2011

Investigating Reaction Schemes for Improving Silica-Based Monomeric Bonded Stationary Phases for Reversed-Phase Liquid Chromatography

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INVESTIGATING REACTION SCHEMES FOR IMPROVING SILICA-BASED
MONOMERIC BONDED STATIONARY PHASES 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, 2011
Michael D. Bair defended this dissertation on October 18th, 2011.

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This work would not have been possible without all the love and support from my family. Many thanks to my sister, Kelly, and her wonderful family for making me a happy uncle. Endless thanks to my mom and dad, whose unwavering faith in me has allowed me to count on their support through every decision I’ve ever made in life. I love you all very much.
ACKNOWLEDGEMENTS

The past five years I’ve spent in Tallahassee as a graduate student at Florida State University have been the most rewarding of my life, and for that I owe many people an enormous debt of gratitude. First and foremost, I must thank my research advisor, Dr. John Dorsey. John, I’ve learned more from you than I could have imagined, including how to be an inspiring and respected teacher, which is a trait I’m sure every one of your students past and present has recognized. But most importantly, you’ve taught me how to be an independent and free-thinking research scientist, which I consider to be the most valuable resource for me to succeed in my future endeavors. Also, many thanks to Dr. Albert Stiegman, whose knowledge of materials science and “real chemistry” I consider to be infinite. I doubt I’d know half as much about silicate chemistry as I currently do without your guidance and expertise. To Dr. Mike Roper, I’m always very impressed with the amazing research that you and your research group continually puts-out. Best of luck to you on gaining tenure – you most certainly deserve it. Finally, to Dr. Salters, I greatly appreciate you sitting on my doctoral committee, and I respectfully welcome your thoughts and opinions on this dissertation.

I would also like to thank all the faculty and staff in the chemistry department. First, I owe a lot of thanks to Dr. Yingfeng Xu at the FSU/NHMFL Stable Isotope Lab for providing %C elemental analysis data presented in this work. To Dr. Andre Striegel, thank you very much for your help and recommendations toward my post-graduate career. Thanks to Dr. Cooper for your discussions with me about chromatography and allowing me to raise interesting questions regarding my research. To Dr. Marshall, you probably taught me more about spectroscopy in general (let alone mass spectrometry) than any other teacher. Thanks to Dr. Tom Gedris and the NMR staff who really know their solid state stuff. To Tom Dusek, you are truly a master of your craft, and I still talk about that amazing Schlenk manifold you made that performed phenomenally. Many thanks to Dave Stewart, Kieth Collins, Kevin Kiley, and Mike Fennel – my research could not have gone anywhere without your technical abilities and your vast knowledge of how to repair my equipment whenever I would invariably destroy it. Thanks to all the administrative staff, in particular a huge thank you to Shellie Camp, whose job of herding all us cats is probably the most trying task I can imagine, but just know that everything you do is
greatly appreciated and nothing around here would get accomplished without your hard work and dedication. Finally, I’d like to thank Dr. Dillon, Dr. Gormin, Dr. Dudley, Dr. Dougherty, my fellow TA’s and all the undergraduate students I’ve taught in general and analytical chemistry courses who have all made teaching at FSU a great pleasure.

I would be greatly remiss if I didn’t thank all the Dorsey Group members, both past and present. First and foremost, a huge thanks to J. Dave Sunseri, whose insightful discussions led directly to me pursuing the salt study. To Catherine Rimmer, if it wasn’t for your help I’d still be trying to pack my HPLC columns. To Steve Allmon, Van Quach, Brad VanMiddlesworth, Wayne Craig, Zahra Alghoul, Qiyu Zhu, Phillip Ogden and Matt Schnippert, I greatly appreciate all the hours working in the lab with you and the vast amounts of knowledge we have shared together on the white board. Each of you has made even the most frustrating days enjoyable. Thanks to Candace McGowan for working with me on the early days of the pre-capping project and helping to get that all-important first round of data, which everyone knows is the hardest to get.

Aside from all the great people I’ve come to know in the chemistry department, I have been blessed with many friends from the greater Tallahassee area. Yes, I’m talking about all those amazing musicians and artists that I’ve met in this humble little burg, and I’m always astounded by the immense talent this town offers. Balancing late nights in the lab with late nights of concerts and campfire jams has kept me sane these past five years. Thanks to Minie, Jen C, Jon, Buda, Amy, and all friends and fans of Missus and the Walking Sticks – you were the best band I’ve ever had the pleasure to play with, and I’ll always cherish our fun times together. Many thanks to Gillian and the rest of the Studio 9 & Adrien Station crew who welcomed me into a home away from home. Thanks to Doc Russell and all the regulars at Warehouse Open Mic, which is the best place in town to spend a Wednesday night. Thanks to Katherine and Tyler, Sara, Jen G, the Clarks and the Millers, Becky and Baker, Kelly and Danny, the 3rd Ave. Drum Shop crew, the Purple Hatters… the list goes on and on. I’m never going to be able to acknowledge all the wonderful people I’ve met in Tallahassee, but just know that the last few years would not have been as enjoyable without y’all. Last but certainly not least, I’d like to thank Jackie, whose patience with me is quite a feat, and whose company is always a treasure.
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ABSTRACT

Central to the advancement of reversed-phase high-performance liquid chromatography (RPLC) is to develop new synthetic strategies for manufacturing stationary phase materials. Methods to improve the efficiency, retention properties, and chemical stability of stationary phases are always being investigated. The work presented focuses on two new synthesis schemes aimed at improving monomeric silica-based stationary phases in these respects.

The first study involves “pre-capping” Type-B silica. Previous work showed that monomeric stationary phases made by pre-treating the silica surface with small amounts of trimethylsilane (TMS) reagents prior to C18 silanization showed vast improvements in the chromatographic efficiency, phase loading, and retention with a maximum at ~5% pre-capping. It was concluded that this pre-capping step improved efficiency by selectively neutralizing the most reactive highly-acidic silanol sites, so-called silanol “hot spots,” producing a more energetically-homogenous surface prior to exhaustive C18 derivatization that subsequently yielded a more evenly-distributed alkyl bonding arrangement. These previous studies were performed on Type-A silica, an older variety of silica gel material containing higher levels of metal impurities than the Type-B silica used today. It has since been argued that metallic impurities are the primary cause of silanol hot-spots, and that pre-capping Type-B silica would have little or no effect, however the experimental evidence has yet to be produced, and there exists the potential for heterogeneous silanol reactivity inherent in the amorphous silica gel regardless of purity. The purpose of the work presented here is to determine the effects of TMS pre-capping on Type-B silica as compared to the previous Type-A results, with the goal of establishing pre-capping protocol for Type-B silica and to form a better understanding of its chemistry.

The current work performed on three Type-B silica substrates of various physical and chemical properties demonstrated optimal TMS pre-capping at approximately 2.5%. The results at this level show only a slight improvement in efficiency for non-polar compounds (< 10% improvement), but a more noticeable improvement (≥ 25%) was observed for some drug compounds and bases under buffered conditions, with the magnitude of the improved efficiencies correlating with metal impurity content and physical parameters of the silica
substrate. Pre-capping also resulted in a slight decrease in retention and hydrolytic stability due to a decrease in bonded phase density. The results lend supporting evidence that metal impurities are the primary source of highly acidic silanols, but they also suggest a means to improve efficiency of basic analytes on certain Type-B silica substrates. It was concluded that TMS pre-capping Type-B silica is best done at low levels ($\leq 2.5\%$) to maximize efficiency while maintaining bonded phase loading and stability.

In the second study, quaternary ammonium salts were added into dichloromethane (DCM) reaction solvent and carbon loading was measured. Phase products showed increased phase modification and faster kinetics for both the primary C18 modification and TMS end-capping reactions upon addition of these salts at low levels (10 mM). The results suggest that this effect is predominately due to the salt’s ability to mitigate the energetic barrier at the interface between the polar silica surface and the non-polar reaction solvent. Larger tetrabutylammonium salt cations induced more of an effect than small tetraethylammonium, indicating that the salt acts to improve steric interactions of the grafted C18 chains at the surface. Also, an increase in DCM/silicate contact angle and decrease in surface tension were observed for DCM upon addition of tetrabutylammonium bromide (TBABr), which indicate a lower surface energy and faster diffusion of reactants and products across the phase boundary. Surprisingly, the density of DCM decreases with TBABr, and an optimization point for calculated capillary pressure gave a maximum for 10 mM TBABr in DCM. A brief investigation into the effects of elevated pressure in the C18 reaction yielded a large increase in bonded phase density at 6.5 atm.

More conclusively, high concentrations of TBABr salt (50 mM) showed a drastic decreased in bonded phase loading and was attributed to ionic suppression and/or shielding of the silica surface. This implies that increased ionic content in the reaction medium is significantly detrimental to silane ligand bonding density. This is a primary concern considering that all silanization reactions that use halosilane reagents result in the formation of ionic products. More study of the effect of ionic strength in the silanization reaction is warranted.
CHAPTER ONE

INTRODUCTION

1.1 Chromatography

1.1.1 Introduction to Chromatography: Definition and History

Chromatography is a molecular separation method by which components of a homogenous mixture can be separated physically by way of each component’s differential preference for one of two distinct phases. It is similar in principle to a liquid-liquid extraction mechanism in which compounds diffusing across a phase contact barrier will concentrate in one of the two immiscible solvents based on differences in their solvation energies between the two phases. Therefore, various components of a mixture can be separated from one another to varying degrees of purity by exploiting differences in their partition equilibria. However, chromatography differs because it is not a single step or series of discrete extraction steps, but rather is a continuous process in which the two phases are in constant motion with respect to one another. This is accomplished by fixing one phase static as the “stationary phase” (SP) and setting it in contact with a flowing “mobile phase” (MP) fluid. The mixture to be separated is introduced into the chromatographic system and is flushed-through the SP region with the MP, causing individual components to elute through the chromatographic region at different velocities depending on differences in their partition equilibria between the two phases. The chromatographic system is chosen to optimize the differences in free-energies of affinity between the SP and MP for each component to allow them to be resolved using the least amount of time and materials possible. The stationary phase is typically a solid sorbent substrate or immobilized fluid, while the mobile phase can be either a gas (gas chromatography – GC), a liquid (liquid chromatography – LC), or a super-critical fluid (SFC). The following dissertation will focus on stationary phase chemistry for use in high performance liquid chromatography (HPLC).

HPLC is the most popular separation technique and is the third most widely used instrumental analytical measurement after pH probing and analytical weighing. The reason for its
popularity is primarily three-fold: 1) the fundamental necessity for separations in chemical analysis, 2) the variation and adaptability of the technique for numerous applications, and 3) the technique’s robustness to provide quality reproducible data. The first modern LC separations were “normal phase” that used a polar SP and a non-polar MPs such as hexane and THF. Today, the most commonly employed HPLC method is “reversed-phase” HPLC (RP-HPLC, or RPLC), which carries-out the chromatographic process by employing a polar mobile phase (usually aqueous based) and a non-polar stationary phase [1]. Typical non-polar RPLC stationary phases are “bonded phases” made by covalently modifying the surface of an inorganic solid substrate with hydrophobic organic ligands, usually alkyl chains of varying lengths (C8, C18, etc.). Great advancements in the production of new and unique stationary phases for RPLC have been made [2-5]. Despite all the various types of SP’s and their properties [6-9], the ultimate goal of any chromatographic stationary phase is a solid material that is chemically and physically robust with fast mass transfer kinetics for efficient separations and an active retentive surface consisting of only the physiochemical interactions preferred for the given application [5].

The history of the development of chromatography in theory and practice has been recently outlined [10,11]. The first example of a chromatographic separation is credited to Russian botanist Mikhail Tswett, who in 1903 described his new method to the Warsaw Society of Natural Sciences as an application for analyzing biological molecules, particularly plant pigments [12]. The next great advancement to the field of chromatography came in 1941, when Martin and Synge introduced liquid-liquid partitioning chromatography and their plate theory for quantifying chromatographic efficiency, as well as proposing the idea for gas chromatography [13]. While Martin and Synge won the Nobel Prize in 1952 for their contributions, their plate theory was incomplete as it did not take into account effects of longitudinal diffusion and rates of phase-transfer. A more complete theory of efficiency encompassing the dynamic and kinetic aspects of chromatography was later developed by Giddings [14], van Deemter [15], Knox [16], and Horváth [17].

The history of HPLC cannot be discussed without addressing silica because the technique owes so much of its conception, development, and implementation to advancements in the design and production of siliceous stationary phase materials. In the 1950’s and 1960’s, diatomaceous earth (DE) was a very popular choice of sorbent phase in packed column GC and LC because its highly rugged and porous structure afforded the surface properties necessary for
fast and efficient separations [1]. However, DE is a very heterogeneous mixture of diatom skeletons, thus its chemical composition and physical characteristics such as mean pore diameter, pore volume, and particle size varied widely in this material. Manufacturing colloidal porous silica from sol-gel chemistry allowed materials to be made with more control over these parameters [18,19]. Like all silicates, the surface of diatomaceous earth and colloidal silica is polar due to the presence of silanols (see Section 1.2.1), thus most LC methods using bare silicates are normal-phase separations. When it was discovered that reversed-phase LC methods offer more efficient separations for most organic analytes than normal-phase methods, organic modifications to the silica surface began to be explored, and bonded phases quickly came into favor [1], with the first example of an LC bonded phase credited to Stewart and Perry [20]. Since then, many improvements to the underlying silica structure have been made, and new and improved means to organically modify the surface have been developed. Today, sales of silica-based bonded stationary phases total approximately $500 million a year [21].

1.1.2 Chromatographic Separations: Fundamental Theory and Measurements

Figure 1.1 shows a typical HPLC system, which is comprised primarily of 3 parts: a mobile phase pump, a stationary phase column, and a detector. The analyte mixture to be separated is introduced into the mobile phase stream and onto the column by way of a 6-port valve, and the effluent is analyzed by a detector, usually UV/Vis absorbance or other optics- based sensing technique, or a mass spectrometer. The resulting signal output versus time trace is called a chromatogram, an example of which is shown in Figure 1.2. The two measurable chromatographic parameters most pertinent to this dissertation work are retention and efficiency.

The retention time ($t_R$) for an analyte component is measured as the location of the peak maximum along the time axis. This parameter will differ with changing column dimensions and flow rates, so the most accurate and unambiguous measure of analyte retention is retention factor ($k'$). Retention factors are calculated from analyte retention times ($t_R$) and the elution time of an unretained void marker ($t_0$) as per Equation 1.1. For RPLC, uracil, thiourea, and nitrate are often used as void markers [22].
Figure 1.1: Instrumentation for a typical HPLC system.

Figure 1.2: Chromatogram showing separation of a 5 component mixture. Mobile phase = 60:40 acetonitrile:water, 1.00 mL/min. Stationary phase = Waters Symmetry 5μm end-capped C18.
In order for analytes to be separated chromatographically they must have different retention factors, the degree of which is called “selectivity.” The value of $k'$ for a given analyte is a purely thermodynamic quantity dependent only on the chemical natures of the stationary phase and mobile phase and how the analyte interacts between the two. In thermodynamic terms, the retention factor is the product of the partition equilibrium constant ($K$), which is the ratio of the analyte concentrations ($C$) in the SP and MP, and the volumetric phase ratio ($\Phi$), which is the ratio of the volumes ($V$) of the two phases, as shown in Equation 1.2.

$$k' = K\Phi = \left( \frac{C_{SP}}{C_{MP}} \right) \left( \frac{V_{SP}}{V_{MP}} \right)$$  \hspace{1cm} \text{Equation 1.2}$$

In reversed-phase liquid chromatography, retention increases with increasing water content of the mobile phase and decreases with increased amount of organic modifier, usually methanol (MeOH) or acetonitrile (ACN). Also, retention usually decreases with increasing temperature. These trends in retention for RPLC pertain to nearly every analyte studied.

