Packed Capillary Columns For Liquid Chromatography

by

William Henry Wilson

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Approved:

H.M. McNair, Chairman

L.T. Taylor

M. Anderson

J.P. Wightman

H.G. Gibson

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Committee Chairman: Harold M. McNair

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(ABSTRACT)

The advantages and disadvantages of packed capillary columns for high performance liquid chromatography are examined. Historically, the advantages are smaller sample and phase consumption, enhancement in sensitivity, easy column synthesis, higher obtainable efficiency, and easier interfacing to other techniques. These points are explored through experiments in microbore ion chromatography, packed fused silica columns, and capillary zone electrophoresis. These studies also address the disadvantages of microscale HPLC which are stringent instrument design, brittle or weak columns, poor column stability, and the lack of commercial instrumentation.

The results of these investigations indicate the following. First, the purported sensitivity enhancement is really attributable to solute focusing and not to column miniaturization. Second, column synthesis is still a

difficult procedure that requires experience. Third, higher efficiencies are realized, especially when the column diameter to particle diameter ratio is optimized. Fourth, interfacing to other techniques is simplified because of the lower volumetric flow rates. Finally, the only real disadvantages are stringent instrument design and brittle columns in some instances. This thesis offers means for circumventing these difficulties.

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Chapter 1

Introduction

Introduction

This dissertation examines microcolumn LC (<4 mm column internal diameter) and assesses its worth as an analytical technique. Presently, this methodology is limited to academic and developmental industrial laboratories. However, microcolumn LC has the potential to fulfill the needs of routine analysis as well. As in the conversion from packed to open tubular capillary gas chromatography, there is an inertia to resist change. The intent of this thesis is to indicate the possibilities of microcolumn LC and promote the applicability of the technique.

In order to establish a common terminology, the various column dimensions and optimal theoretical flow rates are given in Table 1. The optimal flow rate values are calculated assuming five micron diameter particles as the stationary phase, the same analyte (benzene), the same linear velocity (or time of analysis), and an optimal packing procedure (1).

The second entry to Table 1 serves as a reference point for liquid chromatography. The name "analytical" results from the comparison of 4 mm internal diameter (id) high pressure columns to both low and high pressure large diameter columns used for preparative chromatography. The

Table 1

HPLC Column Comparison

Column Type	ID (mm)	Optimal Flow (µl/min)*
Analytical	4-4.6	480-500
Microbore	1-2	30-120
Micropacked	0.10-0.53	0.31-7.5
Packed Microcapillary	0.040-0.080	0.020-0.050
Open Tubular	0.004-0.035	0.0017-0.010

*Assumes 5 µm particles

smaller column was for those instances where the bands were not to be collected, merely "analyzed". The internal diameter was chosen from a study by Kirkland in 1976 (2). This investigation showed similar efficiencies for columns with internal diameters from 4 to 9 mm. The lower end of the range was chosen as it demonstrated the best compromise of performance, convenience, and required stationary phase. Thus, 4 mm id columns serve as the industry standard.

By decreasing the column internal diameter, the volumetric flow drops as a function of column radii ratio to the second power. Therefore, a 1 mm id column requires 1/16 of the flow through an analytical scale column in order to maintain the same linear velocity. Other parameters, such as sample loadability and tolerable extracolumn volume, also vary by the same relationship. These factors are crucial to the chromatographic system since they affect the total resolving power. Note the flow rates required by the packed capillary and the smaller internal diameter columns. For these columns no waste reservoir is necessary since the mobile phase evaporates before liquid can accumulate.

The term "microbore" describes columns whose internal diameter range from 1 to 2 mm. Column materials such as stainless steel, glass, and polymeric plastics like polytetrafluoroethylene are the most common. Most chromatographic systems are capable of using microbore

columns with only minor adjustments. Packed capillary columns vary from 20 to 530 microns in internal diameter and consist of very dense chromatographic beds. In essence, they are true miniatures of the larger bore columns and as such, they generate significant backpressures and require high pressure packing techniques. Micropacked capillary columns are loosely packed and range from 40 to 80 microns in diameter. These columns are very efficient but require excessively long analysis times to achieve such results. Finally, a few diligent research groups have made open tubular LC a reality (66,67). Column internal diameters start at 4 microns and cease to function at 30 microns. As in the previous category, large plate numbers are possible but not easily obtained.

The last three microcolumn types have several aspects in common. First, they all require special equipment such as small volume injectors and modified detection systems. A standard HPLC system will not be able to exploit these columns to their fullest capabilities. Next, they all tend to use fused silica tubing as the column material. The advantages of a smoother, less active wall and easily obtained internal diameters make this a logical denominator. Primarily, these techniques are believed to offer the potential for high efficiency liquid chromatography.

Because resolution is the main goal of chromatography, the effects of miniaturization on each parameter should be examined. Chromatographic resolution, R_S, depends on three variables (3) and is given by {1}

$$R_S = (1/4)\ [k/(k+1)]\ [(\alpha-1)/\alpha]\ (N^{1/2}) \qquad \{1\}$$

where k = capacity factor
α = selectivity
N = number of theoretical plates

The capacity factor, k, is a thermodynamic function. It depends on the equilibrium partition coefficient and the ratio of mobile phase to stationary phase in the column (better known as the phase ratio, ß). Upon size reduction, the former is unchanged and the quantity of phases change proportionately so that the latter is essentially constant. Consequently, k is independent of column dimensions. Similarly, selectivity is also a thermodynamic property and is not a function of system size. The final term, efficiency, is a kinetic parameter and indicates solute transport efficacy.

In order to maintain congruence with standard theory, the concept of plate height (H) is introduced. If N represents the number of theoretical plates in the column, then the height of each plate is given by equation 2 (4).

$$H = L/N \qquad (2)$$

where H = plate height

L = column length

N = number of theoretical plates

Similar to N, H is a transport or flow dependent variable. Plots of H versus the flow velocity, typically called van Deemter plots, illustrate the variables that influence H. Presently, no form or variation of the van Deemter equation for HPLC shows a dependence of H on column dimensions. From a preliminary examination, N seems unaffected by miniaturization. However, a more rigorous treatment is required before conclusions can be drawn.

Why bother miniaturizing? As in most analytical methodologies, the process of reducing the size of the instrumentation leads to disadvantages and advantages. The disadvantages of microcolumn LC are stringent instrument design requirements, brittle or weak columns, poor column bed stability, and the lack of commercial instrumentation. The advantages of microcolumn LC over analytical scale LC are smaller sample and phase consumption, purported sensitivity improvement, easy column synthesis, higher obtainable efficiency, and easy interfacing to other techniques (5). Additional advantages are given but these five appear to be the most commonly cited. However, these

advantages have not been found to be universal. Apparently, some investigators find column synthesis routine while others never succeed in their efforts. This selective ability to manufacture columns has substantially slowed the wide scale investigation and application of microscale LC.

The objective of this dissertation is to examine the advantages of microscale LC and to determine the veracity and value of each point. To achieve this goal, the literature of microcolumn LC is reviewed and the theoretical and experimental findings are summarized. With these models developed, experiments are designed and performed and their findings discussed. Finally, the value and potential of this work is evaluated.

Chapter 2

Historical Review

Literature

The inception of microscale liquid chromatography is difficult to determine chronologically since its beginnings are part of the development of HPLC. The first HPLC system was commercially introduced in 1958 (6). From that point until the late 1970's, the growth of HPLC was based on both theoretical and empirical knowledge. Leading the theoretical advances were Giddings, Huber, Knox, and Guichon while the empirical investigations were led by Kirkland, Halasz, and Horvath. In order to appreciate this two pronged development, a quick review of the historical development will be useful.

In 1952, A.J.P. Martin proposed and demonstrated the workings of gas liquid chromatography and his plate theory. By 1956, gas chromatography had gained international popularity and led to the landmark work of van Deemter and his co-workers at the Shell Research laboratories in the Netherlands as well as the introduction of capillary columns by Marcel Golay at Perkin-Elmer. Van Deemter and his team used experimental data to derive the equation that bears his name (7). In its original form, the dependence of plate height on velocity is given by (3)

$$H = 2ld_p + 2gD_{gas}/u + (8/p^2)[k/(k+1)](d_c^2/D_s)u \quad \{3\}$$

where l = packing factor
d_p = particle diameter
g = tortuosity
D_{gas} = diffusion in the mobile phase
k = capacity factor
d_c = column diameter
D_s = diffusion in the stationary phase
u = linear velocity
p = pi

This equation shows a direct dependence of plate height on column internal diameter, suggesting that miniaturization is one key to high efficiency chromatography. The work of Golay only serves to reinforce this concept. His 1957 paper on open tubular gas chromatography demonstrated the same dependence of plate height on column diameter (8). Since the principles of gas chromatography appeared to be universal to all chromatographic techniques, the ramifications of these two studies were extended to the field of liquid chromatography.

In this time frame of 1961-1965, the separation theoreticians, namely J.C. Giddings and J.H. Knox, began to examine the effects of changing the mobile phase from a gas to a liquid. Van Deemter's as well as Golay's equation indicate the influence of diffusion on the separation

efficiency. Giddings reported that if diffusion were the only variable in converting from GC to LC, the optimal velocity for LC would be 10,000 fold less than the speed of an identical GC analysis (9). However, Giddings cites that the greatly reduced risk of explosion in LC would allow smaller particles and higher pressures to be used. Because H is a direct function of the particle diameter to the second power, small particles would allow much shorter column lengths to be used.

In addition, Giddings indicated that the van Deemter equation overlooked the interaction of the eddy diffusion and the mobile phase mass transfer terms (10). The Dutch equation assumed that these factors are independent and therefore additive. From a physical point of view, this does not seem reasonable. Mass transfer in the mobile phase not only occurs parallel with the column flow but in all directions to the same extent. The phenomena of eddy diffusion assumes that flow stream interchange is minimal. However, lateral diffusion minimizes flow inhomogeneities and the sum of these two effects is less than the van Deemter equation predicted, particularly at high flow rates (11). Similar to electronic circuits, the contributions of eddy diffusion and mobile phase mass transfer add in parallel not in series. The verification of this new

equation, denoted as the coupled equation, is what led Knox to his column diameter studies.

While Giddings' approach to enhancing LC performance involved altering only the particle size, Knox began to investigate the effects of column miniaturization. In a paper presented at the Third International Symposium in Advances in Gas Chromatography (Houston, 1965), Knox demonstrated evidence for the coupling of the eddy diffusion and resistance to mass transfer terms (12). To examine this phenomena, he used a standardized lot of glass beads and packed them into various diameter tubes. Solutions of potassium permanganate were injected onto the column and eluted with 10% aqueous potassium nitrate. Detection was achieved with a photo-voltaic system. The column internal diameter ranged from 3 to 6 mm.

The results from this work indicate several parameters for consideration. At high linear velocities in either GC or LC, the coupled equation is more accurate than the traditional van Deemter equation. However, Knox suggested a more rigorous, integral form of the coupled equation (see Appendix) and Giddings concurred (14). Furthermore, Knox concluded that the column diameter to particle diameter ratio (later to be named the Knox-Parcher ratio) had an effect on plate height. Knox was reluctant to place full confidence in this observation because he was unsure of the

particle size distribution effect on this phenomena. Finally, the benefits of turbulent flow were examined and deemed impractical.

Meanwhile, the empiricists kept abreast of the theoretical developments and applied the results to their own work. Stimulated by the theoretical studies, the first distinguishable microcolumn research was performed. Horvath, Preis, and Lipsky investigated the possibility of LC in the time frame of GC separations (13). After a brief review of the prevalent theories, these workers undertook the separation of nucleotides by microcolumn LC. Because of the extremely slow stationary phase mass transfer for large biomolecules, they employed surface derivatized solid particles for their support. An ion exchange moiety was then grafted onto the surface and the dried stationary phase was packed into 1 mm internal diameter stainless steel tubing. This microbore column "was chosen to lessen transcolumn effects detrimental to column performance". This quotation alludes to one of the five discrete sources of flow inhomogeneities proposed by Giddings (14). The transcolumn effect, perhaps the most dominant of these effects for microscale chromatography, refers to the difference in flow permeability between the center and near the wall of the column. This particular point is vital to some of the work presented in this thesis and will be developed in greater

detail in the Results and Discussion section. Thus, the first true microcolumn LC technique was used as a consequence of theory.

Another of the empiricists, J.J. Kirkland, examined the configurational parameters required for high speed liquid chromatography in 1969 (15). The trend of most research in the late 60's was to remove the stigma that HPLC was an intractably slow separation technique. Kirkland corroborated the work of Horvath et al. by using a controlled surface porosity stationary phase and he demonstrated the possibility of pesticide separations in 5 minutes. More importantly, the effect of column internal diameter was studied. While the theoreticians employed unretained species to simplify their mathematical models, Kirkland performed real separations and examined the kinetic behavior. He found that a reduction in column internal diameter did improve efficiency but there was a limit to the minimum usable diameter. A 2.1 mm id column outperformed a 3.2 mm id column packed with the same stationary phase. However, a 1.6 mm id column with the same phase gave consistently poorer columns than either of the larger columns. Kirkland acknowledged that extracolumn effects were the probable cause but he also claimed that extensive efforts to minimize these effects failed. In addition, he suggested that the actual packing structure or "wall effects" could have been the culprit.

From this study, Kirkland suggested that useful column id should range from 2 to 4 mm while Scott advised on closer to 4 mm (16). Kirkland's employer, Dupont, then used the results of this study to initiate commercial column preparation. This work coupled with Kirkland's study on porous particles in 1976 set the industry standard for column diameter (2). Additionally, Kirkland's co-worker, J.P. Wolf showed that large bore columns (>4 mm id) could lead to a four fold improvement in efficiency over analytical scale columns (17).

With the outlook for microcolumn HPLC becoming bleak, Knox, still in search of the true kinetic equation, followed up his work from 1965. In 1969 (18), Knox and Parcher not only determined the best form of the coupled plate height equation to date, but, discovered an intriguing, contradictory phenomenon to be discussed shortly. The experiments examined 150 cm columns of various internal diameters with several diameters of glass beads used as stationary phases. To simplify the mathematics of the plate equation, an unretained solute (acetone) was employed as the test probe. Plots of reduced plate height (h) versus reduced velocity (v) or versus the Knox-Parcher ratio (p = column id/particle diameter) were prepared from the numerous possible configurations. The column internal diameter ranged from 3.3 to 11.1 mm while the packing material predominantly

used was 0.48 mm in diameter. Although the columns constructed exceed the microcolumn designation, the impact of this work on microcolumn LC is critical.

Knox and Parcher drew five significant conclusions from this investigation. First, they verified the coupled equation proposed by Giddings (10). At high velocities, the eddy diffusion and resistance to mobile phase mass transfer terms do interact and add in a parallel fashion. Second, plots of log h versus log v were constant with Knox-Parcher ratios less than 6 and were higher but constant for ratios greater than 8. Thus, some physical, not chemical, interaction was affecting the performance of the column. Third, a hexagonal column packed with spherical beads could only show good efficiency if the hexagonal unit cell was maintained. Any deviation from a uniform, cross-sectional bed disrupted the homogeneous flow streams. Fourth, by introducing the sample in the core of an "infinite diameter" column, the plate height was lower at high velocities than in a smaller diameter column. Finally, the concept of wall and core packing structures and their effects on efficiency were examined.

The points of interest to the micro chromatographer are the second and the final conclusions. For most of the work, the column diameter was altered while the particle diameter was fixed. In essence, Knox and Parcher repeated Kirkland's

work. In this instance, however, decreasing column internal diameter improved performance. For Knox-Parcher ratios less than 6, a constant, smaller value of reduced plate height was obtained when compared to ratios greater than 8 (see Figure 1). Recall that if particle diameter is fixed, h is really H. By direct comparison the smaller internal diameter columns were more efficient than their larger counterparts. The final conclusion is linked to this point since it macroscopically explains this heretofore contradictory phenomena. The packing process will orient the particles as close to each other as possible in the center of the tube. Such packing density is not possible throughout the tube's cross-section since "the walls impose their structure on about four layers of bends in contact with the walls." (18).

The correlation of Knox and Parcher's work to microcolumn LC becomes apparent after some consideration. As column diameters decrease, the advantages afforded by this phenomena could be exploited. More efficient separations in the same analysis time could be obtained by optimizing the column diameter. Technological advances in LC packing material synthesis, however, lessened the impact of this work. Recall from the van Deemter equation that more plates are achievable with smaller particles. The synthesis of microparticulate (< 20 μm) silicas

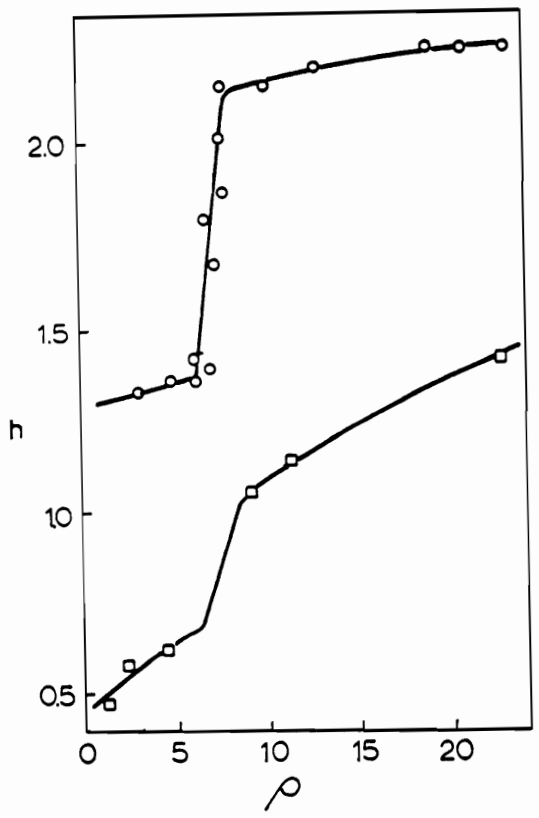


Figure 1. Knox-Parcher Plot from reference 18

Reduced Plate Height (h) vs.

Knox-Parcher Ratio (ρ)

revolutionized LC and diverted interest from the findings of Knox (19).

Advancements in microcolumn LC were minimal from 1967 to 1974. The reduction of particle diameter and its dramatic impact on column efficiency overshadowed the potential of microcolumn LC. The first investigations in miniaturized LC were presented by Daido Ishii in a JASCO technical report late in 1974 (20). This work was further developed and published in a series of articles starting in 1977 (21,22,23). Since Ishii's work is the forerunner of present microcolumn LC, this first paper warrants further examination.

Although standard column diameter LC systems were available, no commercial vendor had engineered a microscale chromatograph. Five specific problems addressed by Ishii and co-workers were packing techniques, micro-flow cell design, detector and pumping improvements, and gradient elution capabilities. In short, all aspects of the liquid chromatograph had to be reexamined.

Steel column packing techniques had been extensively examined by the mid 1970's (24,25). In general, the best columns were achieved with "balanced density" slurries of packing material and typically halogenated, high density solvents like carbon tetrachloride. The suspension was then placed in a reservoir which in turn was connected to a high

pressure pump on one end and the column blank on the other end. Instantaneous pressurization at 10,000 to 15,000 psi was applied for at least 30 minutes (see Figure 2). This procedure could produce columns with 70 to 90% of theoretical efficiency. For Ishii's first microcolumns (21), the tubing material consisted of easily deformed Teflon^{TM}; consequently, high pressure packing was not a feasible technique. To obtain the dense beds believed necessary, Ishii prepared a dense slurry of packing and sucked the slurry into the column blank with a gastight syringe. When 2 to 3 times the desired final column length had been filled, one end was sealed with glass wool and the inlet to the column was connected to a microfeeder syringe pump. Several compressions of the bed were performed until no further settling occurred. This syringe technique, while contrary to the popular conception that the higher the packing pressure the better the column, was and is capable of making columns with 50 to 80% of theoretical efficiency (see Figure 3).

