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Part I: Isocratic and Gradient Elution Reversed-Phase Liquid Chromatography for the Estimation of the Hydrophobicity Parameter Log K'W: Applications to Newer Generation Stationary Phases. Part II: Planar Electrochromatographic Instrumental Design and Results

Peter Anthony Tate
PART I: ISOCRATIC AND GRADIENT ELUTION REVERSED-PHASE LIQUID CHROMATOGRAPHY FOR THE ESTIMATION OF THE HYDROPHOBICITY PARAMETER LOG K’W: APPLICATIONS TO NEWER GENERATION STATIONARY PHASES. PART II: PLANAR ELECTROCHROMATOGRAPHIC INSTRUMENTAL DESIGN AND RESULTS.

By

PETER ANTHONY TATE

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The members of the Committee approve the dissertation
of Peter Anthony Tate defended April 11, 2005

___________________________
John G. Dorsey
Professor Directing Dissertation

___________________________
J. Joseph Cronin Jr
Outside Committee Member

___________________________
William T. Cooper
Committee Member

___________________________
Alan G. Marshall
Committee Member

___________________________
Oliver Steinbock
Committee Member

Approved:

___________________________
Naresh S. Dalal, Chair
Department of Chemistry and Biochemistry

The Office of Graduate Studies has verified and approved the above named committee
members.
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ABSTRACT

The first half of this dissertation (Chapters 1-3) will discuss reversed-phase liquid chromatographic (RPLC) estimations of hydrophobicity. The RPLC retention values in purely aqueous mobile phases are used as measures of hydrophobicity. Unfortunately the accepted way of deriving these estimations is inaccurate. These values are normally obtained by performing isocratic chromatography through a limited range of mobile phase modifier concentrations (somewhere between 30%-70% modifier) and using the linear relationship between the logarithm of retention and modifier concentration to extrapolate a retention value for a purely aqueous mobile phase ($\log k'$). This linear relationship breaks down at low concentrations of modifier resulting in extrapolated values not representative of the actual chromatographic values. We have investigated newer types of RPLC columns using polar-embedded and polar-endcapped stationary phases for their ability to produce a more linear relationship throughout the range of modifier concentrations, therefore reducing error from extrapolation procedures. This work includes comparisons to other chromatographic retention modeling equations, as well as using gradient RPLC to obtain these estimations of hydrophobicity.

The second half of this dissertation (Chapters 4-7) describes the development of instrumentation for planar electrochromatography (PEC). PEC is a new methodology for thin layer chromatography (TLC) where the solvent is pushed through a TLC plate by electroosmotic flow (EOF) rather than capillary action. This allows faster flow rates and better-predicted chromatographic figures of merit. Initial instrument design had major problems due to Joule heating, solvent evaporation and a vertical format. Instrumentation has evolved with horizontal formats, closed systems to eliminate solvent evaporation, and external pressurization for correct voltage profiles across a TLC plate. We now understand the reasons for early instrumental hindrances and have continually redesigned the instrument to combat each obstacle. The latest design has achieved the predicted linear voltage drop of the applied field and hence, consistent EOF. Further proving the efficacy of this technique, initial studies with a fluorescent marker visually prove the correct solvent flow profiles determined with our voltage readers.
CHAPTER 1

INTRODUCTION TO CHROMATOGRAPHIC DETERMINATION OF HYDROPHOBICITY

Octanol-Water Determination of Hydrophobicity

The term “hydrophobicity” has been given many definitions by many different authors. From a phenomenological standpoint, hydrophobicity is the tendency for a non-polar solute to prefer a non-aqueous phase compared to an aqueous phase. Definitions from a physical chemistry standpoint state the solvation of a non-polar solute in water is thermodynamically unfavorable compared to the solvation of the same solute in a more non-polar solvent. Analysis of this statement by the Gibbs equation

\[ \Delta G = \Delta H - T \Delta S \]  

(1.1)

reveals that the transfer of a non-polar solute from an aqueous phase to an organic phase is energetically unfavorable in enthalpy (\( \Delta H > 0 \)) but the entropy change is more favorable (\( \Delta S > 0 \) or, -T\( \Delta S < 0 \)) so the overall Gibbs free energy process is favorable (\( \Delta G < 0 \)). This entropically dominated process leads to the spontaneous expulsion of non-polar solutes from an aqueous environment. IUPAC has suggested the following definition: “hydrophobicity is the association of non-polar groups or molecules in an aqueous environment which arises from the tendency of water to exclude non-polar molecules” [1].

Another term associated with hydrophobicity is “hydrophobic effect”. Dill [2] states that “hydrophobic effect” has three common definitions; (1) the transfer of a non-polar molecule into an aqueous solution, (2) the transfer of a non-polar molecule into an

1
aqueous solution while observing some particular temperature dependence, and (3) a particular molecular model which involves the ordering of water molecules around some non-polar solute molecule. Here, we will refer to “hydrophobicity” as the quantitative measure of a solute’s “phobia” or thermodynamic dislike of an aqueous phase, where the “hydrophobic effect” is the driving force for this partitioning process. Historically, a compound’s hydrophobicity was determined by a biphasic partitioning process between immiscible phases. In order to quantify this process the concentration in each phase at equilibrium must be known. The ratio of the concentrations yields the partition coefficient $P$,

$$P = \frac{[S]^y}{[S]^x}$$

(1.2)

where $[S]^y$ is the equilibrium concentration of solute $S$ in the non-polar phase and $[S]^x$ is the equilibrium concentration of $S$ in the polar phase. Since Overton [3] and Meyer [4] first discovered a correlation of the partition coefficient with potency of anesthetic action at the end of the 19th century, scientists have been correlating the partition coefficient with various biological processes such as membrane permeation and biotransportation. However, it wasn’t until 1964 that Hansch [5-8] characterized the logarithm of the partition coefficient ($\log P$) as a descriptor for hydrophobicity. They used octanol-water shake-flask partitioning data of many compounds to derive the hydrophobic scale. The choice of 1-octanol as the organic phase was because most pharmaceutical compounds had a similar solubility parameter to 1-octanol [9] and its lipophilic-hydrophilic balance, where the n-octyl chains give a lipophilic character, and the ability of the hydroxyl end to hydrogen-bond gives a hydrophilic character [8]. Today, there are thousands of compounds for which octanol-water partitioning data (log $P_{oct}$) exist. Due to the extensive data available, it has become the *de facto* standard for the measurement of hydrophobicity. A compound’s ability to partition between aqueous and organic media is an important property for many different disciplines. One of the most important is pharmacological activity, where the knowledge of a drug’s ability to permeate lipid bilayers, cell membranes, and other biological barriers is essential. The correlation of these properties with solute structure has been formed into its own discipline called
quantitative structure-activity relationships (QSARs), and is now a substantial part of pharmacological sciences. Other such important information correlated to these partition coefficients ranges from concentration of environmental pollutants in animals and fish, groundwater transport of PCBs and other pollutants through soil, polymer permeation, and solubility estimation [10].

The octanol-water partitioning model of hydrophobicity (log $P_{oct}$) is by no means a perfect system. First, an inherent problem exists when trying to model biopartitioning; that is, the biopartitioning system is a dynamic equilibrium, whereas the shake flask system is a static equilibrium. According to Dorsey and Khaledi [11] these two types of partitioning are based on opposite thermodynamic driving forces. Biological partitioning is generally driven by entropy whereas bulk-phase hydrocarbon-water systems are generally driven by enthalpy. Also, n-octanol is an isotropic liquid, so the size and shape of the solutes are not a factor in the partition process, which is contrary to that of a typical biomembrane that this system is trying to model [12]. While much data were obtained from these experiments, shake flask measurements have severe limitations. This analytical technique is tedious and time consuming, and once equilibrium is reached there needs to be an analytical method available for determination of the partition coefficient. Day to day reproducibility is tough to achieve, and lab-to-lab reproducibility is even tougher. Any impurities in either bulk phase or the solute can lead to error in detection. The limited dynamic range of traditional shake-flask method does not allow for the quantitation of highly hydrophobic or highly hydrophilic compounds. Any micro-emulsions can also lead to gross errors, so the experiment must be carried out below the critical micelle concentration (CMC), which can be as low as $10^{-5}$ M for some compounds. Lipophilic, basic compounds can also adhere to the glass wall of the separatory funnel causing misleading data [13]. For these reasons it can be seen why there is such ambiguity in some of the octanol-water partition coefficients that are reported in the literature. Even though there seems to be a multitude of problems with the traditional shake-flask method of determining hydrophobicity, log $P_{oct}$ it is still the most widely used model of hydrophobicity. This is due in part, to the magnitude of data available, and that there have been numerous approaches to model log $P_{oct}$ either through computational, or chromatographic approaches.
Chromatographic Partitioning

The advantages of reversed-phase liquid chromatography (RPLC) over traditional “shake-flask” measurements are numerous. Instrumental advantages include speed and reproducibility. There is now the ability to obtain these values in a reasonable time (less that 24 hours) with greater precision than the traditional method, which could take several days just to mutually saturate the two phases before the introduction of the solute. Increased reproducibility is two-fold. First, instrumental advantages of liquid chromatography (LC) such as limited sample handling and online detection allow for better reproducibility. Second, the elimination of impurities, solute instability, irreversible adsorption to the separatory funnel, and other such problems described earlier are not present. RPLC has a greater dynamic range, allowing highly hydrophobic / hydrophilic compounds to be investigated. The amount of solute necessary for measurement is reduced (~1 mg) and degradation products of the solutes are typically avoided (since chromatography is inherently a method of separation). Problem solutes such as those that create micro-emulsions are also eliminated.

The premise for using chromatographic data as an estimation of octanol-water partitioning is that retention in RPLC is due primarily to partitioning between the stationary and mobile phases. The following relationship illustrates the correlation of the chromatographic retention factor $k'$ to the partition coefficient $K$, through the volume phase ratio, where $V_s$ and $V_m$ are the volumes of the stationary and mobile phases respectively.

$$k' = K \frac{V_s}{V_m}$$

(1.3)

According to Collander [14], if partitioning is the dominant interaction, then a linear relationship will exist between the partition coefficients of differing systems

$$\log P_1 = S \log P_2 + I$$

(1.4)
where $P_1$ and $P_2$ are the partition coefficients of the different systems and $S$ and $I$ are the slope and intercept, respectively. This linear relationship exists for any two-phase aqueous-organic partitioning system as long as hydrophobic interactions are the dominant driving force [11]. It is important to note that typical RPLC experiments are carried out under mixed mobile phase conditions where the preferential solvation of the organic modifier results in a loss of the hydrophobic effect, which can lead to the breakdown of the Collander relationship. Coupling this limitation with the unstandardized multitude of data led to the need for homogeneity of the chromatographic conditions for these relationships. These relationships can be drawn at any composition of mobile phase as long as it is held constant. The reversed-phase chromatographic retention in pure water ($k'_{w}$) is generally agreed to be the standard retention factor for chromatographic hydrophobicity measurements because it has no effects due to organic modifiers, so that the only solvophobic effect is the hydrophobic effect. This makes retention dependent on the solute’s structure and functional groups. In RPLC, using pure water as the mobile phase with a non-polar alkyl stationary phase most truly represents the polar / non-polar partitioning of the organic / water system that it is trying to model.