While the locations of the analyte peaks are defined thermodynamically by the retention factor, the term “efficiency” refers to width and shape of the peaks and is determined primarily by the kinetics of the separation process. The efficiency of an eluted analyte band is measured in number of theoretical plates ($N$), which is defined as the ratio of the retention time squared over peak variance ($N \equiv t_R^2 / \sigma_t^2$) and can be calculated using peak width by Equation 1.3,

$$N = 16 \left( \frac{t_R}{w_t} \right)^2$$  \hspace{1cm} \text{Equation 1.3}$$

where $w_t$ is the baseline peak width measured in the same time units as $t_R$, thus making $N$ a dimensionless number. Calculating peak efficiencies from this equation is common however it is a poor indicator of the true performance of the chromatography as it assumes peak shapes are
symmetrical and Gaussian. The most accurate way to measure efficiency is to use statistical moments to describe the analyte band distribution as per Equation 1.4.

\[ N = \frac{m_2}{m_1^2} \]  
\[ \text{Equation 1.4} \]

Here \( m_1 \) and \( m_2 \) are the first and second statistical moments defined as the center of mass and the variance of the peak distribution, respectively. They can be calculated digitally by summing the discrete data for signal response as a function of time, \( h(t) \), as per Equations 1.5 and 1.6.

\[ \overline{m}_1 = \frac{\sum_{i=0}^{\infty} t_i \cdot h(t)_i}{\sum_{i=0}^{\infty} h(t)_i} \]  
\[ \text{Equation 1.5} \]

\[ \overline{m}_2 = \frac{\sum_{i=0}^{\infty} (t_i - \overline{m}_1)^2 \cdot h(t)_i}{\sum_{i=0}^{\infty} h(t)_i} \]  
\[ \text{Equation 1.6} \]

While accurate, statistical moments calculations require a lot of computing power and become especially daunting with chromatograms containing numerous components. An empirically-derived efficiency calculation that models peaks as exponentially-modified Gaussian (EMG) distributions was proposed by Foley and Dorsey [23] and shown in Equation 1.7.

\[ N = \frac{41.7 \left( \frac{t_R}{w_{0.1}} \right)^2}{1.25 + B / A_{0.1}} \]  
\[ \text{Equation 1.7} \]
Here $w_{0.1}$ and $B/A_{0.1}$ are the peak width and asymmetry factors, respectively, measured at 10% peak height. This calculation takes into account the non-ideality in peak shapes typically seen in practice with the $B/A_{0.1}$ asymmetry term and accurately reflects the true peak variance as calculated by the statistical moments method up to asymmetry factors of approximately 2.76.

Measurements of plate count ($N$) rarely assess how well a chromatographic system performs relative to theory as this number is dependent on the length of the stationary phase column, the size of the particulate SP packing material in the column, and other system parameters. Therefore, plate counts are often normalized to the length of the column to calculate a theoretical plate height ($H$) by $H = L / N$, where $L$ is the length of the column. The value of $H$ is simply the length of column ($\mu m$) required to produce one theoretical plate. Also, the diameter of the stationary phase particles ($d_p$) affects the efficiency of the column, with smaller particles affording higher efficiency. Thus, plate height can be normalized to the size of the packing material to calculate a reduced plate height ($h$) by $h = H / d_p$. The value of $h$ is then the number of particle diameters necessary to produce one theoretical plate. Ideally it’s best to have the smallest plate height possible, but in practicality most packed-bed stationary phase columns give reduced plate heights between approximately 2 and 5.

### 1.1.3 Measuring Thermodynamics and Band Dispersion in Chromatography

As previously stated, retention in liquid chromatography is determined by the thermodynamics of analyte partitioning between the stationary phase and mobile phase. To elucidate the enthalpic and entropic contributions governing retention, a van’t Hoff analysis is performed, which involves running the chromatography experiment at various temperatures. Plotting $\ln k'$ versus inverse absolute temperature $1/T$ (1/K) yields the van’t Hoff relationship as per Equation 1.8,

$$\ln k' = -\frac{\Delta H^o}{RT} + \frac{\Delta S^o}{R} + \ln \Phi$$  \hspace{1cm} \text{Equation 1.8}

where $R$ is the gas constant, $\Phi$ is the volumetric phase ratio, and $\Delta H^o$ and $\Delta S^o$ are the standard enthalpy and entropy of transfer for an analyte diffusing from the mobile phase into the stationary phase. If the van’t Hoff relationship is linear then it can be assumed that these
quantities do not change over the observed temperature range. Thus, the standard enthalpy ($\Delta H^\circ$) of solute partitioning can be calculated absolutely by multiplying the observed slope by -R.

The y-intercept of a van’t Hoff plot comprises both the $\Delta S^\circ/R$ and the ln$\Phi$ terms. The phase ratio cannot be measured directly, but a method to derive entropic information was proposed by Coym and Chester [24]. Assuming two analytes that differ only by a methylene group (-CH$_2$) experience the same phase ratio, subtracting-out the y-intercept term of two homologous compounds removes the phase ratio term, and the standard entropy of partitioning for a single methylene group ($\Delta S^\circ_{-CH2}$) can be calculated by Equation 1.9.

$$
\Delta S^\circ_{-CH2} = \left[ \left( \frac{\Delta S^\circ_1}{R} + \ln \Phi \right) - \left( \frac{\Delta S^\circ_2}{R} + \ln \Phi \right) \right] * R \quad \text{Equation 1.9}
$$

It is important to note that the entropy of methylene transfer calculated from this method cannot be considered an absolute value, but it does offer a quantitative number by which to compare the relative entropies of solute partitioning between similar chromatographic systems.

As for efficiency, while there are arguments for how analyte peak variance can be dependent to some extent on thermodynamic causes [25], the overall consensus is that kinetics is the predominant factor that determines efficiency in HPLC. Thus, efficiency will depend on the rate at which the chromatography experiment is run. Figure 1.3 shows how efficiency measured in plate height (H) depends on the average linear flow velocity ($u$) of the mobile phase calculated from the volumetric flow rate (F), the column cross-sectional area (A), and total column porosity ($\varepsilon_T$) as per $u = F/A\varepsilon_T$. Graphs of H versus $u$ are called van Deemter plots and can be modeled mathematically using the van Deemter equation [15,26] shown in Equation 1.10.

$$
H = A + \frac{B}{u} + Cu \quad \text{Equation 1.10}
$$

The three terms in the van Deemter equation simply denote the band-broadening processes that are independent of flow velocity (A), inversely dependent on flow velocity (B), and directly proportional to flow velocity (C). The combination of these three parameters results in a curve in which there is an optimization point where the efficiency is maximum (H is minimum) at a
certain optimal linear flow velocity \( (u_{opt}) \). The van Deemter model only accounts for on-column band-broadening and does not include extra-column dispersion effects from the HPLC instrumentation. The A, B, and C terms can be calculated by a least-squares fit of the van Deemter model to the experimental data of efficiency at various flow rates.

The van Deemter terms have been assigned to certain diffusion processes and other physical causes that are the primary contributors to each band-broadening parameter. The A-term is referred to as the “eddy diffusion” term, and results from the nature of the packed particle bed. As a fluid moves through a porous medium, the individual analyte molecules in the elution band can travel through different channels, each of varying lengths and streamlet velocities. The A-term is heavily influenced by the quality of the packing in the column, with poorer packing yielding higher A-term dispersion.

The B-term is the “longitudinal diffusion” term and arises solely from the fact that a concentrated analyte band will diffuse outward as it travels down the column. The more time an analyte band spends on the column the more it will diffuse, thus giving the B-term an inverse-dependence on flow velocity, with faster flow velocities yielding lower B-term dispersion. In
practice, the B-term is often inconsequential as typical chromatographic separations run at flow velocities fast enough so that B-term broadening is minimal compared to A-term and C-term.

The C-term is the “resistance to mass transfer” term and is dependent on the rate at which analytes diffuse into and out of the stationary phase. In practical terms, the C-term is most important as it is the band-broadening variable that most limits the speed at which chromatographic separations can be run. For example, as demonstrated in Figure 1.3 [27], there is currently a great interest in stationary phase packing materials with small particle diameters [28], as these phases offer faster mass-transfer kinetics at higher linear flow velocities by minimizing the diffusion distance necessary for the analyte to travel to interact with the stationary phase surface.

1.2 Silica-Based Chromatographic Stationary Phases

1.2.1 Chemistry of Amorphous Porous Silica

For years, columns packed with silica-based bonded phases have been the workhorse of reversed-phase liquid chromatography for several reasons. First, the silica support is economical, highly porous with a large surface area per volume ratio, compatible with a number of different solvents, and has a high mechanical stability able to withstand extremely high pressures necessary in today’s instruments. Secondly, the silica surface can be easily modified with any number of desired chemical functionalities, giving silica-based bonded phases the potential to be designed specifically for just about any application. Last but not least, the chemistry of porous silica is well understood, and the manufacturing processes that dictate the physical parameters of porous silica particles can be easily controlled. Thus, the end-use properties including particle diameter, pore diameter, pore volume, surface area, etc., can be precisely tailored [29-31].

The chemical and physical properties of silica have been thoroughly described in two fundamental texts by Iler and Unger [30,31]. Silicates exist as both crystalline and amorphous solids, the latter of which is used for chromatographic stationary phase supports. Dissolved silica exists as several forms of silicic acid monomers and dimers, including ortho-silicic acid ($\text{Si(OH)}_4$) for example. All silicic acids are readily soluble in basic solutions. Figure 1.4 shows the hydrosilation reaction, describing the polycondensation process for making amorphous silica
sol-gels from monosilicic acids (forward), as well as the dissolution of solid silica via water hydrolysis (reverse).

\[ \text{Si(OH)}_4(aq) \xrightleftharpoons{H^+,H_2O} \rightarrow \text{SiO}_2(s) + H_2O(l) \]

Figure 1.4: The hydrosilation reaction.

According to this reaction, high temperature and high pH favor the dissolved state on the left, whereas the equilibrium shifts to the solid state on the right at low temperatures and in acidic media. Amorphous silica gel is made via a sol-gel process [29-33] that begins with silicates dissolved in a basic solution that is slowly neutralized with acid to form the silica hydrogel, a corpuscular solid network in which the pores are infused with solvent. Drying of the hydrogel under vacuum produces the shrunken xerogel.

Type-A and Type-B silicas differ in the starting material used in this sol-gel process. Type-A silica utilizes silicic acids as in Figure 1.4, forming water as byproduct. Silicic acids are weak acids capable of leaching and solvating metal cations that then get incorporated into the siloxane polymer network, resulting in contamination of the final silica gel product. Conversely, Type-B silica involves the polycondensation of alkoxysilanes as per Figure 1.5 using tetramethoxysilane (R = -CH₃) or tetraethoxysilane (R = -C₂H₅), forming the alcogel as byproduct. This process incorporates far fewer metallic impurities into the silica gel product as compared to the Type-A method, and as a result Type-A materials have fallen out of favor in the last two decades [34].

\[ \text{Si(OR)}_4(aq) \xrightleftharpoons{H^+,H_2O} \rightarrow \text{SiO}_2(s) + ROH(l) \]

Figure 1.5: Hydrosilation reaction for making silica sol-gels from alkoxysilanes.

To produce spherical porous particles for packed-bed HPLC columns, gelling of the silica takes place in an immiscible solvent emulsion, or more commonly, the forming hydrogel is
aspirated into a drying oven. Both processes are used extensively in large-scale manufacturing and result in spheres as small as 1.7 μm in diameter, and various sieving processes can isolate particle fractions with a very narrow size distribution [29-31,33,35]. Examples of a spherical porous silica particles are shown in Figures 1.6 and 1.7 [33,36]. The electron micrograph images clearly show the particle shape and size distribution (Fig. 1.6), and the random globular structure that is formed from the coalescence of millions of silica sol nanoparticles, resulting in a highly porous spherical silicate packing material (Fig. 1.7). Further detail regarding the physical characteristics of porous silica is discussed in Section 1.2.2.

Figure 1.6: SEM of porous silica microspheres made from TEOS. (Adapted from Fig.1d in J. Hanrahan, et al. 2007). [36]
The surface chemical features of amorphous silica are depicted in Figure 1.8a. Of most importance to the synthesis and chromatographic properties of bonded SPs are silicon-bound hydroxyl groups called silanols, of which there are three varieties – (i) free, (ii) vicinal, and (iii) geminal. Silanols are present on the silica surface at a concentration of approximately $8\pm1 \mu\text{mol/m}^2$, the majority of which are vicinal pairs. Isolated and geminal silanols are generally more reactive than the hydrogen-bonded vicinal variety. Siloxane bridges (iv) present on the silica surface are considered to be inert in terms of reactivity and analyte interaction [31].

Silanols are responsible for the attachment of the organic moieties to the surface via the silanization reaction shown in Figure 1.8b, in which silane reagents are covalently bonded to the silica surface at silanol sites via siloxane bonds (Si-O-Si), yielding the modified silica product and an acid byproduct. Of the three alkyl side chains (-R) on the silane reagent, one is the desired phase modification ($R = n$-octadecyl for a C18 phase, $R = n$-octyl for a C8 phase, etc.).
Figure 1.8: a) Surface chemical features of native (bare) silica showing (i) free, (ii) vicinal (bound), and (iii) geminal silanols, as well as (iv) siloxane bridges. b) Monomeric silanization reaction showing siloxane (Si-O-Si) attachment of the organic silane to the silica surface at a single silanol site.

Monomeric RPLC stationary phases are made by organically modifying the silica surface in which a monofunctional silane (a silane reagent with a single leaving group “X,” typically a halide) is covalently bonded to the surface at a single silanol site via one siloxane bond. Various leaving groups can be used, but halosilanes typically yield higher bonding than alkoxy- and amino- silanes. A weak non-nucleophilic base (e.g. pyridine) is employed as a scavenger in the synthesis process to neutralize the acid byproduct and promote a more complete surface coverage. However, despite all attempts to produce RPLC phases with the highest bonded phase coverage possible, a common monomeric C8 or C18 derivatization will only convert roughly 20 - 50% of all surface silanols to alkyl silanes [5,8,30,31,37-39].

Silica has been called a “living polymer” because its surface features are constantly in flux with water. Water can be strongly absorbed physically to the surface in a hydrogen bonded
network several layers thick. All but the final monolayer of adsorbed water can be removed under vacuum at room temperature, while this last layer requires temperatures of ~150-200ºC for complete dehydration [29-31,37,40]. Silanols have been dubbed “chemically absorbed water” because they can be removed under vacuum at very high temperatures in a process called “dehydroxylation.” Water loss from H-bonded vicinal silanols happens at relatively low temperatures beginning at roughly 150ºC, but removal of geminal and free silanols requires much higher temperatures (approx. 800-1200ºC) in a process called “scintering” that destroys the porous structure [29-31,37,40]. Loss of physically and chemically absorbed water can be measured via thermogravimetric analysis. Figure 4a shows the dehydration and dehydroxylation processes [37], and Figure 4b shows examples of resulting thermogravimetric traces [41].

![Diagram](image)

**Figure 1.9:** a) Dehydration and dehydroxylation of the silica surface. (Adapted from Fig. 4 in J. Nawrocki 1997) [37]. b) Thermogravimetric traces of loss of water vs. temperature and first-derivative rate. (Adapted from Fig. 1 in S. Ek, et al. 2001) [41].
1.2.2 Physical Parameters of Porous Silica and Chromatographic Consequences

The physical structure of porous silica - its defining characteristics and the methods used to measure them - are extensively outlined in Chapter 2 of Unger [31]. The three most important physical parameters describing the overall pore characteristics are pore diameter (D), pore volume (\(V_p\)), and surface area (S), and each of these parameters imparts certain effects on the synthesis and performance of LC stationary phases. As shown in Fig. 1.7, the porous structure of silica sol-gels arises from the conglomeration of millions of silica sol particles, each having a diameter on the order of 1-100nm, typically. Such a complex porous system is difficult to model, but generally these three parameters are interrelated as per the proportion in Equation 1.11.

\[
S \propto \frac{V_p}{D}
\]  

Equation 1.11

The most important aspect of this proportionality is that the surface area is inversely proportional to the pore diameter and directly proportional to the pore volume. Thus, porous silicates with a wide average pore diameter generally have a smaller surface area.