The miniaturization of the column dictated that the extracolumn volumes introduced by the injector and detector systems be minimized. Ishii and his co-workers addressed the latter aspect in detail. In the introduction of this thesis, the tolerable total extracolumn volumes were cited. Ishii's

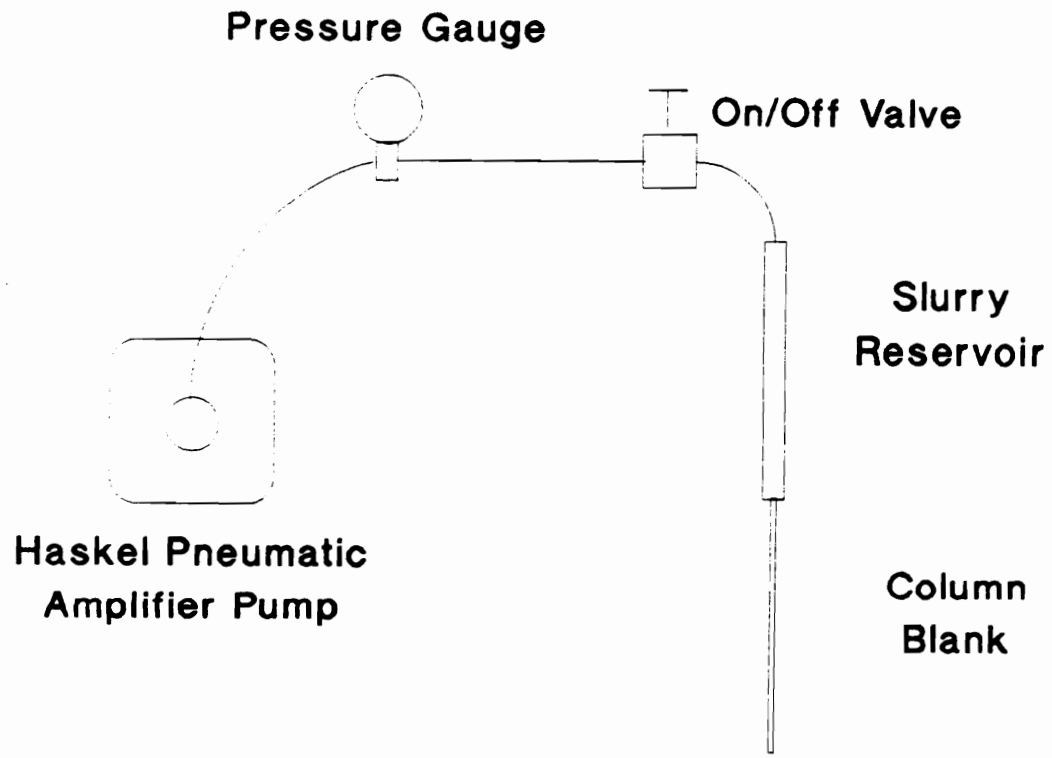


Figure 2. High Pressure Packing Apparatus

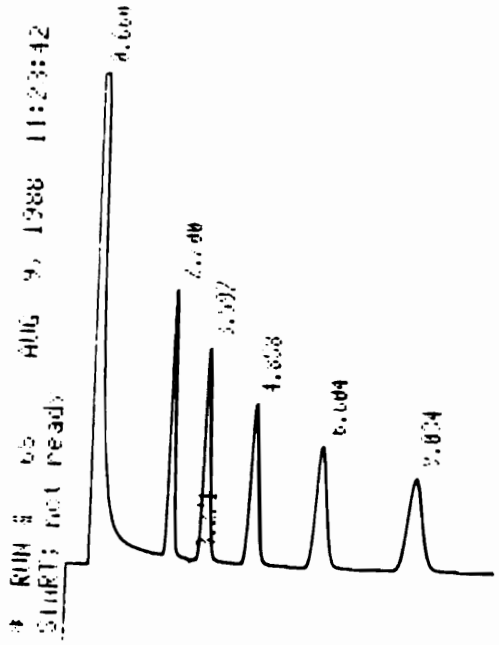


Figure 3. Supercritical Fluid Chromatography on a Syringe Packed Column, 100 X 0.53 mm, 5 μm ODS, Sample C12-C16 in C5, 100 C, 1200 psi, CO2 mobile phase, CCS restrictor (Ref 90)

columns were typically 0.5 to 1.0 mm id and could thus tolerate between 0.8 and 3.2 μl of dead volume, respectively. Spatially, the column terminus had to be in close proximity to the detector cell. His group solved this by connecting a piece of quartz tubing to the column outlet and using that as the flow cell. Ishii noted that this resulted in low sensitivity since the optical pathlength was only as long as the tube diameter (this particular problem still plagues separation scientists using "on column" detection in μLC and CZE). The detector optical bench made the micro cell easily accessible for minimum extracolumn volume and this design is still popular today. High intensity sources and better slit design also aided in simplifying the micro LC system.

The flow rate of mobile phase delivered to these microcolumns for best performance was far below that of any commercial pump (see Table 1 for optimum flow rates). Ishii and his group opted to modify their microfeeder with a smaller syringe and more divisions on the stepper motor assembly. This syringe pump could easily produce the 6 to 30 μl/min flow necessary for optimal performance. In addition, the small volumes of mobile phase consumed in a typical analysis allowed small syringes to be used.

Because of the small volumes pumped in a typical analysis, a new gradient forming system had to be devised.

The sophistication of proportioning valves and gradient mixers was still too primitive to accurately blend several microliters of mobile phase per minute. Ishii realized that for one microscale run, a few hundred microliters would suffice. Therefore, they constructed a two syringe gradient former where the syringes were 180 degrees out of phase. While one syringe was adding the strong mobile phase to a mixing vessel, the other syringe was withdrawing a continuous amount from the mixing vessel to a tube. The result is that the eluting strength of the mobile phase varied as a function of displacement in the collection tube. By connecting the tube to both the injector and the pump, gradient chromatography was possible. To adjust the rate of change in the mobile phase, a smaller or larger syringe was used for the first syringe.

With these initial problems solved, the ensuing publications dealt with applications of microscale LC. At this time, gel permeation stationary phase was quite costly and the reduction in packing material used presented a real advantage for microcolumn LC. Ishii and his group demonstrated similar results to larger bore columns but with tremendous savings in sample, solvent, and stationary phase consumption (22). The following paper examined the development of a "microprecolumn method" for ppb analysis of water contaminants (23). The water sample was pumped through

a precolumn which trapped most organic moieties. These analytes were then displaced in a sharp band by a strong solvent and chromatographed on a microcolumn. This phenomenon is referred to as solute focusing and makes trace analysis much easier.

While Ishii explored Teflon™ tubes for microcolumns, Scott and Kucera performed some preliminary work in 1 mm id stainless steel columns for size exclusion chromatography (26). The focus of the paper centered on using microporous silica as a stationary phase for size based separations. In an attempt to get very high efficiencies, ten one meter segments of 1 mm id stainless steel tubing were packed with 20 μm porous silica. The goal was achieved in that the column generated 250,000 theoretical plates, the maximum number possible for the given particle size and column length. Although this was quite an accomplishment, the authors failed to reiterate its potential value in the conclusion of the paper. Perhaps the authors initially failed to see the possibilities with microbore columns; however, this publication served as the basis for their investigations in early 1979.

Although Ishii's and, later, Scott's work made microcolumn LC a potential analytical technique, high performance LC in 4 mm id columns was firmly established. The turning point in the choice of small versus large

diameter columns was illustrated in a paper by Unger (27). With the help of one of the major HPLC suppliers (Merck), Unger investigated spherical and irregular silica from 1 to 10 microns in diameter. The performance of the microparticulate silicas in terms of efficiency, speed, and resolving power verified the predictions of the 1960's theoreticians (8,10). Columns of 3 μm material could generate 30,000 plates for a 20 cm long column. Additionally, the optimum volumetric flow for these small particles was faster than their larger counterparts. While smaller particles meant higher pressure requirements, it seemed like a reasonable price to pay for GC-like efficiencies. Unger found that particles smaller than 2 μm did not behave as expected and that efficiency became worse at predicted flow optimums. However, 3 and 4 μm particles showed excellent performance and suggested the possibility of high speed liquid chromatography. The consequences of these developments plus the relative ease of using large diameter columns served to suppress micro LC advances.

Nevertheless, the need for a higher resolution liquid based separation technique surfaced again and column miniaturization was reexamined. In this instance, though, the development of open tubular capillary columns for LC was fervently investigated. Since they had already devised microcolumn LC components, Ishii's group naturally began the

endeavor (28). The other research group was headed by Milos Novotny at Indiana University. Because Novotny is regarded today as one of the foremost authorities on microcolumn chromatography, it is interesting to note his first contribution to the technique (29). Novotny not only studied open tubular columns but tried a unique approach for increasing column loadability. Before drawing out the glass preform, silica particles were packed tightly inside. The process of drawing then caused not only tube miniaturization but the incorporation of silica particles into the tubing wall, thus increasing the surface area of the column.

The findings of both groups were quite similar. In order to attain theoretical efficiencies, column diameters from 10 to 80 μm had to be employed. Furthermore, injection and detection schemes required even more miniaturization. Concentration detection limits were easily three orders of magnitude worse than in packed column LC and run times were typically several hours. However, high efficiencies were possible. Theoretical plate numbers of >100,000 were demonstrated and complex mixtures could be resolved to a greater extent. In this instance, though, the difficulties outweighed the advantages and a new solution was needed.

In 1979, R.P.W. Scott reentered the picture and began to publish work on microbore (1 mm id) HPLC columns for complex sample analyses. As is the case with most new

methodologies, feasibility and engineering considerations served as the focus of the first paper (30). Scott had previously published articles on extracolumn effects (31) in LC and, consequently, was well aware of the problems with miniaturization. The authors first addressed the detection and injection systems, minimizing the contribution of these devices to extracolumn dispersion. Next, they modified the pumping system with an external function generator to allow independent drive motor control. In this way, flow rates as low as 1 μl/min were practical. Finally, column packing techniques were developed. A "balanced density" system was employed with 25,000 psi packing pressure delivered from a pneumatic amplifier pump.

With the construction considerations sufficiently optimized, Scott and Kucera then utilized the system to examine the effects of column internal diameter, sample loading, column coiling, particle diameter, and ultrahigh efficiency systems. The column diameter study yielded an optimum for 1 mm id columns with 20 μm particles; however, the authors did not indicate that this was a universal optimum. The capacity experiments demonstrated that columns from 0.010 to 0.050" could tolerate from 1 to 50 μg, respectively. Due to the long lengths of tubing necessary to achieve high efficiencies, the authors examined the effects of column coiling. Significant losses in efficiency due to

the "racetrack effect" (32) occurred when the coil diameter was less than 12 cm. The results of the particle diameter study showed that in terms of reduced parameters, 20 μm was better than 10 μm and 10 μm was better than 5 μm. Because of the long column lengths used and the difficulty in packing small (<20 μm) particles densely, these results were not surprising. The paper concluded with some ultrahigh efficiency applications. A 14 m X 1 mm column generated 650,000 plates when used in a size exclusion mode. Unfortunately, the last peak eluted nine hours after the injection. In addition, the theoretical limit for this column was 1,400,000 plates while the actual attained efficiency was only 46%.

Similar to Ishii's approach, the next several publications by R.P.W Scott involved the utilization of microbore LC. The first of these demonstrated the use of microbore LC for biological separations (33). The high complexity of most biological samples instigated the development of a small dwell volume, microbore gradient system that was modified, through firmware, to deliver sub milliliter per minute flow. By placing the pumps, the mixing "T", and the injection valve in close proximity to the column, the dwell time for the gradient changeover was minimized. To test the repeatability of the gradient former, five replicate injections of a complex mixture were made

with column rejuvenation cycles in between each analysis. Retention times exhibited less than 1.0% relative standard deviation while peak areas and heights typically varied by 5.0% RSD.

A second aspect of this paper examined mass sensitivity enhancements in microbore LC. The authors stated that in terms of concentration detection, microbore columns were no more sensitive than normal scale columns when all of the external volumes (i.e. injection, detection) were appropriately sized. For mass sensitivity, though, the microbore column was more sensitive. What they failed to say was that this was due to a smaller volume of sample being injected onto the system. This particular example was typical of some proponents of microscale LC. Experimental work in this thesis will demonstrate clearly the actual gains in sensitivity. Finally, the authors showed a focusing effect similar to Ishii (23) but for microbore LC. They were able to detect 1 ppb of acetophenone in water by concentrating 1 ml of sample. This effect was also used to analyze blood serum and compared an abnormal to a typical serum sample. The "fingerprint" of each was then examined to determine the compounds responsible for the abnormality.

Another paper by Scott and co-workers examined fast separations by microbore LC (34). Once again, the majority of the paper dealt with equipment development. The detector

required modification in order to decrease its time constant and allow the peaks to be "seen". Data acquisition also needed work to allow a real time plot of the analysis. An impressive separation of seven compounds in under 30 seconds was presented (Figure 4). For "on the fly" process stream analysis, such a system might have merit. The advantage of using microbore LC was that fast flows were possible with small solvent consumption rates. A summary of Scott's initial contributions appeared the following year in a review article (35).

While Scott and Ishii were busy working on steel and plastic microcolumns, a research group at Hewlett-Packard, led by Ray Dandeneau, developed flexible fused silica tubing (36). The impact of this material on gas chromatography revolutionized the industry. However, the availability of smooth, inert, small internal diameter tubing provided the microscale LC investigators an interesting possibility.

Two research groups initiated the activities in packed fused silica columns for microscale HPLC. As perhaps anticipated, Ishii and co-workers were one team while Frank Yang, then working for Varian Associates, was the other major investigator. In fact, Yang has the distinction of being the first scientist to publish on microscale LC in fused silica columns.

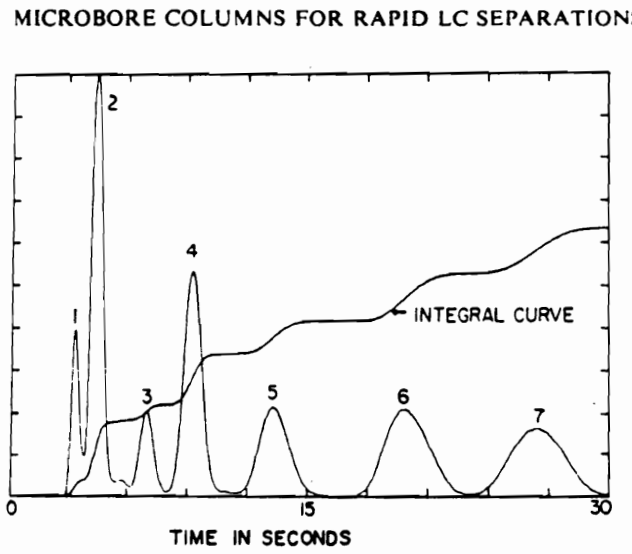


Figure 4. Fast Microbore HPLC (Ref 34)

This first paper by Yang examined fused silica tubing for open tubular LC (37). Columns of 100, 50, and 30 μm id with chemically bonded octadecylsiloxane stationary phase were successfully prepared. Injections were made by a split technique and mobile phase flow was delivered by a FAMILIC™ (Jasco Scientific Co., Osaka, Japan) syringe pump. Perhaps the greatest contribution of this publication was the detector design. Yang demonstrated that the fused silica tubing could serve as the flow cell and thus, extracolumn volume from the detection cell was essentially eliminated (Figure 5). In an ensuing paper, Yang coined the term "on-column" detection and showed its application to packed fused silica columns (38). The latter publication also examined mathematically the improvement in detection when using an on-column system. By stacking the typical values in favor of the on-column system, the author was able to show an increase in sensitivity of 20 fold over conventional systems.

The comparison of detection limits is indicated by a factor, f, which is given in equation 4.

$$f = S \times E = (S/N_{micro})/(S/N_{conv}) \times (EV_{conv}/EV_{micro}) \quad \{4\}$$

where S/N = signal to noise ratio for the microscale or conventional system

EV = the elution volume of the model compound

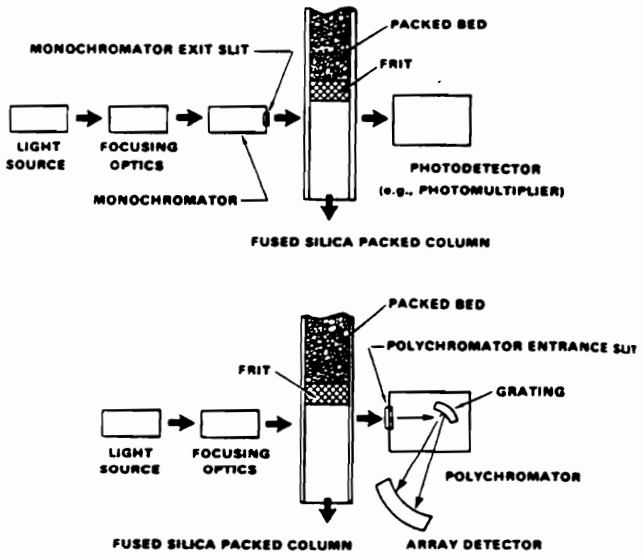


Figure 5. On-Column Detection Cell (Ref 38)

S is further defined as

$$S = (b_{micro}/b_{conv}) \times (n_{conv}/n_{micro}) = B \times N \quad (5)$$

where b = pathlength of the detection cell

n = noise measured for the detection system

For a 50 μm on-column cell and a 1 cm conventional cell,

$$B = 50\ \mu m/1\ cm = 1/200$$

Yang measures N as

$$N = 10^{-4}/1.5 \times 10^{-5} = 6.67$$

Therefore,

$$S = 1/200 \times 6.67 = 0.0330$$

E is arbitrarily chosen as 625

Thus,

$$f = 0.0330 \times 625 = 20.8$$

From the author's point of view, these assumptions are not realistic. The noise observed in most UV/Vis detectors is fairly consistent, regardless of whether it is used for on column or standard cells. Consequently, S should remain at 1/200. In addition, Yang assumes that the elution volume of a peak from a 150 X 2 mm column is 100 μl. From the capillary column results he employed, the capacity factor of the probe was 0.88. A 150 mm column packed with 5 μm particles has a dead time of approximately 1.2 min when operated at an optimum flow of 120 μl/min (1). If the column attains theoretical efficiency (15,000 plates), the volume of the peak is easily calculated.

$$N = (t_R/\sigma)^2 \qquad (6)$$

$$15{,}000 = (2.26/\sigma)^2 \; ; \; \sigma^2 = 0.018 \text{ min}$$

A typical chromatographic peak is contained in five standard deviations from the mean. Thus,

$$5\sigma = 0.09 \text{ min}$$

Multiplying by the flow rate yields

$$0.09 \text{ min X } 120 \; \mu\text{l/min} = 10.8 \; \mu\text{l}$$

Keeping Yang's original volume of the capillary peak at 0.16 μl, the final calculation gives

$$E = 0.16/10.8 = 67.5$$

$$f = 1/200 \text{ X } 67.5 = 0.338$$

This indicates a three fold improvement in sensitivity for the conventional system. Experimental work in this thesis will show that similar detection limits are achievable with matched components (eg, detector and injector volumes)

Ishii and Takeuchi (39) took advantage of the new fused silica tubing to verify an effect they reported previously (40). In that publication, they discovered that Pyrex™ glass tubing was superior to stainless steel and Teflon™ tubing with regards to efficiency. A similar comparison was made in the following study and fused silica surpassed all other column types. Efficiencies comparable to standard HPLC were obtained in similar time frames. Injection volumes onto a 9.8 cm X 250 μm column were examined and no deleterious effects were noticed for a 0.05 μl injection of moderately

retained solutes (k>2). Up to 0.1 μl was injected onto a 30.0 X 250 μm column without disrupting performance.

The following year, Yang published a paper on packed fused silica columns that would become one of the most frequently cited articles (5). Yang showed a 1 m long column that generated 110,000 plates in 25 minutes. Furthermore, he demonstrated a priority pollutant separation of 16 polycyclic aromatic hydrocarbons (PAHs) on a 50,000 plate column. These examples were the first to show high efficiency separations with standard HPLC-like capacities and selectivities. The remainder of the paper examined sample solvent and volume effects on micro LC. Once again, the concept of solute focusing was deemed a powerful sampling technique.

Until this time, Yang was the only researcher capable of making high plate count "HPLC" columns. Ishii and others had been able to make fairly efficient, short columns (<30 cm); consequently, the Japanese workers decided to couple several well-packed columns in series to attain high efficiency (41). A similar approach had been tried by Verzele and Dewaele (42) and yielded some success. They suggested that the better thermal dissipation by microbore would allow concatenation to work. Ishii and Takeuchi showed an almost linear additivity of column efficiencies. This was

achieved by a miniaturized column union and, thus, reduced plate heights approached their theoretical limits.

The major drawback of Ishii's latest work was the need to pack the columns individually and then to make microscale connections. It seemed only logical that someone would try to optimize the procedure for continuous fused silica columns. Novotny and his students were responsible for just such a study (43). This 1983 paper proved to be one of the most useful references for in house column preparation. By using the column performance parameters proposed by Knox and Bristow (44), Novotny could make the first direct comparison of standard bore and packed capillary LC in terms of permeability and other physical properties.

The first task was to optimize the packing procedure. The ratio of packing material (g) to solvent volume (ml) was chosen as a value typical of conventional packing technology. With this parameter fixed, the effect of slurrying and displacement solvent was examined. The least viscous solvent, acetonitrile, was slightly more effective and substantially less time consuming. The slurry ratio was then varied and the densest slurry (3.8 to 1) proved to be the best. Novotny and his group also corroborated Ishii's claim that fused silica was superior to glass, steel, and plastic tubing. The performance parameters such as separation impedance, column resistance factor,

interparticle porosity, and specific permeability all indicated that packed capillary column beds were less dense than standard bore columns. One of the studies in this thesis indirectly supports this concept and will be examined later.

One of the strong proponents of micro LC, Dr. Kiyokagatsu Jinno, entered the field at this time (45). His initial work demonstrated long packed glass columns, up to 5 m. Particles of 3, 5, and 10 μm and column diameters of 80 to 250 μm were examined. The results echoed Yang's indication that 200 μm was the best diameter for packed capillary columns. Perhaps the most interesting aspect of this work was the concept of temperature programmed LC. While the thermal mass of standard bore columns produced temperature gradients across the column, uniform thermal fields were possible with packed capillaries and, later, microbore columns (21,40,47). An increase in column temperature improved both mass transfer and partitioning in a way such that solvent gradient-like analyses were plausible with temperature programming. Jinno has examined this prospect recently and has continued its development (48).

The research activities from 1983 to the present can be broken down into three categories: column synthesis, detector/injector improvements, and applications. Although

the previous publications covered these aspects of microcolumn LC, improvements on established technology in the hopes of simplification were attempted. In most cases, the investigators were hoping to make microcolumn LC a practical option.

Studies on column synthesis can be further broken down into packing techniques, column resiliency, and column termination. Hartwick and Meyer (49) extended the usable particle diameter for microbore columns down to 5 μm. Novotny and workers studied the preparation of packed fused silica columns with normal phase sorbents (50). A student of Novotny, D.C. Shelly, attempted to elucidate the mechanism of column packing (51,52) and to optimize the process. Some Italian workers claimed a novel dry packing technique for microparticulate silica based material (53). Kennedy and Jorgenson published methodologies for packing 5 μm particles into 20 μm columns (54). Several workers addressed the noted disadvantage of packed fused silica columns in that they occasionally become brittle. Armstrong and Han (55) used microscopy to examine the formation of cracks in the fused silica tubing. Verzele and researchers investigated the application of silicone polymers to the inner tubing wall to improve stability (56). One study in this thesis examines the potential of surface pretreatment. Finally, the design of the column termination warranted experimentation. The

critical nature of injector/column and column/detector connection prompted several solutions. Glass wool frits had already been optimized (21) but a more durable screen was desired. Novotny, Shelly, and Gluckman (57) utilized a porous Teflon™ disc <1mm in length and cored to match the inner diameter of the column. A smaller diameter tube was cemented behind the disc to hold it in place. Perhaps the most elegant frit design belonged to Cortes and his peers at Dow Chemical. Using the company's advanced knowledge of ceramics, Cortes was able to grow a silicate frit in the column end (58). This frit was reasonably permeable and required no exterior support to keep it in place (up to 8000 psi).

