing material was used to pack each of the columns examined in this study; therefore, there should not be any significant difference between the C term values for these columns unless the structure of the packed bed is different. The formation of a more homogeneous bed within the columns packed using the surfactant modifier in the slurry and pushing solvents is a possible explanation for the differences observed in the performances of the columns examined in this study.

Table 4.6: van Deemter term values obtained from regression using SDAS in conjunction with excel.

<table>
<thead>
<tr>
<th>Term</th>
<th>Control</th>
<th>Radially Spun</th>
<th>Surfactant Modifier</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>.0013012</td>
<td>.0001518</td>
<td>.0007629</td>
</tr>
<tr>
<td>B</td>
<td>.0001350</td>
<td>.0003587</td>
<td>.0001105</td>
</tr>
<tr>
<td>C</td>
<td>.0043045</td>
<td>.0052905</td>
<td>.0030214</td>
</tr>
</tbody>
</table>
There are several advantages that arise from increasing column efficiency. The first of which is an increase in resolution. The fundamental resolution equation (equation 4.1) relates column efficiency, selectivity, and retention to overall resolution between two solutes in a given separation. $\alpha$ is the selectivity, which is the column’s ability to distinguish between two solutes. $N$ is the column efficiency, and $k'$ is the capacity factor. An increase in column efficiency results in an increase in the resolution by square root of $N$. Increasing the column efficiency to gain improvement in resolution is usually viewed as the brute strength approach because the general approach to increase $N$ is to increase the length of the column used to perform the separation. This results in an increased analysis time. In this study, the column efficiency was increased without increasing column length, and the gain in resolution came at no price.

The other advantage from an increase in column efficiency is an increase in the peak capacity (equation 5.2). Peak capacity is the number of peaks that can be separated isocratically with a given amount of resolution in a given amount of time. Just as resolution increased by the square root of column efficiency, so does the peak capacity. In this case peak capacity would increase by 5 peaks, and again this increase came at no expense.

### 4.3 Column-to-Column Reproducibility

#### 4.3.1 Different slurry and pushing solvents

The column-to-column reproducibility for columns packed with the same batch of stationary phase and different solvents depending upon which packing method was used were investigated in this study. The columns packed by the
traditional slurry packing procedure were packed using pure chloroform as the slurry solvent and a mixture of 50/50 by volume chloroform/methanol, pure methanol, and a 50/50 by volume mixture of methanol/water as the pushing solvents. The columns packed by the radially spinning method were packed using a 50/50 by volume mixture of chloroform/methanol as the slurry solvent and a mixture of 40/60 methanol/water as the pushing solvent. The solvents used to pack columns by the traditional slurry packing procedure with a surfactant modifier were a 45/45/10 by volume mixture of chloroform/methanol/2-butoxyethanol as the slurry solvent and a 35/55/10 by volume mixture of methanol/water/2-butoxyethanol as the pushing solvent. The average %RSD in the retention times for three columns packed by each method are shown in figure 4.8. It is clear that the columns packed by the radially spinning method provided the most reproducible results, showing percent RSD values of less than half a percent for retention time. As indicated in figure 4.8, the columns

![% RSD in Retention Time](image)

Figure 4.8: Average %RSD in retention time for three columns packed by each method. Mobile phase was 30/70 by volume acetonitrile/water.
packed in-house by the traditional slurry packing procedure exhibited the highest percent RSD in retention time. The same trend is also seen in the average percent RSD’s for the capacity factor ($k'$) for three columns packed by each method, figure 4.9. The columns packed by the radially spinning slurry packing procedure exhibited the best percent RSD in the capacity factor while the columns packed in-house by the traditional slurry packing procedure exhibited the worst reproducibility in the capacity factor. More reproducible bed formation during the packing process is a possible explanation for the results obtained in this study.