Current HPLC packing materials are spherical (Fig. 1.6) and fully porous with the pore structure running throughout the entire particle. However, “superficially porous” materials made with a solid core and a porous shell have garnered recent interest [42-44]. Regardless, it’s agreed that the vast majority of a porous silica microsphere’s surface area comes from the porous structure inside the particle and not on the external surface, which can be several orders of magnitude less than the internal surface area. The surface area determines the maximum amount of silane derivatization possible. For RPLC bonded phases in which alkylsilanes are used to modify the surface, the bonding density (\(\alpha\)) of silane ligands in \(\mu\)mol/m\(^2\) can be calculated by Equation 1.12 from the mass-percent carbon (%C) measured by elemental analysis [45,46],

\[
\alpha = \frac{\%C \times 10^6}{12.011n_c S \left[100 - \left(\frac{\%C}{12.011n_c}\right)(M - L)\right]}
\]  

Equation 1.12
where \( S \) is the surface area (m\(^2\)/g) of the silica substrate, \( n_c \) is the number of carbons on the silane ligand, \( M \) is the molar mass (g/mol) of the silane reagent used, and \( L \) is the molar mass of the leaving group. Stationary phases with a larger surface area almost always have a higher stationary phase volume, thus they usually provide more retention and sample loading capacity.

Of the three physical parameters mentioned above, pore diameter is most interesting in that it has many dramatic effects in both the synthesis of the phase products and their resulting chromatographic properties [47-50]. Many of these pore size effects are still being investigated. Most silica-based HPLC stationary phases are mesoporous with average pore diameters ranging from 6 nm to 30 nm. Some macroporous silica materials (\( D_{\text{avg}} > 50 \) nm) are used for separating macromolecular mixtures like peptides and polymers, while microporous (\( D_{\text{avg}} < 2 \) nm) substrates are typically not used in LC as their highly-porous structure yields slow mass transfer kinetics and poor efficiency, and their narrow pores restrict silane derivatization and analyte intrusion. The porous characteristics can be controlled in the sol-gel process. Temperature, rate of neutralization, solvent viscosity, ionic strength and presence of metal cations such as Na\(^+\) and Ca\(^{2+}\), and certain ripening and aging processes performed during and/or after the gelation step can be used to affect the size of the sol nanoparticles, which in turn determine the pore size [30,31,35].

The pore size is affected by the surface modification, and vice versa. Upon derivatization, the bonded phase layer acts to narrow the pores. This effect is often minimal for monomeric phases on substrates with an average pore diameter greater than 100 Å, but can become more problematic for smaller pore materials and polymeric surface modifications (see Section 1.2.3) [48]. More importantly, pore size affects the derivatization reaction and the carbon loading. Narrow pores restrict silane reagents from accessing the silica surface in these regions, resulting in a decrease in bonded phase density. This effect is most significant with pore diameters less than 140 Å and with longer alkyl ligand reagents like octadecylsilanes [47,49,50].

Pore diameter also affects how fluid is transported through a porous network by way of capillary pressure (\( p_c \)) as describe by the Young-Laplace equation in Equation 1.13,

\[
p_c = \frac{-2\gamma \cos \theta}{r} \tag{Equation 1.13}
\]
where \( r \) is the capillary radius, \( \gamma \) is the surface tension at the liquid-vapor interface, and \( \theta \) is the contact angle between the fluid and the solid at the surface interface.

The fluid interface is most often a liquid-gas barrier, although a liquid-liquid interface between two immiscible fluids is also possible. The contact angle defines whether the fluid in question is a wetting phase (\( \theta < 90^\circ \)) or a non-wetting phase (\( \theta > 90^\circ \)). At every fluid-fluid interface in a capillary, one fluid (liquid or gas) is considered wetting and one considered non-wetting, unless the contact angle is exactly 90°. The negative sign denotes the direction in which the capillary pressure acts, with wetting fluids yielding pressure into the capillary (\( p_c = - \)), while a non-wetting solvent is pushed out of the capillary (\( p_c = + \)). Note that \( p_c \) is inversely proportional to the capillary diameter, thus any capillary pressure effects are most relevant in a porous medium with very narrow pores. Capillary pressure and other effects in porous media are outlined in great detail in a text by Pinder and Gray [51].

The Young-Laplace Equation is a derivation of the Young Equation shown in Equation 1.14 and describes a three-phase equilibrium where the surface tensions are a minimum at the three interfaces: solid-liquid (SL), liquid-vapor (LV), and solid-vapor (SV).

\[
\gamma_{SV} = \gamma_{SL} + \gamma_{LV} \cos \theta \tag{Equation 1.14}
\]

Based on this equation, if the solid-vapor system is kept constant (\( \gamma_{SV} = 0 \)), and changes are made to the liquid that affect both contact angle and liquid-vapor interfacial tension, then corresponding changes to the solid-liquid interfacial tension can be calculated by Equation 1.15.

\[
\Delta \gamma_{SL} = -\Delta(\gamma_{LV} \cos \theta) \tag{Equation 1.15}
\]

Capillary pressure can be determined by measuring the height of fluid imbibation up a vertical capillary (\( h \)) and the solution density (\( \rho \)) by \( p_c = \rho gh \), where \( g \) is the gravitational acceleration constant (9.8 m/s\(^2\)). However, this assumes that height is solely determined by capillary pressure forces (e.g. interfacial tension) and not due to bodily gravitational forces. To qualify whether the measured effects are acting within the “capillary region” and thus \( p_c = \rho gh \) is an appropriate approximation, the Eötvös or “bond number” (\( B_0 \)) as calculated by Equation 1.16.
must be as small as possible ($B_0 \ll 1$). For this reason, narrower capillaries give more accurate capillary pressure measurements.

\[ B_0 = \frac{dgr^2}{\gamma} \]  \hspace{1cm} \text{Equation 1.16}

Capillary pressure in porous RPLC stationary phases is an important consideration in both the chromatography experiment and the surface modification reaction. In RPLC, the aqueous-based mobile phase is often non-wetting with respect to the alkyl-derivatized silica surface [52], thus requiring pressure to force the solvent into the porous silica network. In a typical HPLC experiment the pumping pressure ranges from ~20-100 atm, and even upwards of 1000 atm in today’s ultra-high pressure (UHPLC) methods. These high pressures are often adequate to overcome the exclusion of aqueous mobile phases from most of the pores in the bonded RPLC stationary phase. However, highly-aqueous mobile phases (>90% water) can be excluded from narrow pores, causing a loss of retention that was once attributed to “phase collapse” [53].

The pressure inside the reaction vessel where the surface silanization of the porous silica takes place is often orders of magnitude less (~1-3 atm) than the phase product will see inside the chromatography column. Thus, it is conceivable that porous areas restricted from solvent intrusion by capillary pressure effects in the low-pressure phase synthesis environment are accessible to analytes in the high pressure environment of the chromatography column. There are several factors that complicate matters. First, the random globular structure of silica sol-gels creates pores of varying diameter and shape. Secondly, the silica surface changes from beginning to end of the reaction, becoming less and less polar as the alkyl surface coverage increases. Thus the contact angle at the solvent-substrate interface is dynamic, and the wetability of the reaction solvent changes over the course of the silanization modification. The effects of capillary pressure on surface modification reactions have not been thoroughly investigated, and only limited information is available at present.
1.2.3 Silica-based Bonded Stationary Phases for Liquid Chromatography

Utilizing porous silica as the backbone support for RPLC bonded stationary phases is not without its downsides. First, the surface chemistry of currently available bonded stationary phases is inherently heterogenous, and residual silanols that remain on the surface can interact with polar and charged analytes resulting in poor peak shapes and reduced efficiency, particularly for basic compounds. Secondly and most importantly, silica-based stationary phases suffer drastically from a lack of chemical stability, which limits column lifetime and prohibits its use at extended temperature and pH ranges. This instability owes itself to residual silanols on the silica surface and to the siloxane bonds that are formed between the surface and the attached organic silane moiety. Currently, there is great interest in improving the performance of silica-based chromatographic stationary phases with respect to these two properties.

The roles residual silanols play in the chromatographic properties of silica-based bonded phases have been extensively reviewed [29,35,37,40,54]. Chiefly, they give rise to “silanophilic” interactions with certain analytes, particularly amines and other bases, as well as peptides. Residual silanols are capable of hydrogen bonding and ionic interactions with these analytes, resulting in a mixed-mode retention mechanism that is both thermodynamic and kinetically different than the predominant single-mode solvophobic partitioning of an alkyl RPLC stationary phase [25,55,56]. Such heterogeneity in partitioning mechanisms gives rise to broader, more asymmetric peaks, resulting in poorer elution band efficiency. Many chromatographic methods have been developed to quantify the strength and abundance of silanophilic interactions for stationary phases [25,57-62], most of them measure peak asymmetry factors of basic analytes and/or their selectivity versus non-basic polar compounds.

The chemical and thermal stability of silica and bonded phases made from it have been thoroughly investigated [30,31,40,63,64]. It’s generally regarded that silica-based phases are stable within a pH range from 4 to 7, with some extending the stable region down to pH 3. Regardless, it’s agreed that acidic conditions cause loss of bonded phase by hydrolyzing the siloxane bond formed between the surface and the silane modifier (reverse reaction shown in Fig. 1.8b), and that high-temperatures and basic conditions dissolve solid silica by the reverse of the hydrosilation reaction shown in Figure 1.4.

Unger and Iler both proposed a dissolution mechanism that is kinetically favored at surface silanol sites over siloxanes [30,31], as shown in Figure 1.10. Such a mechanism is autocatalytic
as it creates more silanols that then accelerate further dissolution. Therefore, silica with a more hydroxylated surface will dissolve faster than silica with a dehydroxylated surface, and silica materials with lower silanol activity will provide improved stability at elevated temperature and high pH. It has been shown that dehydroxylated silica shows improved stability at high pH [65], but suffers in its chromatographic properties [40]. Figure 1.11 illustrates the chemical stability of native silica and commercially available silica-based bonded phases [9,63,65].

![Chemical structure](image.png)

Figure 1.10: Proposed mechanism of hydroxide catalyzed dissolution of silica in water. Dotted line represents the interface between silica on the left and water on the right. (Adapted from Fig. 1.11 in R.K. Iler, 1979) [30].

Several techniques can be employed to remedy the negative effects of residual silanols by changing either the mobile phase or stationary phase [54,66]. The simplest method is to suppress ionic interactions by adjusting the pH of the mobile phase. At high pH, basic analytes remain uncharged, and at low pH silanols stay protonated. This will alleviate the strongest ionic silanophilic interactions, but the pH regions required for ionic suppression almost always lie outside the stability range for silica-based bonded phases. Often times mobile phase modifiers like trifluoroacetic acid (TFA) and triethylamine (TEA) are added at low concentration (~0.1% v/v) to compete with analytes for silanophilic interactions and are used routinely to improve peak shapes for basic analytes and peptides [58,59].
Figure 1.11: a) Mass of silica dissolved vs. eluent volume of 50:50 methanol / carbonate buffer 0.1M pH 10.0 for several commercial columns. (Adapted from Fig. 8 in H. Claessens, M. van Straten. 2004) [63]. b) Mass of silica dissolved vs. eluent volume of aqueous phosphate buffer 0.25M pH 7.0 at 40°C and 60°C. (Adapted from Fig. 19 in H. Claessens, M. van Straten. 2004) [63]. c) Chromatograms of pharmaceuticals on a Phenomenex Luna column with a 45:55 acetonitrile/phosphate buffer 20mM pH 10. (Adapted from Fig. 4 in J. Nawroki, et al. 2004) [9]. d) Dissolution of dehydroxylated and hydroxylated silica with pH 11.5 pyrrolidine buffer. (Adapted from Fig. 8 in J. Sunseri, et al. 2003) [65].
Figure 1.12: End-capping reaction on a monomeric C18 substrate.
A better way to limit silanol effects is to improve the stationary phase modification. Commonly employed in monomeric phase synthesis is “endcapping,” a process in which a smaller and less sterically-incumbered silane reagent like trimethylchlorosilane (TMCS) is added after the phase modification to derivatize the remaining silanols that are missed due to steric hinderance of the larger primary phase attachments like octadecylsilane, thus “filling-in” some of the silanol “gaps” that inevitably remain. However, as illustrated in Figure 1.12, end-capping only converts a fraction of the remaining silanols, and residual silanols are always present in the final monomeric bonded phase product [2]. Another method employed in the production of monomeric bonded phases is the use of silane reagents containing bulky side-groups like isopropyl or t-butyl that help shield the silica surface better than a methyl group [67]. Both of these methods at best still only derivatize roughly half of all surface silanols [4,7,35].

Two other synthesis methods for producing organically-modified silica are polymeric surface modifications and bulk-phase modifications [68-71]. Polymeric derivatization is similar to monomeric derivatization in which a functional silane is covalently bonded to a native silica surface, but it differs in that surface polymerization utilizes polyfunctional silanes (ex.: dichloro- or trichloro- silanes) to enable polycondensation of the silane reagent into a polymeric network that can then be grafted to the silica surface at more than one point. This is achieved by one of two pathways shown in Figure 1.13: 1) solution or “vertical” polymerization, and 2) self-assembled monolayer (SAM) or “horizontal” polymerization. Both reactions require a catalytic amount of water to initiate the polymerization but differ with respect to what order the water and the silane reagents are introduced into the reaction. Both methods yield a much higher surface ligand density compared to monomeric phases, but a common problem with oligomer phases is restriction of the pore network and higher-than-optimal ligand density, particularly for SAM polymerization. Often, the silica surface is pre-treated with short-chain silane “spacers” to better control the density of the polymerized stationary phase product [72].

Bulk modification methods utilize organically-modified silicate monomers in the sol-gel process itself, thereby incorporating organic functionalities into the siloxane polymer support prior to surface modification. An example of a common bulk modification is sol-gel polycondensation of a mixture of alkoxysilanes (ex.: TEOS) and alkyl-substituted alkoxy silanes (ex: triethoxymethylsilane). Such “hybrid” silica supports exhibit dramatically improved stability and have been demonstrated to be reliable in high temperature superheated water.
chromatography (SWC) applications at temperatures up to 200°C [68,70,73]. Another interesting type of bulk modification is polysiloxane immobilized ligand systems, in which the modified functional group of choice (ex.: C18) is present in the gelling siloxane (ex.: n-octadecyltriethoxysilane), thus the stationary phase product is made in the sol-gel process without the need for surface modification [5,69]. Common concerns with bulk modified stationary phases include batch-to-batch reproducibility and structural rigidity.

Figure 1.13: Mechanisms of polymeric surface modification reactions. A C8 modification is shown as example.

Bulk modification and polymeric surface derivatization methods for producing organically-modified silica-based stationary phases drastically reduce silanol interactions and improve stability, but they have yet proved to completely eliminate all deleterious silanol effects. There are dozens of new and interesting syntheses methods to improve bonded phases and reduce residual silanols that are outlined in recent publications [4,6,35,38,74-76].
Of course, the most obvious way to eliminate the problems caused by silanols is to avoid the use of silica altogether as a stationary phase support. Several other supports have been investigated, and each has its advantages and disadvantages. For example, polymer substrates are inexpensive, easy to manufacture to tailored specificity, and provide a hydrophobic surface without the need for derivatization, however they are typically not as rugged as silica and tend to shrink or swell depending on the pressure, temperature, and organic solvent environment [7].

A very promising material for stationary phase supports is zirconia (ZrO$_2$), a transition metal oxide that provides mechanical ruggedness and surface area comparable to silica but with remarkably improved stability, especially at temperatures as high as 200ºC. The only setback of zirconia-based bonded phases, aside from their higher cost, is that the surface chemistry is more complicated than that of silica, and further understanding of its surface features is required before broader implementation. Zirconia contains bare Zr ions that act as strong Lewis acid sites that may irreversibly bind analytes. Zirconia bonded phases usually require continuous conditioning with mobile phase additives like phosphates and other oxyanions to permanently shield these sites from binding with Lewis base analytes [8,9,77].