The problem with using a pure water mobile phase is that the elution strength is too weak for most solutes to be eluted in a reasonable time. To solve this problem, isocratic retention values at mobile phases with different amounts of organic modifier are determined. These data are then plotted as the log of the retention factor ($\log k'$) vs. fraction of organic modifier ($\Phi$), and then the data is fit with a mathematical function ($\log k' = f(\Phi)$) and extrapolated to a mobile phase where the fraction of organic modifier is zero, which corresponds to the pure water retention factor ($\log k'_{w}$). This intercept is the most commonly used RPLC term in the Collander equation when correlating RPLC with $P_{oct}$, and the equation becomes

$$\log P_{oct} = S \log k'_{w} + I$$  \hspace{1cm} (1.5)$$

where all the terms are as described above. This is the standard model for relating the two partition processes for the purpose of characterizing hydrophobicity. This is now a
“model of a model”; RPLC is used to model $P_{oc}$, which is used model hydrophobicity.

Some authors believe that the chromatographic model alone is an accurate estimation of hydrophobicity [11,12]. In order to validate the use of reversed-phase liquid chromatography to estimate hydrophobicity, the next section is devoted to explaining several different retention models in chromatography.

**Retention Models in RPLC**

**Solvophobic Model**

One of the first attempts to model retention in reversed phase chromatography was by Horvath and Melander [15] who interpreted the solvophobic theory of Sinanoğlu [16-19]. This model described the retention process by a compilation of energy changes. It stated that retention was controlled by a solute’s hydrophobic volume (the size of the cavity in the mobile phase needed to surround the molecule), the volume of the mobile and stationary phases, the dielectric constant and surface tension of the mobile phase, and the differences in partial electrostatic charges. The combination of these parameters yielded a model that argued the retention process was largely controlled by the energy liberated from the re-ordering of solvent molecules after the expulsion of the non-polar solute. However, this theory neglects any influence of the stationary phase to retention, such as chain length or surface density of the bonded phase. This model has rarely been used as a fitting function for chromatographic retention vs. mobile phase composition for the determination of $\log k’_w$, but it is important to mention since there have been many retention models based on this work.

**Linear Solvent Strength (LSS) Model**

The simplest model of retention in RPLC is a linear dependence between the amount of organic modifier in the mobile phase (solvent strength) and retention factor,

$$\log k’ = \log k’_w - S \Phi$$

(1.6)
where \( \Phi \) is the fraction of organic modifier in the mobile phase, \( S \) is the slope of the equation (which is dependent on the system and solute), \( k' \) is the retention factor and \( k'_w \) is the retention factor in pure water. This was originally based on experimental observations that there was linear behavior over limited range in percent modifier [20]. This model has been further researched, and the \( S \) term better defined as a function of various structural properties and experimental conditions [21,22]. Investigation of the entire range of \( \Phi \) reveals that most solutes have non-linear behavior at the extremes, such that linear behavior is limited to approximately 0.2-0.8 \( \Phi \). This non-linear behavior as \( \Phi \) approaches zero is a common phenomenon. Although the actual reason for this curvature is still debated, it is commonly assumed that the curvature at low percentages of organic modifier is due to some change in the stationary phase solvation. Many researchers will accumulate data within the linear range and use the LSS model (eqn. 6) to extrapolate back to a “hypothetical” \( k'_w \). These researchers assume that if the retention mechanism does not change (i.e. stationary phase changes), there will be no curvature in the log \( k' \) vs. \( \Phi \) plots. This assumption does not change the fact that curvature is observed, but is ignored by these extrapolations.

**Solubility Parameter Model**

The solubility parameter model [23,24] attempts to better describe the curvature seen in the log \( k' \) vs. \( \Phi \) plots by a chemically valid model. This model describes retention in terms of the differences in the solubility parameters of the solute, mobile phase and stationary phase. The equation is:

\[
\log k' = A\Phi^2 + B\Phi + E\sqrt{\Phi} + \log k'_w
\]  

(1.7)

where \( \Phi \) is the fraction of organic modifier and \( A, B, E, \) and \( \log k'_w \) are the fitting coefficients from the log \( k' \) vs. \( \Phi \) plots. These coefficients are all expressed in terms of solubility parameters (for detailed derivations see ref 23). The \( A\Phi^2 \) term in this equation accounts for the curvature seen at high concentrations (>80%) of organic modifier, whereas the \( E\sqrt{\Phi} \) term accounts for curvature due to low percentages (<20%) of organic modifier. The fundamental limitation for applying this model to RPLC is that the
solubility parameters describe the enthalpy effects of mixing but neglect the entropy effects. Since retention is related to the Gibbs free energy, the exclusion of the entropy term forces this model to be only semi-quantitative. The usefulness of this model is that it can fit experimental data with great accuracy throughout the entire range of mobile phase composition. The more data points taken at the extreme ranges of %B, the better the fitting parameters will accurately model the observed data and the less error upon extrapolation to a log \( k' \) value. If no chromatography is performed below 20% B, there will be little or no gain in using the solubility parameter model versus the LSS model, since the curvature is not available to be modeled. Accordingly, the closer to 0% that data is taken, the shorter the extrapolation distance, which lowers the inaccuracy upon extrapolation and increases the confidence in that value.

**ET-30 Model**

This model is based on the \( \lambda_{\text{max}} \) shift of the ET-30 probe molecule 2,6-diphenyl-(2,4,6-triphenyl-N-pyridino)-phenolate (see figure 1.1). This molecule has a charge transfer absorbance with a strong dependence on the polarity of the solvent system, with a solvatochromic shift from 453nm in pure water to 810nm in diphenyl ether. This strong dependence on polarity allows quantitation of RPLC mobile phase polarity, and an independent polarity scale was created known as the E\(_t\)(30) polarity scale [25]. The scale was based on the energy of the charge transfer of the probe molecule, and the

![Figure 1.1. Structure of the probe molecule ET-30.](image)
values of the scale were set as

$$E_t(30) = \frac{28,592}{\lambda_{\text{max}}}$$

where the constant (28,592) is the product of the speed of light, Plank’s constant and Avogadro’s number, $\lambda_{\text{max}}$ is the wavelength of maximum absorption, and the $E_t(30)$ units are kcal/mol. This scale was used by Dorsey’s group [26] to examine 332 sets of data. They showed that plots of log $k’$ vs. $E_t(30)$, were more linear then plots of log $k’$ vs. $\Phi$. Another study examined the estimation of the log $k’_\infty$ hydrophobicity parameter by use of this scale [27]. The nature of the ET-30 shift vs. $\Phi$ accounts only for the changing mobile phase polarity, and since the polarity measurement of the solvent systems are completely independent from the retention process, this allows for deconvolution of changes in the stationary phase structure [28]. This model, like Schoenmaker’s solubility parameter model, was not intended to be quantitative to the extent of a priori retention prediction, but rather to better fit the data to a chemically valid model. This model was also successful in deconvolving some curvature inherent in retention dependence on solvent strength. Park et al. [29] tested another compound similar to ET-30, called ET-33 (2,6-dichloro-(2,4,6-triphenyl-N-pyridino)-phenolate) where the only difference is the phenyl groups at the 2,6 positions on the phenoxide ring are replaced with chlorines to lower the $pK_a$. The lowering of the $pK_a$ from 8.65 to 4.38 by the substitution of the electron withdrawing chlorines allows this molecule to be applicable to acidic conditions, where most chromatographic conditions exist. This work shows no conclusive data that one polarity scale is better than the other, but rather reinforces the idea that there will not be any “all encompassing” single parameter retention model for solvents that have the ability to hydrogen bond.

**Other Models**

There have been many other proposed models of the retention mechanism in RPLC, but a complete review would be too extensive. There are however, some others that should be discussed. One retention model uses linear solvation energy relationships (LSERs), and is now commonly called the “LSER” model. Introduced by Sadek et al.
the notation of the equation has changed through the years but the terms have stayed the same. The equation is

\[
\log k' = \log k'_0 + mV_x + s\pi^\text{HI}_2 + a\Sigma\alpha^\text{HI}_2 + b\Sigma\beta^\text{HI}_2
\]  

(1.9)

where \(V_x\) is the McGowan’s molecular volume [31], \(\pi^\text{HI}_2\) is the solute’s dipolarity/polarizability term, and \(\alpha^\text{HI}_2, \beta^\text{HI}_2\) are the hydrogen bond acidity and basicity, respectively. The subscript 2 represents that term as a property of a solute. The parameters \(\pi^\text{HI}_2, \alpha^\text{HI}_2, \beta^\text{HI}_2\) are solvatochromic parameters generally obtained from Abraham’s work [32]. The fitting coefficients \(m, s, a, b, \) and \(\log k'_0\) are calculated based on a least squares regression of the data. These coefficients (including the intercept) are characteristic of the mobile and stationary phase conditions, and are representative of the difference of each parameter in the mobile and stationary phase. So, the above equation can be written as to include the parameters not only of the solute, but also of the chromatographic phases.

\[
\log k' = \log k'_0 + M(v_s - v_m)V_x + S(\pi^*_s - \pi^*_m)\pi^\text{HI}_2
\]

\[
+ A(\alpha_s - \alpha_m)\Sigma\alpha^\text{HI}_2 + B(\beta_s - \beta_m)\Sigma\beta^\text{HI}_2
\]  

(1.10)

All the variables are the same as the previous equation but the subscripts \(s\) and \(m\) represent stationary and mobile phase, respectively. The fitting coefficients \(M, S, A, \) and \(B\) in this equation are now independent of the chromatographic phases and the solute [33]. The parameters that control retention to the greatest extent in this model are the solute molar volume and hydrogen bond acceptor basicity [34]. It is no surprise that the molar volume (size) term is of such importance, since the well-known solvophobic theory was based on the expulsion of a non-polar solute from a polar solvent by the closing of a solute sized cavity in the mobile phase. The hydrogen bond acceptor (HBA) basicity of the solute is complimentary to the hydrogen bond donor (HBD) acidity of the solvent, and since water is a very large HBD acid it easy to recognize that the \(\beta^\text{HI}_2\) term will also be important in modeling retention.
This model has received much attention not only because of its ability to model retention based on specific individual parameters of each phase and solute but also for its ability to predict retention. This model works well with non-polar solutes in the linear region of mobile phase composition, but actual chromatography cannot yet be predicted by the summation of these linear relationships. This is especially true for solutes with more than one polar functionality where the solvatochromic parameters $\pi^H_2$, $\alpha^H_2$, $\beta^H_2$ become less reliable due to non-additive behavior in some of the estimation procedures (some values are mathematically determined). Estimating a log $k'_w$ value from the dependence of retention on %B for this model is not practical, as solving this equation for each solute at each mobile phase would be tedious and time consuming. This model will not be evaluated in our examination of chromatographic hydrophobicity, but is mentioned to illustrate the current knowledge and ongoing science into the evaluation of a precise retention model.

One other model important to mention is the statistical-mechanical model [35]. This model describes interactions in the stationary phase more than any other model of reversed-phase retention. It states that instead of the stationary phase chains being treated as passive “picket fences” acting just as a medium for mass transfer, it acts as an interphase much like surfactant aggregated micelles, monolayers, or micro-emulsions. These interphases are made of chains that are free at one end and planted at the other, with surface bonding density high enough for steric effects to severely limit chain configurations. This gives rise to an orientational disorder gradient where the planted end is much more ordered than the free end, and this disorder depends on the depth of the chain. The organization of these interphases is determined by the surface density, alkyl chain length, and ability of the solvent to solvate the interphase ligands. In accordance with the 2nd law of thermodynamics, these interphases will become as disordered as possible within their constraints. This model derives all the relevant terms for the entire retention mechanism, but focuses on the stationary phase properties that have been previously ignored, mostly due to the complexity in obtaining data. This is too complicated a model to use in defining a relationship between retention and %B for the purpose of obtaining a hydrophobicity value. This is an important model to mention because in our opinion, a complete understanding of the retention mechanism in RPLC
will not be available until the contributions of the stationary phase are understood. Many earlier models of retention mechanism have neglected the stationary phase or considered it constant, focusing on the interactions of the solute and mobile phase, since these properties are readily measurable.