![Graph showing %RSD in k']

Figure 4.9: Average %RSD in capacity factor for three columns packed by each method. Mobile phase was 30/70 by volume acetonitrile/water.
4.3.2 The same slurry and pushing solvents

This study is similar to the previous study discussed, with one major exception, the slurry and pushing solvents were of the same composition throughout the study. The slurry and pushing solvents used to pack columns according to both the traditional slurry packing and the radially spinning slurry packing procedures were 50/50 by volume chloroform/methanol as the slurry solvent and 40/60 by volume methanol/water as the pushing solvent. Of course, these percentages were adjusted accordingly to allow for the use of 10% by volume surfactant modifier for columns packed by the traditional slurry packing procedure using the surfactant modifier. Again, the radially spinning slurry packing procedure produced columns that were more reproducible in both retention time and capacity factor than those by the other two packing methods as shown in figures 4.10 and 4.11.

Figure 4.10: Average %RSD in retention time for three columns packed by each method. Mobile phase was 30/70 by volume acetonitrile/water.
Figure 4.11: Average %RSD in capacity factor for three columns packed by each method. Mobile phase was 30/70 by volume acetonitrile/water.

4.4 Pyconometry study

There are a number of methods that can be used to find the void volume of chromatographic columns. One of the more commonly used methods for this purpose is pyconometry. The solvent pair selected for this study was acetonitrile and chloroform. This pair was chosen for the significant difference between their densities: acetonitrile has a density of 0.7138 g/mL and chloroform has a density of 1.484 g/mL. The data, table 4.7, indicate that the columns packed by the radially spinning slurry packing method have the largest average void volume. The
Table 4.7: Average void volumes and standard deviations for three columns packed by the traditional slurry packing procedure, radially spinning slurry packing procedure, and the traditional slurry packing procedure using a surfactant modifier.

<table>
<thead>
<tr>
<th>Packing Method</th>
<th>Average Void Volume (mL)</th>
<th>SD (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Traditional</td>
<td>0.8809</td>
<td>0.0263</td>
</tr>
<tr>
<td>Radially Spun</td>
<td>0.9294</td>
<td>0.0298</td>
</tr>
<tr>
<td>Surfactant Aided</td>
<td>0.8803</td>
<td>0.0239</td>
</tr>
</tbody>
</table>

The traditional slurry packing method and the slurry packing method using a surfactant modifier produced columns with essentially the same average void volume, which is slightly less than the void volume for the columns packed by the radially spinning slurry packing procedure. The larger average void volume for the radially spun columns suggests that there is not as much stationary phase present within the columns. This may be due to the loss of a small amount of stationary phase during the process of removing the end fitting assembly that is used to connect the column to the electric motor for the radially spinning slurry packing process and placing an end fitting assembly on the column outlet that is suitable for use in HPLC. The fact that the average void volumes for columns that are packed by the traditional slurry packing procedure with and without surfactant present are essentially the same indicates that differences observed in the van Deemter plots arose from differences in the bed structure within the columns.

4.5 Spinning rate study

There are many variables that can be utilized to achieve the optimum packing conditions, and one variable of extreme interest is the spinning rate. The optimum
column efficiency should be observed when the centripetal force created by spinning the column during the packing process is equal to the downward force applied by the air driven fluid pump to the stationary phase particles during the packing process. A rough approximation of the rotational rate needed to have the two forces acting upon

![N vs. rotation rate graph](image)

Figure 4.12: Column efficiency (N) as a function of the rotation rate in RPM.

the stationary phase particles during the radially spinning slurry packing procedure offset each other indicated that an extremely high rotational rate is needed. The force acting upon the stationary phase particles during the slurry packing process was calculated using equation 4.1. The downward force produced while slurry packing