1.2.4 Effects of Metal Impurities and Pre-capping

Residual silanols on the silica bonded-phase surface primarily contribute two negative consequences to the quality of the chromatographic stationary phases: 1) silanophilic interactions with certain analytes resulting in poor efficiency, and 2) decreased hydrolytic stability at high pH and temperature. Furthermore, the presence of metal cations in the silica matrix exacerbates these effects by increasing the acidity of proximal silanols. Silanols are weakly acidic, with most having pKa’s ranging from approximately 3-7, however a small concentration of silanols with pKa’s as low as 1-2 have been shown to exist on the silica surface [9,59,78]. These highly acidic silanols are dubbed “hot spots” since they are more reactive and prone to ionization.

While the nature of the amorphous silica surface is quite complex, with various silanol species interacting with each other on a highly curved surface, the source of silanol hot-spots is mostly attributed to the presence of metal ion impurities, most notably Fe and Al, but also Zn, Cr, Cu and Zr. These metal impurities can be leached from the surface by acid-washing the silica in a strong inorganic acid solution (0.1M to 1.0M of HCl, H$_2$SO$_4$, or HNO$_3$) [79]. However, metal cations residing deeper within the silica matrix cannot be removed. As a result, they will
inductively draw electron density away from the surface, causing the silanol species in the vicinity of the metal impurity to be more acidic and thus have an increased activity toward analyte interaction and hydrolytic dissolution of the silica substrate [80].

Variation in silanol reactivity also has effects on the silanization reaction used to synthesize bonded phases. In 1983, Lochmüller et al. used fluorescent pyrene ligands to probe bonded silane distributions on the silica surface and identified clusters of the bonded phase at varying density on the derivatized silica surface [81]. It was suspected that the energetic heterogeneity of the surface silanols led to a less evenly-distributed ligand arrangement, with the bonded phase clustering in higher density around the highly reactive silanol hot-spots.

In the years shortly following, D. B. Marshall and Lochmüller showed that pretreatment of the bare silica with small amounts of trimethylsilane (TMS) reagents prior to C18 derivatization resulted in stationary phases with improved chromatographic properties [82,83]. Trimethylsilanes were chosen due to their small size, thus minimizing steric hindrance of neighboring silanol sites for further C18 attachment. They were able to conclude that TMS was selectively neutralizing the most reactive silanols first, leaving a more energetically homogenous surface that then caused the subsequent C18 ligands to bind in a more random, evenly distributed fashion. As a result, the C18 stationary phase products improved analyte efficiency by a factor of 2 to 3 upon “pre-capping” with TMS at approximately 5% to 7.5%, as shown in Table 1.1 [82].

Table 1.1: Table V from Marshall, Cole, & Connolly 1986 showing analyte efficiency upon TMS “initial deactivation” pre-capping. (Adapted from Table V in Marshall, et al. 1986) [82].

<table>
<thead>
<tr>
<th>Solute</th>
<th>Initial deactivation (%)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Phenol</td>
<td>1855</td>
</tr>
<tr>
<td>Aniline</td>
<td>1530</td>
</tr>
<tr>
<td>3-P-1-P</td>
<td>1880</td>
</tr>
<tr>
<td>Benzene</td>
<td>2400</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>2370</td>
</tr>
<tr>
<td>Anthracene</td>
<td>2305</td>
</tr>
</tbody>
</table>

* Values of \( m_2 \) were too close to \( m_3 \) for sodium nitrate: spuriously high or negative values of \( N_{adj} \) were obtained.
Pre-capping also has effects on polymeric phase synthesis [72,84], although the mechanism and purpose to do so are slightly different than for monomeric phase synthesis. For polymeric phases, the effects of precapping have less to do with the initial deactivation mechanism and more to do with spacing and density arrangement of the polymerized ligands. The addition of TMS and other somewhat bulkier ligands (e.g. isopropylidimethylsilane) acts as a stop-gap on the silica surface to limit the extent of polymerization, thereby allowing a means to control the phase density of the bonded phase product. Increased amounts of pre-treatment lead to a less-dense polymeric phase [72]. Pre-capping in polymeric phase applications have been performed on both Type-A and Type-B silica, but until now pre-capping monomeric phases has only been investigated on Type-A silica.

1.2.5 HPLC Packed Columns

In order for the stationary phase to be used in a liquid chromatography instrument, it must first be contained in a space in such a way as to allow mobile phase to permeate it without being dispersed or otherwise physically disturbed. In HPLC, the stationary phase is usually tightly packed into a stainless steel column equipped with specially designed end-fittings containing plumbing hook-ups and porous frits to contain the packed material while allowing mobile phase flow through the length of the column. The interior walls of HPLC columns are chrome plated and high-mirrored polished to prevent channeling of the mobile phase around the packed stationary phase bed. The most commonly used method to pack stationary phases into HPLC columns is *slurry packing*. This involves suspending the particulate phase material in a solvent, then pushing this slurry into the column with liquids at very high pressures (5000 – 20000 psi).

There have been a number of scientific investigations into proper methods of slurry packing HPLC columns for maximum performance [85-88]. However, the fact remains that packing one’s own columns requires a developed technique, a fine touch, and a sense of timing. Hence, there is an “art” to packing good HPLC columns. There are several parameters that can be adjusted to optimize packing of a certain stationary phase material, the most important of which are the choice of slurry and pushing solvents. There is no universal choice of solvents, as different solvents will provide the best packing for different materials [88], so a lot of trial-and-error is usually required to find the best solvent in every case. This combined with the fact that...
packing quality severely impacts the efficiency and overall performance of the column [89,90], methods that commercial HPLC manufacturers use to pack their column products are considered highly-secret proprietary information.

The total porosity (εₜ) of a packed stationary phase column, defined as the fraction of free space in the column available to be filled with mobile phase, can be measured chromatographically and calculated by Equation 1.17,

\[
εₜ = \frac{Ft₀}{AL}
\]

where F is the volumetric flow rate (mL/min), t₀ is the void time of an unretained solute marker (min), A is the column cross-sectional area (cm²), and L is the length of the column (cm). A typical packed column will have a total porosity of ~0.50 to 0.65, in which most of that porosity (~0.40) is interstitial space between the packed particles, while the remaining porosity (~0.10-0.25) is intrapartical porosity [91].

1.3 Thesis Statement of Purpose

The research presented in this dissertation will focus on two related aspects of the silanization reaction used to synthesize monomeric bonded stationary phases. The goal of the first project is to determine the effect of TMS pre-capping on the measurable chromatographic properties of C18 monomeric phases made from Type-B silica. To date, all investigations into the effects of pre-capping on silica-based monomeric bonded phases have been performed on Type-A silica, the older variety of silica containing higher levels of metal impurities. It has since been argued that the presence of silanol hot-spots and their effects on bonded ligand density heterogeneity is a direct result of the presence of metal contaminates [59,78-80,92], and because Type-B materials lack these sites to any considerable degree, any pre-capping effects on Type-B substrates should be minimal. However, no experimental data regarding pre-capping Type-B silica phases has yet been reported, but it warrants investigating because it potentially offers a
simple and economical way to improve the chromatographic properties of current silica-based bonded phases.

In this first study, three different Type-B silica supports were pre-treated with varying amounts of trimethylchlorosilane (TMCS) prior to exhaustive C18 modification. Stationary phase products were then slurry-packed into standard HPLC columns and the efficiency and thermodynamic properties of the bonded phases were measured. Also, the chemical stability of the phases was tested by forced degradation at elevated pH and temperature.

The second project is an investigation into how the presence of organic-soluble salt and pressure in the reaction medium affect the ligand bonding density in the synthesis of monomeric C18 stationary phases. This study involves adding quaternary ammonium salts into the dichloromethane solvent for C18 derivatization on porous silica substrates and analyzing the SP products for %C. Various organic-soluble salts and silica substrates were studied, and time-point samples of the reaction slurry were obtained to gain information as to the reaction kinetics. Quite surprisingly, there has been no evidence published in the chromatographic literature pertaining to the influence of ionic species in the synthesis reaction and how these factors affect the bonded phase products. Ionic strength is a consideration in most silanization reactions as most reactions use halosilane reagents. Halogens are better leaving groups and make for more complete derivatization, and halosilanes reagents produce ionic species as product (see Fig. 1.8b). Therefore, studying the effect of ions in the reaction will potentially lead to insights into improving the synthesis process. Also, the effect of elevated pressure was briefly investigated using a Teflon-lined high-pressure reaction bomb under several atmospheres of pressure.
CHAPTER TWO

EXPERIMENTAL

2.1 Materials

Four Type-B chromatographic-grade porous silica substrates were obtained from two different manufacturers: Symmetry® 5 μm and Spherisorb® 10 μm from Waters Corp. (Milford MA, USA), and AstroSil® 14 μm and 7.3 μm from Stellar Phases Inc. (Yardley PA, USA). Specifications for these silica products as supplied by the manufacturers’ Certificates of Analysis are listed in Table 2.1. All %C data presented is corrected by subtracting the %C of the bare silica reported in Table 2.1.

Table 2.1: Physical parameters and metal impurity content of the native silica materials used.

<table>
<thead>
<tr>
<th></th>
<th>Stellar AstroSil</th>
<th>Stellar AstroSil</th>
<th>Waters Symmetry</th>
<th>Waters Spherisorb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particle diameter (μm)</td>
<td>14</td>
<td>7.3</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Particle size distribution (dp90 / dp10)</td>
<td>1.6</td>
<td>&lt;1.5</td>
<td>1.49</td>
<td>NA</td>
</tr>
<tr>
<td>Avg. Surface area (m² / g)</td>
<td>325</td>
<td>350</td>
<td>341</td>
<td>150</td>
</tr>
<tr>
<td>Avg. Pore diameter (Å)</td>
<td>106</td>
<td>95</td>
<td>92</td>
<td>300</td>
</tr>
<tr>
<td>Avg. Pore volume (mL/g)</td>
<td>0.9</td>
<td>0.8</td>
<td>0.88</td>
<td>NA</td>
</tr>
<tr>
<td>%C&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.6</td>
<td>0.4</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Metal Impurity Content (ppm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na</td>
<td>12</td>
<td>18</td>
<td>&lt; 1</td>
<td>NA</td>
</tr>
<tr>
<td>Mg</td>
<td>&lt; 9</td>
<td>&lt;9</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Ca</td>
<td>14</td>
<td>&lt;9</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Al</td>
<td>11</td>
<td>15</td>
<td>&lt; 1</td>
<td>NA</td>
</tr>
<tr>
<td>Fe</td>
<td>&lt; 9</td>
<td>&lt;9</td>
<td>1</td>
<td>NA</td>
</tr>
</tbody>
</table>

<sup>a</sup>The %C was measured in this study and not provided on the vendors’ certificates of analysis.
Trimethylchlorosilane (TMCS) and n-octadecyldimethylchlorosilane 70% v/v in toluene (ODCS) silanizing reagents were purchased from Gelest Inc. (Tullytown PA, USA). Anhydrous pyridine and dichloromethane (DCM) reagents were purchased from Sigma-Aldrich Corp. (St. Louis MO, USA). Quaternary ammonium salts tetrabutylammonium bromide (TBABr), tetrabutylammonium chloride (TBACl), and tetraethylammonium bromide (TEABr) were purchased from Sigma-Aldrich, while tetrabutylammonium hexafluorophosphosphate salt (TBAPF₆) was purchased from TCI America (Portland OR, USA). HPLC grade acetonitrile (ACN) and methanol (MeOH) were obtained from Mallinckrodt Baker (Phillipsburg, NJ, USA). Deionized (DI) water was purified to a resistance of approximately 18 MΩ/cm using a NANOPure II water purification system from Barnstead (Debuque IA, USA). Analyte solutes were obtained from various commercial sources.

2.2 Effects of Trimethylsilane Pre-capping on Type-B Monomeric C18 Phases

2.2.1 Stationary Phase Synthesis and Column Packing

The three silica substrates used in this study were the Symmetry® 5 μm and the AstroSil® 14 μm and 7.3 μm materials. The native silica was acid-washed in 0.1M HNO₃ at 80ºC for 4 hours, thoroughly rinsed with DI water until the filtrate was pH neutral, dried under vacuum at 150ºC for at least 96 hrs., then stored in a dessicator. Great care was taken to avoid water and to ensure quantitative TMCS pre-capping in synthesizing the stationary phases. All synthesis glassware was presilanized with a 5% solution of TMCS in anhydrous DCM, dried at 150ºC under vacuum, then assembled hot onto a Schlenk gas/vacuum reaction manifold under dry N₂ as shown in Figure 2.1. In order to minimize error in batch-to-batch synthesis reproducibility, all phases were made in a single-pot fashion, with reagents added sequentially onto the substrate without intermediate isolation.

Approximately 5 g of silica was accurately weighed, loaded into a 250 mL flask equipped with a side-arm and septum, then dried on the manifold at 150ºC under vacuum for at least 40 hours. Approximately 50 mL of DCM was cannelated onto the dry silica and begun stirring and refluxing at 34ºC. TMCS pre-capping was conducted between 0% and 10% silanol equivalents.
calculated using the exact mass of dry silica (g), the manufacturer-specified BET surface area (m²/g), and assuming 8.0 μmol/m² surface silanol concentration, resulting in 100% silanol equivalents of 2.7 mmol/g for the Symmetry 5 μm material, and 2.6 mmol/g and 2.8 mmol/g for the AstroSil 14 μm and 7.3 μm materials, respectively.

![Reaction set-up for performing silanization reactions.](image)

Figure 2.1: Reaction set-up for performing silanization reactions.

For the TMS pre-capping step, a 1:50 v/v TMCS:DCM (anhyd.) solution was freshly prepared and the appropriate amount was introduced dropwise to the stirring/refluxing reaction slurry via gas-tight syringe, followed immediately by an excess amount ( >120% silanol equivalents) of anhydrous pyridine. This was allowed to react for 6 hours prior to exhaustive C18 silanization with ODCS for 18 hours. Most stationary phases were subsequently endcapped with an excess amount of TMCS for 24 hrs. A 2.5% “dilution phase” (2.5%dln) was also synthesized in which the C18 and TMS reagents were added simultaneously at a ratio of 97.5 : 2.5 ODCS:TMCS and reacted for 24 hours, then endcapped with excess TMCS, in order to observe the effect of alkyl ligand dilution previously investigated by Marshall [82].
Derivatized silica products were filtered and washed with ample volumes of DCM, methanol, 50:50 water:methanol, and diethyl ether, then dried under vacuum at 125°C for 6 hours. The resulting monomeric C18 stationary phase products were slurry-packed into standard 4.6 x 150mm stainless steel HPLC columns (SciCon or equivalent) at approximately 6000 psi using a Haskel DSTV-122 air-driven pressure amplifier (Burbank CA, USA) with isopropanol (IPA) used as both the slurry and pushing solvents.

2.2.2 Liquid Chromatography Instrumentation

The van Deemter analysis was performed on an optimized HPLC system designed to minimize extra-column volume and consisted of an LC-10AT VP pump (Shimadzu Manufacturing Inc. Canby OR, USA), a Waters 486 Tunable Wavelength Detector (Milford MA, USA), and a Valco manual 6-port injection valve (Houston TX, USA) with a 10 μL injection loop. van Deemter chromatograms were collected and processed using TotalChrom 6.2.1 software. All other chromatographic experiments were performed on an automated Shimadzu system consisting of an LC-10ADvp pump and DGU-14A mobile phase degasser, SPD-10Avp UV/Vis detector, SIL-10A auto-injector, and a SCL-10Avp system controller. Chromatograms were collected and processed with CLASS-VP v.5.03 software. All analytes were injected at 5.0 μg amounts under isocratic elution conditions at 1.0 mL/min with uracil as a void marker, unless otherwise noted. The UV detection was at 254 nm with a sampling rate of 5 Hz or greater. Temperature-controlled experiments utilized an Isotemp Refrigerated Circulator Model 9100 (Fisher Scientific, Pittsburg, PA) and a column jacket. Data analysis and graphing were done in Microsoft Excel.