Advances in injection/detection systems were also the focus of numerous research endeavors. Static flow splitting was performed by Jorgenson and Guthrie (59) and allowed nanoliter volumes of sample to be loaded. An ensuing graduate student of Jorgenson, R.T. Kennedy, developed a pneumatic device that equalled the performance of its predecessor and eliminated the excessive rinsing times typical of static splitting (60). The development of the moving injection interface in SFC (61) was also applied to microcolumn LC. Developments in detection were mostly aimed at successfully modifying existing detectors for microscale usage. Verzele and his group (62) and Verweij and Kientz

(63) described the necessary modifications for a UV/Vis detector. Jinno et.al. (64) and Pawliszyn (65) employed refractive index systems for detection. Jorgenson (66) and Caprioli (67) demonstrated the coupling of microscale LC and FAB/MS. The growth of capillary electrophoresis has recently aided microchromatographers since the extracolumn considerations are even more stringent for CE. A recent breakthrough in UV/Vis detection was introduced at the 10th International Symposium on Capillary Chromatography (68). A joint effort between LC Packings, a supplier of packed capillaries, and Kontron, a European instrumentation company, yielded a possible solution. A piece of small id fused silica tubing was bent in a Z configuration. The source was focused on one turn with the photodetector placed at the other. A longer pathlength resulted and detection limits improved by 3 to 4 orders of magnitude.

The applications of microcolumn LC have been fairly limited. Most applications have come from the biological realm. Separations of steroids in blood and urine (69), prostaglandins (70), catecholamines (71), and biopolymers (72,73) have been performed. The high efficiency of micro LC columns has proved invaluable. Perhaps the other most widely used applications entailed multidimensional chromatography and/or detection. Micro LC coupled to capillary GC (74,75), mass spectrometry (76), capillary

electrophoresis (77,78), and flame based detectors (79,80) were some of the configurations demonstrated. Other separations included coal products (81) and PAH analyses (82).

Chapter 3

Experimental Materials and Methods

Experimental

Column Construction

Micro Ion Chromatography Columns

Microbore IC columns were prepared from standard 2 mm id Teflon™ tubing. An old Dionex AS4A anion exchange column that had been broken in a previous thesis (83) served as a source for the low capacity anion resin. The packing material was washed in 100 mM NaOH followed by a rinse with deionized water before use. The tubing was cut so that after flaring the ends, the total length was 250 mm. A standard Dionex high pressure minimum dead volume union was fitted with a piece of 0.45 μm Teflon™ filter material to serve as the column termination. This filter was removed from a standard syringe filter and cut to the appropriate size.

The stationary phase was prepared by slurrying it in deionized water at a slurry ratio of 1 g of packing to 30 ml of water. The suspension was then sonicated for 10 minutes and placed in a syringe that was adapted to fit the column inlet. Manual depression of the plunger displaced the slurry into the blank and caused the bed to accumulate. The chromatograms shown in this thesis employed this type of column, but this packing technique proved to be both painful and time consuming. In order to accelerate the process and

potentially increase the bed density, an automated system was constructed. A schematic of the apparatus appears in Figure 6. A high pressure LC pump (EM Science MACS 700, Cherry Hill, NJ) delivered high purity water to a stirred slurry reservoir (Micromeritics model 705, Norcross, GA). This device contains a small magnetic stirring bar that maintains a suspension of the packing material while it is being displaced into the column blank. With this packing setup, column synthesis was possible in 15 minutes compared to the several hours required by the manual method.

Packed Fused Silica Columns

Several processes were developed to achieve high performance packed fused silica columns. For the work examining the wall pretreatment effects (84), high pressure slurry packing was employed. The packing material was slurried at a ratio of 1 g to 4 ml of isopropanol (42). The suspension was then sonicated for 10-15 minutes and placed in a high pressure reservoir constructed of 1 mm id stainless steel tubing. The column blank was attached to the other end of the reservoir by a Kel-F^{TM} capillary high pressure fitting (Upchurch Scientific, Oak Harbor, WA). The outlet of the blank was connected via a similar fitting to a minimum dead volume union fitted with a 2 μm steel screen (VICI, Houston, TX). The slurry was immediately transferred

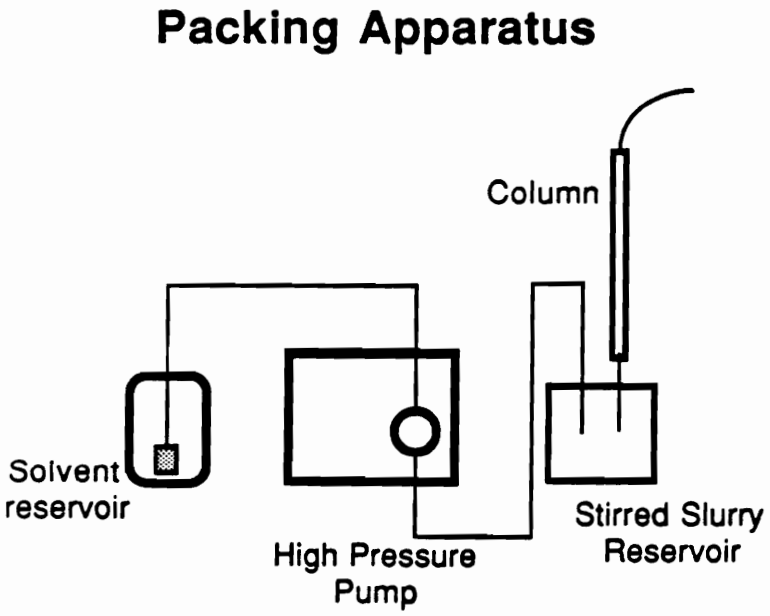


Figure 6. IC Column Packer

to the reservoir and 3000 psi of liquid pressure was provided by a Haskel pneumatic amplifier pump (Haskel Engineering, Burbank, CA). The bed consolidated typically in 15 to 30 seconds but the driving force was maintained for at least 20 minutes.

The column internal surfaces compared in this study included untreated, polymethylhydrosiloxane (PMHS) deactivated, and coated fused silica. The untreated tubing was obtained from the Avondale Division of Hewlett-Packard (Avondale, PA). The PMHS deactivated tubing was prepared by a method similar to Lee (85). The coated column material was a commercially available GC column produced by Hewlett-Packard and sold under the trade name "Ultra 2".

The syringe technique of column packing (21) was also examined. A dense slurry of stationary phase was prepared and the column blank (with no terminus) was inserted into the slurry. A gastight syringe was attached to the protruding end of the column and the slurry was sucked up to fill the blank. High pressure (4000-6000 psi) was then applied to increase the density of the bed. Such conditions yielded moderate efficiency (50 to 60% of theoretical) and good column permeability. The ramp technique proposed by Bowermaster and McNair for microbore columns was also investigated (86). This method was identical to the high pressure procedure described in the previous paragraph but

varied in the application of the high pressure. Instead of using immediate high pressure, the initial packing was displaced with moderate pressure (1000 psi) and the driving force was increased by 1000 psi a minute up to the final pressure. While this method allowed higher final pressures to be used, column performance was not superior.

Columns for the Knox-Parcher experiment (87) where the effect of column id to particle diameter was studied were prepared in two manners. Because such large particles were predominantly used for the study, dry packing (53) was performed. An appropriate weight of sorbent was added to a large volume (20 ml) steel reservoir which was attached to the column blank and the second stage of a nitrogen regulator. Initially, 20 psi of pressure was applied and then quickly ramped to 80 psi. The reservoir and column were vibrated with an engraving tool to aid in settling. After the column was filled, 80 psi pressure was maintained for 30 minutes. The column was then consolidated by pumping methanol through the column at 100 μl/min for several hours. The alternate procedure employed a high pressure slurry technique to help improve the performance of the small particle (5 and 20 μm) columns. The packing was suspended and displaced by hexane at 2000 psi. The larger particles showed no improvement in performance but the smaller particles showed a slight enhancement.

As part of the column packing investigations, fused silica columns of 50 and 100 μm id were packed to test the universality of the packing technique. Several modifications of the standard fused silica method were required to achieve this goal. First, the slurry reservoir had to be placed in an ultrasonic bath to maintain the dispersion of sorbent. Next, the column required occasional vibration to reinitiate the flow of packing. Finally, 1.5% of a surfactant (Triton X-100) had to be added to the suspension and displacement solvent (acetonitrile) in order to minimize aggregation. While these steps seem unimportant to traditional packing techniques, they are crucial to the success of stable, efficient beds in very small diameter columns (88,89).

The most routine columns prepared in this work were columns of 200, 250, and 320 μm id packed with 5 μm spherical particles (90). In addition, these were the easiest columns to prepare. A dense slurry (1g/4ml) of sorbent in acetonitrile was sonicated for 10 minutes, placed in the reservoir, and packed at 5000-6000 psi for 20 minutes. Columns with reduced plate heights of 2.1-2.6 were reproducibly constructed.

Column Termination

The manner by which the packing is retained in the column was no trivial matter for these small diameter separators. Extracolumn band broadening in injector/column and column/detector connections presented significant problems. The flow dynamics through tubes and connection devices has been characterized by Sternberg (91). A schematic of the expected flow deviations appears in Figure 7.

The simplest column connection device, the zero dead volume (ZDV) union, is either the worst or most difficult way to join the column and peripherals. Problems with unions that are not truly zero dead volume are encountered because of the relatively small connecting passage between the tubing seats. This "small" volume serves as a large mixing chamber for packed fused silica columns. A true ZDV that is designed to connect two pieces of tubing intimately also creates problems. In this case, perfect alignment of the ferrule holes is required for a viable union and this is no easy task.

The next union is the most practical for neophytes. The column blank is inserted through a piece of glass fiber paper several times until a small plug of glass wool is obtained. A piece of tubing whose outer diameter is smaller than the column blank's internal diameter is inserted to

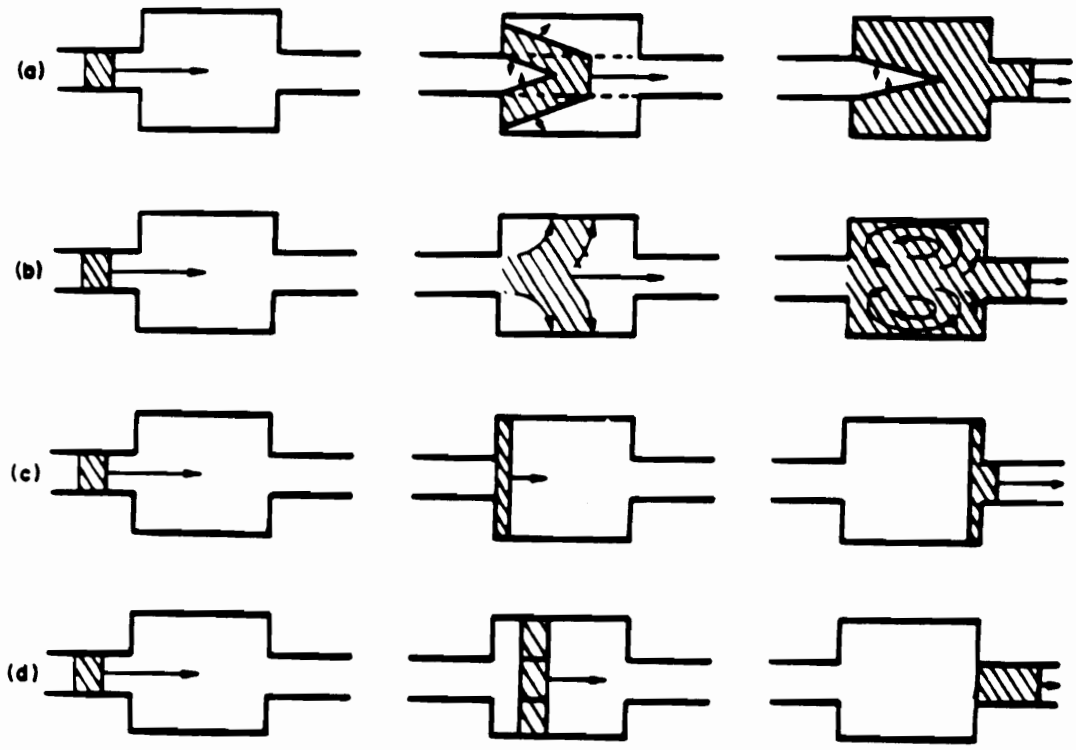


Figure 7. Possible Flow Profiles

Through a Larger Diameter Union (Ref 91)

hold the plug and is glued into place. Thus, a transfer line is now an integral part of the column. While this is quite convenient for the larger sizes (>250 μm) of tubing, it is not practical for the entire range of microcolumn LC.

Two column terminus designs that are compatible with very small diameter columns are the sintered frit and the ceramic frit. The former is easily prepared in columns of 100 μm id or less. The column blank is poked into a pile of 5 μm silica particles numerous times. Approximately 1 mm of packing will be forced into the column. The end of the column is then subjected to an electrical discharge device (92) or a cool blue flame. The ceramic frit is prepared according to a method developed by Cortes et al (58). A solution of potassium oxide and silicon dioxide in a weight ratio of 2.5 to 1 (commercially available as KASIL #1, PQ Corporation, Lafayette Hills, PA) is mixed in a 4 to 1 ratio with formamide (Kodak, Rochester, NY). The resulting gel is then drawn by capillary action into the column blank. Two to three centimeters is necessary to ensure a rugged frit. The column blank is then placed into an oven and heated at 125 C for one hour. The frit is then washed with acetonitrile at 200 kg/cm^2 to remove any unreacted components. The frit is then cut to approximately 1 mm in length and the column is packed according to the previous methods.

Chromatographic Systems

Microbore Ion Chromatography

A Dionex (Sunnyvale, CA) 4000i ion chromatograph was used for the microbore ion separations. The system consisted of glass mobile phase reservoirs, Teflon™ and Tefzel™ plastic tubing, a metal free dual piston reciprocating pump, a metal free injection valve, a homemade microbore separation column, a micromembrane chemical suppressor, and a conductivity cell (Figure 8). The only modifications to the system were the construction of various volume sample loops. Tefzel™ tubing of 0.020" inner diameter was cut to the appropriate length to give sample loops of 20.0, 50.0, 94.1, and 207.1 μl. The detector output was collected with a Varian 4270 recording integrator.

Chemically suppressed ion chromatography was invented in 1975 by researchers at Dow Chemical (93). The stationary phase is synthesized such that the number of exchange sites is relatively small compared to the older, high capacity resins. This allows a weaker buffer to elute even well retained solutes. The unique feature of chemically suppressed or dual column IC is the eluant suppressor device that immediately follows the separation column. The micromembrane suppressor used in this work is illustrated in Figure 9. The column effluent flows into a sandwiched

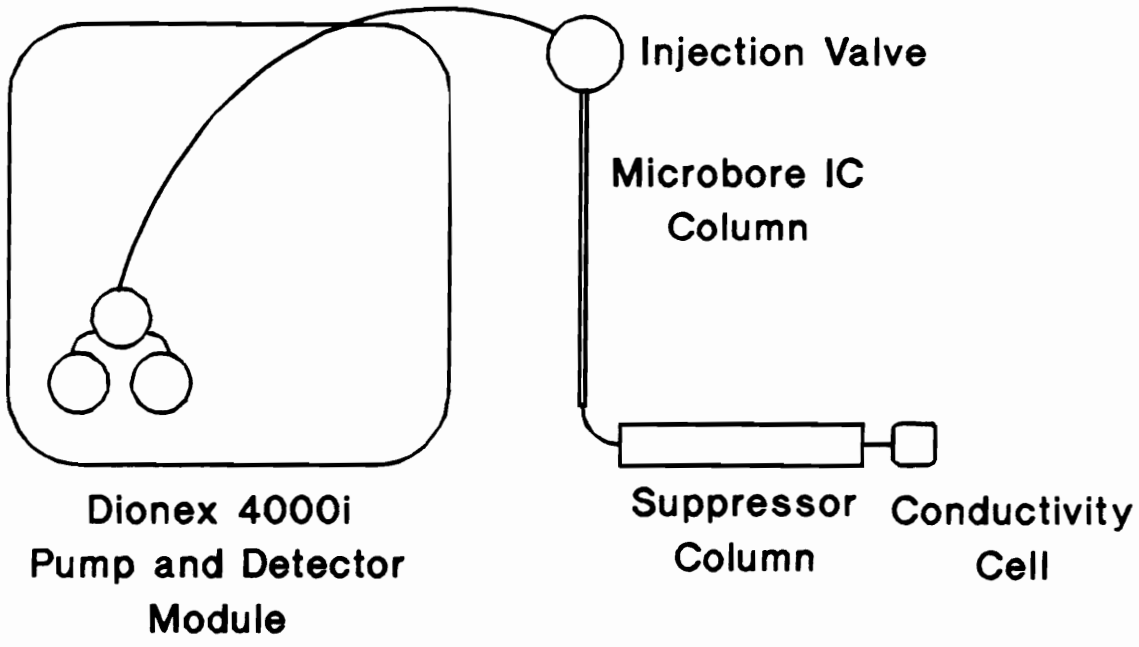


Figure 8. Microbore IC System

membrane that allows cations to pass through into the countercurrent flow but retards anions via Donnan exclusion. Thus, in this example, sodium ions pass into the stream of sulfuric acid as a result of a concentration gradient and the dissociated protons enter the column effluent. Two advantageous effects occur. The conductive sodium hydroxide mobile phase is converted into relatively nonconductive water. This lowers the background conductance of the stream entering the conductivity cell and enhances the signal to noise ratio. In addition, the analytes (chloride and sulfate) are converted from sodium salts to acids. Since conductivity is a measure of ion mobility and the hydrogen ion is three times more mobile than the sodium ion, a three fold increase in sensitivity is observed. The combination of these effects makes dual column IC more attractive.

Packed Fused Silica Chromatographic Systems

While a commercial system could be easily adapted for microbore IC, this was not the case for smaller diameter fused silica columns.

Studies on surface pretreatment effects involved perhaps the simplest configuration of equipment. A MACS 700 microbore pump capable of delivering 5 µl/min at 6000 psi was used to pump the mobile phase. A VALCO (Houston, TX) CI4W 4 port injection valve with a 60 nl internal volume

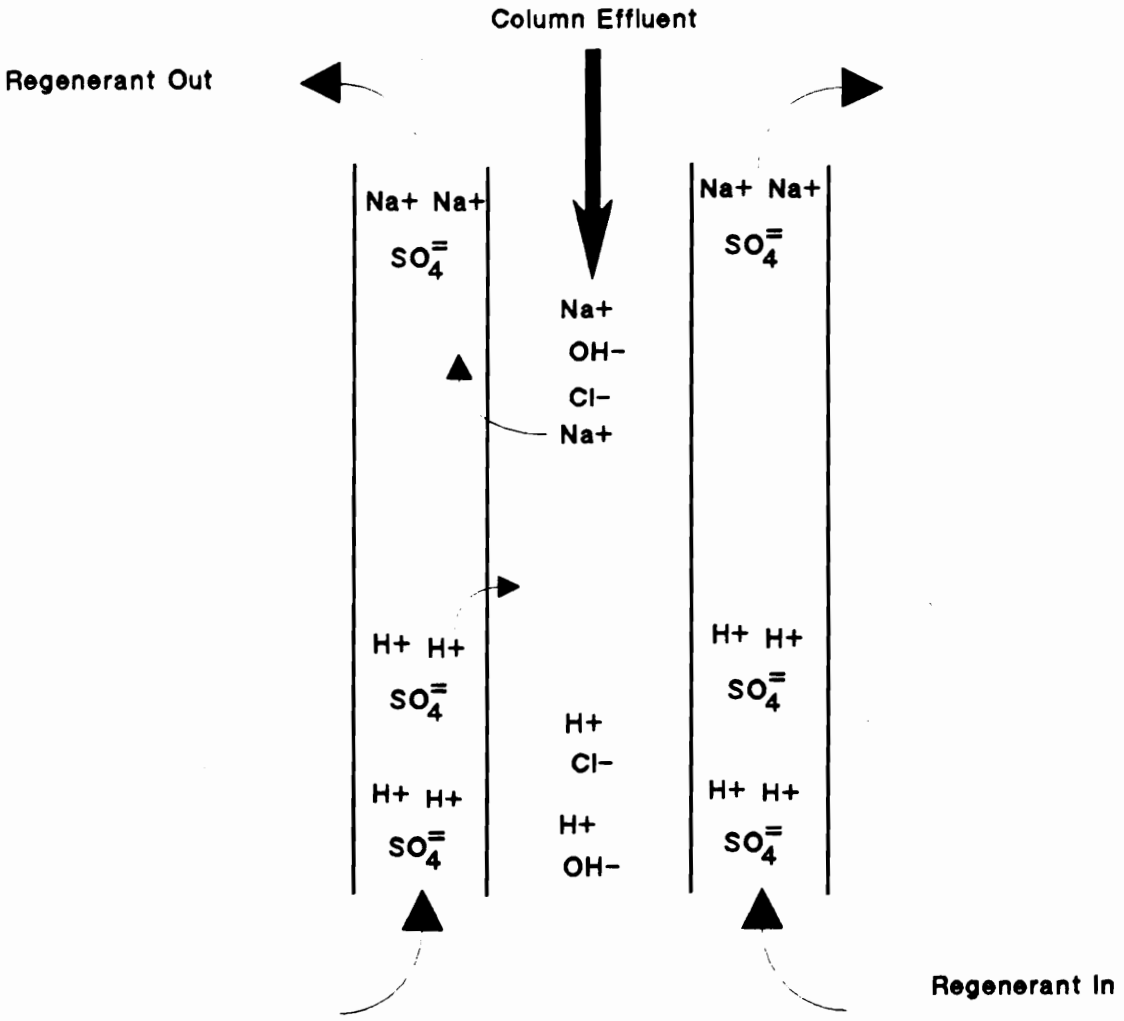


Figure 9. Expanded View of the Suppressor Column

allowed sample introduction. The columns were connected to this valve with Upchurch capillary ferrules drilled to match the outer diameter of the column. The outlet of the column was attached to the homebuilt detection cell through a VALCO column end fitting union equipped with a 2 μm stainless steel screen. While this union created considerable extracolumn volume, it is sufficient for this study. The homemade detection cell, illustrated in Figure 10, consisted of a standard MACS 500 UV cell that had been fitted with a mounting bracket and cardboard slits. The analog signal was fed to a Hewlett-Packard 3390A integrator.