Equation 4.1: \( F = p \pi r^2 \)
under 6,000 psi of pressure was found to be 688 newtons. Several assumptions were made initially to aid in the calculation of the rotational rate needed to have the centripetal force produced during the radially spinning slurry packing process equal the downward force acting upon the stationary phase particles during the traditional slurry packing process. The first assumption was that these calculations can be based upon the characteristics of a “good” column, and a “good” column has a total column porosity of approximately 0.40. Based upon this, it was assumed that 60% of the column was packed. The other assumption made was that the column was packed with approximately 1.10 g of stationary phase. A column of the dimensions used in this study has a volume of 1.662 cm$^3$. If 60% of that volume is occupied by packing material, then the volume of stationary phase is 0.9972 cm$^3$. The number of stationary phase particles within the column was found by dividing the volume of packing (0.9972 cm$^3$) by the volume of one stationary phase particle (6.54*10$^{-11}$ cm$^3$). Based upon the assumption made previously there are 1.525*10$^{10}$ stationary phase particles in the column. Assuming the mass of stationary phase in the column is approximately 1.10 g, the mass of one stationary phase particle is roughly 7.214*10$^{-11}$ g. The rotational rate needed to create a centripetal force equal to the downward force acting upon the stationary phase particles was calculated with equation 4.2 and determined to be 6.147*10$^8$ RPM. After calculations were done to Equation 4.2: $F_c = \frac{mv^2}{r}$ find the optimum conditions, it was determined that this rotational rate is well outside the working range of the current radially spinning slurry packer set-up. The only way to have the two forces counteract would be to significantly reduce the pressure used during the packing process, but the double action air driven fluid pump is already operating at its lowest limit of pressure for proper functioning. Therefore, with the current design of the radially spinning slurry packer set-up it is not possible to have the centripetal force equal the downward force applied to the stationary phase particles. But, there may be a maximum in column efficiency observed over the range of rotational rates possible using the current radially spinning slurry packer set-up. These columns were packed according to the radially spinning slurry packing procedure at rotation speeds of 3000, 6000, and 10000 RPM. As indicated by the
plots in figure 4.12, the highest column efficiencies were observed with the column that was packed while spinning at 6000 RPM. The optimum operating rotation rate for the current radially spinning slurry packer set-up is 6000 RPM. This rotation rate was arbitrarily chosen for use at the onset of this work.
CHAPTER 5

SUMMARY AND CONCLUSIONS

Alternative methods for packing RPLC columns have been developed for the preparation of columns that have a more homogeneous bed structure than columns packed by the traditional slurry packing procedure. The first of these methods, the radially spinning slurry packing procedure, was indeed a challenge as the development of a coupling that could handle both high pressure and a high rate of rotation was required. The main problem with the development of this coupling was choosing the type of material for the seal to function properly in this application. There is not a seal available that is well suited for creating a sufficient seal at both the high rate of rotation and the high pressure used during the packing process. A number of manufacturers make seals that can handle the high pressure or the spinning during the radially spinning slurry packing procedure but not both at the same time; therefore, it was necessary to try seals made from several different materials. The first material selected for use as an o-ring seal in the coupling was Teflon. Three Teflon o-rings were placed in the seal capture assembly, figure 2.4, to seal between the rotor and the inlet shaft of the upper housing of the coupling. These seals failed frequently and allowed solvent and stationary phase to escape into the housing of the coupling. The stationary phase/solvent mixture acted like fine sandpaper during the packing process and created a flat surface on the Teflon o-rings as the shaft within the coupling spun causing the seals to fail. The second type of o-ring used did hold the pressure during the radially spinning slurry packing procedure. These o-rings were made from Viton and can be purchased at any hardware store. The Viton o-rings were placed in the seal capture assembly, which aided in maintaining the shape of the o-rings and the formation of a better seal between the rotor and inlet shaft of the upper housing. The Viton o-rings performed much better than the Teflon o-rings, and
the success rate for packing columns was much higher with the Viton o-rings than with the Teflon o-rings. It was also necessary to utilize solvents that were compatible with the Viton o-rings during the radially spinning slurry packing process. A mixture of 50/50 (v/v) chloroform and methanol was found to work well as the slurry solvent, and a 40/60 (v/v) mixture of methanol and water was used as the pushing solvent.