2.3 Effect of Organic-Soluble Salts in the Bonded Phase Synthesis Reaction

2.3.1. Stationary Phase Synthesis

The two HPLC-grade silica substrates used in this study were the AstroSil® 14 μm and the Spherisorb® 10 μm materials (see Table 2.1). A third non-HPLC grade 60Å angular silica
substrate (EMD Silica gel 60. 230-400 mesh ASTM. \(d_p = 40-63 \mu m\). \(S_{BET} = 480-540 \text{ m}^2/\text{g}\) was also investigated. The native silica was acid-washed and dried as previously described in section 2.2.1, and all glassware was similarly presilanized. Approximately 2 g of silica was accurately weighed, loaded into a 250 mL flask equipped with a side-arm and septum, then dried on the manifold at 150ºC under vacuum for at least 40 hours.

Four quaternary ammonium (QA\(^+\)) salts were investigated: three tetrabutylammonium (TBA\(^+\)) salts including TBABr, TBACL, and TBAPF\(_6\), and tetraethylammonium bromide (TEABr). Solutions in anhydrous DCM solvent were made at three levels of salt concentrations: 0 mM, 10 mM, and 50 mM. In order to ensure that the synthesis conditions and reagent concentrations were identical for each reaction, a simple recipe was followed that consisted of precisely 10 mL of DCM/salt solution per gram of silica, and 120% silanol equivalents of both the silane reagent and the pyridine base. These reagents were introduced dropwise via a graduated gas-tight syringe. It was noted that the addition of salt to DCM only raised the reflux temperature minimally, ranging from ~33.5ºC up to ~36ºC for pure DCM and the 50 mM TBABr solutions, respectively. Time-point samples were made by withdrawing 4 mL of the reaction slurry via a gas-tight syringe and a wide-gauge needle. The silica samples were then filtered and washed repeatedly on-filter with ample volumes (4 x 30mL) of DCM, methanol, 1:1 methanol:water, methanol, then diethyl ether. Finally, the silica samples were dried under vacuum at 150ºC for 2.5 hours then stored in a desiccator until submitted for percent carbon elemental analysis.

2.3.2. Flow Conductivity Measurements

Sample batches of stationary phase products made in Section 2.3 were tested for residual tetraalkylammonium salt by soaking 100 mg in 1.0 mL of both DCM and 80:20 MeOH:H\(_2\)O for 40-90 hours, filtered with 0.45 \(\mu m\) Nylon filters (Gelman Sciences. Pensacola FL, USA), and the leachate was analyzed for residual ions via flow conductivity measurements with a Waters Millipore® Model 430 Conductivity Detector and a Waters 501 HPLC pump (Milford MA, USA). Samples were injected using a Valco 6-port valve with a 20 \(\mu L\) sample loop into a carrier mobile phase at 1.0 mL/min flow rate. For DCM solvent samples, the carrier mobile phase was 100% isopropanol, while a 80:20 MeOH:H\(_2\)O carrier was used for the similar sample leachate. No ionic separation or suppression was performed. Conductivity signal was acquired at 25 Hz,
gain = 0.001, range = 50, and processed using TotalChrom 6.2.1 software. Also, TBABr sorption studies were conducted on both a bare AstroSil 14 silica substrate and a C18 bonded phase product. Approximately 100 mg of each material was soaked in 1.0 mL of 10 mM TBABr in DCM, both with and without 25 μL pyridine, for 48 hours, and the supernatant was filtered and analyzed for ion conductivity.

2.3.3. Capillary Measurements

Kimax-51® borosilicate open-tube capillaries (Kimble Products. Vineland NJ, USA) of two inner diameters (0.75 and 1.5 mm) were used to measure contact angles of DCM solvent at three concentrations (0, 10, and 50 mM) of TBABr. A pair of capillaries was C18 surface derivatized by soaking in DCM/ODS/pyridine (10 / 1.0 / 0.1 mL) overnight at room temperature. Standard-resolution (640 x 480 pixels) digital images were taken on a CCD camera equipped with a 3x magnifying objective. Capillary heights and contact angle measurements were performed using ImageJ 1.44p image processing software (NIH. USA. http://imagej.nih.gov/ij) as per Figure 2.2.

Figure 2.2: ImageJ® processing used to measure contact angles and capillary heights.
CHAPTER THREE
RESULTS AND DISCUSSION

3.1 Effects of Trimethylsiline Pre-capping on Type-B Monomeric C18 Phases

3.1.1 Stationary Phase and Packed Column Characteristics

Table 2.1 provides information on the silica substrates used in this study, and Table 3.1 provides information on the C18 stationary phases synthesized from them. Note that the Symmetry silica contains considerably less metal impurities than the AstroSil silicas. The batch of AstroSil 14 μm material was available in ample quantity (>500 g), but the AstroSil 7.3 μm and the Symmetry 5 μm batches were both limited (20 – 30 g). Therefore, more experimentation of the AstroSil 14 substrate was possible, and this material was employed in the early stages of the study to develop column packing procedures and chromatography methods. In total, one batch of the 0% pre-capped (non-precapped) and two batches each of the 2.5%, 5%, 7.5%, and 10% TMS pre-capped phases were made on the AstroSil 14 silica, and multiple columns (1-3) of each batch were successfully packed. Data from these multiple columns were used to gauge the reproducibility of the column manufacturing process.

Table 3.1 shows the carbon loading of the synthesized C18 phases and the total porosity ($\varepsilon_T$) of each column packed (Eq. 1.17). Each phase showed a decrease in carbon loading with increased pre-capping, which is a consequence of the diminished number of silanol sites available for the subsequent C18 addition. Note that the 2.5% pre-capped phase showed the lowest discrepancy in the %C measured for the two batches synthesized at ± 0 %C, while the 10% phase showed the greatest discrepancy at ± 0.5 %C. This suggests that increased pre-capping may introduce greater batch-to-batch variation in carbon loading, but overall the effect is minimal, especially at low levels of TMS pretreatment. The 2.5% “dilution phase” (2.5%dln) was made from AstroSil 14 μm material in a “co-capping” fashion where the TMS and C18 silanes were added concurrently, not sequentially, in order to determine what effects are due to pre-treatment and which can be attributed to a simple dilution of the alkyl ligand density.
Table 3.1: List of all C18 stationary phases synthesized, their pre-capping levels, presence or absence of end-capping, measured carbon loading as determined by elemental analysis, and total porosity of all columns packed.

a Corrected %C = measured %C of phase product – measured %C of bare material listed in Table 2.1. Four repeated measurements of a separate standard C18 phase gave a standard deviation of ±0.3 for the %C elemental analysis error. 

b Average and range of %C for two separate synthesis batches. Each batch was within ± 0.5 %C of the reported mean, indicating a low batch-to-batch variability of the synthesis process.

<table>
<thead>
<tr>
<th></th>
<th>Amount of Pre-capping (%TMS)</th>
<th>End-capped (Y/N)</th>
<th>%C (corrected)</th>
<th>Total Porosity eT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AstroSil</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14μm</td>
<td>0%</td>
<td>Y</td>
<td>19.2</td>
<td>0.52, 0.54, 0.56</td>
</tr>
<tr>
<td></td>
<td>2.5%</td>
<td>N</td>
<td>17.5</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td>5.0%</td>
<td>Y</td>
<td>17.2 ± 0.0b</td>
<td>0.51, 0.51, 0.54</td>
</tr>
<tr>
<td></td>
<td>7.5%</td>
<td>Y</td>
<td>16.2 ± 0.3b</td>
<td>0.51, 0.52, 0.51</td>
</tr>
<tr>
<td></td>
<td>10.0%</td>
<td>Y</td>
<td>16.2 ± 0.5b</td>
<td>0.51, 0.51</td>
</tr>
<tr>
<td></td>
<td>2.5%dIn</td>
<td>Y</td>
<td>16.9</td>
<td>0.53</td>
</tr>
<tr>
<td><strong>AstroSil</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.3μm</td>
<td>0%</td>
<td>Y</td>
<td>16.8</td>
<td>0.48</td>
</tr>
<tr>
<td></td>
<td>2.5%</td>
<td>N</td>
<td>15.8</td>
<td>0.52</td>
</tr>
<tr>
<td><strong>Symmetry</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5μm</td>
<td>0%</td>
<td>Y</td>
<td>16.5</td>
<td>0.53</td>
</tr>
<tr>
<td></td>
<td>2.5%</td>
<td>Y</td>
<td>14.7</td>
<td>0.54</td>
</tr>
<tr>
<td></td>
<td>10.0%</td>
<td>Y</td>
<td>12.6</td>
<td>0.55</td>
</tr>
</tbody>
</table>

To ensure adequate column packing quality of the stationary phases, the columns were screened by running an isocratic method to measure the chromatographic efficiency of various aromatic compounds. All efficiencies were calculated using the Foley-Dorsey equation [23] as shown in Equation 1.7. The measured efficiency for non-polar analytes on all endcapped columns is shown in Figure 3.1. Here again, the results reported for the AstroSil 14 phases are the average of several columns packed with different SP batches at each pre-capping level, while the Symmetry 5 and AstroSil 7.3 show one column from one batch. The method involved isocratic elution with 40:60 H2O:ACN at 1.00 mL/min and ambient temperature, 5 μL injection.
of 1 mg/mL toluene and ethylbenzene, 0.6 mg/mL naphthalene, and 0.04 mg/mL anthracene in 1:1 H₂O:ACN diluent. For the Symmetry 5 and the AstroSil 7.3 phases, error bars show the standard deviation of 5 replicate injections on one column. For the AstroSil 14, error bars show standard deviation of 5 injections from multiple packed columns: 0% = 3 columns total from 1 batch of synthesized phase; 2.5%, 5% and 7.5% = 3 columns total from 2 batches; and 10% = 2 columns total from 2 batches.

Figure 3.1: Measured efficiency of ethylbenzene (■), toluene (▲), naphthalene (♦), and anthracene (●) vs. amount TMS pre-capping for all endcapped packed columns.
The efficiency variability for the multiple AstroSil 14 columns packed at each pre-capping level averaged ± 11% RSD, with a maximum of ± 15% RSD. The column-to-column reproducibility is the principle source of error in this study, thus any difference in efficiency less than ± 15% is considered to be within experimental error. TMS pre-capping offered little or no improvement in the efficiency for non-polar analytes on any phase. The efficiencies on the Symmetry 5 and AstroSil 7.3 phases varied < 10%, and peak asymmetry remained between 1.05 and 1.15. For the AstroSil 14 phase, no significant change was seen at the 2.5% pre-capped level. At 5% and 7.5%, there is an approximately 20% decrease in efficiency, with a slight increase in peak tailing but with asymmetry still <1.2 on average. However, the 10% pre-capped phase showed a drastic 60% decrease in efficiency and a sharp increase in tailing (>1.5). None of the other packed columns showed this trend. The decrease in efficiency upon increased pre-capping observed for the AstroSil 14 phases is most likely due to poor column packing [89]. It is believed that the IPA slurry packing method employed is less suitable for larger particle phases with lower carbon loading, and aggregation and/or sedimentation occurs more rapidly during the packing process producing a packed-bed with a higher degree of radial heterogeneity.
A statistical moments analysis (Equations 1.4 – 1.6) was performed on all columns to correct for variances in column packing. Adjusted plate numbers \( (N_{adj}) \) were calculated by subtracting the variance exhibited by the uracil void-marker \( (\sigma_{void}^2) \) from the analyte peak variance \( (\sigma_{anal}^2) \), giving \( N_{adj} = \frac{t_R^2}{(\sigma_{anal}^2 - \sigma_{void}^2)} \) \cite{82,93}. The variances in the uracil void peak are plotted in Figure 3.2. An increase in \( \sigma_{void}^2 \) indicates poorer packing and was only seen for the AstroSil 14 phases. The uracil peak variance changed little for the Symmetry 5 and AstroSil 7.3 phases and their \( N_{adj} \) values reflect those shown in Figure 2a. For the AstroSil 14 phases, the calculated \( N_{adj} \) showed a slight maximum at 2.5% pre-capping (6-8 % over non-precapped), and the ratio of \( \sigma_{anal}^2 / \sigma_{void}^2 \) was a minimum at this level as well. The \( N_{adj} \) values at the 10% precapped level were improved considerably, but overall the trend was the same as that calculated by the Equation 1.7 and shown in Figure 3.1. While void variance adjustment couldn’t correct for all of the decrease in efficiency observed for the AstroSil 14 phases at 5% pre-capping and higher, this would eventually be considered inconsequential as work began to focus on the 2.5% level as the optimal pre-capping amount to be studied.

### 3.1.2 Efficiency

A flow-rate dependent analysis using toluene as the probe analyte was conducted on the endcapped phases to gain more insight into the kinetics governing each phase’s efficiency. The results were modeled using the van Deemter equation (Eq. 1.10). The van Deemter analysis results are presented in Figure 3.3 and Table 3.2. Several trends can be seen in the van Deemter data. First, the A-term tends to increase while the B-term decreases with increased pre-capping for the AstroSil 14 phases; both are an indication of poorer packing quality. For practical evaluation, the A-term should be less than a factor of 2 larger than the particle diameter for a well packed column \cite{2}. Only the 10% precapped AstroSil 14 column failed this criterion.

More importantly, the calculated C-term for all three silica substrates was a minimum at the 2.5% pre-capped level, regardless of packing quality. As a result, the 2.5% pre-capped phases generally had the lowest minimum plate height \( (H_{min}) \) and the highest optimal linear flow velocity \( (\mu) \) on all three silica substrates. The van Deemter results confirmed what was observed repeatedly throughout the study, which is that the 2.5% phases generally showed an
improvement in efficiency and peak shape over the non-precapped phase for each silica substrate studied. This improvement was often negligible (< 10%) for the non-polar analytes but more significant for polar analytes.

Figure 3.3: van Deemter plots for toluene on Symmetry 5 (♦), AstroSil 7.3 (▲), and AstroSil 14 (■) C18 phases at various pre-capping levels. Isocratic elution with 40:60 H₂O:ACN from 0.05 mL/min to 2.50 mL/min at ambient temperature, with uracil as void marker. Error bars show the standard deviation of triplicate injections of 10 μL at 1.0 mg/mL of toluene in 1:1 H₂O:ACN
diluent. Smooth line represents the least-squares fit of the van Deemter model from Equation 3. All stationary phases were endcapped.

Table 3.2. Calculated van Deemter parameters for toluene. From Figure 3.3.

<table>
<thead>
<tr>
<th>Table 3.2</th>
<th>Calculated van Deemter parameters for toluene. From Figure 3.3.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Precapping Level (%TMS)</td>
</tr>
<tr>
<td>AstroSil 14μm</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>2.5%</td>
</tr>
<tr>
<td></td>
<td>5.0%</td>
</tr>
<tr>
<td></td>
<td>7.5%</td>
</tr>
<tr>
<td></td>
<td>10.0%</td>
</tr>
<tr>
<td></td>
<td>2.5%dln</td>
</tr>
<tr>
<td>AstroSil 7.3μm</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>2.5%</td>
</tr>
<tr>
<td>Symmetry 5μm</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>2.5%</td>
</tr>
<tr>
<td></td>
<td>10.0%</td>
</tr>
</tbody>
</table>

In practice, improved efficiency and peak shape are most relevant to drug compounds because their polar and often basic structures give rise to broad, tailing peaks [54,66]. Several pharmaceutical analytes were studied under buffered conditions using a 15 mM phosphate buffer at both low pH (2.75) and high pH (6.90). Also, non-endcapped phases at 0% and 2.5% pre-capping were synthesized on the AstroSil materials to compare the relative effects of TMS pre-capping and end-capping on peak shape and efficiency.