The work on wall ordering effects (referred to as the Knox-Parcher study) employed several chromatographic systems. The majority of the work was performed on a modified Hewlett-Packard 1050 liquid chromatograph (Figure 11). Since the pump was incapable of delivering the desired flows directly, a splitter system was constructed. A 200 X 4 mm Hypersil column (Hewlett-Packard, Avondale, PA) packed with 5 μm particles and a 200 X 0.32 mm packed capillary column formed the heart of the splitter. With the particle size and column lengths identical, the split ratio is approximately 150 to 1. The majority of columns evaluated generated very small backpressures; thus, the flow could be estimated from the aforementioned ratio and then gravimetrically determined. The appropriate flow was then

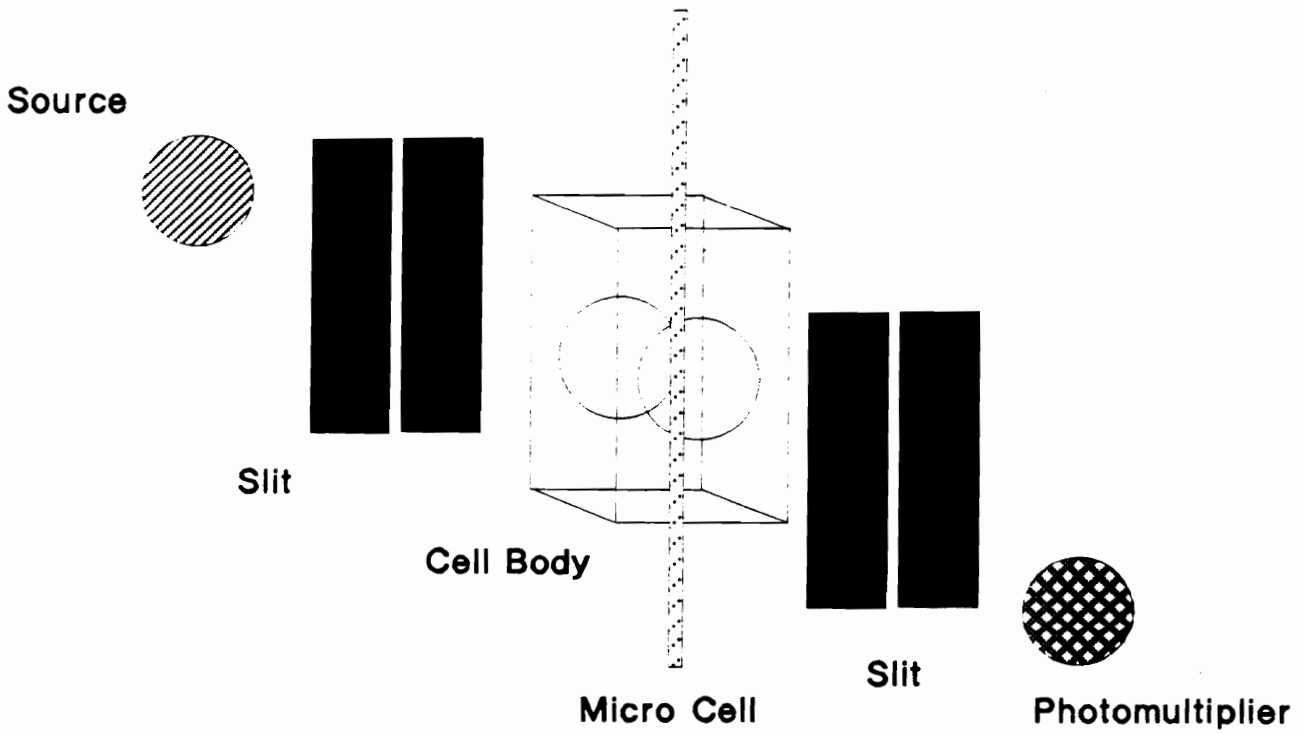


Figure 10. Homemade UV Detection Cell

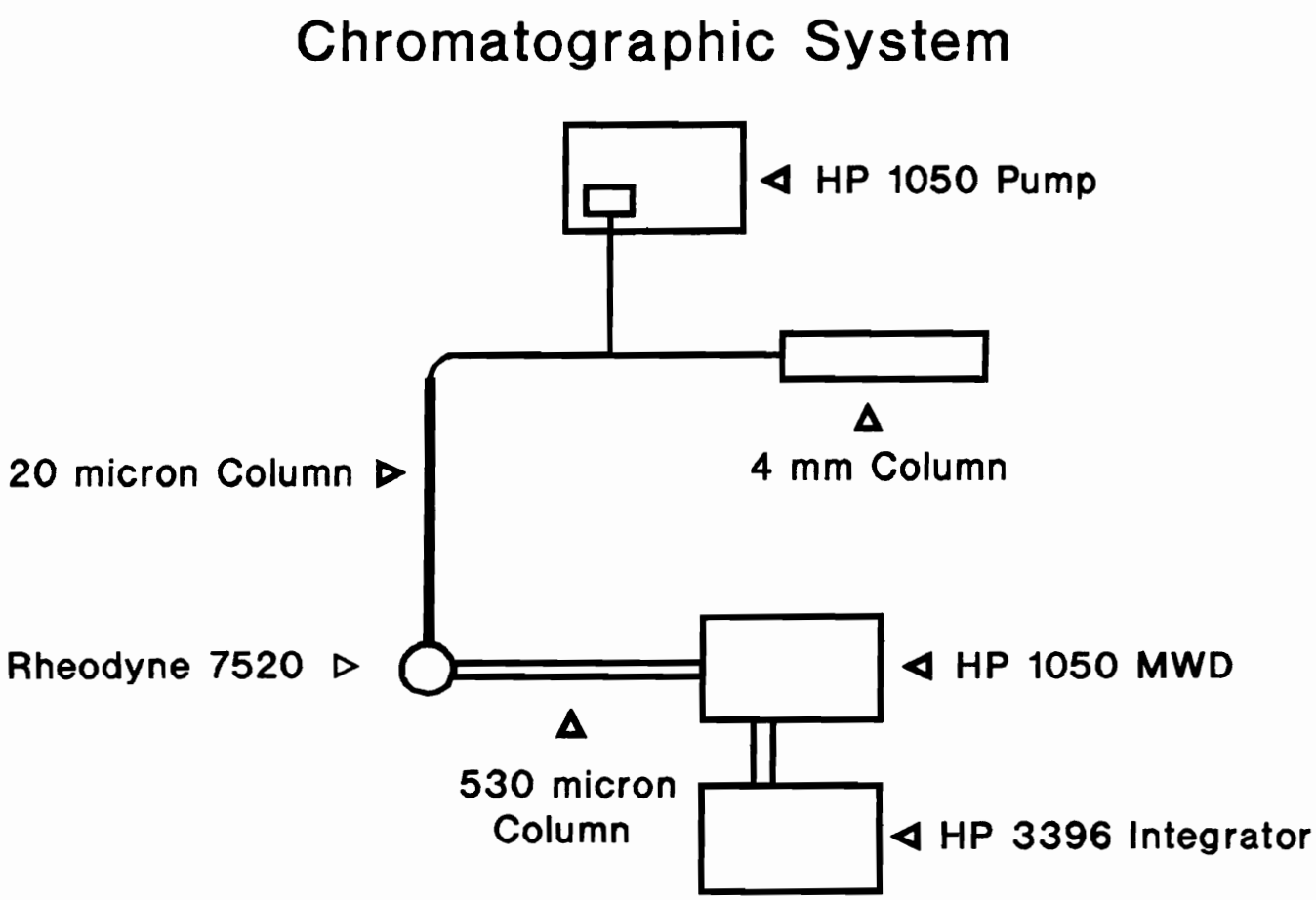


Figure 11. Split Flow System for Knox-Parcher Study

fed to a Rheodyne (Cotati, CA) 7520 injection valve equipped with a 0.2 μl rotor. The columns were connected directly to the injection valve and to a column end fitting previously described with Upchurch fittings. This end fitting was connected to the inlet of a 0.6 μl flow cell designed for the HP 1050 detector. The analog data was recorded by a HP 3396A integrator.

Additional work was performed on a modular system. Flow was delivered by an Isco μLC 500 syringe pump (Lincoln,NE) to a Valco 60 nl injection valve. The columns were connected to the valve as described previously; the detector and connections were different. Detection was accomplished with a Linear (Reno, NV) model 200 capillary UV detector fitted with a piece of 50 μm id tubing. The column and this "cell" were connected by a piece of Teflon™ tubing. By drilling the tubing to match the outer diameters of the column and the cell, an almost zero dead volume union was achieved.

The chromatographic system used in the multi-dimensional work was comprised of pieces from the previous units. Pumping was again performed by the Isco μLC 500 and sample introduction via the Valco valve. The column effluent then passed to a second valve or a tubing interface and into the capillary zone electrophoresis system (Figure 12). The CZE, designed and assembled by H. Rasmussen,

consisted of a Spellman high voltage supply (Plainview, NY), a Plexiglas interlock box, platinum electrodes (Aldrich, Milwaukee, WI), and an on-capillary Isco CV4 detector. The data were collected by a HP 3390A integrator.

Reagents/Samples

Chromatographic Evaluations

Microbore IC columns were examined with industrial samples of ultrapure water and standards of chloride and sulfate at various concentrations. Standards were prepared from triply distilled water and master stock solutions obtained from Fisher (Raleigh, NC). The chromatographic mobile phase was 50 mM NaOH carefully prepared to minimize the formation of carbonate and maintained under a helium environment. The regenerant was 25 mN sulfuric acid prepared from concentrate (Fisher). In all cases triply distilled water was used for any dilution or cleaning.

As mentioned previously, the sorbent was Dionex AS4A anion exchange resin. This material has a mean particle diameter of 15 μm and has been prepared in several steps. The core particle consists of a polystyrene/divinylbenzene copolymer that is highly crosslinked to yield an essentially fluid impermeable sphere. Next, the surface of the particle was reacted to give a sulfonic acid coating and this product

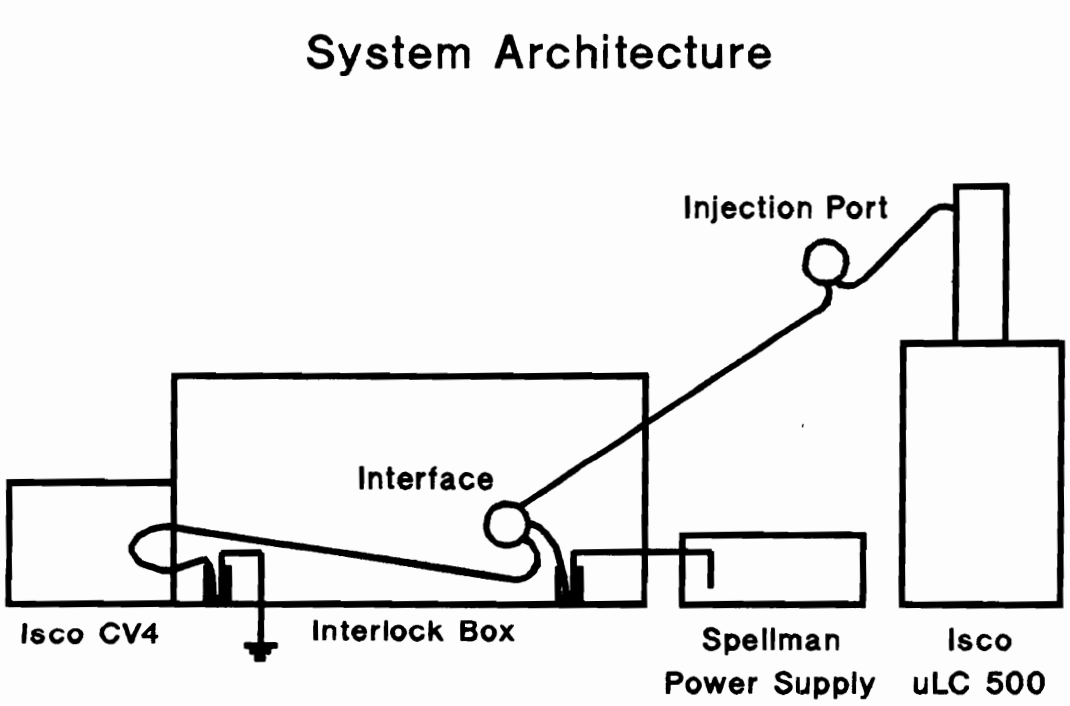


Figure 12. Multidimensional μLC-CZE System

functions as a cation separator. Anion separators are formed by pumping an aminated latex suspension through the prepacked cation column. The small latex particles are held by coulombic attraction on the surface of the sulfonated polystyrene sphere. This thin surface layer has a low exchange capacity and good mass transfer characteristics. The combination of these two properties permits fast ion separations with dilute buffer concentrations. Additionally, the latex particle is so strongly held that the column can be used from pH values of 0 to 14 without significant loss of stationary phase.

Packed fused silica columns with different wall pretreatments were evaluated for their chemical and physical properties. These columns were tested by chromatographing sodium nitrate (a void volume indicator) and several other components with 60/40 acetonitrile/water at 5 μl/min. The physical properties were tested on a Chemco (Osaka, Japan) Econopacker at several pressures and conditions. These conditions included a high pressure test of the column blank and a high pressure packing test to illustrate the role of the packing in column weakening. The solvents used in this and all other investigations were HPLC grade from Burdick and Jackson (Muskegon, MI), EM Science (Cherry Hill, NJ), JT Baker (Phillipsburg, NJ), or Fisher (Raleigh, NC). The

probes were obtained from either Aldrich or Kodak. The stationary phase was 5 μm spherical Hypersil ODS (Hewlett-Packard, Avondale, PA).

The Knox-Parcher work employed specially prepared spherical silica particles from Phase Separations Ltd.(Queensferry, UK). Particles of 5, 20, 40, 70, and 120 μm with similar pore sizes were used without further treatment. Fused silica tubing of 530 μm id was obtained from Hewlett-Packard (Avondale, PA). Methanol was used for the mobile phase and the probe was benzene prepared at 0.025% (v/v).

The multidimensional studies required some of the columns prepared for the surface treatment studies. The tubing used for all but the 50 and 100 μm id material was obtained from Hewlett-Packard (Avondale, PA). The other tubing was thick wall material obtained from Polymicro Technologies (Phoenix, AZ). The stationary phase was 5 μm Hypersil ODS. The CZE capillary was prepared from 50 μm id fused silica tubing by a procedure developed by Rasmussen (94). The 0.02 M phosphate buffer (pH = 7) used for the electrophoresis medium was prepared by standard methods. The polyimide cladding on the capillary was removed with a cool flame so that an on-column window was formed. The switching valves used for the interface were a Valco CI4W 60 nl 4 port and a Rheodyne 9125 metal free 6 port that had been modified

to function as a 4 port valve. Removal of the internal collar allowed the valve to be turned more than 60 degrees. The fused silica tubing was connected to the valves with Upchurch capillary ferrules. An initial experiment was performed to determine the effects of different organic modifier concentrations in the sample and the buffer on the CE separation. Dimethylsulfoxide (0.1% v/v) was the probe and acetonitrile was used as the modifier, both from Burdick and Jackson.

Chapter 4

Results and Discussion

Results and Discussion

Ultrapure Water Analysis by Microbore Ion Chromatography

Trace contaminants in ultrapure water present a severe problem to several industries, such as electroplating and power generation companies. The presence of trace anions in high purity water can affect the production of semiconductors by doping the nonconductive material with conductive ions. In the case of power generation from nuclear reactors, trace anions can cause rapid deterioration of the emergency cooling water vessels. Since these vessels are at elevated temperatures and pressures, 10 ppb of chloride in the water will cause the stainless steel vessel to corrode in several hours. For these reasons, qualitative and sensitive techniques are required for trace ion analysis. Previous methodologies employ electrochemical detection or preconcentration steps leading to liquid chromatography (95).

Another approach to trace ion analysis is the use of microbore columns in chemically suppressed ion chromatography systems. Since most IC detectors such as conductivity are concentration dependent, a decrease in volumetric flow rate provides an enhanced signal. By maintaining the same volumes of injection and detection, a

reduction in column diameter allows lower detection limits. This work examines the theoretical and practical ramifications of using microbore ion chromatography for trace analysis.

Figure 13 shows the chromatograms obtained on the standard and microbore IC columns. The chromatographic conditions are identical for both columns with the exception of volumetric flow. To maintain the linear velocity at a constant value, the microbore column has only one quarter of the volumetric flow (0.5 ml/min for the microbore, 2.0 ml/min for the standard bore). Notice that the speed of analysis is preserved while an increase in sensitivity is apparent for the microbore column. A numerical comparison is presented in Table 2. In terms of peak area, a 1.6 fold improvement is observed for both chloride and sulfate. Since the same total amount of the analytes is loaded onto the column, this indicates that the peak shape of the microbore separation is more easily identified by the integration algorithm. In most trace analysis, however, peak height is used for quantitation. Peak heights are improved by 2.5 and 4.1 fold for chloride ($k'=0.63$) and sulfate ($k'=2.9$), respectively. The reason for this improvement is simple. By fixing the injector and detector volumes, the microbore column elutes the same mass analyte in one quarter the volume of mobile phase.

The effect of extracolumn volume on efficiency is well documented (96). As injection (or detection) volume is increased, the eluting peaks are broadened. From theory, efficiency is measured by N, the number of theoretical plates.

$$N = t_R^2/\sigma^2 \qquad (6)$$

where t_R = retention time of component

σ = standard deviation of the chromatographic band

Recall that the variance (σ^2) is a measure of the peak broadening introduced by the chromatographic system. Because variance is an additive function, the individual variances caused by the system can be broken down to its respective parts (97). Mathematically, this is expressed by equation 7.

$$\sigma^2_{sys} = \sigma^2_{inj} + \sigma^2_{col} + \sigma^2_{det} + \sigma^2_{conn} + \sigma^2_{other} \qquad (7)$$

where sys = system

inj = injector

col = column

det = detector

conn = connections

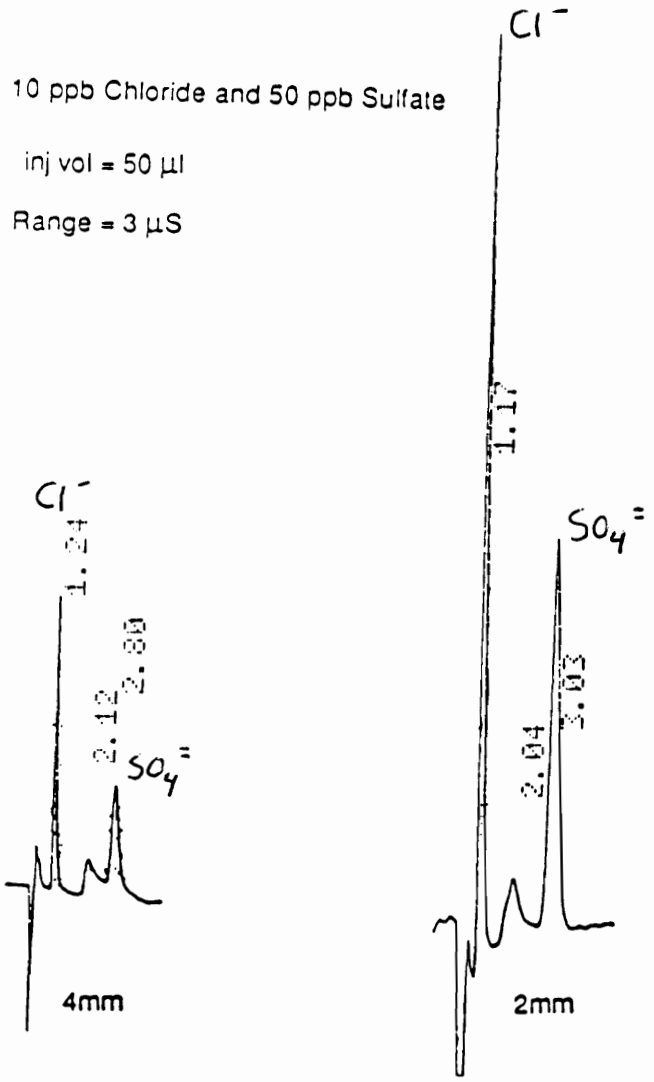


Figure 13. Comparable IC Chromatograms from Standard (4 mm) and Microbore (2 mm) Columns

Table 2

Numerical Comparison of Standard and Microbore Columns

Peak Area	4 mm	2 mm	Improvement
Chloride	6036	10020	1.66 to 1
Sulfate	5339	8547	1.60 to 1
Peak Height			
Chloride	4.9 cm	12.1 cm	2.5 to 1
Sulfate	1.4 cm	5.7 cm	4.1 to 1

For the purpose of this study, these can be reduced to injector contributions and a composite contribution (denoted $\sigma^2_{residual}$) consisting of the column, detector, connections, and other causes (equation 8).

$$\sigma^2_{sys} = \sigma^2_{inj} + \sigma^2_{residual} \qquad (8)$$

By fixing all other variables, the injection volume effect on efficiency can be studied. To simplify the experimental system, a commercially available 4 mm AS4A (Dionex) column was used. In order to establish a reference point, it can be shown that a 2 to 20 μl injection on a 4 mm column makes a negligible contribution to the total system variance. Thus, equation 8 reduces to

$$\sigma^2_{sys} = \sigma^2_{residual} \qquad (9)$$

Experimentally, a 20 μl injection onto the standard Dionex system gives N = 4900 and t_R = 125.4 seconds. Solving equation 8 for the system variance gives:

$$\sigma^2_{sys} = \sigma^2_{residual} = 3.21 \text{ sec}^2$$

Repeating the same process but with a 50 μl injection yields

$$\sigma^2_{sys} = 3.76 \text{ sec}^2$$

With the values for the residual variance and the residual plus 50 μl injection variance, the variance introduced by the 50 μl injection can be obtained.

$$\sigma^2_{inj} = \sigma^2_{sys} - \sigma^2_{residual} \qquad (8)$$

$$\sigma^2_{inj} = 3.76 \text{ sec}^2 - 3.21 \text{ sec}^2 = 0.55 \text{ sec}^2$$

Recall that a typical chromatographic peak is Gaussian in nature and has a base width of four times the standard deviation (95% of total area).

$$4\sigma_{inj} = 2.96 \text{ sec}$$

For a flow rate of 1 ml/min, the injection volume that caused this dispersion is

$$2.96 \text{ sec} \times 1 \text{ ml/60 sec} = 49.6 \ \mu\text{l}$$

For six standard deviations (99.3% of total area), the injection volume is 55.0 μl. Thus, theory and experiment agree very well. The results from additional injection volumes of 94.7 and 207.1 μl of 1 ppm chloride and 5 ppm sulfate are given in Figures 14 and 15, respectively.