**Stationary Phase Considerations**

There are numerous chromatographic hydrophobicity scales based on differing chromatographic media including reversed phases [10,34,36-46], polymeric phases [13,47], micellar phases [48-50] and micro-emulsion phases [51-54]. This text only investigates reversed-phase stationary phases, in particular octadecyl-silane stationary phases (ODS, also noted as C\textsubscript{18}) since these are the most common chromatographic stationary phases in use.

Numerous attempts to improve the C\textsubscript{18} stationary phases have been made within the last two decades. The most sought after goal of stationary phase improvements is the elimination of residual surface silanols, which are responsible for the severe peak tailing of basic and ionizable solutes. These silanols are, in part, responsible for the observed curvature in the dependence of retention on %B (i.e. the non-linear behavior due to a competing retention mechanism other than partitioning) for some solutes. This cannot be the only reason for the observed curvature since non-polar / non-ionizable solutes, which are not affected by the residual silanols, also demonstrate some degree of non-linearity.

One of the strategies to reduce residual silanols is increasing the bonding density of the alkyl chains, but this quickly becomes a problem due to steric hindrance. Another approach to reduce the unwanted silanols uses a technique called “endcapping”. This procedure uses a secondary reaction scheme where a small alkyl chain is allowed to react with the residual silanols from the initial C\textsubscript{18} bonding reaction. These small ligands can reach most of the residual silanol sites without much barrier from the bulky C\textsubscript{18} ligands.

In an attempt to control the heterogeneous surface of the silica particles, Waters\textsuperscript{®} has developed a hybrid organic-inorganic porous particle synthesized by a mixture of organosilanes that form siloxane and methylsiloxane groups throughout the particle. This arrangement produces a stationary phase surface that has silanol and methylsiloxane units
on the surface of the particle. This helps to eliminate the inhomogeneity of the surface [55]. This surface is then the anchor point for derivatization with the C₁₈ ligand. The main advantage of this procedure is that the hybrid particle can be controlled to allow only a certain number of surface silanols available for reaction. This then allows for reaction of the surface to the degree desired. For example, if the particle is controlled as to allow only 2 µmol/m² of surface silanols, then the bonding reaction could replace most all the silanols with ligands and leave very few residual silanols. Another benefit of this procedure is that the silanols that are left on the surface are not as acidic as conventional surface silanols, so if unwanted surface silanols are present, their interactions become less problematic.

Another notable problem with conventional mono-functional C₁₈ phases is that they become desolvated under highly aqueous conditions. One method to solve this problem includes the introduction of polar groups within the bonded phase. These “water-friendly” columns are designed for chromatography with highly aqueous mobile phases. They are supposed to help keep the non-polar C₁₈ phase from expelling the mobile phase under highly polar conditions. This is important because expulsion of mobile phase reduces the effective volume of the stationary phase, and hence the retention factor (see eqn.1.3). This desolvation is also dependent on Φ, and introduces some degree of non-linearity on the dependence of retention with Φ. Two popular strategies for the inclusion of polar groups have emerged.

The first uses polar groups that are bonded as part of an endcapping step. There is no change to the C₁₈ ligand, but rather a second reaction is run after the initial bonding of the C₁₈ ligand. This second reaction bonds a small chain alcohol to the residual silanols that were not reacted in the first step. This is called “polar endcapping”, and its purpose is two-fold. This first improvement, reduction of residual silanols, is the same as traditional endcapping and improves the chromatographic phase, but doesn’t help the aqueous instability. The introduction of the hydroxyl group at the end of this endcapping ligand helps the stationary phase solvate the highly aqueous mobile phase with the ability to hydrogen bond.

The second use of polar groups is to include them in the C₁₈ ligand, and these are called “polar embedded group” ligands. The polar groups used in this arrangement are
usually ureas, carbamates, or amides [56-58]. These bonded ligands are then able to hydrogen bond to each other via the polar group, creating a stabilizing network in aqueous environments. This arrangement has no further steps to reduce residual silanols such as endcapping since the hydrogen bonding of the polar groups hinders any solutes from diffusing to the surface of the silica, therefore reducing the exposure to residual silanols [59].

This study focuses on hydrophobicity estimations by reversed-phase liquid chromatography, specifically with C\textsubscript{18} stationary phases. We investigate four different types of C\textsubscript{18} stationary phases consisting of a polar endcapped, polar embedded, hybrid based polar embedded, and a conventional C\textsubscript{18} column (see figure 1.2). The goal is to understand whether newer generation C\textsubscript{18} stationary phases are better at estimating hydrophobicity (log k'\textsubscript{w}). The first experiments, detailed in Chapter 2, examine the isocratic determination of log k'\textsubscript{w} by plotting retention vs. $\Phi$ and seeing whether these

![Diagram of four reversed-phase ODS binding chemistries](image-url)

Figure 1.2. Schematic representation of the four reversed-phase ODS binding chemistries studied. A) Typical mono-functional ODS stationary phase. B) Polar endcapped ODS (Here shown as C\textsubscript{3} endcapping ligand). C) Polar embedded ODS (Here shown with amide as polar group). D) Hybrid based particle (Xterra manufactured by Waters\textsuperscript{®}) bonded with the polar embedded ligand.
newer phases show more linear trends compared to a conventional reversed-phase column. The data for all columns will be evaluated using the LSS, Solubility Parameter, and ET-30 chromatographic models of retention. This elucidates the best column/modeling pair for most accurate estimations of chromatographic hydrophobicity. Chapter 3 discusses another means of determining chromatographic hydrophobicity by use of gradient RPLC. These experiments reveal whether fewer, faster gradient experiments can give the same hydrophobicity estimations as the isocratic experiments.
CHAPTER 2

ISOCRATIC REVERSED-PHASE LIQUID CHROMATOGRAPHY FOR ESTIMATION OF HYDROPHOBICITY

Introduction

Newer reversed-phase column technologies that incorporate polar groups either by an endcapping procedure or by embedding them into the stationary phase ligand have been receiving much attention in the literature for their robustness when highly aqueous conditions are needed. We are investigating their ability to accurately determine the chromatographic hydrophobicity value \( \log k' \). The non-linear deviations of isocratic retention data as mobile phase conditions approach zero are a large source of error when extrapolating to \( \log k' \) values using the linear solvent strength model. Here we compare a conventional reversed-phase stationary phase with others that have incorporated either polar embedded or polar endcapped phases, along with a hybrid based particle derivatized with a polar embedded ligand. Our first experiments investigate which column is best suited for these determinations by using analytes of differing polarity, as well as different organic modifiers. After this, we investigate the Linear Solvent Strength model, Solubility Parameter model, and the ET-30 model as to determine which modeling equation yields the least error in extrapolation. This gives a complimentary set of information for choosing a reliable method of obtaining hydrophobicity values by chromatography. We make no attempt to correlate these \( \log k' \) values to the \( \log P_{\text{oct}} \) values. It is our belief that the chromatographic scale of hydrophobicity parallels the octanol / water scale in the same relative fashion.
Experimental

The HPLC system consisted of a Spectra-Physics Model 8800 ternary mixing pump (Spectra-Physics, Mountain View CA, USA), a Valco six port injector (Valco, Houston TX, USA) equipped with a 20 µL sample loop, and a Spectroflow 757 variable UV-Vis detector (Kratos Analytical, Chestnut Ridge NY, USA). Chromatograms were recorded with TurboChrom 4.0 software (Perkin Elmer, Wellesley MA, USA). All columns were thermostated with a water-jacket and TE-7 Tempette (Techne, Cambridge, England) temperature controller.

The columns used in this study are listed in table 2.1 with specifications provided by the manufacturers. All columns are reversed-phase C18 columns measuring 15 cm in length and 4.6 mm I.D. containing 5-µm diameter particles. The 4 columns studied differ in their bonding chemistries. The Zorbax SB-C18 column (Hewlett Packard, Wilmington DE, USA) is a conventional C18 bonded phase, the YMC ODS-AQ column (YMC Inc.,

<table>
<thead>
<tr>
<th>Column Type</th>
<th>Dimensions</th>
<th>Pore Size (Å)</th>
<th>Surface Area (m²/g)</th>
<th>% Carbon</th>
<th>Bonding Density (µmol/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zorbax SB-C18 (Mono-functional)</td>
<td>4.6 X 150 mm</td>
<td>80</td>
<td>180</td>
<td>10 %</td>
<td>2.66</td>
</tr>
<tr>
<td></td>
<td>5µm dp</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YMC-Pack ODS-AQ (Polar Endcapped)</td>
<td>4.6 X 150 mm</td>
<td>120</td>
<td>300</td>
<td>14 %</td>
<td>~3.2-3.5^a</td>
</tr>
<tr>
<td></td>
<td>5µm dp</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Symmetry Shield RP-18 (Polar Embedded)</td>
<td>4.6 X 150 mm</td>
<td>90</td>
<td>332</td>
<td>17.38 %</td>
<td>3.31</td>
</tr>
<tr>
<td></td>
<td>5µm dp</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xterra RP-18 (Hybrid Polar Embedded)</td>
<td>4.6 X 150 mm</td>
<td>120</td>
<td>175</td>
<td>14.76 %</td>
<td>2.31</td>
</tr>
<tr>
<td></td>
<td>5µm dp</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

^a Estimated total bonding density as a sum of C18 density (2.0-2.2 µmol/m²) and endcapping agent density (1.2-1.4 µmol/m²), assuming a three carbon chain endcapping agent.
Wilmington, NC, USA) is a polar (hydrophilic) endcapped C18 phase, the Symmetry Shield RP-18 column (Waters Corp. Milford CT, USA) is a polar embedded bonded phase, and the Xterra RP-18 (Waters Corp. Milford CT, USA) is a hybrid particle made of an organic/inorganic matrix that is bonded with polar embedded ligands.

Acetonitrile and methanol were HPLC grade (Fisher Scientific, Fair Lawn NJ, USA). The water used for mobile phases was purified with a Barnstead NANOPure II system (Barnstead International, Dubuque IA, USA). All test analytes are reagent grade or better. Acetone, methyl-ethyl ketone, diethyl ketone, benzyl alcohol, and benzene were from Fisher Scientific (Fisher Scientific, Fair Lawn NJ, USA), nitroethane was from Sigma Aldrich (Sigma Aldrich, Milwaukee WI, USA), and deuterium oxide from Cambridge Isotope Labs (Cambridge Isotope Labs, Andover MA, USA). 1-propanol (Fisher Scientific, Fair Lawn NJ, USA) was used as an additive to help solvate the test analytes.

Analytes were chromatographed at mobile phases containing 50, 40, 30, 20, 15, 10, 8, 6, 4, 2 and 0 percent (v/v) organic modifier. The HPLC system was operated at 1 ml/min with the detector set at 254 nm and an injection volume of 20 µL. All columns were thermostated at 30.0 ± 0.2 °C. The void time (t_o) of the system was measured by injecting D_2O. Samples were prepared in water with 1% 1-propanol added for solvation. All chromatograms were run in triplicate and these data were used to construct plots of the logarithm of the retention factor (log k’) vs. percent organic modifier. The fitting functions were calculated using the regression tools in Microsoft Excel 2000 (Microsoft, Redmond WA, USA) and Scientific Data Analysis Software (Prentice Hall, Upper Saddle River NJ, USA) add-on for Excel.