A total of ten drug compounds at two buffered pH conditions were screened on the AstroSil 14 and Symmetry 5 phases from 0% to 10% pre-capping, as shown in Figure 3.4. Ketoprofen, Lidocaine, and Procaine were analyzed at both pH conditions. Error bars show the standard deviation of triplicate injections. For the Symmetry 5 phases, all analytes at both pH conditions showed an insignificant (< 15%) improvement in peak efficiency upon TMS pre-capping, with the exception of quinine that showed a 20% improvement upon 10% pre-capping at pH 2.75. Conversely, all analytes on the AstroSil 14 phases showed a maximum efficiency at the 2.5% pre-capping level under both pH conditions, with the exception of Ketoprofen at pH 6.90. In summary, the AstroSil 14 material, which contains a higher level of metal impurities, showed
small but noticeable improvements in efficiency for some polar compounds, while the highly-
pure Symmetry material showed no significant improvement for almost all analytes studied.
Figure 3.4. Efficiency vs. TMS pre-capping for ten drug analytes on a) Symmetry 5, and b) AstroSil 14 C18 phases at pH 2.75 (solid line) and pH 6.90 (dashed line). Isocratic elution at 1.00 mL/min at the MP conditions listed (15 mM aqueous phosphate buffer : ACN), 1:1 H2O:ACN sample diluent, and ambient temperature. All phases endcapped.

Figure 3.5. Efficiency (upper plot, wide bars) and peak asymmetry at 10% peak height (lower plot, narrow bars) of eight drug analytes for a) AstroSil 14 and b) AstroSil 7.3 C18 phases. Stationary phases were both endcapped (solid) and non-endcapped (striped) at 0% pre-
capping (red) and 2.5% pre-capping (blue). The 2.5% dilution phase “2.5dln” is shown for AstroSil 14 (light blue). Same conditions as listed for Fig. 3.4. Error bars show the standard deviation of triplicate injections.

Figure 3.5 shows how the efficiency and peak shape vary with both pre-capping and end-capping for the two AstroSil materials. For the 14 μm phases (Fig. 3.5a), pre-capping at 2.5% showed an equivalent or improved peak shape (B/A closer to 1) for both the end-capped and non-endcapped phases, and improvements were seen in the efficiencies as well. In general, end-capping outperformed pre-capping alone, but the combination of 2.5% pre-capping and endcapping improved efficiency over end-capping alone by ≥ 25% for all analytes shown. However, some of these same effects were seen on the 2.5% dilution phase, indicating that gains in efficiency and peak shape upon pre-capping for many of these analytes can be attributed in part to a more dispersed C18 alkyl arrangement and not necessarily a result of the TMS-pretreatment’s neutralization of reactive silanols prior to C18 derivatization.

Many of the trends seen for the AstroSil 14 μm phases were less apparent on the 7.3 μm phases shown in Figure 3.5b. Improvements in peak shape were less noticeable on 7.3 phases, and the combination of pre-capping and endcapping only showed an insignificant (< 10%) improvement in efficiency versus endcapping alone. A potential reason for this can be seen in Tables 2.1 and 3.1. While both AstroSil phases are chemically similar in terms of their metal impurity profiles, the 7.3 μm material is more similar to the Symmetry 5 μm material physically in terms of particle diameter, surface area, and most importantly average pore diameter. Narrow pores restrict silane reagents from accessing the silica surface, resulting in a decrease in overall carbon loading for silica materials with a smaller average pore diameter. This effect is most significant with pore diameters less than 140 Å and with longer alkyl ligand reagents like octadecylsilanes [47,50].

The carbon loading data in Table 3.1 correlates well with the mean pore diameter data reported in Table 2.1 for this reason. Thus, if some of the improvements in peak shape and efficiency observed in the pre-capped phases can be attributed to a diminished C18 bonding density as evident by the 2.5%dln phase, then those phases with an already low bonding density should exhibit less improvement upon pre-capping, as evident by the Astrosil 7.3 and Symmetry 5 phases. It is now hypothesized that metal impurity content is not the sole indicator of whether
TMS pre-capping will improve efficiency on Type-B silica, but physical parameters such as mean pore diameter and how they affect bonded phase density should also be considered.

3.1.3 Retention

Pre-capping Type-B silica was shown to decrease carbon loading (Table 3.1), and this will undoubtedly affect the retention properties for the C18 phase products. Figure 3.6 shows the retention factors (k’) of ethylbenzene and toluene as a function of pre-capping under thermostatted conditions. Retention of these analytes was maintained upon 2.5% pre-capping, however a sharp decrease in k’ was observed at higher levels. Other non-polar compounds analyzed including benzene, naphthalene, and anthracene showed similar retention loss. This result is in stark contrast to the trend observed on Type-A silica, which typically showed an increase in carbon loading and retention up to ~5% pre-capping [82].
Figure 3.6: Retention factor vs. pre-capping of ethylbenzene (▲) and toluene (■) for a) AstroSil 7.3 (red), b) AstroSil 14 (blue), and c) Symmetry 5 (green) C18 phases. Isocratic elution with 40:60 H₂O:ACN at 1.00 mL/min at 25.0 ±0.05°C, 1:1 H₂O:ACN sample diluent, and uracil void marker. Error bars shows standard deviation of triplicate injections. All phases were endcapped.

To obtain retention thermodynamic data, a temperature-dependent van’t Hoff analysis was conducted and analyzed as per Equations 1.8 and 1.9. The results of the van’t Hoff analysis are shown in Table 3.3. In general, the retention enthalpy for phenol, toluene, and ethylbenzene decreased in correlation with the carbon loading reported in Table 3.1. Most of the enthalpic changes for the two AstroSil phases were within the calculated error, but the Symmetry 5 material showed a more significant decrease. An interesting trend was observed in the entropy data, which consistently showed an increase in entropic favorability of retention with increased pre-capping. This effect was larger for the AstroSil 14 phase than the other two phases, with the 2.5%dln phase showing an identical entropy value as the 2.5% pre-capped phase. However, the calculated difference in retention entropies were minimal and within error of the van’t Hoff linear regression.

Table 3.3: van’t Hoff analysis results for phenol, toluene, and ethylbenzene reported with standard error. Isocratic elution with 40:60 H₂O:ACN at 1.00 mL/min with uracil void marker. Temperature range from 15°C to 55°C (±0.05°C) at 10°C intervals, with triplicate injections at each temperature. All van’t Hoff plots were linear with R² ≥ 0.99. All phases were endcapped.

<table>
<thead>
<tr>
<th>% TMS Pre-capping</th>
<th>Phenol</th>
<th>Toluene</th>
<th>Ethylbenzene</th>
<th>Ethylbenzene / Toluene</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ΔH° (kJ/mol)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AstroSil 14μm</td>
<td>0%</td>
<td>-0.17 ± 0.10</td>
<td>-7.89 ± 0.18</td>
<td>-8.26 ± 0.23</td>
</tr>
<tr>
<td></td>
<td>5.0%</td>
<td>-0.28 ± 0.07</td>
<td>-7.82 ± 0.12</td>
<td>-8.17 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>7.5%</td>
<td>-0.19 ± 0.10</td>
<td>-7.79 ± 0.11</td>
<td>-8.03 ± 0.16</td>
</tr>
<tr>
<td></td>
<td>10.0%</td>
<td>-0.20 ± 0.08</td>
<td>-7.94 ± 0.11</td>
<td>-8.16 ± 0.17</td>
</tr>
<tr>
<td></td>
<td>2.5%dln</td>
<td>-0.15 ± 0.08</td>
<td>-7.87 ± 0.11</td>
<td>-8.10 ± 0.16</td>
</tr>
<tr>
<td>AstroSil 7.3μm</td>
<td>0%</td>
<td>-0.52 ± 0.05</td>
<td>-7.62 ± 0.18</td>
<td>-7.97 ± 0.21</td>
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<tr>
<td></td>
<td>2.5%</td>
<td>-0.39 ± 0.04</td>
<td>-7.48 ± 0.16</td>
<td>-7.80 ± 0.21</td>
</tr>
<tr>
<td>Symmetry 5μm</td>
<td>0%</td>
<td>-0.01 ± 0.14</td>
<td>-7.44 ± 0.11</td>
<td>-7.77 ± 0.16</td>
</tr>
<tr>
<td></td>
<td>2.5%</td>
<td>-0.49 ± 0.19</td>
<td>-7.05 ± 0.07</td>
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</tr>
<tr>
<td></td>
<td>10.0%</td>
<td>-0.40 ± 0.17</td>
<td>-7.10 ± 0.06</td>
<td>-7.37 ± 0.11</td>
</tr>
</tbody>
</table>
3.1.4 Stability

The limited temperature and pH range in which silica is chemically stable is often cited as a primary limitation of silica-based bonded phases. The rate of dissolution of the silica substrate under high pH and temperature conditions is directly related to the concentration of surface silanols accessible to hydrolysis by the solvent. In theory, if pre-capping yields a more evenly-distributed bonded layer, this should shield the silica substrate more effectively than a heterogeneous bunching-type arrangement.

The AstroSil 14 columns were subjected to forced degradation conditions with a 70% 0.20M carbonate buffer (pH 10) and 30% methanol solution at 40ºC. At regular intervals, the columns were flushed-out with 50:50 H₂O:ACN, and aniline, toluene, and ethylbenzene were injected. The ethylbenzene results of this stability study are shown in Figure 3.7. Retention decreased linearly (R² > 0.996), and the rate of retention loss was faster on phases with higher pre-capping. The efficiency for all phases was maintained until approximately 370-400 mL of degradation solution was flushed through, then it decreased drastically. Toluene and aniline exhibited identical trends in the loss of retention and efficiency upon degradation. These results indicate that pre-capping has a negative effect on the retention stability of silica bonded phases, most likely due to a decrease in carbon loading.
Figure 3.7: AstroSil 14 pre-capping stability results showing a) change in efficiency (▲), and b) change in retention (■) of ethylbenzene vs. volume of degradation solution [0.20M carbonate buffer pH 10 : methanol (70:30)]. C18 phases at 0% (red), 2.5% (green), 5% (blue), and 7.5% (orange) pre-capping levels. Isocratic elution with 1:1 H₂O:ACN at 1.00 mL/min at 40.0 ±0.05°C, 1:1 H₂O:ACN sample diluent, and uracil void marker. Error bars show the standard deviation of triplicate injections. All phases were endcapped.
3.2 Effect of Organic-Soluble Salts in the Bonded Phase Synthesis Reaction

3.2.1 C18 Silanization on Bare Silica with TBABr

Quaternary ammonium (QA⁺) salts are often used in multi-phase reactions as phase-transfer catalysts [94]. Most commonly used QA⁺ salts contain n-alkyl ammonium cations such as tetrabutylammonium (TBA⁺) and tetraethylammonium (TEA⁺) with halide (Cl⁻, Br⁻) or hexafluorophosphate (PF₆⁻) counter-anions. Some ammonium salts (e.g. CTAB) also exhibit surfactant properties. These salts are soluble in both organic and aqueous solvents and aid in the transport of ionic reactive species across immiscible solvent phase boundaries. QA⁺ salts have been used extensively in liquid-liquid dual phase reactions, however the use of such salts in mediating reactions at liquid-solid interfaces, particularly surface silanization reactions on porous silica substrates in SP synthesis applications, has yet to be studied.

![Graph](image)

Figure 3.8: Carbon loading of monomeric C18 phases on AstroSil 14 silica at various TBABr concentrations in DCM.
The first reaction investigated in this study involved monomeric octadecyldimethylchlorosilane (ODS, or C18) grafting onto bare LC-grade porous silica (Stellar AstroSil, 14 μm particle diameter, 10.6 nm avg. pore diameter) in dichloromethane (DCM) and pyridine at various TBABr concentrations (0, 10, and 50 mM). The reflux temperature of DCM changed less than 3°C from 0 mM (~33.5°C) to 50 mM (~36°C). The results shown in Figure 3.8 indicate that low concentrations (10 mM) of TBABr yield a modest improvement in the extent of bonded C18 onto the silica surface. Repeated trials of the 0 mM and 10 mM batches demonstrated good reproducibility. The increase in %C observed averaged 0.45 ± 0.22 and corresponds to a ~3% increase in bonding density (α) from 3.00±0.04 to 3.10±0.01 μmol/m². The ±0.22 error in %C was the highest observed in the repeated trials and is henceforth considered the limit of experimental error throughout the rest of the study.

At higher levels of salt (50 mM), the resulting phase showed a dramatic decrease in bonded carbon. This is most likely due to ionic suppression. As shown in Figure 3.9, silanization of halosilanes in the presence of a base like pyridine (pyr) produces ionic species as product. According to the Le Chatlier principle, increasing the ionic strength of the reaction medium will push the equilibrium left toward the reagent side, resulting in less bonding.

\[
Si - OH + X_n Si(R)_{4-n} + pyr \longrightarrow Si - O - Si(R)_3 + nHpyr^+ X^-
\]

Figure 3.9: Silanization reaction in presence of base.

In addition, a decrease in carbon loading at higher salt concentrations may also be due to oiling-out of the C18 reagent, or more likely due to dielectric shielding of the silica surface. At increasing concentrations, the salt may be forming a dielectric bi-layer, with a layer of TBA\(^+\) sorbing onto silanols followed by an anion layer of bromide, which acts to shield the surface from reacting with the chlorosilane reagent. Despite the various reasons that may explain why the presence of salt can inhibit silanization of C18 onto bare silica at high concentrations, a modest yet reproducible increase in carbon loading upon addition of 10 mM TBABr salt was evident.
3.2.2 Quantitation of Salt on Bare Silica and C18 Phases via Flow Conductivity

The difference in %C between the 0 mM and 10 mM TBABr-treated batches is attributed to an increase in C18 phase loading. However, it is conceivable that residual TBA\(^+\) contamination on the samples is skewing the %C results. For the average 0.45 %C difference between the 0 mM and 10 mM TBABr batches at 24 hours, this would require that the phase product retain 23 \(\mu\)moles of TBA\(^+\) per gram silica (approximately a quarter of the total TBA\(^+\) added) throughout the wash/dry cycles of the samples. Previous studies showed that adsorption of TBABr onto C18 silica phases increases with ionic strength [95] and decreases with organic modifier [96]. Given isotherm adsorption data under buffered aqueous conditions and assuming 300 m\(^2\)/g with 2.3 g packing material for the LiChrosorb C18 column used [95], the maximum TBA\(^+\) sorption at 10 mM predicted for the AstroSil material in this study would be approximately 70 \(\mu\)mol/g. While possible, TBA\(^+\) contamination in the final product is unlikely due to the high batch-to-batch reproducibility of the %C results indicating that these samples were thoroughly washed and dried. Each gram of product is washed with approximately half a liter total of wash solvent, the weakest of which is 1:1 MeOH:H\(_2\)O.

In order to ensure that the increase in %C observed is accurately attributed to increased C18 phase bonding and not to residual TBA\(^+\), the 24 hour product samples in Figure 3.8 were measured for residual ions by a flow conductivity experiment. The 0, 10, and 50 mM TBABr treated samples were prepared in duplicate by soaking 100 mg of the silica phase products in 5.0 mL of 80:20 MeOH:H\(_2\)O for 48 hours at room temperature in glass scintillation vials. The leachate was filtered with 0.45 \(\mu\)m Nylon filters and diluted 1:100, then injected in triplicate at 20 \(\mu\)L into an 80:20 MeOH:H\(_2\)O flow stream at 1.0 mL/min into a flow conductivity detector. No ion separation or suppression was performed. An example of the resulting ion response trace is shown in Figure 3.11. Also, a calibration of TBABr in MeOH:H\(_2\)O is shown in Figure 3.10. Results are listed in Table 3.4.
Figure 3.10: Calibration for TBABr conductivity in 80:20 MeOH:H₂O.

Figure 3.11: Conductivity responses of 80:20 MeOH:H₂O leachate for the 24 hour C18-derivatized silica phases treated with 0, 10, and 50 mM TBABr.
Table 3.4: Results of 80:20 MeOH:H₂O residual ion leachate quantitation of the 24 hour C18-derivatized phases treated with 0, 10, and 50 mM TBABr.