With the problems of seal failure and solvent compatibility solved, it was time to begin packing and testing columns. A rotational rate of 6,000 rev/min was arbitrarily chosen for the radially spinning slurry packing procedure. It was later determined that this was indeed the optimum rotational rate within the capabilities of the radially spinning slurry packing system, figure 4.12. The procedure for packing columns while spinning radially was to use approximately 1.2 g of stationary phase suspended in 46 mL of the 50/50 (v/v) mixture of chloroform and methanol used as the slurry solvent, place the slurry in the pre-column assembly, turn on the electric motor, set the spinning rate, and pressurize the system by opening the valve of the regulator to allow the compressed air (supplied at 50 psi) to operate the pump and force the pushing solvent (40/60 (v/v) methanol and water) through the system. The system was held under pressure for 30 minutes. The column was then removed and end fittings and frit assemblies were placed on both the column inlet and outlet. Columns packed by this method were then tested and the results were compared with results of columns packed in-house by the traditional slurry packing procedure as well as columns packed by Dr. Kate Rimmer at NIST using the traditional slurry packing procedure.

The initial column performance study was performed using stationary phase that was derivatized by Karyn Usher. The bonding density of the C18 ligand on the surface of the silica particles was not known. The columns packed by the traditional slurry packing procedure were packed using pure chloroform as the slurry solvent and pushing solvents of 50/50 (v/v) chloroform and methanol (200 mL), pure methanol (100 mL), and 50/50 (v/v) methanol and water (200 mL). There was approximately a thirty percent increase in the average column efficiency observed for the columns packed by the radially spinning slurry packing procedure when compared to the columns packed by the traditional slurry packing procedure.
The results of the initial column performance study prompted further investigation of columns packed by the radially spinning slurry packing technique. A stationary phase with a bonding density of 3.46 µmol/m² was used to pack columns in-house by both the radially spinning and traditional slurry packing procedures. The same stationary phase was also used by Dr. Kate Rimmer to pack columns by the traditional slurry packing procedure for use as a control group of columns. The control columns were packed using isopropanol as both the slurry and pushing solvent. After testing, the columns packed by the traditional slurry packing procedure performed better than columns packed by the radially spinning slurry packing procedure and the control columns. The average column efficiency for the columns packed by the radially spinning slurry packing was less than the average column efficiency for columns packed by the traditional slurry packing procedure but greater than the average column efficiency for the control columns. The reproducibility in terms of column efficiencies for the columns packed by the radially spinning slurry packing procedure was better than that of the columns packed by the traditional slurry packing procedure.

The different solvents utilized during each of the packing procedures could have led to the differences observed in the average column efficiencies for columns packed by the different packing techniques. Therefore, another study was performed that looked at the average column efficiencies for columns that were packed by the different packing methods using the same solvents. The resulting column efficiencies for the columns packed by the traditional slurry Packing procedure were essentially the same as those obtained previously and were still better than the average column efficiencies obtained for columns packed by the radially spinning slurry packing technique. The differences seen in the column efficiencies for columns packed by the different techniques are not related to the different solvents that were used in the previous study.

It was thought that the use of a surfactant modifier in both the slurry and pushing solvent used when packing columns by the traditional slurry packing procedure might reduce particle-particle and particle-wall friction and allow for the stationary phase to be better wetted and dispersed in the slurry and pushing solvents;
therefore, 2-butoxyethanol was used as a surfactant modifier when packing columns by the traditional slurry packing procedure for a final column performance study. A ten percent by volume addition of the surfactant modifier did give better efficiency for two of the three test solutes and produced a column that showed better efficiency. Columns were then packed by the traditional slurry packing procedure using a 45/45/10 (v/v/v) mixture of chloroform, methanol, and 2-butoxyethanol as the slurry solvent and a 35/55/10 (v/v/v) mixture of methanol, water, and 2-butoxyethanol as the pushing solvent. The columns packed with the surfactant modifier present in both the slurry and pushing solvents performed better and had better reproducibility in terms of efficiencies than the columns packed by the other methods studied. This indicated that a more homogeneous bed might have been formed within the column during the packing process.