Results and Discussion

Column Comparison

For the column comparison, we use a modified version of Schoenmaker’s solubility parameter model eliminating the quadratic term (AΦ^2) from the equation. The quadratic term most accurately models the curvature at high fractions of modifier
concentrations (Φ>0.8). Baczek et al. showed that there was only a statistical difference between the linear and quadratic models for acetonitrile systems, not methanol systems for the 20-95% modifier range [60]. In this work we are investigating the low range of modifier concentrations and therefore do not require the quadratic term. This can be seen when examining data containing a high degree of curvature and inspecting the differences in the correlation coefficients (r²). The correlations shown are the average of all four columns for the solute diethyl ketone using acetonitrile as modifier, since this solute/modifier pair consistently showed the most curvature. The equations are listed according to their decreasing correlations coefficients where $A\Phi^2 + B\Phi + E\sqrt{\Phi} + \log k'_w$ (r² = 0.9989), $B\Phi + E\sqrt{\Phi} + \log k'_w$ (r² = 0.9986), $A\Phi^2 + B\Phi + \log k'_w$ (r² = 0.9793), $B\Phi + \log k'_w$ (r² = 0.9321). From this data we can see that the addition of another term to the linear equation is quite beneficial (from r² = 0.9321 to 0.9793 or 0.9986) and that the quadratic term is less efficient at modeling the data than a square root term. We can also see that the addition of both terms to the to the linear equation does not increase the correlation to any significant value when compared to the linear equation with the square root term (from r² = 0.9986 to 0.9989). We expect to see some increase in correlation since we are adding another term to the equation and decreasing the degrees of freedom in the modeling system, but the added correlation is inconsequential. For this reason we have chosen not to show the fitting data for all of these equations, but just the linear (it is the most used and correlated equation) and linear w/ square root equation (it models the curvature very accurately).

In order to see whether the newer chemistry stationary phases are more accurate in determination of the log k’w value, a basis for comparison is needed. The conventional column used was the Zorbax SB-C18 column, and the chromatographic data were as anticipated. Hsieh and Dorsey have shown that more polar solutes have greater upward curvature (concave) in the range of 0<Φ<0.1, and that non-polar solutes have curvature in the opposite fashion (convex) and that there is less curvature with methanol as a modifier than with acetonitrile [61]. As expected, our data (figure 2.1) follows the same general trends. Table 2.2 gives quantitative regression information about these plots (as well as measured values for log k’w). There are also slight discrepancies in the measured pure water retention data for the two modifier systems; however, the two retention values
Figure 2.1. Chromatographic data for the Zorbax column. (A) acetonitrile as the organic modifier and (B) methanol as modifier. Legend: (x) nitroethane, (Δ) diethyl ketone, (*) benzyl alcohol and (●) benzene.
### Table 2.2. Fitting parameters for Zorbax column

<table>
<thead>
<tr>
<th>Solute</th>
<th>Modifier</th>
<th>Formula</th>
<th>Equation</th>
<th>log k’w</th>
<th>Correlation (r²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diethyl ketone</td>
<td>ACN</td>
<td>N/A</td>
<td>Chromatographically Determined</td>
<td>1.63</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>$-0.029\Phi + 1.30$</td>
<td>1.30</td>
<td>0.9061</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>$0.2248 [E_T(30)] - 12.806$</td>
<td>1.37</td>
<td>0.9481</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>$0.0024\Phi - 0.24\Phi^{0.5} + 1.65$</td>
<td>1.65</td>
<td>0.9978</td>
</tr>
<tr>
<td>Nitroethane</td>
<td>ACN</td>
<td>N/A</td>
<td>Chromatographically Determined</td>
<td>0.49</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>$-0.011\Phi + 0.45$</td>
<td>0.45</td>
<td>0.9879</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>$0.0847 [E_T(30)] - 4.8684$</td>
<td>0.47</td>
<td>0.9729</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>$0.0096\Phi - 0.012\Phi^{0.5} + 0.47$</td>
<td>0.47</td>
<td>0.9895</td>
</tr>
<tr>
<td>Benzyl Alcohol</td>
<td>ACN</td>
<td>N/A</td>
<td>Chromatographically Determined</td>
<td>1.54</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>$-0.034\Phi + 1.32$</td>
<td>1.32</td>
<td>0.9500</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>$0.2678 [E_T(30)] - 15.48$</td>
<td>1.40</td>
<td>0.9832</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>$-0.0097\Phi - 0.20\Phi^{0.5} + 1.61$</td>
<td>1.61</td>
<td>0.9921</td>
</tr>
<tr>
<td>Benzene</td>
<td>ACN</td>
<td>N/A</td>
<td>Chromatographically Determined</td>
<td>1.97</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>$-0.026\Phi + 1.89$</td>
<td>1.89</td>
<td>0.9941</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>$0.2053 [E_T(30)] - 10.999$</td>
<td>1.94</td>
<td>0.9902</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>$-0.022\Phi - 0.037\Phi^{0.5} + 1.95$</td>
<td>1.95</td>
<td>0.9940</td>
</tr>
<tr>
<td>Diethyl ketone</td>
<td>MeOH</td>
<td>N/A</td>
<td>Chromatographically Determined</td>
<td>1.60</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>$-0.029\Phi + 1.48$</td>
<td>1.48</td>
<td>0.9817</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>$0.3015 [E_T(30)] - 17.532$</td>
<td>1.52</td>
<td>0.9935</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>$-0.016\Phi - 0.11\Phi^{0.5} + 1.63$</td>
<td>1.63</td>
<td>0.9983</td>
</tr>
<tr>
<td>Nitroethane</td>
<td>MeOH</td>
<td>N/A</td>
<td>Chromatographically Determined</td>
<td>0.52</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>$-0.017\Phi + 0.43$</td>
<td>0.43</td>
<td>0.9753</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>$0.1751 [E_T(30)] - 10.615$</td>
<td>0.45</td>
<td>0.9863</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>$-0.0083\Phi - 0.071\Phi^{0.5} + 0.53$</td>
<td>0.53</td>
<td>0.9978</td>
</tr>
<tr>
<td>Benzyl Alcohol</td>
<td>MeOH</td>
<td>N/A</td>
<td>Chromatographically Determined</td>
<td>1.56</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>$-0.029\Phi + 1.46$</td>
<td>1.46</td>
<td>0.9894</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>$0.2921 [E_T(30)] - 16.96$</td>
<td>1.50</td>
<td>0.9957</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>$-0.019\Phi - 0.078\Phi^{0.5} + 1.57$</td>
<td>1.57</td>
<td>0.9991</td>
</tr>
<tr>
<td>Benzene</td>
<td>MeOH</td>
<td>N/A</td>
<td>Chromatographically Determined</td>
<td>1.85</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>$-0.022\Phi + 1.85$</td>
<td>1.85</td>
<td>0.9961</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>$0.2199 [E_T(30)] - 12.026$</td>
<td>1.87</td>
<td>0.9849</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>$-0.024\Phi + 0.014\Phi^{0.5} + 1.83$</td>
<td>1.83</td>
<td>0.9966</td>
</tr>
</tbody>
</table>
should be the same. It has been shown that when changing mobile phase compositions to pure water some small amount of modifier can become trapped in the pores of the stationary phase and minor effects from this modifier can be observed [62].

The data for the YMC Pro-Pack ODS-AQ can be seen in figure 2.2 and table 2.3. From the data we can see that there is much similarity between this column and the conventional C$_{18}$ column. The four test solutes show the same trends in curvature, regression coefficients, and log $k'_w$ values for the different modeling equations between these two columns. This holds true for both methanol and acetonitrile modifiers. This was not anticipated. Since the surface of this silica was secondarily reacted with the polar endcapping agent, we expected differences in retention behavior—especially in the curvature of the graphs. The purpose of the polar endcapping is to create an environment in the stationary phase solvation layer that is more tolerable to highly aqueous milieus. We anticipated that there would be less change to the stationary phase solvation layer with highly aqueous mobile phases, which would result in less curvature in the range 0 - 0.1 $\Phi$. Unfortunately it does not exhibit the reduced curvature that we expected. Although we did not expect the retention and trends to be this close, it has been shown that these two column types do not exhibit many differences. Layne studied these types of stationary phases and showed that there were no significant differences in hydrophobicity, hydrogen bonding capacity or methylene selectivity of either the polar/hydrophilic endcapped or conventional RPLC column [63]. Our data are in agreement with his, and others [64-66].

In contrast to the polar endcapped YMC column investigated, the polar embedded Symmetry Shield RP-18 column does exhibit noticeable differences in the data when compared to the conventional (Zorbax) column. These differences are represented in figure 2.3 and table 2.4. It is easy to notice that diethyl ketone and benzene have reduced intercepts (log $k'_w$) for both modifiers. We believe the shift to lower retention values is representative of the increased polarity of the stationary phase. This change in polarity of the stationary phase solvation layer makes the stationary and mobile phases thermodynamically closer, that is, $\Delta G$ is closer to zero. This belief is further evidenced

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* Data for acetone, methyl ethyl ketone and diethyl ketone were obtained, but since they all have the same retention characteristics, only the highest retained ketone is discussed.
Figure 2.2. Chromatographic data for the YMC column. (A) acetonitrile as the organic modifier and (B) methanol as modifier. Legend: (x) nitroethane, (Δ) diethyl ketone, (*) benzyl alcohol and (●) benzene.
Table 2.3. Fitting parameters for YMC column

<table>
<thead>
<tr>
<th>Solute</th>
<th>Modifier</th>
<th>Formula</th>
<th>Equation</th>
<th>log k’w</th>
<th>Correlation ($r^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diethyl ketone</td>
<td>ACN</td>
<td>N/A</td>
<td>Chromatographically Determined</td>
<td>1.60</td>
<td>N/A</td>
</tr>
<tr>
<td>A</td>
<td>-0.027Φ + 1.27</td>
<td>1.27</td>
<td>0.8989</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>0.21 [Eₜ(30)] - 11.902</td>
<td>1.34</td>
<td>0.9410</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0.0034Φ – 0.24Φ⁰.⁵ + 1.62</td>
<td>1.62</td>
<td>0.9975</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitroethane</td>
<td>ACN</td>
<td>N/A</td>
<td>Chromatographically Determined</td>
<td>0.53</td>
<td>N/A</td>
</tr>
<tr>
<td>A</td>
<td>-0.0097Φ + 0.47</td>
<td>0.47</td>
<td>0.9614</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>0.0743 [Eₜ(30)] - 4.1986</td>
<td>0.49</td>
<td>0.9455</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>-0.0069Φ – 0.023Φ⁰.⁵ + 0.50</td>
<td>0.50</td>
<td>0.9682</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzyl Alcohol</td>
<td>ACN</td>
<td>N/A</td>
<td>Chromatographically Determined</td>
<td>1.58</td>
<td>N/A</td>
</tr>
<tr>
<td>A</td>
<td>-0.033Φ + 1.34</td>
<td>1.34</td>
<td>0.9450</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>0.2569 [Eₜ(30)] - 14.78</td>
<td>1.41</td>
<td>0.9787</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>-0.0071Φ – 0.21Φ⁰.⁵ + 1.64</td>
<td>1.64</td>
<td>0.9949</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzene</td>
<td>ACN</td>
<td>N/A</td>
<td>Chromatographically Determined</td>
<td>1.95</td>
<td>N/A</td>
</tr>
<tr>
<td>A</td>
<td>-0.026Φ + 1.85</td>
<td>1.85</td>
<td>0.9897</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>0.1975 [Eₜ(30)] - 10.548</td>
<td>1.90</td>
<td>0.9767</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>-0.023Φ – 0.024Φ⁰.⁵ + 1.89</td>
<td>1.89</td>
<td>0.9909</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diethyl ketone</td>
<td>MeOH</td>
<td>N/A</td>
<td>Chromatographically Determined</td>
<td>1.57</td>
<td>N/A</td>
</tr>
<tr>
<td>A</td>
<td>-0.029Φ + 1.43</td>
<td>1.43</td>
<td>0.9785</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>0.2915 [Eₜ(30)] - 16.946</td>
<td>1.47</td>
<td>0.9915</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>-0.015Φ – 0.11Φ⁰.⁵ + 1.59</td>
<td>1.59</td>
<td>0.9984</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitroethane</td>
<td>MeOH</td>
<td>N/A</td>
<td>Chromatographically Determined</td>
<td>0.53</td>
<td>N/A</td>
</tr>
<tr>
<td>A</td>
<td>-0.016Φ + 0.44</td>
<td>0.44</td>
<td>0.9727</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>0.1666 [Eₜ(30)] - 10.071</td>
<td>0.46</td>
<td>0.9851</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>-0.0075Φ – 0.071Φ⁰.⁵ + 0.54</td>
<td>0.54</td>
<td>0.9973</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzyl Alcohol</td>
<td>MeOH</td>
<td>N/A</td>
<td>Chromatographically Determined</td>
<td>1.57</td>
<td>N/A</td>
</tr>
<tr>
<td>A</td>
<td>-0.028Φ + 1.47</td>
<td>1.47</td>
<td>0.9882</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>0.2864 [Eₜ(30)] - 16.592</td>
<td>1.51</td>
<td>0.9956</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>-0.018Φ – 0.080Φ⁰.⁵ + 1.58</td>
<td>1.58</td>
<td>0.9990</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzene</td>
<td>MeOH</td>
<td>N/A</td>
<td>Chromatographically Determined</td>
<td>1.77</td>
<td>N/A</td>
</tr>
<tr>
<td>A</td>
<td>-0.020Φ + 1.77</td>
<td>1.77</td>
<td>0.9953</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>0.2062[Eₜ(30)] - 11.228</td>
<td>1.80</td>
<td>0.9835</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>-0.022Φ + 0.014Φ⁰.⁵ + 1.75</td>
<td>1.75</td>
<td>0.9959</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 2.3. Chromatographic data for the Symmetry Shield column. (A) acetonitrile as the organic modifier and (B) methanol as modifier. Legend: (x) nitroethane, (Δ) diethyl ketone, (*) benzyl alcohol and (●) benzene.
Table 2.4. Fitting parameters for Symmetry Shield column