In addition, the theoretical injection volume that would give the same plate height as the experimentally observed value is plotted. The theoretical trend is noted by the dotted line. Since variance is a squared function, the parabolic increase in plate height is expected as injection volume increases. Experimentally, though, a sharp deviation from theory occurs. This curve is designated by the solid line. From these data, an actual injection volume of 207.1 μl
only gives the dispersion corresponding to a theoretical volume of 90.9 μl. It is apparent that some sort of peak compression or focusing is occurring in the column. In IC, water can only weakly displace sample anions from the exchange resin. Because the sample matrix can not move the solutes, they are concentrated on the head of the column. Only when the sample matrix has been displaced by the mobile phase will chromatography begin. A similar effect is observed for sulfate. This effect also explains the discrepancy in peak height improvement. Due to sulfate's higher affinity for the AS4A resin (83), it preferentially binds and requires more mobile phase to remove it. The lack of migration reduces most of the variance introduced by the injector and the sulfate ion achieves the expected 4 to 1 improvement. The chloride ion, on the other hand, is less

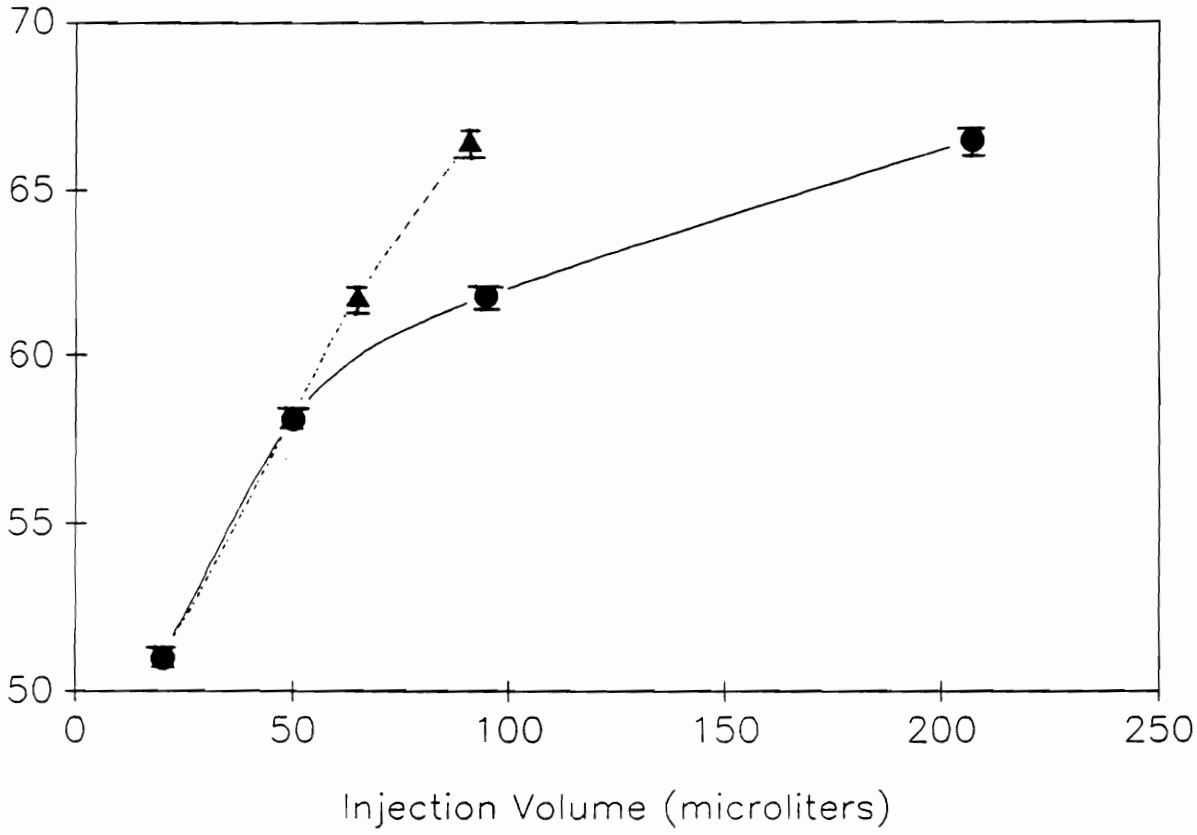


Figure 14. Plate Height vs. Injection Volume for Chloride. Circles = Experimental Results (triplicate measurements), Triangles = Theoretical Values

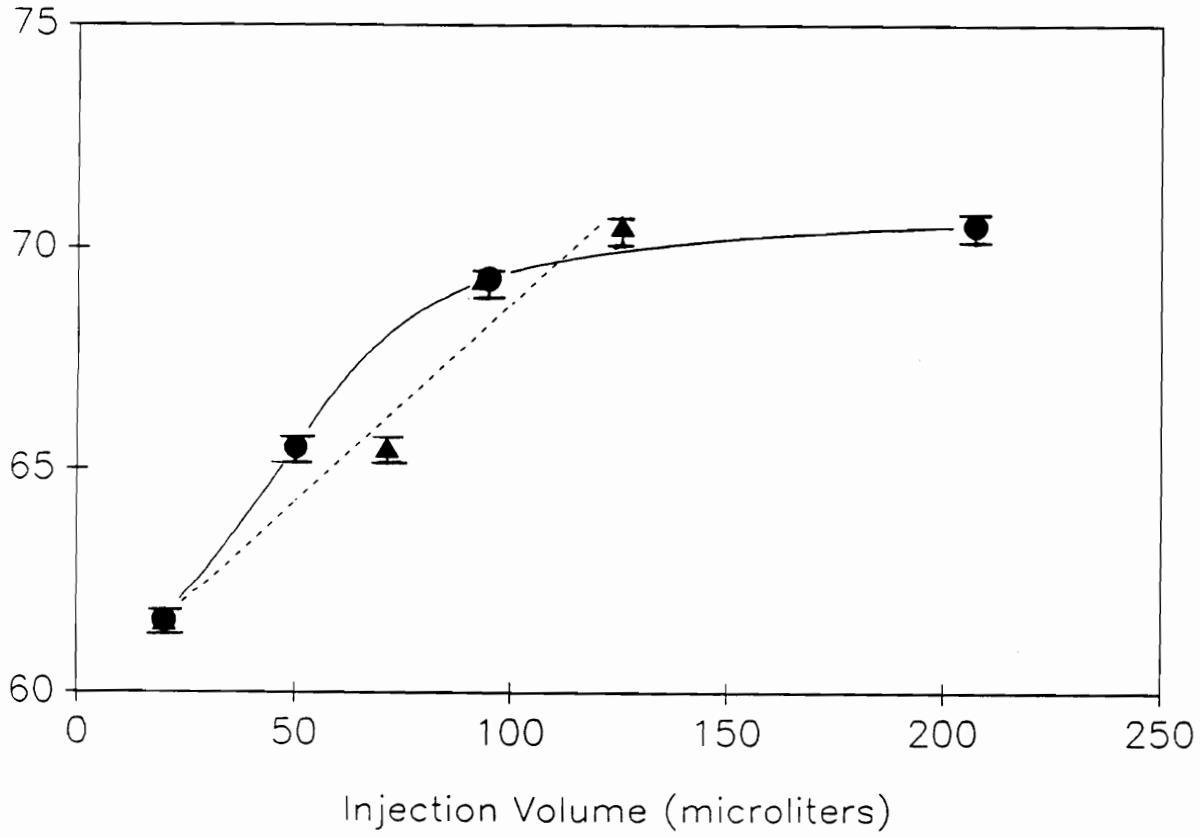


Figure 15. Plate Height vs. Injection Volume for Sulfate. Circles = Experimental Results (triplicate measurements), Triangles = Theoretical Values

retained and the result is a broader initial band on the column. This leads to a noticeable loss in peak height and an increase in plate height. The 2 mm column shows precisely the same trend as the standard bore column. It is the interplay of dispersion and focusing of which the analyst must take advantage.

The real power of this technique is the improved sensitivity and the minimization of sample handling. The former is an artificial effect but one that can provide real detection gains. The latter is crucial to good reproducibility and quantitation. The use of microbore columns with standard chromatographic systems is a viable solution to trace analysis. However, it can not be stressed enough that the scale down of the chromatographic components to match the column diameter reduction does not improve sensitivity.

Surface Pretreatment Effects in Micropacked Fused Silica Columns

In transferring steel column packing technology to fused silica columns, a major problem is encountered. The most popular steel column packing method (24) employs a large flow rate, constant pressure pump. Steel columns are typically packed at 8000 to 12000 psi. This packing pressure

is applied for a designated period of time (30-60 min). The high pressure is required to create a stable, highly efficient column bed. Fused silica, however, fails at substantially lower pressures.

Microscopic viewing of the packing process is presented schematically in Figure 16. The flow of the slurry can be approximated by a laminar profile rather than a turbulent one. Observations made by the author while packing 100 μm id tubes support this approximation. Since flow is fastest at the greatest distance from the wall, the center of the column always packs first. When the central region has accumulated enough packing to become unstable, the core collapses and fills in the wall region of the blank. Slurry flow is not completely laminar; true laminar flow approaches zero at the liquid/wall interface. From observations, the slurry at the wall is moving at a substantial velocity. Thus, interaction between the slurry and the wall is possible and will prove essential later on.

The most efficient chromatographic beds are usually those that form in a very short period of time (24). The slurry moves at the highest possible velocity and packs as a whole with no time for sizing or sieving to occur. Under the microscope, this is indicated by a very short accumulation zone of packing in the center of the column. The wall regions are quickly formed and feel the highest possible

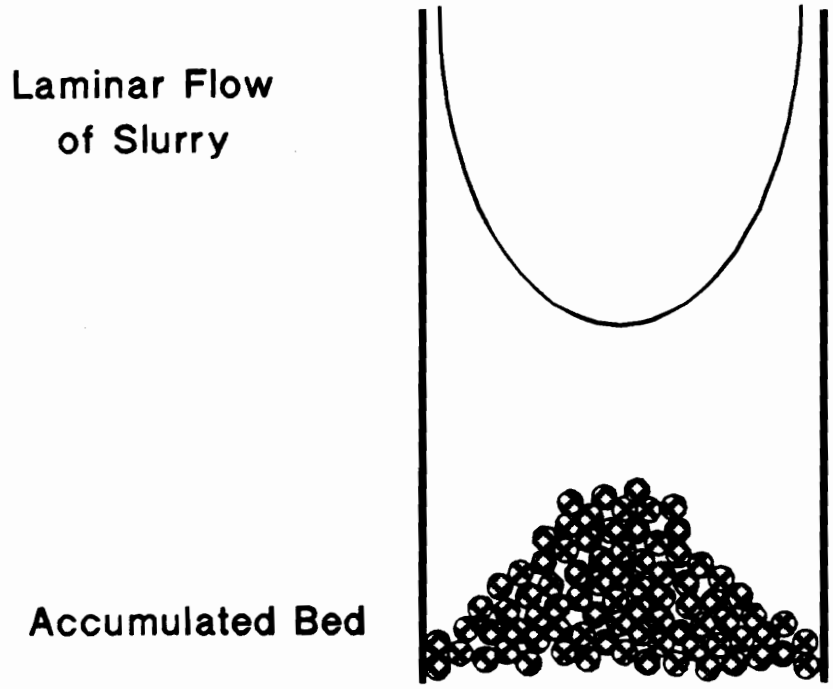


Figure 16. Schematic of Packing Accumulation

driving force. Consequently, the best beds are typically made with very high packing pressures (or high flow rates).

With this in mind, initial attempts to prepare packed fused silica columns were prone to failure. Packing pressures as "mild" as 6000 psi easily fractured 15 columns. Since this pressure was applied instantaneously (designated slam packing), other packing methodologies were explored. These techniques are the syringe (21) and the ramp methods (86). The syringe technique is a modification of Ishii's method where a dense slurry is drawn by suction into the column blank. Subsequent pressure treatment compacts the bed for chromatographic use. The advantage of this method is the relatively gentle manner in which the packing fills the column. Reasonably (h = 4 to 5) efficient columns are obtainable after extended usage, suggesting the bed is not stable after preparation. The ramp technique is a compromise between the slam and the syringe methods. Bowermaster and McNair reasoned that the sudden, high strain of slam packing could crush the packing material and form fines that would plug the column. They thought that a smooth ramp of applied pressure could pack the particles with the same driving force independent of their position in the bed. Again, fairly efficient columns are obtained but the theoretical optimums of h equal to two particle diameters is still unachieved.

From this line of thought, pretreatment of the column blank was first considered to improve resiliency. Concomitant with that deduction was the consideration that the fused silica wall might be contributing to the surface adsorption in the column. Thus, the concept of surface coating the column held two advantages, one chemical and the other mechanical. The purpose of this investigation is to determine the chemical effects of deactivating the wall and the potential interferences created by adding polymer to the analyte flow path. The other aspect of this study is to assess the practicality of wall treatment in terms of column durability and stability.

Figures 17, 18, and 19 represent the three surfaces examined in this study. Untreated fused silica is comprised of acidic surface silanols (both geminal and vicinal), inert siloxane bridges, and highly acidic hydrogen bonding sites. The poly-methylhydrosiloxane (PMHS) surface consists of a thin film of 50% dimethyl, 50% dihydrogen siloxane polymer that can be chemically bound to the surface or crosslinked via the reactive Si-H bonds. Because of the relatively thin, multilayer film and the possibility of unreacted surface silanols, the PMHS material may still be capable of polar interactions with the solutes. The final material is a commercial GC column that consists of a crosslinked film of 5% phenyl-methylpolysiloxane (OV-73).

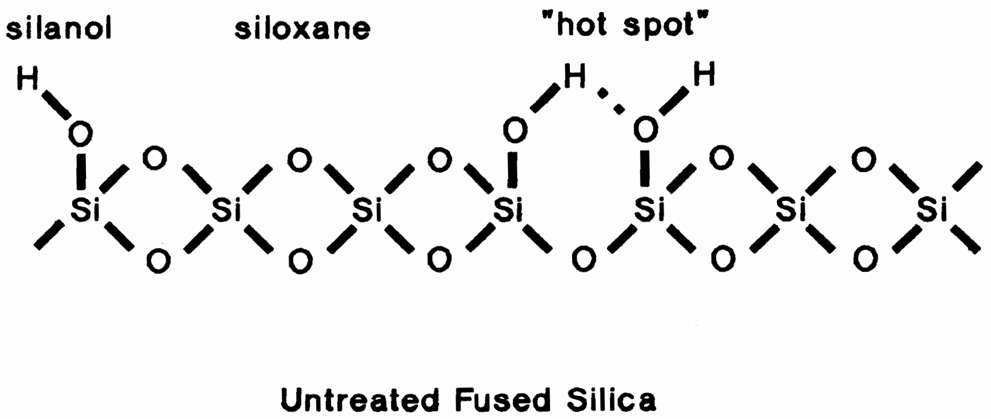
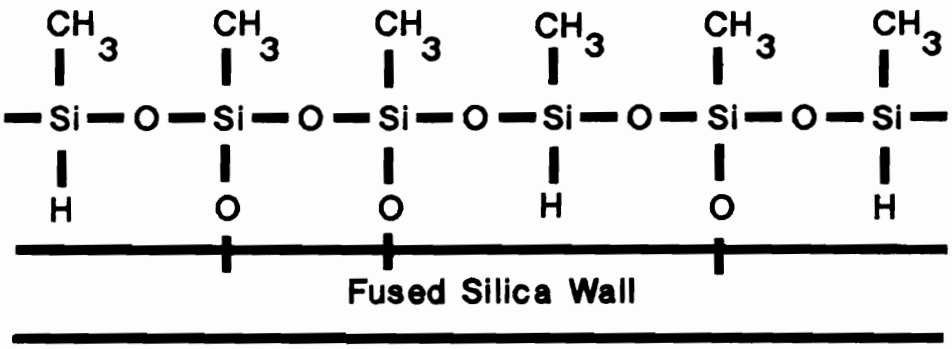


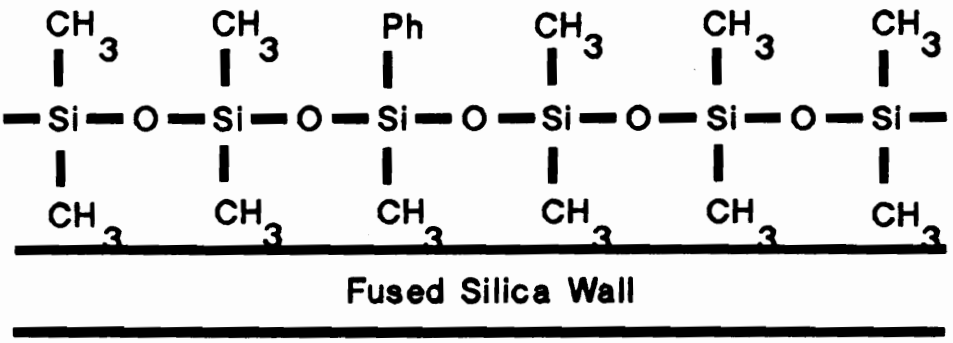
Figure 17. Schematic of the Fused Silica Surface



PMHS Deactivated Tubing

Figure 18. Schematic of the PMHS Surface

(A multilayer film is actually formed)



Polymer Coated Column (SE-54)

Figure 19. Schematic of the "Ultra 2" Surface

(A multilayer film is actually formed)

To determine the native activity of the various column materials, gas chromatography with an adsorbable probe was performed. The results of this experiment are given in Table 3. A statistically significant difference is obtained for each column type. The raw fused silica has a higher degree of activity than the thin PMHS film and the PMHS film is slightly more active than the thick polymer film. However, the difference is minimal and in the transition from GC to HPLC, several factors serve to further reduce the wall contribution. First, the decrease in solute diffusion coefficient by three to four orders of magnitude minimizes the chance for solutes to interact with the wall. In addition, the presence of acetonitrile and water as the mobile phase decreases the wall activity. These polar compounds can adsorb to the active sites on the surface and eliminate them. Perhaps the greatest effect occurs when the column is packed with silica based material.

Ratio of silanol groups on the packing versus the wall in a packed fused silica column

For standard 5 μm silica particles (Hypersil), the surface area of such particles is usually reported at 300 m^2/g (from BET measurements). The amount of packing in a 250 X 0.320 mm

Table 3

Adsorptivity of the Column Blanks

Column Blank Activity by GC

Sample: 1-octanol

Col Temp: 90 C

Inj Temp: 175 C

Det Temp: 250 C

Split: 190 to 1

Column Material	Peak Asymmetry
Untreated	1.22+/-0.03
PMHS	1.16+/-0.04
Ultra 2	1.09+/-0.03

column is approximately 25 mg. The resulting surface area of the stationary phase is simply

$$300\ m^2/g\ X\ 0.025\ g = 7.5\ m^2$$

The surface area of the column blank is also easily calculated.

$$0.25\ m\ X\ (0.000320\ m/2)\ X\ 4\ X\ \pi = 0.00050\ m^2$$

For silica gel particles, it is accepted that there are 8 $\mu mol/m^2$ of silanols (24). Thus, the number of silanols on bare silica gel is

$$7.5\ m^2\ X\ 8\ \mu mol/m^2 = 60\ \mu mol$$

With a standard reverse phase packing, the amount of organic substrate bound to the surface is as high as 50%. Thus, the actual number of silanols is only 30 μmol. The number of silanols per square meter on the fused silica tubing is lower than on silica gel but for uniformity, assume that it is the same as silica gel. The number of silanols for the tubing is

$$0.00050\ m^2\ X\ 8\ \mu mol/m^2 = 0.004\ \mu mol$$

The ratio of residual silanols on the packing versus the silanols on the wall is

$$30/0.004 = 7500 \text{ to } 1$$

Clearly, the residual activity on the packing outweighs the possible effects of the tubing activity.