van Deemter analysis was performed on columns packed by the radially spinning slurry packing procedure, the traditional slurry packing procedure with a surfactant modifier, and one of the control columns to determine if any change in bed structure existed between the columns. The columns packed by the radially spinning slurry packing technique and the traditional slurry packing technique with a surfactant modifier performed better than the control column. The major differences between the columns packed in-house and the control column were observed in the A term (eddy diffusion) and the C term (mass transfer) regions of the van Deemter plots. The shape of the van Deemter plots in the eddy diffusion region for the radially spinning slurry packed columns and traditional slurry packed columns with a surfactant modifier were very similar. The A term values obtained from regression were also similar for the radially spinning slurry packed columns and the columns packed by the traditional slurry packing procedure with a surfactant modifier, and these values were almost an order of magnitude lower than the A term for the control column. The reduction observed in the A term values indicate that a more uniform spacing between the stationary phase particles within the packed bed of the column was achieved. A more uniform spacing between the stationary phase particles would result in a more homogeneous mobile phase velocity across the column and allow solute molecules to travel down the column at the same rate. This would result in less
band broadening as the solute band travels down the column leading to better peak shape and column efficiency. There was also a notable difference between the slopes of the van Deemter plots in the region corresponding to mass transfer (C term). The C term value for columns packed using the surfactant modifier is 30% less than that observed for the control column. Knox indicated that an improvement in the C term region of the van Deemter plot would be possible with an improvement in the structure of the packed bed within the column [40]. The reduction of the slope of the van Deemter plot for the columns packed by the traditional slurry packing procedure using a surfactant modifier indicates that a more homogeneous bed was formed during the packing process than the bed of the control column. Increased column efficiency can lead to several advantages: an improvement in resolution would be seen, and an increased peak capacity would be observed.

Column-to-column reproducibility was studied for columns packed by each method with and without the same slurry and pushing solvents. The retention times and capacity factors of columns packed by the radially spinning slurry packing procedure were far more reproducible than columns packed by the other methods when using different slurry and pushing solvents. The columns packed in-house by the traditional slurry packing procedure were the worst in terms of the reproducibility of the retention times and capacity factors. When comparing columns packed by each method utilizing the same slurry and pushing solvents, the radially spinning slurry packing method again produced columns that had more reproducible retention times and capacity factors than columns packed by the other methods.

Pyconometry was utilized to determine the void volume of columns packed by the traditional slurry packing procedure, the radially spinning slurry packing procedure, and the traditional slurry packing procedure with a surfactant modifier. The columns packed by the traditional slurry packing method with and without a surfactant modifier present had almost identical void volumes and the standard deviations for these columns were very similar as well. The average void volume for columns packed by the radially spinning slurry packing procedure was the largest of all the columns indicating that there was not as much stationary phase present in the columns packed by this technique. A small fraction of stationary phase was lost.
when removing the fitting used to mate the column to the electric motor during the radially spinning slurry packing process. This loss of stationary phase accounts for a portion of the difference between the void volumes of the columns packed by the traditional slurry packing procedure with and without a surfactant modifier.

It appears that there are benefits to using either of the two novel packing methods developed during this work. The radially spinning slurry packing technique produces columns that are very reproducible in terms of column efficiency, retention time, and capacity factor. Columns packed by the traditional slurry packing procedure utilizing a surfactant modifier in both the slurry and pushing solvents have greater column efficiencies than columns packed by the traditional slurry packing procedure or the radially spinning slurry packing procedure. Due to the difficulty of packing columns by the radially spinning technique, the use of a surfactant modifier when packing RPLC columns according to the traditional slurry packing procedure would be much more appealing to column manufacturers.
REFERENCES


BIOGRAPHICAL SKETCH

James Paul McCall was born in Statesville, NC on March 4, 1977. He grew up in Hays, NC and received his undergraduate education at Western Carolina University in Cullowhee, NC where he obtained a B.A. in chemistry in the spring of 1999. Upon the completion of his undergraduate studies, he began his graduate work in chemistry at The Florida State University in Tallahassee, FL. He enrolled in the Ph.D. program in chemistry and joined Dr. John G. Dorsey’s research group to study chromatography. Paul received his Ph.D. on October 7, 2004 and is currently employed as a research scientist with Mylan Pharmaceuticals (Morgantown, WV).