<table>
<thead>
<tr>
<th>Solute</th>
<th>Modifier</th>
<th>Formula</th>
<th>Equation</th>
<th>log k'w</th>
<th>Correlation (r²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diethyl ketone</td>
<td>ACN</td>
<td>N/A</td>
<td>Chromatographically Determined</td>
<td>1.27</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>A -0.024Φ + 1.07</td>
<td>1.07</td>
<td>0.9511</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>B 0.1743 [E₄(30)] - 9.8678</td>
<td>1.30</td>
<td>0.9769</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C -0.0052Φ - 0.14Φ⁰.⁵ + 1.27</td>
<td>1.27</td>
<td>0.9995</td>
</tr>
<tr>
<td>Nitroethane</td>
<td>ACN</td>
<td>N/A</td>
<td>Chromatographically Determined</td>
<td>1.31</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>A -0.029Φ + 1.17</td>
<td>1.17</td>
<td>0.9749</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>B 0.223 [E₄(30)] - 12.822</td>
<td>1.24</td>
<td>0.9952</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C -0.014Φ - 0.118Φ⁰.⁵ + 1.35</td>
<td>1.35</td>
<td>0.9971</td>
</tr>
<tr>
<td>Benzyl Alcohol</td>
<td>ACN</td>
<td>N/A</td>
<td>Chromatographically Determined</td>
<td>1.65</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>A -0.028Φ + 1.53</td>
<td>1.53</td>
<td>0.9888</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>B 0.2143 [E₄(30)] - 11.924</td>
<td>1.59</td>
<td>0.9958</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C -0.018Φ - 0.080Φ⁰.⁵ + 1.65</td>
<td>1.65</td>
<td>0.9999</td>
</tr>
<tr>
<td>Benzene</td>
<td>ACN</td>
<td>N/A</td>
<td>Chromatographically Determined</td>
<td>1.72</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>A -0.024Φ + 1.78</td>
<td>1.78</td>
<td>0.9871</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>B 0.1827 [E₄(30)] - 9.6956</td>
<td>1.82</td>
<td>0.9557</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C -0.032Φ + 0.066Φ⁰.⁵ + 1.69</td>
<td>1.69</td>
<td>0.9970</td>
</tr>
<tr>
<td>Diethyl ketone</td>
<td>MeOH</td>
<td>N/A</td>
<td>Chromatographically Determined</td>
<td>1.28</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>A -0.024Φ + 1.14</td>
<td>1.14</td>
<td>0.9691</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>B 0.244 [E₄(30)] - 14.246</td>
<td>1.17</td>
<td>0.9851</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C -0.0097Φ - 0.11Φ⁰.⁵ + 1.30</td>
<td>1.30</td>
<td>0.9984</td>
</tr>
<tr>
<td>Nitroethane</td>
<td>MeOH</td>
<td>N/A</td>
<td>Chromatographically Determined</td>
<td>1.32</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>A -0.023Φ + 1.24</td>
<td>1.24</td>
<td>0.9899</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>B 0.2385 [E₄(30)] - 13.804</td>
<td>1.27</td>
<td>0.9961</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C -0.016Φ - 0.062Φ⁰.⁵ + 1.33</td>
<td>1.33</td>
<td>0.9992</td>
</tr>
<tr>
<td>Benzyl Alcohol</td>
<td>MeOH</td>
<td>N/A</td>
<td>Chromatographically Determined</td>
<td>1.66</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>A -0.025Φ + 1.56</td>
<td>1.56</td>
<td>0.9891</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>B 0.2589 [E₄(30)] - 14.768</td>
<td>1.59</td>
<td>0.9951</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C -0.017Φ - 0.070Φ⁰.⁵ + 1.66</td>
<td>1.66</td>
<td>0.9991</td>
</tr>
<tr>
<td>Benzene</td>
<td>MeOH</td>
<td>N/A</td>
<td>Chromatographically Determined</td>
<td>1.72</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>A -0.018Φ + 1.72</td>
<td>1.72</td>
<td>0.9953</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>B 0.1861 [E₄(30)] - 10.017</td>
<td>1.74</td>
<td>0.9835</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C -0.020Φ + 0.016Φ⁰.⁵ + 1.70</td>
<td>1.70</td>
<td>0.9963</td>
</tr>
</tbody>
</table>
by the fact that this column has the largest phase ratio (product of surface area and bonding density) so it should have the greatest retention.

The solutes benzyl alcohol and nitroethane have increased intercepts on the polar embedded column, contrary to the other solutes, to which we attribute their hydrogen bonding ability. Benzyl alcohol’s hydrogen donating ability creates more interactions with the polar embedded group of the stationary phase increasing retention compared to the conventional column. As for nitroethane, not only is there the ability to hydrogen bond, but also the polar group embedded in the stationary phase is a carbamate, so there are N-N lone pair interactions that increase the retention of nitrogen-containing solutes.

For acetonitrile-modified systems we also see reduced curvature in the low Φ region. This is also true for the methanol systems, but is not as obvious in the figures. The curvature in the system is represented by the coefficient (E) in the equation

$$\log k' = \Phi E \sqrt{\Phi} + \log k'_w.$$  

The larger absolute value of the coefficient means greater curvature. The sign of the coefficient implies the direction of curvature, where a (+) coefficient implies convex (downward) curvature and a (-) coefficient implies concave (upward) curvature. Looking at the acetonitrile data, benzyl alcohol shows an E coefficient decrease from 0.20 for the conventional phase to 0.08 for the polar-endcapped phase (tables 2 and 4), translating into a 60% reduction of curvature. There is also a large decrease in curvature for 3-pentanone (actually all the ketones had this reduction in curvature, but only 3-pentanone is listed). Benzene had seven-fold less curvature than the polar ketones or alcohol (Zorbax column, ACN modifier). When comparing the Zorbax and Symmetry Shield columns benzene shows an E coefficient increase (from 0.04 to 0.07), respectively. This small change could be due to experimental error. The curvature for nitroethane (in ACN) actually increases by an order of magnitude. This is again attributed to the N-N lone pair interactions between the stationary phase and solute. Overall, the trend seems to be that of increasing linearity of the plots. These observations are reinforced by the correlation coefficient ($r^2$) of the linear equations. The less curvature will produce a better correlation to the linear equation.

The hybrid Xterra RP-18 column has the same polar embedded ligand as the Symmetry Shield column just discussed, but a different base particle. Investigation of the regression data in table 2.5 shows a shift of all solutes to decreased retention values.
compared to the Symmetry Shield column. The decreased retention is believed to be a result of the lower surface area (175 m$^2$/g) of the hybrid phase compared to the Symmetry Shield column (332 m$^2$/g). This belief is reinforced by the similarities in the coefficient of the linear term ($B\Phi$) of the modified Schoenmaker’s equation. All the solute/modifier pairs have approximately the same slope as they did when chromatographed using the Symmetry Shield column.