Packed capillary columns were then prepared and their chromatographic performance was compared in terms of activity and permeability. At this time, some corollary work had been published. Maurice Verzele's research group at the University of Ghent reported on several aspects of precoated packed fused silica columns (98). Verzele's group used only coated GC columns for the tubing blanks and varied the type of phase immobilized on the fused silica surface. The effects of the polymer layer on asymmetry, retention, and plate height are illustrated in Table 4.

The coated polymers are of different polarities, ranging from a non-polar polydimethylsiloxane (SE-30 or RSL-150) to a slightly polar 5% phenyl (RSL-300) to a polar polyethylene glycol (Superox 20M). Note that the capacity factor for each solute does not change with the different polymers. In addition, the asymmetry factors for each probe are quite similar on the various columns. The largest

Table 4

Verzele's Data on Different Polymer Coated Packed Capillary Columns

Wall Coating Effects on Chromatography

	Superox-20M			RSL-300			RSL-150		
Compound	k′	As	H	k′	As	H	k′	As	H
Phenol	0.47	1.65	0.044	0.47	1.80	0.037	0.48	1.74	0.037
Benzald	0.84	1.49	0.041	0.84	1.32	0.037	0.86	1.64	0.033
NNdpT	1.11	1.46	0.031	1.10	1.56	0.026	1.13	1.51	0.027
Toluene	2.64	1.21	0.014	2.62	1.26	0.012	2.71	1.28	0.011
EtBenz	3.99	1.18	0.012	3.87	1.25	0.011	4.03	1.30	0.010

Adapted from M. Verzele, C. Dewaele, M. DeWeerdt, and S. Abbott, Proceedings of the Ninth Symposium on Capillary Chromatography, Monterey 1988 p341

relative standard deviation (RSD) for peak asymmetry is only 10.8% for benzaldehyde. All other RSDs are below 5%. While the wall coating is different for each column, the type and strength of interaction between solute and micropacked column (both wall and stationary phase) are relatively constant. Finally, a comparison of plate height for ethylbenzene on each column shows that, theoretically, optimum efficiency is attainable. For 5 μm particles, the reduced plate height, h, should give a value of 2. The average value for these columns is 2.25. Consequently, the use of an inner wall coating is not significantly detrimental to the chromatographic performance of the microcolumn.

The column resistance factor, Φ, is also a good measure of the performance of a column and indicates physically the packing structure of the column. Column resistance is defined by Snyder and Kirkland (24) as (10)

$$\Phi = \frac{0.1\ P\ t_0\ d_p^2}{n\ L^2\ 10^9} \qquad (10)$$

where P = pressure drop (bar)

t_0 = dead time (sec)

d_p = particle diameter (microns)

n = viscosity (centipoise)

L = column length (meters)

To make a qualitative comparison, the values of Φ for each column type are calculated and normalized to the value obtained for the untreated fused silica column. The results are given in Table 5. The untreated and PMHS columns are extremely similar in terms of column resistance. Since the film thickness is so small in the PMHS column, it is anticipated that its contribution to backpressure will be negligible. In the polymer coated column, however, a much thicker film is present. Most siloxane polymers used as GC phases are very viscous and present a considerable effect on the column backpressure. The crosslinked polymeric layer creates drag and thus, could retard the passage of mobile phase. This is supported by the experimental results obtained as the column resistance for the polymer coated column increases by 30% over either the untreated or the PMHS columns. An additional effect that could decrease column permeability is the elasticity of the polymeric film. As suggested by Verzele and co-workers (98), the packing particles may be able to press into the column coating and obtain a greater particle density at the walls. Normally, the wall imposed structure is less dense than the particle imposed structure (18); therefore, flow velocities near the

wall are greater than in the center of the column. A third possibility is that the force of packing strips the phase from the wall and it accumulates at the outlet frit. However, such an occurrence should affect the performance of the column in terms of efficiency. This work as well as Verzele's shows no significant plate difference between untreated and polymer coated columns. In any case, the use of a coated, crosslinked GC column for the blank material will increase the column resistance. For short columns, this poses no significant problem. Packed capillaries, though, are typically used in long lengths for greater efficiency. In this instance, an increase in column backpressure may not be so tolerable.

The second aspect of this study involves physical testing of the column types to elucidate the effect of surface pretreatment. Two specific tests were performed: a blank column pressure test and a column packing test. After each test was performed an empirical assessment of flexibility was made.

The pressure test results are given in Table 6. The untreated fused silica repeatedly failed at 8250 psi. Since the stainless steel HPLC fittings are standardized to withstand up to 6000 psi, the absolute strength of the fused silica appears to be sufficient. In terms of flexibility, the fragments break upon bending but the polyimide remains

Table 5

Relative Column Resistance Measurements

Microcolumn Resistance (Φ)

Column Material	Relative Resistance
Untreated	1.00 ± 0.04
PMHS	1.01 ± 0.04
Ultra 2	1.34 ± 0.04

intact. The PMHS material also failed at this pressure. When the pieces are flexed, they break crisply. Not only is the fused silica weakened but the polyimide exterior coating is also brittle. Because the film is too thin to provide additional mechanical stability, the column blank fails at the same pressure as the untreated fused silica. The difference in the two types is that the PMHS tubing underwent thermal treatment at 300 C. This factor explains the different polyimide characteristics of the flexed tubing. The polyimide becomes brittle at high temperatures and breaks crisply. The polymer coated column blanks, however, demonstrate far greater tensile strength under high pressure. At 9000 psi none of the test columns failed. In terms of flexibility, the Ultra 2 column is as resilient as an untested piece of the same material.

The packing test results are given in Table 7. Ten columns of each type of material were made by typical packing conditions and the percentage of failures are listed. The untreated fused silica had a very high failure rate. The reason behind this is partially explained by the work of Armstrong and Han (55). Electron microscopy shows that cracks are formed by the packing process. Armstrong suggests that the cracking could be caused by particle induced scoring of the inner walls, the high pressure of

Table 6

Pressure Test Results

Fused Silica Blank Test

Conditions: Acetonitrile as pumping fluid

Ramp from 3000 to 9000 psi

@ 1500 psi/min and hold at 9000 psi

Column Material	Highest attainable P
Untreated	8250 psi
PMHS	8250 psi
Ultra 2	>9000 psi

packing, or localized high pressure points. If the cracks are formed by the high pressure of packing, then the pressure test should give 6000 psi as the highest attainable pressure. Since this is not the case, this explanation can be ruled out. If the cracks are caused by localized high pressure points, then the untreated and PMHS columns should have similar failure rates. While 90% of the untreated columns failed, only 30% of the PMHS columns failed. These column materials have the same highest attainable pressure but not the same column success rate. The thin deactivation layer provides some protection from particle induced scoring but not enough. Thus, the scoring phenomenon appears to be the main culprit of column failure. Essentially, the packing particle scratches the inner wall and the hydrated fissure then propagates radially. The relatively thick film of the Ultra 2 column almosts eliminates this problem as evidenced by its high success rate.

As tubing diameter decreases, the resiliency and durability of the fused silica increases. Therefore, it is possible to pack smaller diameter columns (<200 μm) with a high driving pressure (6000 to 7000 psi). The work of Novotny, Jinno, and the author are some instances of this improved column integrity. Consequently, this investigation indicates that surface pretreatment is useful for fused

Table 7

Packing Test Results

Packing Test

Conditions: Constant Pressure Packing at 6000 psi

Slurry Ratio - 1g/4ml

Slurry Solvent - Isopropanol

Displacement Solvent - Acetonitrile

Column Material	% Failures
Untreated	>90
PMHS	30
Ultra 2	10

silica columns larger than 320 μm or for columns that must endure constant high pressure.

Effects of the Knox-Parcher Ratio in Microcolumn LC

In 1969, Knox and Parcher examined the effects of column internal diameter (d_c) and packing particle diameter (d_p) on the efficiency of liquid chromatography columns (18). They determined that the ratio of column id to particle id, now termed the Knox-Parcher ratio and designated by p, reached a minimum in reduced plate height (h) at $4<p<6$. For $6<p<8$, a sharp increase in h was observed before reaching a relatively constant value at p greater than 8 (see Figure 1). Knox and Parcher theorized that a nonuniform cross-sectional packing structure at high values of p resulted in several flowpaths of greatly different permeability. Thus, the solute molecules traverse the column with a wider range of velocities and peak dispersion increases. For low values of p, only the wall ordered packing structure is possible; therefore, path dispersion is reduced.

A recent study by Kennedy and Jorgenson (54) showed similar results for columns of 20 to 50 μm id packed with 5 μm spherical particles. Once again, an increase in reduced plate height is observed as p increases from 6 to 8. Kennedy

and Jorgenson offer three explanations for the improved efficiency in small Knox-Parcher ratio columns. The first theory is based on Knox's original study, namely that the mobile phase flow inhomogeneities are decreased. The second reason involves a decrease in retention inhomogeneities. The minimized transcolumn variations in packing density result in more uniform capacity factors (k') and zone capacity factors (k"); thus, peak broadening is reduced. The final explanation states that the smaller column diameter allows the solute to enter the various flow streams more rapidly. Consequently, the flow inhomogeneities are time averaged and the system is allowed to diffusionally relax.

As the interest in microcolumn liquid chromatography grows, column theory and technology must also be developed and modified. Common internal diameters for fused silica range from 200 to 530 μm. Typical HPLC packing material consists of small diameter (<10 μm), monodispersed particles. While these are the easiest column and particle diameters to obtain, they may not be the best suited for optimal performance in microcolumn LC.

This study examines the Knox-Parcher ratio in packed fused silica columns of 530 μm internal diameter. While sitting in the audience, listening to Kennedy present his results, it occurred to the author that Knox-Parcher ratio could readily be altered by changing particle diameter

rather than column diameter. The ramifications of this action are explored later in this section. Spherical silica particles of 5 to 120 μm in diameter are packed and evaluated under identical conditions. Whereas Knox and Parcher used an unretained probe and Kennedy and Jorgenson used a reverse phase system, this study employs normal phase chromatography to examine solute dispersion. A comparison of column permeability, efficiency, and particle size provides insight into the capabilities of 530 μm id fused silica packed columns.

The extracolumn variance for this study is determined by the linear extrapolation method. In this technique, the column is removed from the system and a small diameter tube is used in its place. The sample is injected into the system at a typical flow rate and the variance of the resulting peak is plotted against the volume of the connecting tube. Several iterations of this with smaller volume connecting tubes are performed and the plot is extrapolated to zero tubing volume to determine the extracolumn volume.

Van Deemter curves for each spherical particle size are illustrated in Figure 20. As anticipated, the 5 μm packed column shows very small changes in plate height as linear velocity is increased. In addition, the 120 μm packed column shows a drastic change in plate height as linear velocity increases. This is a result of the greater mean free path

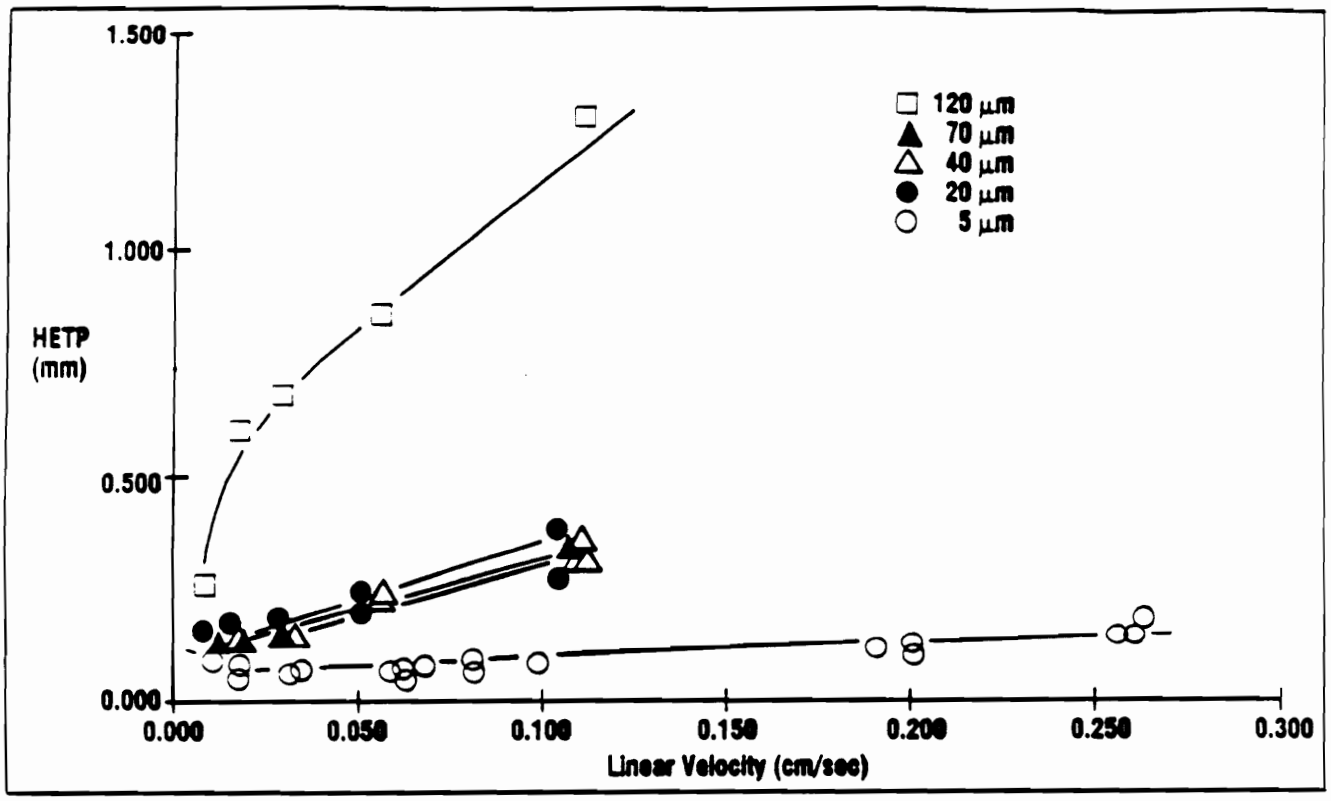


Figure 20. Van Deemter Curves for the various Particle Sizes. Conditions on pg 66. (All experimental data is plotted)

the analyte must traverse before coming into contact with the stationary phase. The flow must be greatly reduced in order to obtain the highest possible efficiency since diffusion is slow in liquids. The most interesting aspect of this figure are the curves obtained for the 20, 40, and 70 μm particles. Note that the data are clustered together and the curves show similar slopes in spite of the large change in particle diameter. Since these columns encompass the transitional range of the Knox-Parcher effect, some reduction in peak dispersion for the larger particle (70 μm) columns is apparent. This is in rough agreement with the results of Kennedy and Jorgenson (54). From their work (Figure 21), a column with a p of 4 showed a lower minimum in reduced plate height and a flatter van Deemter curve than a column with a p of 10. In this study, these gains are equally balanced by the use of larger particles. The net result is that a 70 μm particle column has similar operating properties (e.g. optimal flow rate) to a 40 μm particle column.

An experimentally derived Knox-Parcher plot is shown in Figure 22. The plotted values are given in Table 8. Initially, the 5 and 20 μm packed columns showed unexpectedly high values of h in the Knox-Parcher plot. By correcting for the extracolumn variance, the h values obtained for these particle sizes showed better agreement

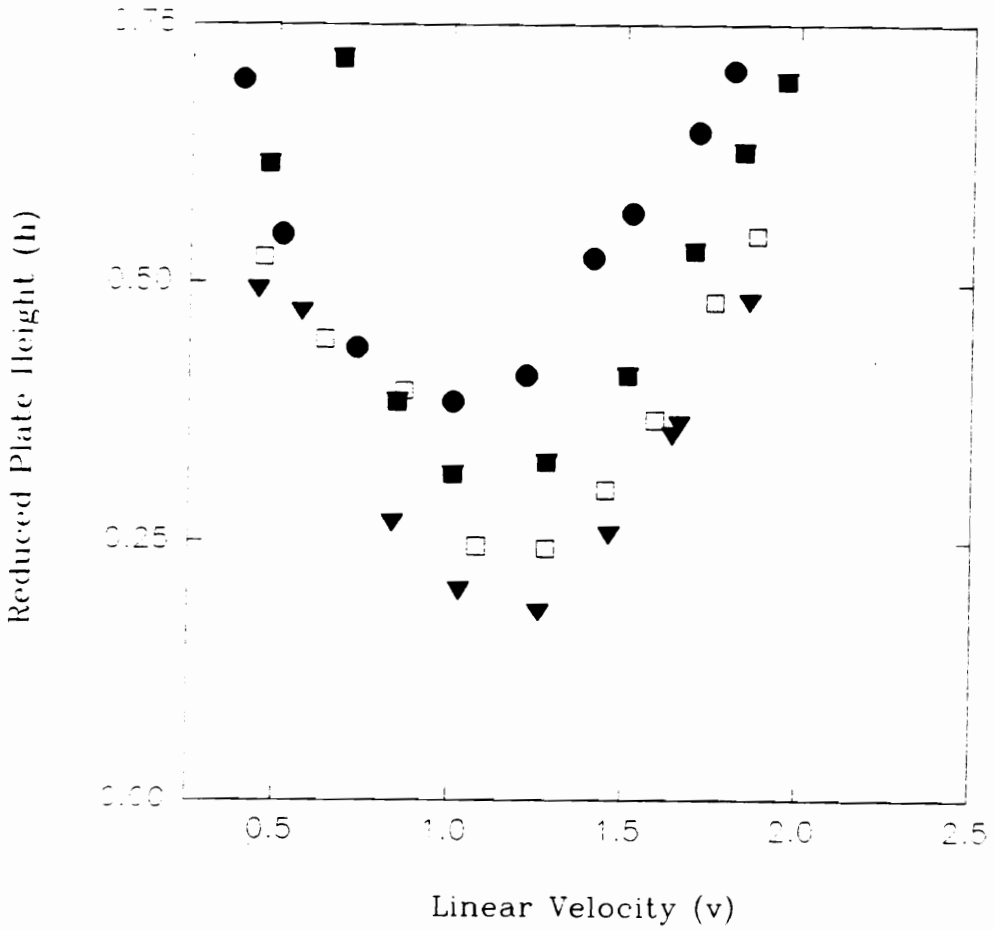


Figure 21. Van Deemter Curves from Kennedy's Work (Ref 54). Circles = 50 μm column, Filled Squares = 42 μm column, Open Squares = 25 μm column, Triangles = 21 μm column

with the anticipated sigmoidal curve. Since the reduced plate height is normalized to the particle diameter, a slight change in H shows a significant change in h for a small particle column. In addition, the inability to densely pack 5 and 20 um particles by gas pressure or low liquid pressure could account for the remaining deviation. For the columns with 4<p<7, other studies (18,54) found a relatively constant value for the reduced plate height. The results of this study show a slight increase in h for p = 4.42. While the 120 um particles appeared to pack densely, the possibility of bridging (14,54) could be the cause of the higher than expected reduced plate height.

The power of the Knox-Parcher effect is its ability to make larger particles behave as efficiently as smaller particles. From this study, consider the following results. For spherical particles,

$$H_{70} = 43.4\ \mu m$$

$$H_{40} = 40.8\ \mu m$$

Thus, the two columns have almost identical plate heights. Recall that pressure drop is a function of $1/d_p^2$. Therefore, a 70 μm particle will give between 1/3 and 1/4 the pressure drop of a 40 μm particle. However, for an equal column length, the 70 μm particle gives the same number of theoretical plates as the 40 μm particle when the column diameter is 530 μm. In terms of constant pressure drop, the

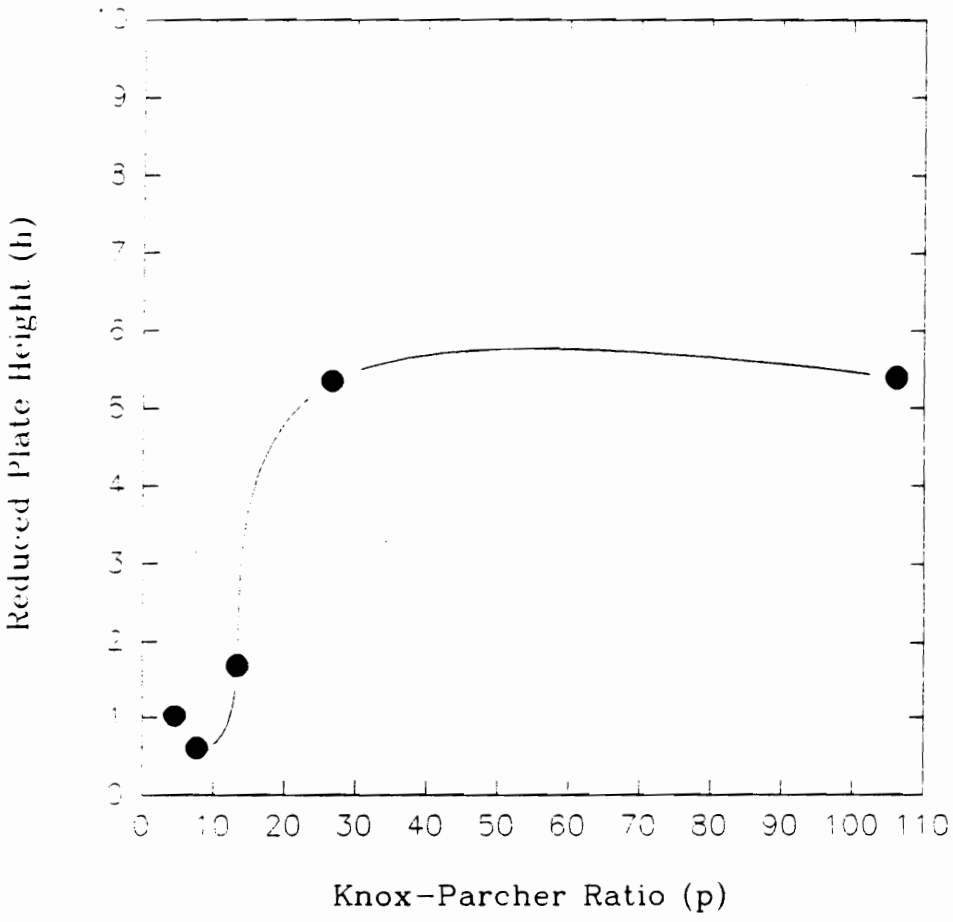


Figure 22. Knox-Parcher plot from this work

Table 8

Numerical Values* for the Knox-Parcher plot before and after correcting for the system variance

d_p (μm)	h (observed)	h (corrected)
5	24.7 ± 0.5	9.4 ± 0.2
20	11.4 ± 0.2	3.8 ± 0.1
40	3.85 ± 0.08	1.80 ± 0.04
70	1.90 ± 0.04	1.24 ± 0.03
120	2.21 ± 0.04	1.31 ± 0.03

* Reduced velocity fixed at 10^{-4} $(cm^2/sec)/D_m$

70 μm particle can give more than three times the number of theoretical plates as the 40 μm can, simply by increasing column length. In addition, the van Deemter curves are quite similar for 40 and 70 μm particles. Thus, the 70 μm particle columns can be used at elevated flow rates and will sacrifice the same amount of efficiency as the 40 μm particle columns but at greatly reduced pressures. These points are illustrated in Figure 23 by the comparable chromatograms obtained with 40 and 70 μm particle columns. The only difference between the chromatograms is the particle size.