<table>
<thead>
<tr>
<th>Conductivity Area Response (μV*s)</th>
<th>Sample 1</th>
<th></th>
<th>Sample 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triplicate injections</td>
<td>0 mM</td>
<td>10 mM</td>
<td>50 mM</td>
</tr>
<tr>
<td>Avg</td>
<td>304871</td>
<td>349600</td>
<td>208868</td>
</tr>
<tr>
<td>std dev</td>
<td>9898</td>
<td>13699</td>
<td>3071</td>
</tr>
<tr>
<td>%RSD</td>
<td>3.27</td>
<td>3.96</td>
<td>1.47</td>
</tr>
</tbody>
</table>

The results show that, if the difference in the conductivity signal between the 0 and 10 mM TBABr treated batches is attributed solely to residual TBA⁺, this would only account for at most 0.17±0.07 %C, which can be considered to be within experimental error. However, it is unlikely that the residual TBA⁺ content in the 10 mM batch is even this high. The conductivity signal in the neat leachate was extremely high for all batches tested, hence the necessity for the 1:100 dilution, indicating that the MeOH:H₂O wash extracted an enormous amount of ions present in all phase products. Also, the 50 mM TBABr-treated batch consistently had the lowest conductivity signal, even less than the 0 mM batch. In fact, the conductivity response correlates better to the calculated bonding density, indicating that acid-catalyzed hydrolysis of the C18 siloxane may be the primary contributor to the ion signal observed in these washes. Also, pH variation of the unbuffered aqueous wash could affect the conductivity signal.
In the interest of obtaining confirmation, a second set of samples was analyzed by washing in a less astringent solvent. As before, 100 mg samples of the 24 hour products were soaked in 1.0 mL of DCM for 90 hours at room temperature. Only organic ions such as TBA$^+$ that are highly soluble in DCM should be observed, and thus a lower background and better specificity for the TBA$^+$ ion is achieved with this method. Samples were prepared in both glass and polyethylene scintillation vials to test for discrepancies between the type of sample vial used. The leachate was filtered and injected neat without dilution at 20 μL into an isopropanol carrier stream at 1.0 mL/min. The DCM calibration is shown in Figure 3.12.

![Figure 3.12: Calibration of TBABr conductivity in DCM.](image)

As a result, there was no detectable signal in the conductivity trace for any of the samples prepared, regardless of amount of salt treatment or sample container. The limit of detection for this method was ~0.1 mM. Therefore, if the extraction efficiency of TBA$^+$ ions from the C18-derivatized silica in DCM is ≥ 5%, it can be concluded that the increase in %C observed for the 10 mM TBABr-treated batches cannot be attributed to residual TBA$^+$ contamination.

Further investigations using the flow conductivity apparatus were performed to study the adsorption of TBABr onto the silica phases. 100 mg samples of bare (AstroSil 14) and C18 silica
(Fig. 3.8, 0 mM, 24 hrs.) were soaked in 1.0 mL of 10 mM TBABr/DCM for 90 hours, while another set was similarly prepared with the addition of 25 μL of pyridine to simulate reaction conditions. The supernatant fluid was filtered and injected, and conductivity responses were calculated using the calibration in Fig. 3.E. Neat samples that yielded conductivity responses beyond the calibration range were diluted 1:20. Area responses for samples containing pyridine were corrected by subtracting the area contribution of pyridine injected at those levels.

For the C18 bonded phase, the results of the TBABr sorption study yielded a 100.9 ± 6.5% and 103.4 ± 3.2% recovery of TBABr in the supernatant for the pyridine and non-pyridine containing samples, respectively. This demonstrates that TBABr is not strongly adsorbed onto a C18 modified silica phase. This can be assumed true for all time-point samples, as the surface coverage after just 1 hour is ~85% complete on average. However, the opposite case was observed for the bare silica, which showed no detectable signal for the presence of TBABr in either of the supernatant liquids. Accounting for the limit of detection, it can be stated that bare silica absorbed > 99% of the TBABr present in the DCM solution.

The results of the ion conductivity experiments provided three important pieces of information. First, there was no measurable amount of TBA⁺ present in any of the product washes that would significantly affect the %C measurements. Secondly, TBABr does not strongly adsorb onto and thus should be easily purged from a C18-derivatized phase with adequate washing. Therefore, it is concluded that the %C measured for salt-treated batches accurately quantify the bonded C18 phase loading and cannot be attributed to residual TBA⁺. Lastly, TBABr has a strong affinity for the bare silica surface, which potentially may give clues as to how it affects the silanization reaction.

### 3.2.3 Trimethylsilane Endcapping on a C18 Phase with TBABr

The presence of TBABr in the reaction solvent also improved the kinetics of trimethylsilane (TMS) modification on a C18 substrate in an end-capping reaction, a technique that is typically employed in monomeric stationary phase production to neutralize residual surface silanols that remain after the primary C18 modification. In this study, one batch of the 24 hour 0 mM TBABr-treated phase products from Figure 3.8 was reacted with TMCS in DCM/pyridine at various concentrations of TBABr for 24 hours.
The results in Figure 3.13 show that TBABr drastically improves the kinetics of the reaction, providing the same TMS loading after one hour at 10 mM TBABr as the non-TBABr treated batch produced after 24 hours. The difference between the 0 mM and 10 mM batch after 24 hours is 0.17 %C and within experimental error, and each batch resulted in an additional 9-11% silanol conversion with TMS. Once again, the 50 mM batch gave the lowest final %C amount but showed some evidence of faster kinetics. From this point on, experiments focused on the 10 mM level as the optimal salt concentration for further study.

![Graph](image)

Figure 3.13: Carbon loading resulting from TMCS end-capping onto a C18 substrate (AstroSil 14) at various TBABr concentrations in DCM. The C18 substrate had 17.78% carbon by mass.

### 3.2.4 Further Investigations: Various Salt Species and Pore Diameters

According to Equation 1.12, the only way to increase %C of a bonded phase is to either increase the density of the silane ligands ($\alpha$), or to increase the surface area (S) available for silane modification. There are three conceivable mechanisms by which these increases can occur as illustrated in Figure 3.14. For one, the salt may act to improve the wettability of the DCM...
solvent on the silica surface, minimizing the surface/solvent interfacial energy and increasing contact area of the reaction solvent by way of decreasing the contact angle ($\theta$) and increasing capillary pressure ($p_c$) into narrower pores (Fig. 3.14a). Also, the TBABr salt may be inducing higher bonding by improving the equilibrium thermodynamics of the reaction by ion substitution, lowering the free energy of the product state (by improved solvation) and/or raising the free energy of the reagent (Fig. 3.14b). A third possibility involves TBA$^+$ displacing silanol protons by way of a cation-exchange mechanism. TBA$^+$ is a larger and more labile cation than H$^+$ [97], and the formation of TBA-silanol adducts (Si-O$^-$TBA$^+$) after deprotonation of the silanols by the pyridine base could theoretically push the bonded C18 ligands further apart, affording subsequent C18 and TMS silanes a faster and less sterically-incumbered access route to silanol binding sights on the surface (Fig. 3.14c).

To study which of these mechanisms most affect the observed %C increase, several more reactions involving different silica substrates and different salts were conducted. These tests involved using QA$^+$ cations of different sizes (TEA$^+$ vs. TBA$^+$) with different counterions (Cl$^-$, Br$^-$, PF$_6^-$) on silica substrates of various pore diameters (60, 106, and 300 Å). It is expected that the chemical identity of the salt should not drastically affect the surface wettability properties of the DCM solvent so long as the salts are all singly charged species, but if capillary pressure is a cause then bonded C18 is expected to increase with decreasing average pore diameter. Also, if the thermodynamic favorability of the salt products formed by the reaction varies with counteranion, then the Cl$^-$ salts should show no improvement for a chlorosilane C18 reagent, and the Br$^-$ and PF$_6^-$ salts should show increased bonding regardless of the cation identity. Finally, if salt mediates steric hinderance of the bonded phase ligands at the silica surface, then a smaller QA$^+$ cation like tetraethylammonium (TEA$^+$) should show a smaller increase in %C upon addition of salt than TBA$^+$. An overview of the reaction parameter adjustments and how they would expect to affect bonding density upon addition of salt at 10 mM are shown in Table 3.5.
Figure 3.14: Proposed mechanisms of increased silane bonding and kinetics upon addition of TBABr. 

a) increased pore inclusion by improved wetting, 
b) increased $\Delta G$ by ion substitution, 
and c) improved steric arrangement of alkyl chains.

$Si-OH + XSi(R)_3 + pyr \rightarrow Si-O - Si(R)_3 + Hpyr^+ X^-$

Free Energy

Reaction Coordinate

$\Delta G_{Cl}$ $\Delta G_{Br}$

$Hpyr^+ Cl^-$ $Hpyr^+ Br^-$

$= H^+$

$= TBA^+$

$= C18$ chains
Table 3.5: Experimental matrix of expected results upon changes in three reaction variables. + = increase in %C expected for 10 mM vs. 0 mM salt; NE = no effect expected.

<table>
<thead>
<tr>
<th>Experimental parameter</th>
<th>Mechanism (A): Pore Inclusion</th>
<th>Mechanism (B): Product Counter-anion Thermo</th>
<th>Mechanism (C): C18 Sterics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pore Diameter (D)</td>
<td>+</td>
<td>NE</td>
<td>NE</td>
</tr>
<tr>
<td>(10 mM vs. 0 mM TBABr)</td>
<td>300 Å</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>80 Å</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salt Counter-anion (X)</td>
<td>Cl-</td>
<td>NE</td>
<td>+</td>
</tr>
<tr>
<td>(0 mM vs. 10 mM TBAX)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Br-, PFB-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salt Cation Size</td>
<td>TEA+</td>
<td>NE</td>
<td>NE</td>
</tr>
<tr>
<td>(0 mM vs. 10 mM TEABr and TBABr)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TBA+</td>
<td></td>
<td>+</td>
</tr>
</tbody>
</table>

Figure 3.15: Calculated bonding density vs. time for three silica substrates. EMD Silica 60 Å, AstroSil 14 106 Å, and Spherisorb 300 Å. Treated with 10 mM TBABr (pink squares) and with no TBABr (blue diamonds).
Results in Figure 3.15 show bonding density ($\alpha$) with respect to time for three silica substrates of varying average pore diameter without salt (0 mM TBABr) and with 10 mM TBABr. Figure 3.16 shows a similar $\alpha$ vs. time profile for the addition of various salt species at 10 mM concentrations on AstroSil 14 106Å silica. The bar graphs in Figures 3.17 and 3.18 highlight the change in bonding density as compared to no salt for the salt treated batches at 1 hour and 24 hours. From these figures, several inferences can be made to determine which of the three proposed mechanisms are most likely involved in the observed %C increase.

![Figure 3.16: Calculated bonding density vs. time for AstroSil 14 106Å silica with various salt treatments at 10 mM.](image-url)

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Figure 3.17: Effect of various salt species on bonding density at 10 mM as compared to no salt treatment (0 mM) after a reaction time of 1 hour and 24 hours. The maximum error observed for the AstroSil 14 106Å silica in this study is ±0.22 %C corresponding to $\alpha = 0.037 \, \mu\text{mol/m}^2$. Differences in bonding density less than this are considered to be within experimental error.

Figure 3.18: Effect of average pore diameter on bonding density upon addition of 10 mM TBABr as compared to no salt treatment (0 mM) after a reaction time of 1 hour and 24 hours.
With the exception of the EMD 60Å silica, the addition of salt to the DCM solvent improved the bonding density on all phase substrates at the end of the 24 hour reaction period. Also, all salt species with the exception of TEABr showed a significant increase in bonding density, including TBACl. Evaluating the data in relation to the presumed effects for each variable described in Table 3.5, it is now believed that Mechanism C (sterics) is the most likely route that produces the increase in bonding density. The larger TBA$^+$ cation regularly gave a significantly increased bonding density, whereas the TEA$^+$ did not, indicating that surface sterics are a potential reason. TBACl gave a significant increase, ruling-out the possibility that the effect can be solely attributed to improved thermodynamics by ion substitution. On the other hand the evidence suggests that the counter-anion may play a role, with Br$^-$ yielding the greatest improvement to phase loading after 24 hours.

Insight into how the addition of salt affects reaction kinetics can be surmised by looking at the 1 hour data in Figures 3.17 and 3.18. Here we see that addition of salt improves the bonding density after 1 hour, with the exception of TEABr and the wide-pore 300Å substrate. This lends supporting evidence that Mechanism C, which also predicts that kinetics would be improved with larger and more labile cationic species, is a valid interpretation. In addition, the TBAPF$_6$ salt showed the greatest increase in bonding density after 1 hour. One mechanism could be that chlorosilane reagent is undergoing dissociative loss of its chloride leaving group in solution and being replaced by PF$_6^-$, which is a better leaving group. Upon finding their way to the surface these hexafluorophosphatesilane reagents react faster with silanols due to decreased activation energy.

The end result of this investigation determined that organic soluble salts act to increase the kinetics and overall bonding density of C18 chlorosilanes in DCM primarily by mediating steric interactions at the surface, as well as potentially improving the thermodynamics by ion substitution of the reactants and/or products. Further discussion of Mechanism A (wetting) is addressed in the following section.

### 3.2.5 Effects of TBABr on Density, Interfacial Tension, and Capillary Pressure of DCM

It was suspected that the increase in bonding and kinetics observed upon addition of TBABr could be explained, in part, by improved wetting and capillary pressure effects. In short, salt is helping DCM wet the porous silica faster and more completely. Evidence in support of this
hypothesis came from observing contact angles at the DCM/silicate/air interface. Open-tube borosilicate capillaries of two different inner diameters (1.5mm and 0.75mm) were placed in TBABr/DCM solutions, and magnified digital photographs were taken to measure contact angles. In addition, a pair of capillaries was C18 surface-derivatized by soaking in an ODS/DCM/pyridine solution overnight at room temperature.

![Graph showing observed contact angles θ (°) and calculated cosθ values (scatter plot, right axis) of DCM solvent on bare (red) and C18-derivatized (blue) borosilicate glass capillaries at three TBABr concentrations.](image)

Figure 3.19: Observed contact angles “θ” (solid bars, left axis) and calculated cosθ values (scatter plot, right axis) of DCM solvent on bare (red) and C18-derivatized (blue) borosilicate glass capillaries at three TBABr concentrations.
The results displayed in Figure 3.19 show a decrease in the observed contact angle with increasing TBABr salt content on both the bare and derivatized capillaries. Lower contact angles were consistently observed for the derivatized surface and decreased less with TBABr as compared to the bare silicate surface. A smaller contact angle is beneficial as it indicates a lower surface energy and interfacial tension at the solid/liquid interface, the implication of which is improved reaction kinetics at the phase barrier due to increased phase-transfer diffusion and/or decreased activation energy. Also, a faster and more complete surface coverage by means of increased capillary pressure ($p_c$) into restricted porous regions in the silica substrate as dictated by the Young-Laplace equation (Eq. 1.13) is conceivable.

However, upon further investigation it appeared that this reasoning was only half correct. While a noticeable decreasing trend in contact angle was observed, surprisingly the capillary pressure of DCM actually varies non-linearly with TBABr, yielding a maximum near the 10 mM level. This was determined by measuring the fluid height levels in the 0.75 mm borosilicate capillaries (photographic image processing using ImageJ®), as well as measuring the density ($\rho$) of the DCM/TBABr solutions using a 2.0 mL Class-A TD volumetric glass pipette (Kimax. Kimble Products. Vineland NJ, USA) and an analytical balance (Fisher Scientific A-250). Typically, solvating salts tends to yield denser solutions, as studies with TBABr in acetone and alcohols have shown [98]. But surprisingly the addition of TBABr into DCM showed a decrease in the measured density as shown in Figure 3.20a. The density of DCM decreases upon solvating ionic liquids [99], but data regarding TBABr/DCM solutions has yet to be found in the literature.