It is also visually evident in figure 2.4 that the curvature in the data seems diminished. Quantitative evidence can again be seen in the $E$ coefficient of the modified solubility parameter model (table 2.5). From the data of the Xterra and Symmetry Shield columns, the deviation from linearity is much less in the Xterra column. For example, the $E$ coefficient for diethyl ketone in acetonitrile-modified systems is reduced from 0.14 on the Symmetry Shield to 0.08 on the Xterra column. This is a 43% reduction in curvature from Symmetry Shield to the Xterra column (67% from Zorbax to Xterra). Each solute has diminished curvature when using the Xterra column compared to the Symmetry Shield column. Since both columns have the same polar embedded ligand, it would seem logical that the reduced curvature is related to the base particle. There are far less surface silanols on the hybrid particle since the surface contains methyl groups in the place of some surface silanols. The reduction of surface silanols will help in the reduction of curvature. Also, there could be effects from the reduced bonding density because there are not as many polar embedded groups for hydrogen bonding interactions with the polar solutes. The hybrid phase has a substantial decrease in the “coefficient of curvature” ($E$ coefficient) compared to the polar embedded phase, and the polar embedded phase has an overall decrease in curvature compared to the conventional and polar endcapped phases, setting the hybrid phase further apart from the others. In the next section we will see how this translates into extraplogating the log $k'_w$ values from isocratic data points.
Figure 2.4. Chromatographic data for the Xterra column. (A) acetonitrile as the organic modifier and (B) methanol as modifier. Legend: (x) nitroethane, (Δ) diethyl ketone, (*) benzyl alcohol and (●) benzene.
<table>
<thead>
<tr>
<th>Solute</th>
<th>Modifier</th>
<th>Formula(^a)</th>
<th>Equation</th>
<th>log (k'_w)</th>
<th>Correlation ((r^2))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diethyl ketone</td>
<td>ACN</td>
<td>N/A</td>
<td>Chromatographically Determined</td>
<td>0.85</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>-0.018Φ + 0.74</td>
<td>0.74</td>
<td>0.9721</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.1353 ([E_T(30)] - 7.7954)</td>
<td>0.77</td>
<td>0.9886</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>-0.0075Φ - 0.08Φ(^{0.5}) + 0.85</td>
<td>0.85</td>
<td>0.9996</td>
<td></td>
</tr>
<tr>
<td>Nitroethane</td>
<td>ACN</td>
<td>N/A</td>
<td>Chromatographically Determined</td>
<td>0.95</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>-0.024Φ + 0.88</td>
<td>0.88</td>
<td>0.9861</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.1867 ([E_T(30)] - 10.838)</td>
<td>0.93</td>
<td>0.9993</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>-0.016Φ - 0.066Φ(^{0.5}) + 0.98</td>
<td>0.98</td>
<td>0.9966</td>
<td></td>
</tr>
<tr>
<td>Benzyl Alcohol</td>
<td>ACN</td>
<td>N/A</td>
<td>Chromatographically Determined</td>
<td>1.35</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>-0.024Φ + 1.25</td>
<td>1.25</td>
<td>0.9912</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.1881 ([E_T(30)] - 10.556)</td>
<td>1.30</td>
<td>0.9951</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>-0.017Φ - 0.062Φ(^{0.5}) + 1.34</td>
<td>1.34</td>
<td>0.9997</td>
<td></td>
</tr>
<tr>
<td>Benzene</td>
<td>ACN</td>
<td>N/A</td>
<td>Chromatographically Determined</td>
<td>1.48</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>-0.022Φ + 1.50</td>
<td>1.50</td>
<td>0.9866</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.1639 ([E_T(30)] - 8.7975)</td>
<td>1.54</td>
<td>0.9554</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>-0.027Φ + 0.044Φ(^{0.5}) + 1.43</td>
<td>1.43</td>
<td>0.9920</td>
<td></td>
</tr>
<tr>
<td>Diethyl ketone</td>
<td>MeOH</td>
<td>N/A</td>
<td>Chromatographically Determined</td>
<td>0.85</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>-0.019Φ + 0.77</td>
<td>0.77</td>
<td>0.9846</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.1941 ([E_T(30)] - 11.467)</td>
<td>0.80</td>
<td>0.9941</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>-0.011Φ - 0.063Φ(^{0.5}) + 0.86</td>
<td>0.86</td>
<td>0.9989</td>
<td></td>
</tr>
<tr>
<td>Nitroethane</td>
<td>MeOH</td>
<td>N/A</td>
<td>Chromatographically Determined</td>
<td>0.95</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>-0.020Φ + 0.91</td>
<td>0.91</td>
<td>0.9966</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.2009 ([E_T(30)] - 11.759)</td>
<td>0.94</td>
<td>0.9969</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>-0.016Φ - 0.027Φ(^{0.5}) + 0.95</td>
<td>0.95</td>
<td>0.9991</td>
<td></td>
</tr>
<tr>
<td>Benzyl Alcohol</td>
<td>MeOH</td>
<td>N/A</td>
<td>Chromatographically Determined</td>
<td>1.35</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>-0.023Φ + 1.29</td>
<td>1.29</td>
<td>0.9950</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.2286 ([E_T(30)] - 13.125)</td>
<td>1.32</td>
<td>0.9958</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>-0.018Φ - 0.038Φ(^{0.5}) + 1.34</td>
<td>1.34</td>
<td>0.9988</td>
<td></td>
</tr>
<tr>
<td>Benzene</td>
<td>MeOH</td>
<td>N/A</td>
<td>Chromatographically Determined</td>
<td>1.44</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>-0.017Φ + 1.45</td>
<td>1.45</td>
<td>0.9880</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.1664 ([E_T(30)] - 9.0407)</td>
<td>1.47</td>
<td>0.9709</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>-0.020Φ + 0.027Φ(^{0.5}) + 1.42</td>
<td>1.42</td>
<td>0.9915</td>
<td></td>
</tr>
</tbody>
</table>
**Estimating log k’<sub>w</sub> by Extrapolation**

In the previous sections we showed how accurately the three fitting functions modeled the data, while using all eleven isocratic retention values. This showed that when using all the data points at low percentages of organic modifier, the modified solubility parameter equation was best at fitting the data (0.9959 ± 0.0058), followed by the [Eₜ(30)] (0.9822 ± 0.0164) and finally the linear equation (0.9767 ± 0.0238), where n = 32 for each fitting equation. The coefficient of curvature for the modified solubility parameter model allowed non-linear deviations to be more accurately modeled than the one-term equations, resulting in the better fitting, and usually gave very accurate log k’<sub>w</sub> values. We also could see that there was reduced curvature in the polar embedded phase and the hybrid-based polar embedded phase when compared to the conventional and endcapped phases.

Most researchers that use these log k’<sub>w</sub> values do not include the extensive data near pure water as in these experiments, but rather take a few data points in the linear range and extrapolate back to the intercept. Since there are no data at low Φ for deducing curvature, there is often great error in the extrapolation, especially for ACN as the modifier. For this reason we have re-evaluated the data for the purposes of extrapolating a log k’<sub>w</sub> value. In this exercise we have discarded all data points lower than 15% organic modifier. This should allow us to see the extrapolation error associated with each column and fitting equation. The five data points in the range of 50-15% modifier are mostly within the linear range of modifiers, so there is a high correlation coefficient for all data and fitting functions (r² = 0.9950 ± 0.0082, n=96), but the resulting intercept and its error is the meaningful piece of information. For this reason the r² values will not be further discussed. As tables 2.6 & 2.7 show, there are rational trends in the data. For the three modeling equations, the modified solubility parameter equation is consistently the worst in extrapolating a log k’<sub>w</sub> value. This is because the fitting equation is allowing non-linearity (modeled by the E term) and with a low number of data points it may be forcing any slight differentiation of the data to be modeled as curvature. Because this is the region that is consistent with linear behavior, any induced curvature at this point has the potential to be grossly unrepresentative of the actual trends at low modifier.
concentrations. The other trend with the modeling equations is that the \([E_t(30)]\) model consistently gives lower error in extrapolation compared to the linear equation for all columns. This data is consistent with results published by Dorsey’s group [26,27]. So the overall ranking of equation (by percent error, \(n=32\) each) is: \([E_t(30)]\) (12.1%) < linear (16.8%) < modified solubility parameter (24.2%). There is also decreased error in extrapolation based on stationary phase type. Note that the modified solubility parameter model is inducing curvature not necessarily representative of the system; so the data for the other two models will be used for the column comparison. The column comparison shows, of decreasing order (in percent error, \(n=16\) each) is: Zorbax (16.7%) ~ YMC (16.9) > Symmetry Shield (13.5%) > Xterra (10.8%). This trend mimics the trend of decreasing curvature in the log \(k^\prime\) vs. \(\Phi\) graphs seen earlier in this paper. This was expected since the non-linearity of the data is what creates the extrapolation error. Also, as expected, the acetonitrile data consistently has a higher error in extrapolation, due to the enhanced non-linearity of these systems.

**Conclusions**

We have attempted to classify reversed-phase packings with respect to their efficacy for the rapid estimation of the hydrophobicity indicator \(\log k^\prime_w\). The columns studied represent the newer style reversed-phase packings as compared to conventional reversed-phase packings. It was observed that there is almost no difference in retention characteristics for the conventional reversed-phase \(C_{18}\) ligand and the polar endcapped ligand. Their data were almost overlapping on most occasions, showing that the polar endcapping was insufficient at creating a more stable environment for highly aqueous milieu. This resulted in the same error in extrapolation for both columns. The polar embedded column, however, did show a decrease in curvature in the data, which did result in an overall decrease of the extrapolation error. The data for this column did show that the ability of the ligand to hydrogen bond can change the retention characteristics for polar solutes, and that the N-N lone pair interactions will also greatly influence the retention of nitrogen containing compounds.
Table 2.6. Extrapolation error for columns and regression equations, in acetonitrile systems. This data shows the extrapolated log k’w value (from 15% to the y-axis), and the error in that value. The average number in bold is the average error of all 4 solutes for each column, and each regression equation. a) Mod. S.P. stands for Modified Solubility Parameter model

<table>
<thead>
<tr>
<th>Column</th>
<th>Experimental log k’w</th>
<th>Linear Extrapolation log k’w</th>
<th>%Error</th>
<th>ET-30 Extrapolation log k’w</th>
<th>%Error</th>
<th>Mod. S.P. Extrapolation log k’w</th>
<th>%Error</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zorbax</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diethyl Ketone</td>
<td>1.63</td>
<td>0.98</td>
<td>40.2%</td>
<td>1.13</td>
<td>30.8%</td>
<td>1.57</td>
<td>4.0%</td>
</tr>
<tr>
<td>Nitroethane</td>
<td>0.49</td>
<td>0.47</td>
<td>3.9%</td>
<td>0.56</td>
<td>12.9%</td>
<td>0.07</td>
<td>85.4%</td>
</tr>
<tr>
<td>Benzyl Alcohol</td>
<td>1.54</td>
<td>0.99</td>
<td>35.8%</td>
<td>1.18</td>
<td>23.1%</td>
<td>2.23</td>
<td>45.1%</td>
</tr>
<tr>
<td>Benzene</td>
<td>1.97</td>
<td>1.87</td>
<td>4.9%</td>
<td>2.08</td>
<td>5.5%</td>
<td>2.80</td>
<td>42.5%</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td></td>
<td></td>
<td><strong>21.2%</strong></td>
<td></td>
<td><strong>18.1%</strong></td>
<td></td>
<td><strong>44.2%</strong></td>
</tr>
<tr>
<td>YMC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diethyl Ketone</td>
<td>1.60</td>
<td>0.98</td>
<td>38.9%</td>
<td>1.11</td>
<td>30.3%</td>
<td>1.44</td>
<td>9.8%</td>
</tr>
<tr>
<td>Nitroethane</td>
<td>0.53</td>
<td>0.50</td>
<td>5.6%</td>
<td>0.57</td>
<td>7.9%</td>
<td>-0.03</td>
<td>105.1%</td>
</tr>
<tr>
<td>Benzyl Alcohol</td>
<td>1.58</td>
<td>1.02</td>
<td>35.3%</td>
<td>1.22</td>
<td>23.3%</td>
<td>2.11</td>
<td>33.3%</td>
</tr>
<tr>
<td>Benzene</td>
<td>1.95</td>
<td>1.91</td>
<td>1.9%</td>
<td>2.11</td>
<td>8.7%</td>
<td>1.94</td>
<td>0.0%</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td></td>
<td></td>
<td><strong>20.4%</strong></td>
<td></td>
<td><strong>17.5%</strong></td>
<td></td>
<td><strong>37.1%</strong></td>
</tr>
<tr>
<td>Sym. Shield</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diethyl Ketone</td>
<td>1.27</td>
<td>0.91</td>
<td>28.4%</td>
<td>1.04</td>
<td>17.9%</td>
<td>1.08</td>
<td>14.3%</td>
</tr>
<tr>
<td>Nitroethane</td>
<td>1.31</td>
<td>0.98</td>
<td>25.8%</td>
<td>1.15</td>
<td>12.3%</td>
<td>1.60</td>
<td>22.0%</td>
</tr>
<tr>
<td>Benzyl Alcohol</td>
<td>1.65</td>
<td>1.44</td>
<td>12.8%</td>
<td>1.63</td>
<td>1.4%</td>
<td>1.68</td>
<td>1.7%</td>
</tr>
<tr>
<td>Benzene</td>
<td>1.72</td>
<td>1.91</td>
<td>10.9%</td>
<td>2.11</td>
<td>23.0%</td>
<td>1.68</td>
<td>2.1%</td>
</tr>
<tr>
<td><strong>Average</strong></td>
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<td></td>
<td><strong>19.5%</strong></td>
<td></td>
<td><strong>13.6%</strong></td>
<td></td>
<td><strong>10.0%</strong></td>
</tr>
<tr>
<td>Xterra</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>0.85</td>
<td>0.64</td>
<td>24.6%</td>
<td>0.75</td>
<td>11.5%</td>
<td>0.73</td>
<td>14.4%</td>
</tr>
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<td>Nitroethane</td>
<td>0.95</td>
<td>0.75</td>
<td>21.5%</td>
<td>0.91</td>
<td>4.8%</td>
<td>1.31</td>
<td>37.3%</td>
</tr>
<tr>
<td>Benzyl Alcohol</td>
<td>1.35</td>
<td>1.19</td>
<td>11.9%</td>
<td>1.36</td>
<td>0.7%</td>
<td>1.37</td>
<td>1.3%</td>
</tr>
<tr>
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<td>1.48</td>
<td>1.62</td>
<td>9.6%</td>
<td>1.81</td>
<td>22.1%</td>
<td>1.30</td>
<td>12.4%</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td></td>
<td></td>
<td><strong>16.9%</strong></td>
<td></td>
<td><strong>9.8%</strong></td>
<td></td>
<td><strong>16.4%</strong></td>
</tr>
</tbody>
</table>
Table 2.7. Extrapolation error for columns and regression equations, in methanol systems. This data shows the extrapolated log $k'w$ value (from 15% to the y-axis), and the error in that value. The average number in bolds is the average error of all 4 solutes for each column, and each regression equation. a) Mod. S.P. stands for Modified Solubility Parameter model