The Knox-Parcher ratio is a measure of flow path and its effect on column transport efficiency. From the previous studies, an interesting result is obtained. By changing column diameter and holding particle size fixed, the Knox-Parcher plot becomes H versus p rather than h versus p. From the most recent work, the conclusion to be drawn is that H is a function of column diameter. The van Deemter equation for HPLC is given by Scott (99) as {11}

$$H = A + B/v + C_m v + C_s v \qquad \{11\}$$

where $A = 2\lambda d_p$

$B = 2\gamma D_m$

$C_m = f_1(k')d_p^2/D_m$

$C_s = f_2(k')d_f^2/D_s$

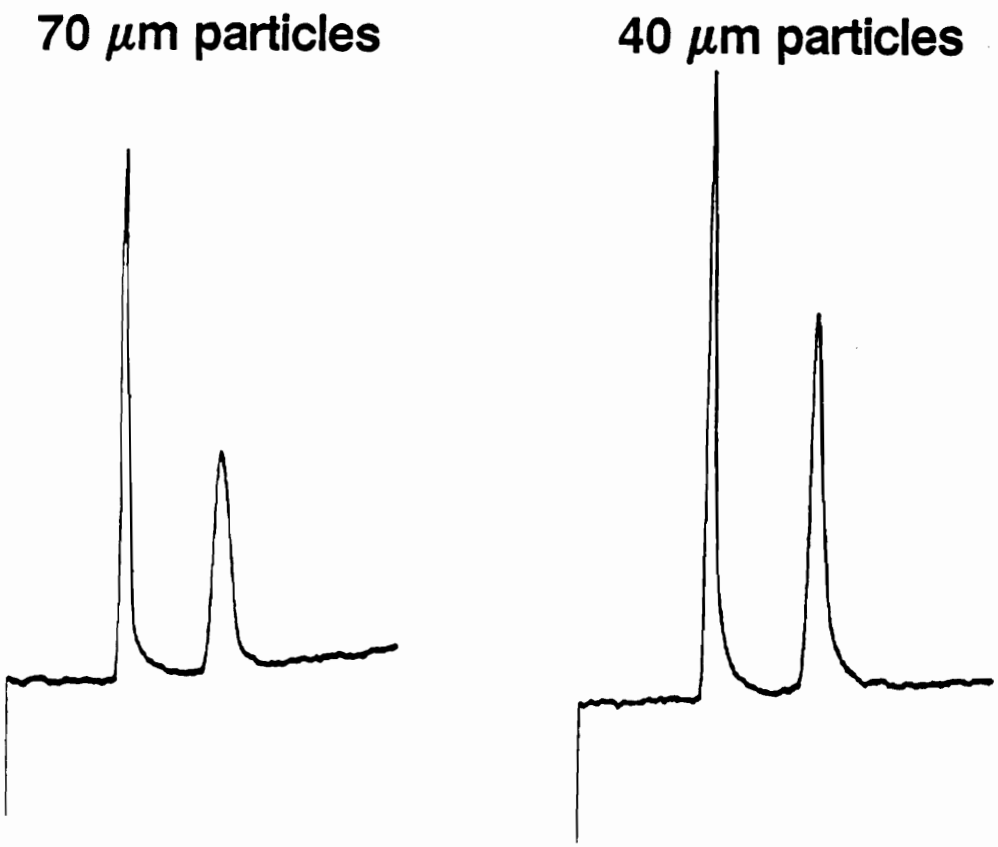


Figure 23. Chromatograms from the 40 and 70 μm silica gel particle columns

The "A" term deals with the multiple flow paths that are possible in a packed column. The "B" term addresses longitudinal diffusion and the "C" terms quantify the resistance to mass transfer of the solute bands between the phases.

Since the column wall "imposes" a structure on the packing material, the column diameter has a direct impact on the "A" term. This term can be further broken down into five discreet sources of differential flow paths (Figure 24). These are transchannel, transparticle, short range interchannel, long range interchannel, and transcolumn effects (14).

The transchannel term arises from the laminar flow profile that occurs between two adjacent particles. Solute molecules that travel in the central region between particles have a higher local velocity than those molecules that travel closer to the particle. This general cause of peak broadening has been well characterized (100). The transchannel effect makes a relatively small contribution to the "A" term since the solute molecules are rapidly interchanging their intraparticle flow velocities.

The transparticle term arises from the flow profiles through the support. Most chromatography supports are high surface area, microporous solids. Flow not only travels around these materials but directly through them. The

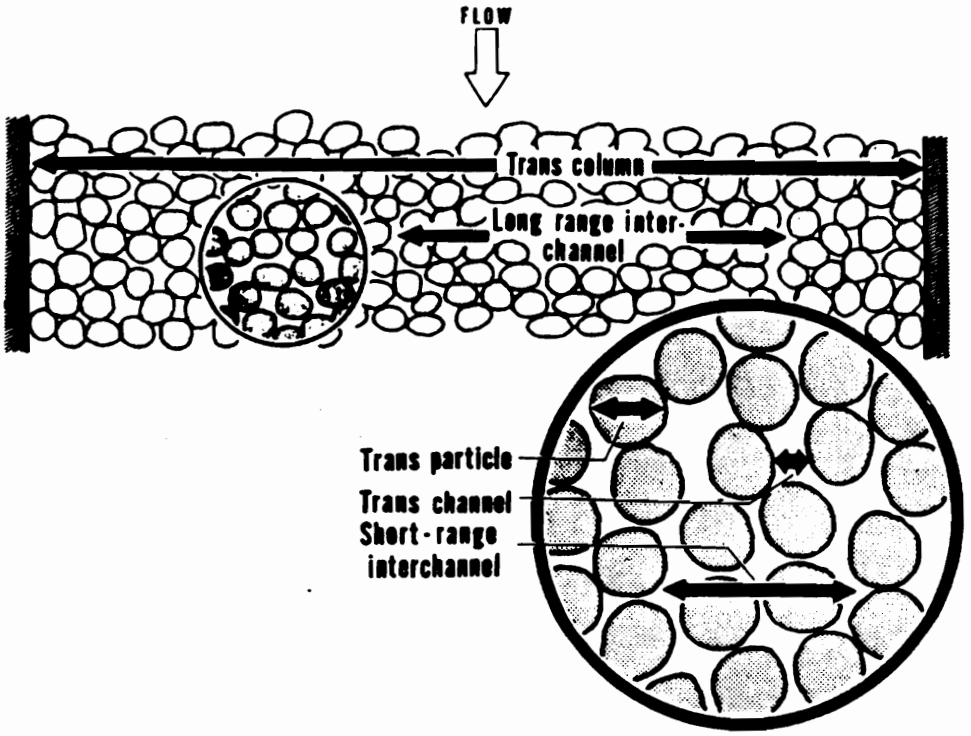


Figure 24. Five Sources of "A" term broadening

(Ref 14)

arrangement of pores is impossible to predict or model since the particles themselves are not homogeneous in size. The large heterogeneity works to some advantage since it is likely that the solute molecules will traverse a similar mean path.

The short range interchannel effect is the first term to deal with the actual packing structure rather than particle anomalies. Within the core of the column, small regions of very tightly packed particles have been observed (14). These regions are typically bordered by loose packing structure. Consequently, a permeability difference exists on a very close local range and flow velocities are not uniform. The entire cluster of tight and loose packing is random and exists throughout the cross section of the column. Since no two clusters have identical geometries, these differences lead to the long range interchannel effect. The contributions of these two terms is essentially impossible to quantify.

The final contributing term, the transcolumn effect, is of significant importance to this research. In the center of the column, the only influence on the arrangement of the particles are the particles themselves. With most packing techniques, the particles are forced tightly together to consolidate the bed and make it unaffected by mobile phase flow. Near the wall of the column, the particles can not be

packed as closely together. This wall imposed structure is much more fluid permeable and creates a large scale difference in flow velocities.

The Knox-Parcher effect examines the composite of the last three effects since the particle differences are averaged over such a large population that a mean value is representative. Initially, the enhancement of efficiency observed by optimizing this ratio appears to effect only the "A" term of the van Deemter equation. By making the flow paths more homogeneous, the multipath effect would obviously diminish; however, an additional improvement is seen in the mass transfer terms. Because the analytes encounter a more uniform stationary matrix, their exchanges between the phases become more reproducible. Consequently, the broadening of the peaks is smaller. This effect is also noted at higher flow rates. Figure 25 illustrates the effect of the Knox-Parcher ratio at optimal and 100 times optimal linear velocity.

Note that at low Knox-Parcher ratios, the increase in plate height is proportional. These are the well packed columns that show theoretical minimums in h. At the high p values, the inability to densely pack the columns is evident. Since the 5 μm particles are not tightly packed, the column has poor performance at both high and low flow rates. The overall trend of this plot indicates that at

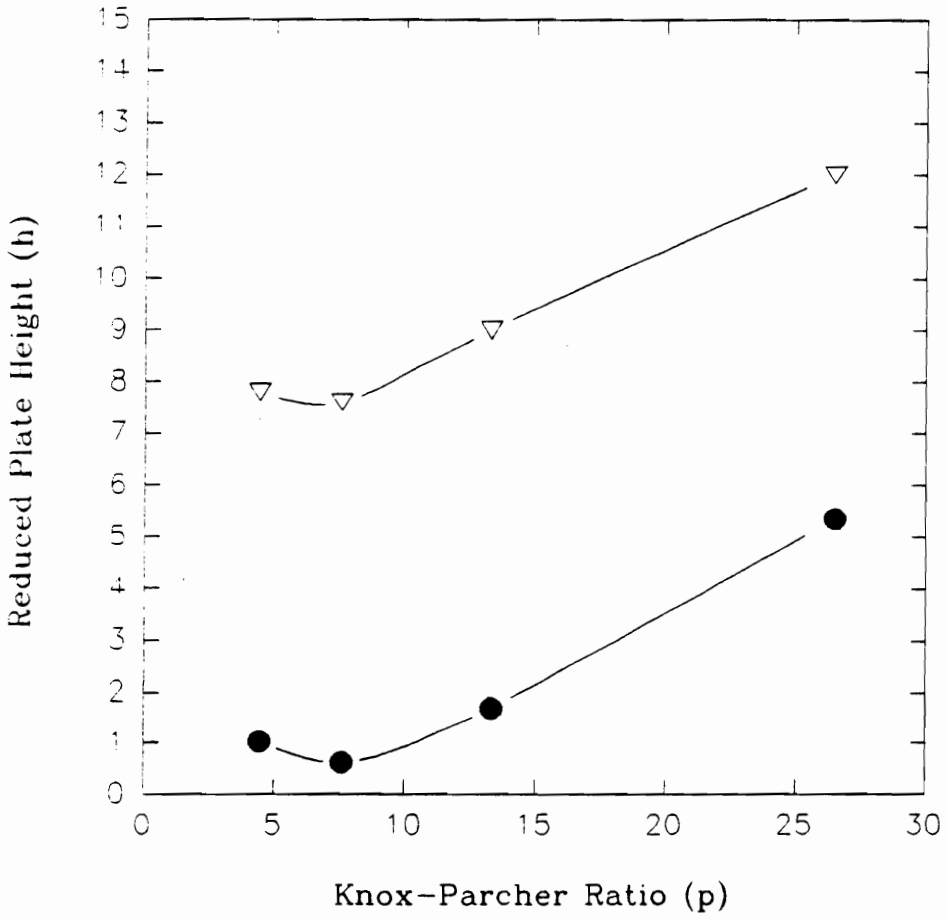


Figure 25. Knox-Parcher Plot at Optimal (Circles) and 100 times Optimal Flow (Triangles) (triplicate measurements)

higher flows, the differences in cross-sectional packing structures and solute transport mechanisms are maintained. At first glance, this is contrary to the concept of diffusional relaxation as an explanation for the improvement in column performance (54). Typical values for liquid diffusion coefficients are 10^{-5} to 10^{-9} cm^2/sec. From Knox (18), however, the column length required for a standard solute to diffuse from the center of the column to the wall is given by (12)

$$d_c^2/L = 2.4\ d_p \qquad (12)$$

For the columns in this study, Table 9 gives the necessary lengths. Even for the longest column length required, the solute will still be able to traverse the column cross-section 11 times. For standard bore columns (4 mm) with the same 5 μm particles, the sample will travel only once from the center to the wall in 16.96 cm of column length. While diffusional relaxation is not practical for large bore columns, it is generally accepted that the analytes never see the wall packing structure but are transported through the dense particle imposed structure. If this is true, then micropacked columns should never be able to attain reduced plate heights similar to their larger counterparts. This work, along with the work of Kennedy and

Table 9

Column length required for Test Solute to diffuse from the core to the wall

p	L (mm)
4.49	0.951
7.57	1.60
13.3	2.81
26.5	5.62
106	22.5

Jorgenson clearly demonstrate that the particle imposed structure is not necessarily the best packing geometry.

Multidimensional μLC/CZE

The development of an interface between microcolumn LC and capillary zone electrophoresis is discussed and the potential of such a system investigated. The use of a multidimensional system should alleviate some of the trace level detection and surface modification problems associated with CZE. The effects of organic solvent in the sample on aqueous free zone electrophoresis are examined. Several interfaces are explored and their merits and shortcomings discussed.

The power of capillary zone electrophoresis has become more apparent since its incipience (102,103). This separation technique can provide plate numbers from 10^5 to 10^6 in minutes with very simple instrumentation. Biological separations that were labor intensive can now be automated. It appears that CZE will provide a great deal of answers for the appropriate users.

Capillary zone electrophoresis is a separation technique where the solutes are resolved by their charge and mobility. A capillary of 20 to 100 μm internal diameter is filled with a buffer solution and the ends of the tube are

immersed in separate reservoirs. The electrodes of a high voltage supply are placed in the reservoirs and a potential of 5 to 30 kV is applied across the capillary. An electrical double layer forms within the tube and, for fused silica tubing, a strong flow develops called the electroosmotic flow. Depending on the pH of the buffer, this flow can be in the anodic or cathodic direction. The flow is so strong that not only do the oppositely species move but the uncharged and similar charged solutes are also migrated towards the outlet. The sum of the electrophoretic and electroosmotic flows permit the separation of charged solutes while all uncharged species move at approximately the same rate.

The sample is introduced by siphon, gas pressure, vacuum, or electrokinetically. While the first three of these methods are obvious, the latter is unique to CZE. An electrokinetic injection is performed by replacing the inlet buffer reservoir with a sample reservoir and a relatively low potential is applied to the system for a short period of time (1 to 10 seconds). A small amount of sample is swept into the capillary by electroosmosis. The sample reservoir is removed, the buffer reservoir is reconnected, and the separation voltage is applied. Detection is typically performed on the separation capillary with a spectroscopic technique although some workers have demonstrated post-column detection (107,108).

Of the difficulties still associated with CZE, two substantial problems are poor trace level detection and modification of the separation capillary by the sample. While mass detection limits are now at the attomole level, the corresponding concentration level is only in the ppm range (104). This means that sample preparation will be required to attain the detection limits achieved by liquid chromatography. The latter problem leads to variations in retention time and potential sample loss (105).

The recent increase in the use of microcolumn LC for various separation systems led the authors to investigate the potential of these two micro techniques coupled together. The advantage of using packed columns in chromatography is their large sample capacity. In addition, it has been demonstrated that solute focusing can enable larger than optimal volumes of sample to be injected without seriously degrading column performance (5,22,92). This suggests that a packed capillary column could act as a preconcentrator for CZE and as such, it would alleviate the first problem cited. Furthermore, microcolumn LC adds an orthogonal degree of selectivity to the separation. With the appropriate operating conditions, the analyst could limit the sample for electrophoresis to slices of the desired solutes. By minimizing the amount of adsorbable material

entering the capillary, shifts in analyte retention should be reduced.

Thus, the objective of this research was to develop a multidimensional interface between microcolumn LC and CZE. Theoretical enhancements are discussed and several interfaces are examined.

Theoretically, two factors influence the concentration of analyte passed between the separation techniques in a heartcutting multidimensional system. They are the sample slice width and time and the sample capacity of the initial separation mode. The former parameter is demonstrated in Figure 26. Since most chromatographic bands can be represented by a Gaussian function, this will serve as a reference point. Assume that the entire band is contained within three standard deviations from the mean and each standard deviation represents one volume slice. Thus, the concentration of analyte determined is a given mass in six volume slices. For plus or minus one standard deviation from the mean, 68.3% of the analyte is present in 1/3 of the solvent. This suggests a concentration increase of 2.0 for the analyte.

In terms of capacity, it has been shown several times that larger than optimal injection volumes are possible in packed capillary LC (5,22,92). An example of this is given in Figure 27. From theoretical calculations, the column

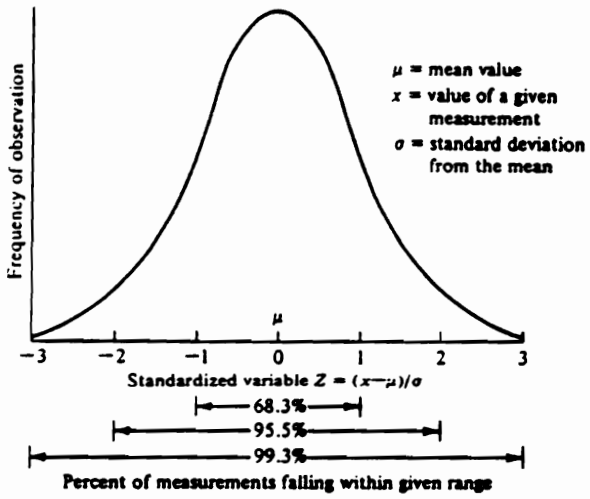


Figure 26. Gaussian Function

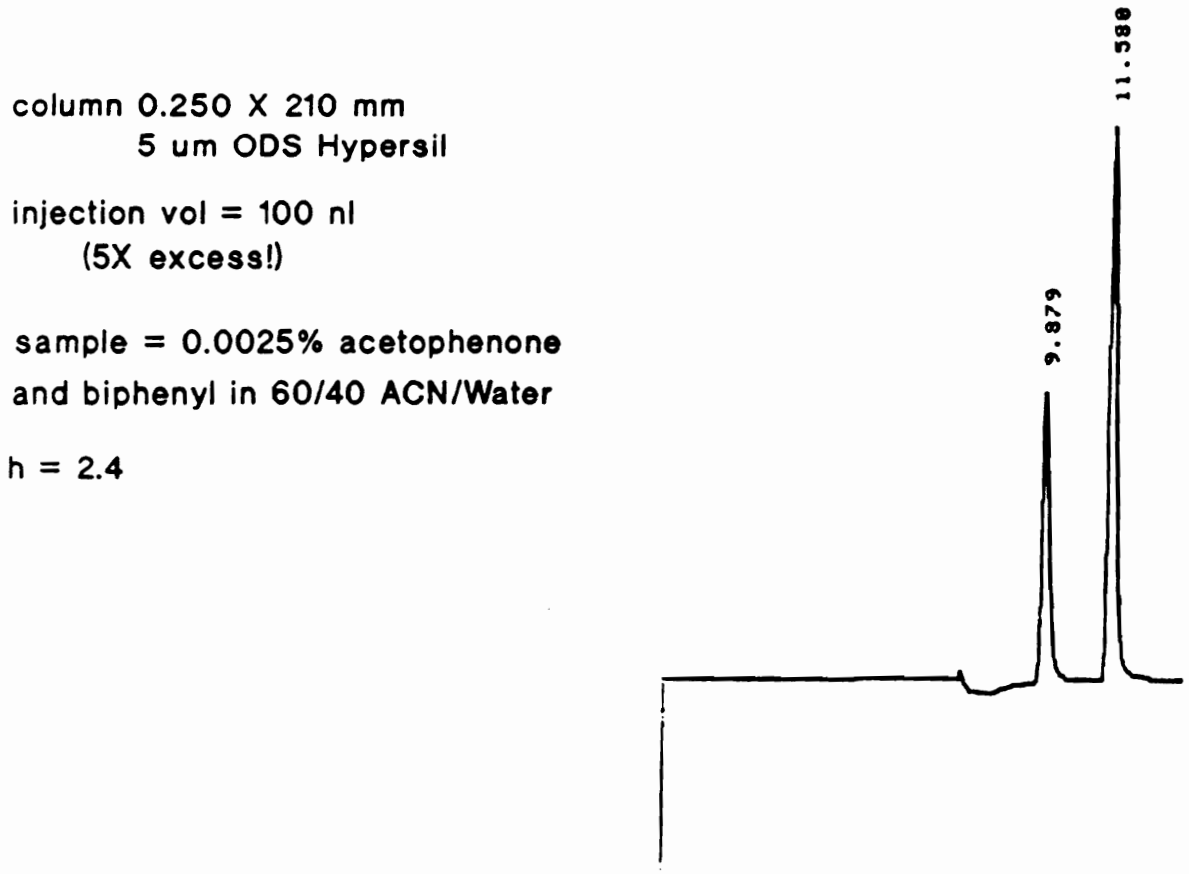


Figure 27. Packed Capillary Chromatogram with 5 times larger than optimal sample volume

should begin to lose efficiency when the injection volume reaches 20 nl. By dissolving the sample in a weaker solvent, the column is able to maintain its efficiency at a five fold increase in injection volume. The combination of the two factors reduces the detection limits by an order of magnitude.