Measuring the density of TBABr/DCM solutions proved difficult especially at low concentrations, giving high variability of the measured mass most likely due to TBABr sorption onto the glassware and the fast rate of DCM evaporation. In order to validate the method, the density of a 10 mM (0.0127 m) TBABr solution in acetone was measured, and Figure 3.20b shows that indeed the resulting solution had a higher density in good agreement with published results [98]. Given the capillary diameter ($D = 0.75$ mm), and measuring capillary height ($h$) and density ($\rho$) of the TBABr/DCM solutions, the capillary pressure ($p_c$) was calculated by $p_c = \rho gh$, and surface tension ($\gamma$) as calculated according to Eq. 1.13. Results are reported in Table 3.6.
Figure 3.20: Density measurements of TBABr solutions. a) Measured density of TBABr/DCM solutions. b) Method validation of measured TBABr/acetone solutions compared to literature reference values [98].

Table 3.6: Capillary pressure ($p_c$) and liquid-air surface tension ($\gamma$) calculated from measured solution densities, capillary heights ($h$) and contact angles on bare and C18-derivatized borosilicate capillaries (0.75 mm ID).

<table>
<thead>
<tr>
<th>[TBABr] in DCM</th>
<th>0 mM</th>
<th>10 mM</th>
<th>50 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solution density ($\rho$ kg/L) at 23.2°C</td>
<td>1.3223 ± 0.0007</td>
<td>1.3206 ± 0.0020</td>
<td>1.3167 ± 0.0010</td>
</tr>
<tr>
<td></td>
<td>Bare capillary</td>
<td>Derivatized capillary</td>
<td>Bare capillary</td>
</tr>
<tr>
<td>$h$ (mm)</td>
<td>10.11 ± 0.11</td>
<td>10.47 ± 0.16</td>
<td>10.31 ± 0.14</td>
</tr>
<tr>
<td>$p_c$ (mmHg)</td>
<td>131.0 ± 1.4</td>
<td>135.7 ± 2.0</td>
<td>133.5 ± 1.8</td>
</tr>
<tr>
<td>$\gamma$ (dyn/cm)</td>
<td>30.9 ± 1.3</td>
<td>29.3 ± 0.9</td>
<td>29.4 ± 1.1</td>
</tr>
</tbody>
</table>

This method of measuring density, contact angle, and capillary height to calculate $p_c$ and $\gamma$ yielded results with good precision ($\leq 4\%$ RSD). Also, the calculated surface tension at each concentration showed good agreement between the bare and derivatized capillary experiments and accurately reflect the value reported in the reference literature (pure DCM $\gamma = 27.2$ dyne/cm at 25°C) [100]. The contact angle measurements on the borosilicate capillary surface gave the
highest error. Measurements of contact angle require a flat and highly smooth surface to get an accurate value, whereas the capillaries used have a rough, curved surface. Furthermore, evaporation caused the fluid level to drop slowly, so measurements reflect a receding contact angle, not a static contact angle. All of these effects result in smaller contact angles [101,102]. Regardless, the measurements were done consistently and on the same capillaries, and ultimately the error is minimal and the observed trends in capillary pressure and surface tension are reliable.

Generally speaking, the surface tension of DCM decreased with increased amount of TBABr, but with a high degree of uncertainty. In addition, the changes in solid-liquid interfacial tension ($\Delta \gamma_{SL}$) calculated by Eq. 1.15 for the TBABr/DCM solutions as compared to pure DCM were all less than or equal to zero for both the bare and derivatized capillaries, but the error was greater than any calculated difference. However, this provides some evidence that a decrease in solid-liquid interfacial tension could explain the improvements in bonded phase density and kinetics seen with the addition of salt. More reliable measurements of these physical quantities should provide a more confident conclusion.

Only the capillary heights measured for the narrower 0.75 mm capillaries were used to calculate capillary pressure due to their consistently lower error and lower bond number. For pure DCM ($\gamma = 27.2$ dyne/cm, $\rho = 1.33$ g/mL), the calculated bond numbers ($B_0$ in Eq. 1.16) for the 0.75 and 1.5 mm I.D. capillaries were 0.067 and 0.27, respectively. Regardless, the DCM solutions regularly showed a maximum capillary height at 10 mM TBABr for both the bare and derivatized capillaries and both capillary diameters. This, along with a decrease in density, results in a maximum calculated capillary pressure at the 10 mM TBABr level.

Overall, the difference in $p_c$ is quite small, approximately a 2% increase of only a few Pascals. However, recall that this capillary pressure was observed on a macroscopic scale using capillary diameters (D) on the order of about a millimeter. Because $p_c$ is inversely proportional to D (Eq. 1.13), in porous silica phases where the average pore diameter is on the order of 100 Å (a decrease in D by 5 orders of magnitude), a difference in capillary pressure of just 1 Pa at 1 mm diameter can be magnified to approximately one atmosphere in porous silica! The results of the pore diameter study (Figs. 3.15 and 3.18) failed to show the inverse correlation between average pore diameter and bonded phase loading expected from an increase in capillary pressure at 24 hours, but did show a substantial increase after 1 hour. This can be attributed to faster wetting of the solvent by increased capillary pressure but not necessarily a more complete wetting.
3.2.6 Effect of Pressure in Stationary Phase Synthesis

The results of the capillary study became a catalyst to briefly investigate the effect of pressure in the reaction vessel. A simple question was posed – if capillary pressure effects in the synthesis process are considerable, then would a considerable increase in the reaction vessel pressure result in a considerable increase in bonded phase? To answer this question, a 100 mL high-pressure Teflon-lined stainless steel reaction bomb was used to run the derivatization reaction under elevated pressure. To the author’s knowledge, this is the first time a silica silanization RPLC bonded stationary phase synthesis has been attempted in a high-pressure reaction bomb. Approximately 1 g of AstroSil 14 silica was dried under vacuum in the Teflon-lined bomb at 150°C in the vacuum oven for at least 40 hours. The oven was purged with N₂ and the bomb was quickly capped and the inlet was sealed with a septum. The bomb was allowed to cool to room temperature under slow N₂ purge, then the DCM and reagents were added as per the recipe reported in section 2.3.1. The reaction was allowed to stir for 24 hours at room temperature. No reflux was performed, no salt was added, and no time point samples were taken. The bomb reaction was done once at high pressure (~6.5 atm) and once at low pressure (~1 atm) in the same vessel. In addition to washing the product on-filter as described in 2.3.1, additional washing of the 6.5 atm batch was done once with ~70 mL DCM and once with ~70 mL MeOH by stirring in the reaction bomb at ~7 atm pressure for about 1 hour to ensure that the penetration of wash solvent was adequate for removing all residual reagents.

Table 3.7: Effect of elevated pressure on bonding density for AstroSil 14 silica in a Teflon-lined high-pressure reaction bomb.

<table>
<thead>
<tr>
<th>Pressure</th>
<th>%C</th>
<th>a  (μmol / m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 atm</td>
<td>8.80</td>
<td>1.27</td>
</tr>
<tr>
<td>6.5 atm</td>
<td>10.42</td>
<td>1.54</td>
</tr>
</tbody>
</table>
The results of the high-pressure reaction are shown in Table 3.7. It is readily apparent that increasing the reaction pressure produced a large increase in carbon loading and bonding density after 24 hours. This result must be approached with caution as the %C loading and the bonding density are only about 40% of that routinely achieved for the same reaction under reflux conditions in a glass Shlenk manifold set-up. Thus, it’s hard to judge whether an increase is truly achieved when the bonding is less than complete. However, it does show that under similar non-refluxing conditions pressure does indeed have a considerable effect. A more thorough study using a pre-silanized glass-lined bomb under reflux conditions at various pressures for silica substrates of various pore diameters should yield more conclusive results.

In summary, TBABr affected the physical properties of the DCM solvent, showing decreased density and contact angle with a silicate surface. These effects resulted in an optimal capillary pressure at or near 10 mM concentration. These results could explain, in part, the slightly improved kinetics and bonding seen in the silanization reaction. More reliable measurements of contact angle on fused silica and surface tension of TBABr/DCM solutions are needed to confirm this hypothesis. Also, elevated pressure provided increased bonded phase loading, although yielding less-than complete coverage under the reaction conditions used.
CHAPTER FOUR

CONCLUSION

4.1 Effects of Trimethysilane Pre-capping on Type-B Monomeric C18 Phases

TMS pre-capping is still a viable method to improve analyte efficiency for Type-B monomeric C18 phases, however the effects on these phases are minimal. Analyte efficiency and peak shape improvements on Type-B phases were less in magnitude and occurred at a lower level of pre-capping as compared to those reported for Type-A silica. This supports the hypothesis that highly-acidic silanols - their abundance, variation in reactivity, and their effects on bonded ligand heterogeneity - are primarily caused by metal contamination. In addition to impurity content, efficiency improvements may be dependant on the physical parameters of the silica substrate and how they affect bonded phase density.

Pre-capping at 2.5% gave a lower C-term for toluene compared to non-precapped phases, but improvements in efficiency were more significant for polar analytes under buffered conditions. However, due to the small difference in observed efficiencies in relation to the wide column-to-column variability, at present any improvements can only be described qualitatively. Also, pre-capped phases showed decreased carbon loading and less retention of non-polar analytes, as well as a slightly faster rate of retention loss under dissolution conditions. Therefore, it is concluded that pre-capping Type-B silica phases is best done at lower levels (~2.5%) in order to maximize efficiency while minimizing loss of retention and stability. Future work will focus on silica phases of various pore sizes, alkyl ligand lengths, bonding densities, and lower pre-capping levels (< 2.5%).

4.2 Effect of Organic-Soluble Salts in the Bonded Phase Synthesis Reaction

The effect of adding quaternary ammonium ionic species into the silanization reaction for monomeric C18 bonded phases has been demonstrated. There is a slight increase in bonding
density at approximately 10 mM, but a sharp decrease at 50 mM was observed. The optimization at low salt concentrations was interpreted to be a result of competing effects, where the presence of salt at high concentration acts to suppress reagent ionization and/or shield the surface from reacting with the chlorinated silane reagent, but at low levels the presence of salt gave modest improvements to both the reaction kinetics and the overall bonding density.

Increases in bonding density observed at 10 mM are dependent on the type of salt used, with larger cations and bromide anions achieving the greatest effect. This improvement is likely due to bulky cations sorbing onto the silica surface and inducing a more spread-out C18 arrangement that improves steric and kinetic interactions at the liquid/solid interface. This proposed mechanism of improved alkyl ligand sterics can be further tested with larger cationic salts and silane reagents of various alkyl chain lengths. If correct, this mechanism predicts that longer alkyl chains would show a greater increase in bonding density upon addition of larger salts than smaller cations and shorter chains, as well as faster kinetics.

It was shown that addition of salt to DCM lowers the surface energy at the DCM/silica surface as demonstrated by measuring contact angles on borosilicate glass capillaries. One of the more unexpected results from this work was the decrease in DCM density upon addition of TBABr, as well as the calculated optimization maximum of the capillary pressure at 10 mM TBABr concentration. However, the results from the ad-hoc capillary study are demonstrative and lack the precision necessary to confidently conclude the effects of TBABr on the physical properties of DCM and how it affects solvent interaction with the silica surface. More reliable measurements of DCM/TBABr surface tension and contact angle are required.

Several more experiments should follow from this work. First and foremost, organic-soluble salt effects in other common silanization solvents (toluene, xylene, hexane, DMF, DMSO, etc.) should be studied. While most solvents such as acetone and n-alcohols show an increase in density upon addition of TBABr [98], the results from this experiment say that DCM is an exception. Thus, effects of salt addition on surface tension and capillary pressure may be drastically different for other solvents. Investigating different solvents can potentially answer whether the proposed pore inclusion mechanism (Mechanism A) is valid. Also, performing a synthesis on a non-porous substrate may provide more information to this end.

In addition, the use of salts in polymeric phase synthesis should be studied. The solution polymerization mechanism requires solution-state oligomers to attach to the surface, which
represents a large steric barrier that could be used to study the validity of the improved sterics mechanism. In addition, the SAM mechanism requires diffusion of the functional silanes across the phase boundary from the organic solvent to the absorbed water monolayer, and improvements in contact angle and surface wettability with the addition of salt may be more pronounced on a hydrated silica substrate as compared to a dry one, thereby exhibiting a greater improvement in surface energetics resulting in a faster and possibly more complete SAM modification. However, one important consideration is that polymerization is catalyzed by ionic hydrolysis intermediates (H⁺, OH⁻), which in the presence of TBABr could diffuse away from the surface faster. Therefore these salts can act as a means to limit the final bonded SAM phase density similar to pre-capping [72].

This work provides a wealth of new and interesting information suggesting that tetraalkylammonium salt in a DCM solvent can improve phase bonding in monomeric silanization reactions, however there are few major considerations worth addressing. First and foremost, the highest improvement in bonding density seen was only 0.1 μmol/m² on average. This is not expected to have much effect on the chromatographic properties of these phases unless this improvement represents a selective derivitization of reactive isolated silanols similar to the TMS pre-capping effect, in which case a ²⁹Si NMR or IR experiment could provide some answers. Also, because the measured %C is small and within the level of carbonaceous salt added to the reaction, it is important to ensure that these reagents are thoroughly purged from the phase product to ensure accurate bonding density determinations. As long as the increased carbon amount of the stationary phase is less than the carbon added to the reaction, caution should always be taken in interpreting higher carbon values as increased phase loading.

Ultimately, the most dramatic and unquestionable finding from this study was the lower C18 bonding at 50 mM TBABr, which was taken to mean that high ionic strength is a hinderance in the phase synthesis. In general, the build-up of ionic species in a typical monomeric halosilane derivatization can be considerable. For example, the ratio of DCM solvent to reagent used in this study will produce pyridinium chloride as high as 100 mM upon completion of ~3.0 μmol/m² bonding after 24 hours. Using polyfunctional silanes in polymeric phase synthesis can result in an ionic strength 2-4 times higher. Ultimately the build-up of ionic species at these high levels will potentially inhibit further bonding by shielding the silica surface or suppressing dissociation of reactive species. An interesting mode to test ionic strength effects is to use alkoxy silane
reagents, which form neutral alcohol as by-product. Addition of salt to this mechanism should not be affected by Le Chatlier’s principle so it can provide a means to study the effects of surface shielding and ionization suppression of reagents and intermediates.

It is commonly accepted that bonding density is limited by steric interactions of the grafted ligands on the silica surface. An interesting question to pose is whether most phase syntheses are truly operating at the steric limit, or perhaps most reactions are limited by ionic strength. It has been shown that isolating a bonded phase product and then repeating the silanization reaction has a significant effect on the bonding density [103,104], indicating that the phase density at the end of the first derivatization was not steric limited. Perhaps this further derivatization isn’t due to the introduction of fresh silane reagent but rather purging ionic products from the reaction. It has also been observed that phase loading tends to be higher in solvents with higher dielectric constants [39] and Lewis acid/base character [105], which could be due in part by these solvents’ ability to better solvate ionic products.

If these speculations are accurate and we can improve bonding density by mediating ionic effects, designing a synthesis scheme in which ionic products can be successfully removed from or neutralized in the reaction solvent may result in increased bonding. Simply precipitating salt out of the reagent solution by introduction of a non-soluble counterion would be unwise, as this would undoubtedly cause the solid to crash-out in the porous silica substrate, effectively halting the surface reaction and possibly fracturing the porous silica. Instead, using an ionic scavenger like crown ethers or introducing electrodes to electrochemically neutralize the charged species may work better.
REFERENCES


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BIOGRAPHICAL SKETCH

Michael Bair was born on April 15th, 1981 in Williamsport, PA to David and Gail Bair. He graduated from Williamsport Area High School in May of 1999. In the fall of 1999 he began undergraduate studies at the Pennsylvania State University in University Park, PA. Along with his undergraduate coursework, Michael researched Mass Spectrometric methods for biological applications. After graduating with a B.S. in Chemistry in 2003, Michael was employed for three years by the Bristol-Myers Squibb Pharmaceutical Research Institute in the Analytical Research and Development division in New Brunswick, NJ. In the fall of 2006, he enrolled in graduate school at the Florida State University Department of Chemistry and Biochemistry, and began his research in Separations Science and Technology under the advisement of Dr. John G. Dorsey in the spring of 2007.