<table>
<thead>
<tr>
<th>Column</th>
<th>Experimental</th>
<th>Linear Extrapolation</th>
<th>ET-30 Extrapolation</th>
<th>Mod. S.P. Extrapolation</th>
</tr>
</thead>
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<td>log $k'w$</td>
<td>log $k'w$</td>
<td>%Error</td>
<td>log $k'w$</td>
</tr>
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<td></td>
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<td></td>
<td></td>
</tr>
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<td>1.33</td>
<td>17.0%</td>
<td>1.45</td>
</tr>
<tr>
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</tr>
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<td>1.50</td>
</tr>
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<td>1.90</td>
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<td><strong>16.6%</strong></td>
<td><strong>10.7%</strong></td>
</tr>
<tr>
<td>YMC</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diethyl Ketone</td>
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<td>1.28</td>
<td>18.6%</td>
<td>1.39</td>
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<td>0.35</td>
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<tr>
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<td>13.1%</td>
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</tr>
<tr>
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<td>1.83</td>
<td>3.3%</td>
<td>1.93</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td></td>
<td></td>
<td><strong>17.4%</strong></td>
<td><strong>12.1%</strong></td>
</tr>
<tr>
<td>Sym. Shield</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diethyl Ketone</td>
<td>1.28</td>
<td>0.98</td>
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<td>1.08</td>
</tr>
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<td>1.27</td>
</tr>
<tr>
<td>Benzyl Alcohol</td>
<td>1.66</td>
<td>1.48</td>
<td>10.5%</td>
<td>1.59</td>
</tr>
<tr>
<td>Benzene</td>
<td>1.72</td>
<td>1.77</td>
<td>3.4%</td>
<td>1.87</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td></td>
<td></td>
<td><strong>12.4%</strong></td>
<td><strong>8.3%</strong></td>
</tr>
<tr>
<td>Xterra</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diethyl Ketone</td>
<td>0.85</td>
<td>0.68</td>
<td>19.4%</td>
<td>0.77</td>
</tr>
<tr>
<td>Nitroethane</td>
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<td>0.89</td>
<td>6.7%</td>
<td>0.97</td>
</tr>
<tr>
<td>Benzyl Alcohol</td>
<td>1.35</td>
<td>1.26</td>
<td>6.3%</td>
<td>1.36</td>
</tr>
<tr>
<td>Benzene</td>
<td>1.44</td>
<td>1.55</td>
<td>7.1%</td>
<td>1.63</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td></td>
<td></td>
<td><strong>9.9%</strong></td>
<td><strong>6.7%</strong></td>
</tr>
</tbody>
</table>

34
The hybrid base particle trademarked as Xterra, with an attached polar embedded ligand produced results that gave the least curvature in the graphs of log k’ vs. Φ, and resulted in the least error in extrapolation.

The three modeling equations revealed that when data points are taken at low modifier concentrations, the modified solubility parameter equation was the best at modeling the data, and that the coefficient of curvature (E term) is the quantitative descriptor of curvature in the low Φ region. It also showed that the linear equation was the worst at modeling the data. When we extrapolated the data to the log k’\textsubscript{w} value it was observed that the modified solubility parameter equation would model curvature in the few data points that are not representative of the data. This is due to the limited data points and even fewer degrees of freedom. This translated into the worst extrapolation error of the three equations. Conversely, the [E\textsubscript{t}(30)] model was the best at extrapolating to the log k’\textsubscript{w} value.

The data presented here illustrates the error associated with linear extrapolations using conventional C\textsubscript{18} columns. It has been shown that newer generation polar-embedded columns show less curvature in the low Φ region. Knowing these polar-embedded phases are adequately solvated at highly aqueous conditions (larger linear range of Φ vs. retention) is especially useful information. Since data are almost never available in the low Φ region, a column that exhibits less change in stationary phase solvation will show less error upon extrapolation to a log k’\textsubscript{w} value. This allows greater confidence in an extrapolation procedure with no retention data at less than 20% modifier. All else equal, the best reduction in extrapolation error would be to use a polar embedded column (here the hybrid based polar embedded column) with methanol as the modifier, and extrapolate (from 15% MeOH) using the [E\textsubscript{t}(30)] model, for our data this resulted in an average 7% error in the data (n=4).
CHAPTER 3

GRADIENT REVERSED-PHASE LIQUID CHROMATOGRAPHY FOR
ESTIMATION OF HYDROPHOBICITY

Introduction

It was seen in Chapter 2 that polar embedded stationary phases for RPLC are more accurate at determining the chromatographic hydrophobicity parameter \( \log k'_{w} \). Although isocratic experiments are much quicker than octanol / water partitioning experiments, they are still lengthy from a chromatographic standpoint. This next section investigates whether or not these newer phases can readily determine the \( \log k'_{w} \) value by use of faster gradient experiments. By performing two experiments in which the mobile phase gradient time (\( t_{G} \)) is significantly different, \( \log k'_{w} \) values can be calculated. These values are compared to the experimentally determined values as well as the isocratic extrapolation values previously determined. These experiments will then be performed again with 3% 1-propanol added to the mobile phases to determine if there are any extra solvation effects on the stationary phases.

Theory

Snyder [67-69] defines the integral of a gradient in liquid chromatography as:

\[
\int_{0}^{V_{G}} \frac{b^{V_{m}}}{k_{o} V_{m}} dV = 1
\]  

(3.1)
where $V_g$ is retention volume in gradient, $b$ is gradient steepness, $V$ is volume, $V_m$ is column void volume, $k_o$ is retention factor equivalent to isocratic separation where the mobile phase composition equals that of the beginning gradient composition. Integration of this yields

\[
V_g = \left(\frac{V_m}{b}\right) \log (2.3k_o b + 1) \tag{3.2}
\]

Substituting gradient time for gradient volume by

\[
t_g = \frac{(V_g + V_m)}{F} \tag{3.3}
\]

and

\[
F = \frac{V_m}{t_o} \tag{3.4}
\]

where $t_g$ is retention time in gradient, $F$ is mobile phase flow rate, and $t_o$ is void time. This substitution yields the desired form of equation 3.2

\[
t_g = \left(\frac{t_o}{b}\right) \log (2.3k_o b + 1) + t_o \tag{3.5}
\]

Gradient steepness is defined as

\[
b = \Delta \phi S V_m / t_G F \tag{3.6}
\]

where $\Delta \phi$ is the total change in mobile phase composition during the gradient run, $S$ is the slope of the Linear Solvent Strength (LSS) model, and all other variables are as previously designated. If two gradient runs are performed with different gradient times ($t_G$) then equation 3.6 reduces to the following
\[
\frac{b_1}{b_2} = \beta = \frac{t_{G2}}{t_{G1}}
\]  
(3.7)

where \( \beta \) is defined as the ratio of gradient times (or gradient steepness) of the two different runs. The numerical subscripts refer to the individual gradient run. For two gradient runs with different gradient times, there will exist two equations for gradient retention times

\[
t_1 = \left( t_o / b_1 \right) \log (2.3k_o b_1 + 1) + t_o
\]  
(3.8)

\[
t_2 = \left( t_o / b_2 \right) \log (2.3k_o b_2 + 1) + t_o
\]  
(3.9)

Once both chromatographic experiments have been executed, the use of equations 3.7-3.9 allows calculation of the three unknown parameters \((b_1, b_2, k_o)\) and ultimately solve for \(\log k'_{w}\) through the following mathematical operations.

1. Estimate \(b_1\)
2. Solve for \(k_o\) by use of equation 3.8
3. Solve for \(b_2\) by use of equation 3.7
4. Using \(k_o\) (step 2) and \(b_2\) (step 3), solve for \(t_2\) using equation 3.9
5. Compare experimentally determined \(t_2\) with calculated \(t_2\). Re-estimate \(b_1\) and repeat steps 1-5 until the calculated and experimentally determined \(t_2\) values are the same. Computer aided iterative processes can easily solve these equations. For this step we used Microsoft Excel and the “Solver” routine.
6. Once the calculated and experimentally determined \(t_2\) values are equal, the three unknowns have been solved \((b_1, b_2, k_o)\). This now allows calculation of \(S\) from equation 3.6 (slope of LSS equation). Also, the gradient parameter \(k_o\) is the isocratic retention value \((k')\) at the starting m.p. composition of the gradient (e.g. if gradient starts at 10% modifier, then \(k_o = k'_{10}\%\) or more appropriately \(\log k_o = \log k'_{10}\%).

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This now allows calculation of log $k'_w$ for the analyte of interest by solving the LSS equation (eqn. 1.6) using the log $k'$ and $S$ values calculated.

**Experimental**

Chromatographic experiments were performed with a Shimadzu LC-10 system [including degasser (DGU-14A), pump (LC10-ADvp), auto injector (SIL-10A), system controller (SCL-10A) and UV-Vis detector (SPD-10A)] controlled via the CLASS-VP software (Shimadzu Corp., Columbia MD, USA). Columns were thermostated using a Fisher Scientific chiller/heater (Fisher Scientific, Pittsburgh PA, USA).

The bonded-phases here are the same as those in Chapter 2, and are shown in figure 1.2. Again, all columns are reversed-phase C$_{18}$ columns (150 X 4.6 mm, 5 µm dp), where the four columns have different bonding chemistries. The Zorbax SB-C$_{18}$ column (Hewlett Packard, Wilmington DE, USA) is a conventional C$_{18}$ bonded phase, the YMC ODS-AQ column (YMC Inc., Wilmington, NC, USA) is a polar endcapped C$_{18}$ phase, the Symmetry Shield RP-18 column (Waters Corp. Milford CT, USA) is a polar embedded bonded phase, and the Xterra RP-18 (Waters Corp. Milford CT, USA) is a hybrid particle made of an organic/inorganic matrix which is bonded with polar embedded ligands.

Acetonitrile and methanol were HPLC grade (Fisher Scientific, Fair Lawn NJ, USA). The water used for mobile phases was purified with a Barnstead NANOPure II system (Barnstead International, Dubuque IA, USA). All solutes were from Fisher Scientific (Fisher Scientific, Fair Lawn NJ, USA) and deuterium oxide from Cambridge Isotope Labs (Cambridge Isotope Labs, Andover MA, USA).

Gradient chromatography was performed at 1 mL/min, 40°C with detection at 254 nm. Gradients started at 10, 5, and 0% organic modifier and went to 80% organic modifier. Gradient times were 15 and 45 minutes. System dwell volume was eliminated by using a delayed injection. Samples were made in water using only enough organic modifier as needed for solvation.