An unknown parameter that required investigation is the effect of the sample solvent composition on CZE. It has been previously demonstrated that as long as the separation buffer and the sample buffer are identical in composition, no significant loss in performance is observed. Most difficult LC separations use gradients to achieve resolution; therefore, a mismatch between buffers is probable. To elucidate the effects of such a mismatch on retention and efficiency, samples with varying amounts of organic modifier were prepared and evaluated. Plots of these results are given in Figures 28 and 29. Note that the scale in Figure 28 is greatly expanded in the retention time axis. While a slight change is observed as % organic is increased, there is statistically no significant difference between conditions. A comparison of efficiency versus % organic (Figure 29) indicates a strong effect of modifier on electrophoretic performance. A 30% change in sample solvent composition results in a 60% loss in theoretical plates. Although the separation performance is still high (>50,000

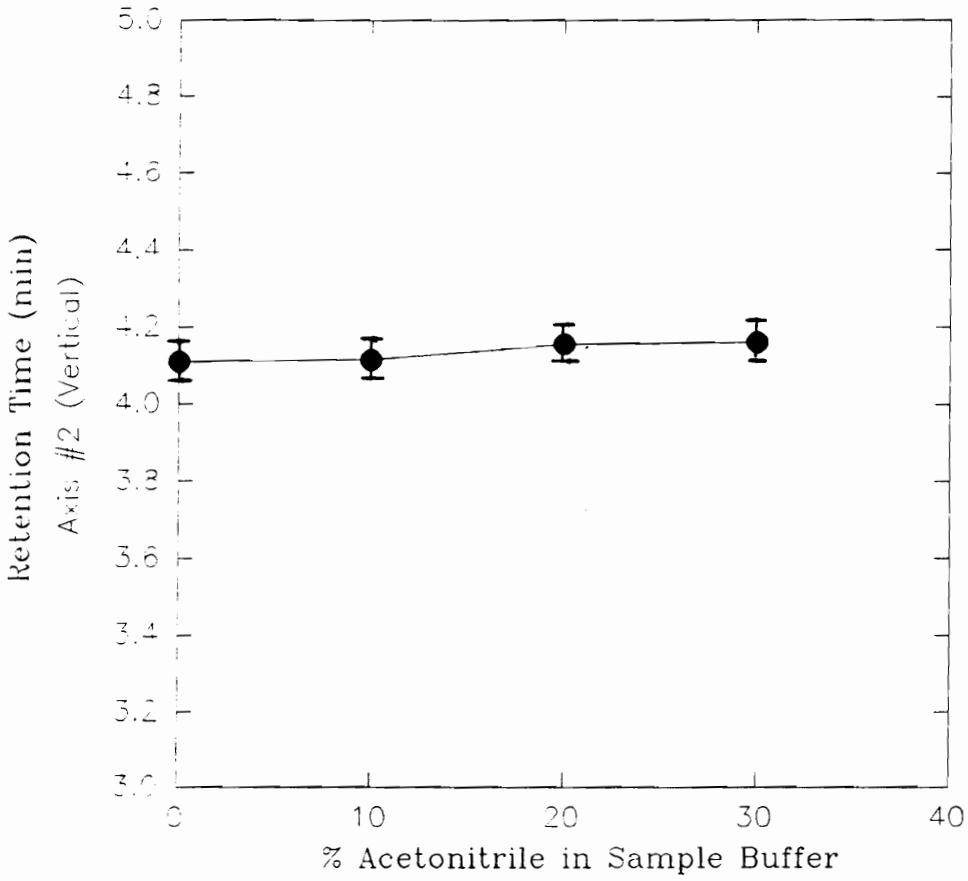


Figure 28. Retention Time vs. % Organic Modifier in the Sample Buffer

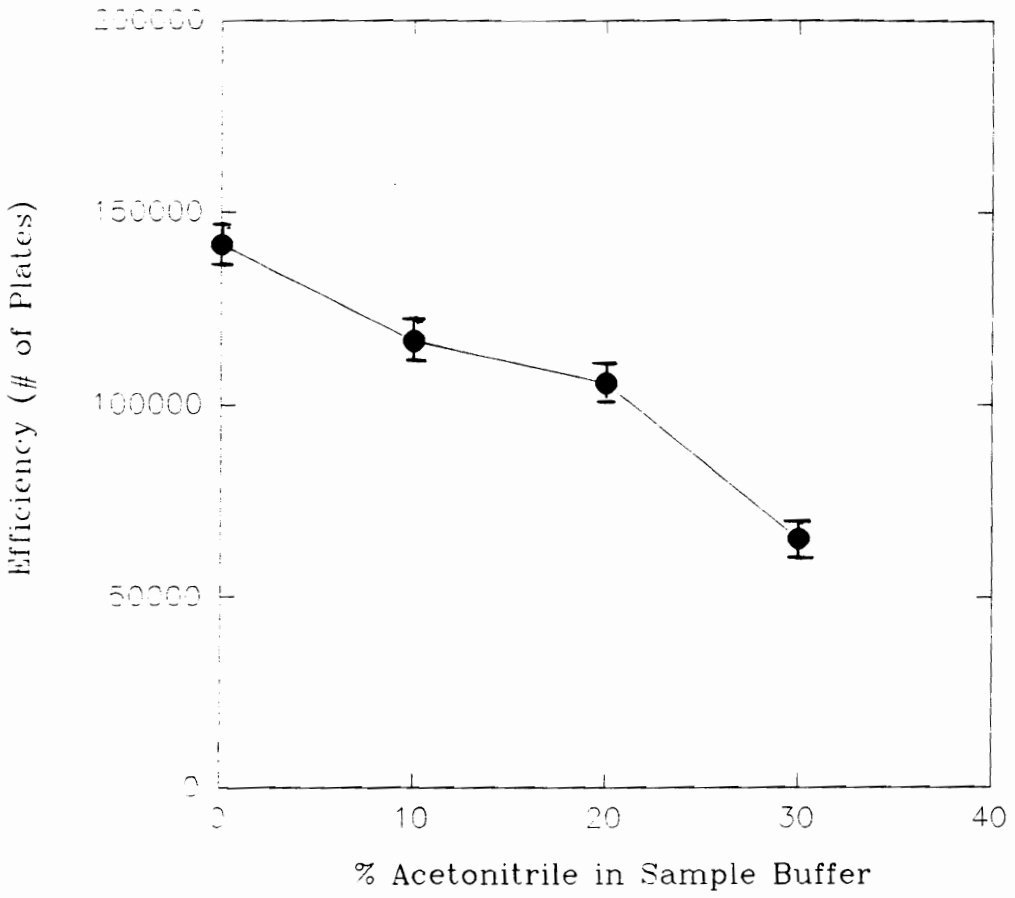


Figure 29. Efficiency vs. % Organic Modifier in the Sample Buffer

plates), complex mixtures may require more plates to achieve resolution.

Because LC and CZE are both liquid based separation techniques, a simple valve arrangement appears to be the easiest interface for the system. In the hopes of potential automation, an actuated valve seemed desirable. A Valco 4 port valve served as a manual switching device that allows very small (60 nl) slices to be sampled. This particular arrangement, however, was quite problematic. With a high impedance multimeter, the measured current lost through the valve constituted almost 75% of the total current in the system. Consequently, a concomitant loss of potential occurs through the capillary such that the electroosmotic flow becomes to slow to be practical. An increase in voltage would reduce the problem but potentials higher than 50 kV would be necessary and the chance of corona discharge would be too great. The steel valve also serves as a catalytic surface for bubble formation. To avoid some of these problems, a metal free 6 port valve was modified to perform like a 4 port valve. Unfortunately, a functioning interface is not possible with this valve due to the relatively large (250 μm) internal passages. These passages allowed bubble formation as a result of the high applied potential.

An additional interface design is given in Figure 30. The sample stream is passed through a Tefzel^{TM} tube. The

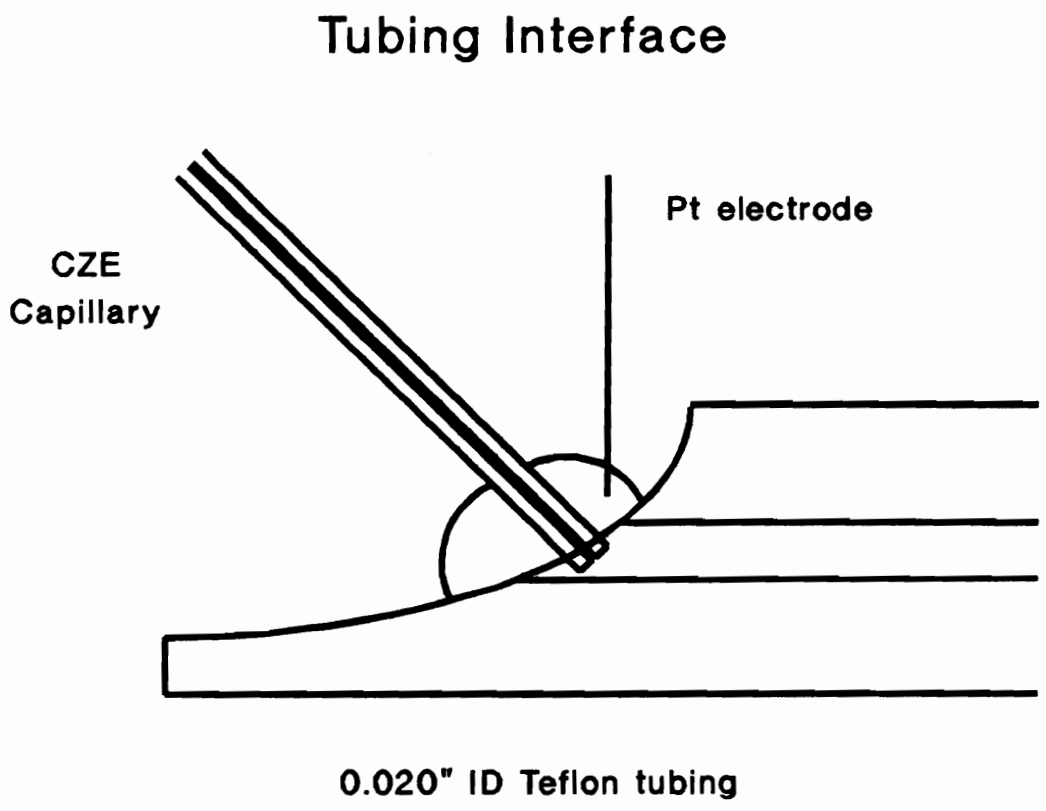


Figure 30. Tubing Interface

inlet to the capillary is positioned in the end of the tubing and a drop of buffer is placed at this junction to allow electrical contact. An electrokinetic injection is made and the capillary inlet is then replaced into the buffer reservoir. A reference electropherogram is given in Figure 31 and a multidimensional result is given in Figure 32. Note how retention time reproducibility is very good (0.4% absolute error). These results also indicate how sampling time can affect the concentration of the sample. In this instance, the sample was not taken from the LC band maximum but from the trailing wing.

Recently presented work by Bushey and Jorgenson (77) showed a similar system with a complementary design. These authors dealt with the interface problem by placing the applied potential at the outlet of the CZE. Thus, bubble formation is eliminated and analysis time is decreased.

A comparison of two standard deviation volume slices for various column internal diameters is given in Table 10. From a consideration of flow rate, peak volume, and tubing interface volume, columns of 1 to 2 mm internal diameter appear to be a reasonable compromise. Columns larger than 2 mm may prove interesting especially if preparative throughput and purity for biological compounds is desired. Columns smaller than 2 mm would more closely match peak volumes to the CZE and make sample slicing more difficult.

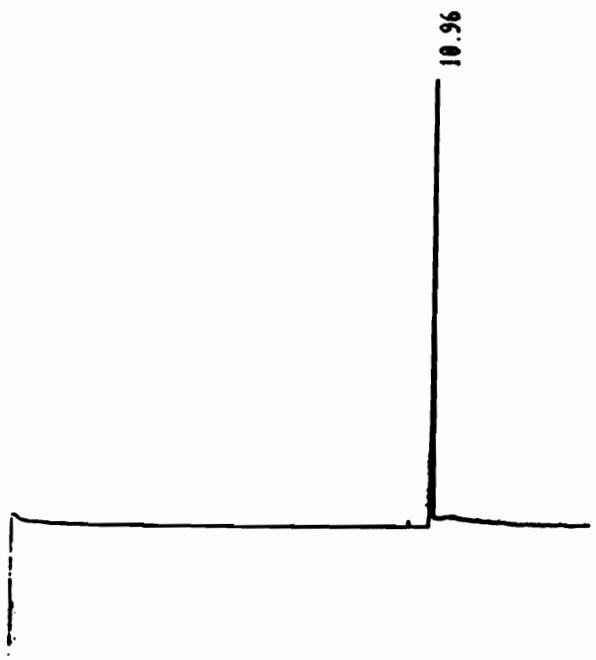


Figure 31. Reference Electropherogram
Caffeine in 0.02 M phosphate buffer pH=7, UV Detection @ 220 nm, 5 sec injection @ 3 kV, 20 kV migration potential

Figure 32. Multidimesional Electropherogram
Same conditions as Figure 31 except sample from Tubing interface (Flow Injection Mode)

This work indicates some of the potential of multi-dimensional microcolumn LC/CZE. While differences in separation buffer and sample buffer do not affect retention time reproducibility, substantial losses in efficiency are observed. Interface placement is critical to the success of the system with the design of Bushey and Jorgenson being superior in this aspect. The tubing interface allows any sampling technique and is easily constructed but is not easily automated. Further investigations should examine the effect of the sample buffer composition on the volumes introduced into the CZE. In addition, a hybrid of this design and Bushey's system should provide greater usability.

Table 10

Peak Volumes (2σ) for Several column diameters

Column Diameter	Optimal Flow Rate	Peak Volume (2σ)
250 μm	1.2 μl/min	0.24 μl
1 mm	30 μl/min	6.0 μl
4 mm	480 μl/min	96 μl
10 mm	3000 μl/min	600 μl

Assumes 10 minute analysis

dp = 5 μm

N = 10,000

Chapter 5

Conclusions

Conclusions

The focus of this dissertation was to examine and investigate the advantages and disadvantages of microcolumn liquid chromatography and assess the veracity and value of these points. The investigations conducted in this thesis explore the prominent fortes and weaknesses of miniaturized LC and the results indicate that some of the common conceptions about these techniques are not founded. Finally, the future of small scale LC is projected.

The five cited advantages of microcolumn LC are the smaller sample and phase consumption, an "increase" in sensitivity, easy column synthesis, higher obtainable efficiency, and easier interfacing to other techniques. The first advantage is intuitively obvious; the remaining points, however, are examined either directly or indirectly in this research.

The purported enhancement in sensitivity is clarified by the microbore ion chromatography studies. With concentration dependent detectors, a smaller diameter column can be more sensitive than a larger diameter column if the volumes of injection and detection are held constant. In such a situation, the smaller column is eluting the same mass of solute in a fraction of the solvent. When the systems are scaled to the column diameter, sensitivity is

identical. The laws of spectroscopy and electrochemistry do not change as a function of column size. However, in trace analysis or sample limited systems (109,110), the reduction in sample required proves beneficial. More than optimal injection volumes can restore the trace level sensitivity needed. In systems with weakly eluting sample solvents, large excesses of sample can be chromatographed without degrading performance.

Microcolumn synthesis is not easy by any measure. The surface pretreatment investigation explores the limits of packed fused silica columns. For those applications where high pressure is employed, pretreatment of the tubing wall will extend the durability of the column. Furthermore, the wall coating will have a negligible effect on the chromatography. For routine applications typical of standard HPLC (5 to 30 cm long columns), gentler versions of traditional packing techniques result in good performance (h=2) columns.

The ability to obtain high efficiency in HPLC will always be shadowed by the inherently poor diffusion properties of the system. However, the optimization of the Knox-Parcher ratio can increase theoretical plates per unit time by 50%. An hour analysis will still take 40 minutes with an optimized system but in high volume analyses, any decrease in time is worthwhile. When the researcher is

willing to wait for high efficiency separations, this phenomenon can provide enormous resolving power. For the microchromatographer, this phenomenon can be a powerful tool. The general conception that narrower columns are inherently more or less efficient than wider columns when operating above the Knox-Parcher transition is simply not true. Theoretical efficiencies are illustrated in this thesis for micropacked columns. It merely depends on the attention to detail such as system design to achieve theoretical plate heights.

The final advantage, easier interfacing to other techniques, has been demonstrated by other workers for a variety of systems. The multidimensional μLC/CZE research was not prompted by this particular point but by the "enhancement" in sensitivity factor. Nevertheless, the strengths of the author's interface when compared to Bushey and Jorgenson's design (77) is the representative nature of the sample. Interaction between the sample and the interface is minimized by matching the peak volumes between μLC and CZE. In this respect, the microcolumn does allow easier interfacing to CZE.

The disadvantages cited are brittle or weak columns, poor column bed stability, stringent instrument design, and the lack of commercial instrumentation. The surface pretreatment study linked the brittleness problem to inner

wall scoring and provides a simple solution. While this thesis did not rigorously examine bed stability, it was observed that in low pressure packed columns, bed compression is present. For high pressure packed columns, though, minimal bed compression is noticed. Well packed columns have always been more of an art than a science. Instrument design is critical and the apparatus must be flexible. Problems with injector extracolumn volume can sometimes be circumvented by the appropriate choice of sample solvent. Detection still presents difficulty. With this last thought in mind, some commercial vendors have managed to introduce micro systems. At time of this writing, Isco (Lincoln, NE), Jasco (Easton, MD), Carlo Erba (Milan, Italy), Brownlee (Santa Clara, CA), and Hewlett-Packard (Waldbronn, Germany) have introduced microscale HPLC components.

What is the future of packed capillary columns in HPLC? Three dominant factors are readily visible. First, the domain of analytical techniques is rapidly expanding. The environmental testing market is growing at a fantastic rate. The need for portable systems capable of routine HPLC analyses is growing. The closer the analytical technique gets to the source, the faster the turnaround of information. Second, the expense of HPLC materials and consumables is so great that major companies have developed

limitation criteria. Chemical giants like DuPont and Ciba-Geigy have internally decreed that HPLC solvent consumption shall be cut in half in five to eight years (111). The number of analyses that these companies plan to perform will obviously not be reduced 50%. Microcolumn LC seems like one of the most logical alternatives since methods will not have to be redeveloped. Finally, the scale of HPLC separations is gravitating towards smaller and smaller sample sizes. For instance, the analysis of neurochemicals and brain chemistry reactions requires appreciably limited sample volumes. Other biological analyses would preferably use small volumes.

With these points demanding the use of high performance liquid chromatography, miniaturization seems like the most logical development. In light of this, microcolumn LC appears to have a promising future.

Chapter 6

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Appendix

Integral form of the coupled equation

$$h = \sum_{1}^{i} (1/2l_i + 1/\infty_i v)^{-1} + 2\tau/v$$

where

h = reduced plate height

l = packing coeffient

∞ = configuration constant

v = reduced velocity

τ = bed tortuosity

Vita

William Henry Wilson was born June 25, 1964 in Doylestown, Pennsylvania. He graduated 22 of 838 from Council Rock High School in June of 1982. In the fall of that year, he began undergraduate studies at Bucknell University in Lewisburg, Pennsylvania. During the University's January Program, he performed synthetic research on the anticancer agent, taxodione, and iron tetraphenylporphyrins. In his senior year at Bucknell, he developed an HPLC experiment for the senior level analytical chemistry course. In addition, he also examined post and precolumn derivatization of biological amines with fluorescent agents. He graduated in June 1986 with a Bachelor of Science degree in chemistry.

In the fall of 1986, he began graduate studies under the direction of Dr. Harold McNair. He spent the summer of 1987 at the Dow Chemical Company in Midland, Michigan. While serving as an intern in the Design Latex and Resin Group, he developed a more quantitative GC method for the kinetic measurement of latex syntheses and renovated an on-line LC system for large scale reactor monitoring. He was employed by the Hewlett-Packard Company in the summers of 1988 and 1989 and worked under Dr. Karen Hyver. His summer investigations examined several aspects of packed fused

silica columns for chromatography. During the school year, he served as an instructor for the American Chemical Society short courses in GC and HPLC and taught numerous in-house courses at IBM, Hewlett-Packard, Merck, Perkin-Elmer, Amoco, and the FDA.

William Henry Wilson completed the requirements for the degree of Doctor of Philosophy in chemistry in September, 1990 and has accepted employment with the Avondale Division of Hewlett-Packard.