Obtaining experimental log $k'_w$ values for such hydrophobic analytes is prohibitively long on a 15 cm column, so these values were obtained by unpacking the 15 cm columns and repacking the stationary phase into 4 cm long (4.6 mm i.d.) column.
blanks (5 cm X 4.6 mm for Xterra phase). Packing the columns was done through
traditional slurry packing procedure with an air driven pump (Haskel Engineering and
Supply, Burbank, CA, USA) at 7000 psi with three 200 mL aliquots of pushing solvents.
First was chloroform then methanol and finally methanol / water (50:50, v/v). The
stationary phase was suspended in chloroform by sonication (Ultrasonic Cleaner FS30,
Fisher Scientific, Pittsburgh, PA, USA) prior to packing. These experiments on the
smaller columns used the previously described experimental conditions, except at a flow
rate of 2 mL/min.

Results and Discussion

These gradient experiments and subsequent calculations to find log k’\textsubscript{w} values
were not without their problems, and unfortunately half of our data is not eligible to be
discussed. This is because the retention of these analytes was not large enough for the
equations to work. It was found that if the calculations do not yield a log k’\textsubscript{w} value
greater than ~1.5, the equations are not adequate. This is due to the small “k\textsubscript{o}b” term
inside the logarithm resulting in an unsolvable set of equations. Therefore our ketone
data (acetone, methyl-ethyl ketone and diethyl ketone) and nitro-alkane data (nitroethane,
nitropropane, and nitrobutane) had to be discarded. The log k’\textsubscript{w} values for the benzene
and paraben homologs (benzene, toluene, ethyl benzene and ethyl paraben, propyl
paraben, butyl paraben) were obtained through the preceding equations with the residual
of the calculations being on the order of 10\textsuperscript{-7}. This assures that the calculated log k’\textsubscript{w}
values have no appreciable error due to mathematics. In order to perform the theoretical
calculations, two gradients must be performed (in triplicate) with different gradient times
(where t\textsubscript{G2}=3 * t\textsubscript{G1}). We used 15 and 45 minute gradient times.

The first experiments looked at the change in calculated log k’\textsubscript{w} value with the
change in gradient starting mobile phase composition. We performed gradients from 10-
80%, 5-80% and 0-80% organic modifier, for both methanol and acetonitrile modifiers.
The results of these experiments are listed in table 3.1. This table show trends between
column / solute pairs for each modifier. The absolute values of
<table>
<thead>
<tr>
<th>Concentration</th>
<th>Butyl Paraben</th>
<th>Propyl Paraben</th>
<th>Ethyl Paraben</th>
<th>Butyl Paraben</th>
<th>Propyl Paraben</th>
<th>Ethyl Paraben</th>
<th>Butyl Paraben</th>
<th>Propyl Paraben</th>
<th>Ethyl Paraben</th>
<th>Butyl Paraben</th>
<th>Propyl Paraben</th>
<th>Ethyl Paraben</th>
</tr>
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<tbody>
<tr>
<td>2.40</td>
<td>2.12</td>
<td>2.52</td>
<td>2.09</td>
<td>2.91</td>
<td>2.89</td>
<td>2.07</td>
<td>2.91</td>
<td>2.89</td>
<td>2.07</td>
<td>2.91</td>
<td>2.89</td>
<td></td>
</tr>
<tr>
<td>2.40</td>
<td>2.12</td>
<td>2.52</td>
<td>2.09</td>
<td>2.91</td>
<td>2.89</td>
<td>2.07</td>
<td>2.91</td>
<td>2.89</td>
<td>2.07</td>
<td>2.91</td>
<td>2.89</td>
<td></td>
</tr>
<tr>
<td>2.40</td>
<td>2.12</td>
<td>2.52</td>
<td>2.09</td>
<td>2.91</td>
<td>2.89</td>
<td>2.07</td>
<td>2.91</td>
<td>2.89</td>
<td>2.07</td>
<td>2.91</td>
<td>2.89</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.1. Log k' values for methanol and acetonitrile experiments for all four columns studied. Percent modifier designates the percentage at the start of the gradient. Pink highlights indicate upward shifts and blue highlights indicate downward shifts.
retention seem to be fairly consistent between the Zorbax, YMC and Symmetry Shield columns, whereas the Xterra column has values consistently less than the others. This is attributed to the lower phase ratio \( \frac{V_S}{V_M} \) of this column. Data for benzene on the Xterra column is shown in red because the retention is too low for adequate solution of the equations.

This table reveals some other, more interesting results. Within many column / solute / modifier data triplets, there are changes in retention with changes in starting composition of mobile phase (10%, 5%, 0%) gradient. We believe this indicates an inability of a stationary phase to adequately solvate highly aqueous mobile phases. If the stationary phase was completely solvated at each starting composition, then we believe that the calculated log \( k'_{w} \) values would be constant with change in starting gradient composition.

In order to evaluate these data (qualitatively) we have determined that any data triplet that has a continuous drift (increasing or decreasing) by 0.04 log units or more over the range of values is very likely an actual reaction of the stationary phase to the different starting mobile phase composition, not just random error. These drifts are highlighted in table 3.1 (pink indicating a drift upward in and blue indicating a drift downward). It is instantly noticeable that the YMC column has the most highlighted data triplets (7/12), followed by the Zorbax column (4/12), then the Symmetry (2/12) and Xterra (1/12). This is believed to be indicative of decreased stationary phase wettability of the conventional (Zorbax) and polar-endcapped (YMC) columns at increasingly aqueous concentrations, as was determined in Chapter 2.

The highlighted data shows other information as well. In table 3.1 all the pink data are from the acetonitrile modified experiments and all the blue are from methanol experiments. Also, all the data triplets highlighted in pink have retention values lower than two and most of the blue values are closer to three, but since these drifts are quite small it seems foolish to try and extrapolate any physical meaning from the direction of the drift (highlight color).

Since most of the drifting data is at the lowest and highest ranges of retention, a more equal evaluation of the four columns would be to eliminate these extreme ranges (triplets below 2.2 and above 2.7) and focus only on that range of data where there are no
obvious data drifts. Unfortunately there is not the same number of data triplets per column that fall within this range, so to normalize the changes in log k’w values per column we have totaled the variances in each data triplet and normalized it for how many data triplets there were for each column. These results are listed in table 3.2. Columns that do not exhibit many changes in log k’w over the three gradients will have lower “Average Variance / Triplet” values. This value allows the columns to be qualitatively ranked by their reliability for obtaining k’w values from these gradient experiments. These results illustrate that the conventional (Zorbax) and polar endcapped (YMC) C18 phases have higher “Average Variance / Triplet” values signifying these are not as “water friendly” as the polar embedded phases. These results are also compared to the “Coefficient of Curvature” value presented in Chapter 2 which represents a column’s robustness in highly aqueous environments; it is a measure of linearity in isocratic data where lower coefficients of curvature translate into better linearity (i.e. better aqueous stability). The “average variance / triplet” and “coefficient of curvature” values both rank the different stationary phases in the same fashion. Conventional and polar endcapped phases are not as reliable as the polar embedded phases. Again, variance in the data triplets are quite small, so no quantitative conclusions can be drawn; but when this data is compared to data from Chapter 2 the trends are to the same.

The next set of experiments includes 3% 1-propanol into all the gradients and recalculates log k’w values and re-evaluates this data. The addition of propanol allows further investigation of stationary phase solvation effects.

<table>
<thead>
<tr>
<th>Column</th>
<th>Total Variance</th>
<th># of Data Triplets</th>
<th>Average Variance/Triplet</th>
<th>Coefficient of Curvature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zorbax</td>
<td>12</td>
<td>6</td>
<td>2.0</td>
<td>0.762</td>
</tr>
<tr>
<td>YMC</td>
<td>17</td>
<td>5</td>
<td>3.4</td>
<td>0.772</td>
</tr>
<tr>
<td>Symmetry</td>
<td>6</td>
<td>6</td>
<td>1.0</td>
<td>0.662</td>
</tr>
<tr>
<td>Xterra</td>
<td>4</td>
<td>4</td>
<td>1.0</td>
<td>0.409</td>
</tr>
<tr>
<td></td>
<td>Butyl Paraben</td>
<td>Propyl Paraben</td>
<td>Ethyl Paraben</td>
<td>Ethyl Benzene</td>
</tr>
<tr>
<td>----------</td>
<td>---------------</td>
<td>----------------</td>
<td>---------------</td>
<td>---------------</td>
</tr>
<tr>
<td>Xterra RP-18</td>
<td>1.94 1.77 1.98</td>
<td>1.94 1.77 1.98</td>
<td>1.94 1.77 1.98</td>
<td>1.94 1.77 1.98</td>
</tr>
<tr>
<td></td>
<td>2.86 2.85 2.83</td>
<td>2.86 2.85 2.83</td>
<td>2.86 2.85 2.83</td>
<td>2.86 2.85 2.83</td>
</tr>
<tr>
<td></td>
<td>2.24 2.24 2.24</td>
<td>2.24 2.24 2.24</td>
<td>2.24 2.24 2.24</td>
<td>2.24 2.24 2.24</td>
</tr>
<tr>
<td></td>
<td>2.16 2.17 2.18</td>
<td>2.16 2.17 2.18</td>
<td>2.16 2.17 2.18</td>
<td>2.16 2.17 2.18</td>
</tr>
<tr>
<td></td>
<td>Butyl Paraben</td>
<td>Propyl Paraben</td>
<td>Ethyl Paraben</td>
<td>Ethyl Benzene</td>
</tr>
<tr>
<td></td>
<td>1.37 1.35 1.36</td>
<td>1.37 1.35 1.36</td>
<td>1.37 1.35 1.36</td>
<td>1.37 1.35 1.36</td>
</tr>
<tr>
<td></td>
<td>2.27 2.28 2.27</td>
<td>2.27 2.28 2.27</td>
<td>2.27 2.28 2.27</td>
<td>2.27 2.28 2.27</td>
</tr>
<tr>
<td></td>
<td>1.98 2.00 2.01</td>
<td>1.98 2.00 2.01</td>
<td>1.98 2.00 2.01</td>
<td>1.98 2.00 2.01</td>
</tr>
<tr>
<td></td>
<td>YMC Pro-Pack ODS-AQ</td>
<td>YMC Pro-Pack ODS-AQ</td>
<td>YMC Pro-Pack ODS-AQ</td>
<td>YMC Pro-Pack ODS-AQ</td>
</tr>
<tr>
<td></td>
<td>2.35 2.35 2.35</td>
<td>2.35 2.35 2.35</td>
<td>2.35 2.35 2.35</td>
<td>2.35 2.35 2.35</td>
</tr>
<tr>
<td></td>
<td>2.71 2.70 2.69</td>
<td>2.71 2.70 2.69</td>
<td>2.71 2.70 2.69</td>
<td>2.71 2.70 2.69</td>
</tr>
<tr>
<td></td>
<td>Zorbax SB-18</td>
<td>Zorbax SB-18</td>
<td>Zorbax SB-18</td>
<td>Zorbax SB-18</td>
</tr>
</tbody>
</table>

Start of the gradient. Pink highlight indicates upward shifts and blue highlights indicates downward shifts.

Table 3.3. Log k' values for experiments modified with 3% 1-propanol for all four columns studied. Percent modifier designates the percentage of the start of the gradient.  Pink highlights indicate upward shifts and blue highlights indicates downward shifts.
The results of these experiments are listed in table 3.3. In comparison to the previous data (table 3.1), most retention times have decreased. This is because propanol decreases the hydrophobic effect within the mobile phase, which decreases the thermodynamic difference (ΔH is closer to zero) between phases, therefore reducing retention. This decrease in retention is also correlated to the highlighted data triplets. There are now more pink highlights because there are more retention values less than two and less blue highlights because there are fewer values near three. In these results the highlighted shifts are more evenly distributed between stationary phase type. We believe that in these experiments all four columns are behaving in the same fashion with the presence of the small surfactant. This is further evidenced in table 3.4. All four columns all have an “Average Variance/Set” value close to unity. For the conventional and polar endcapped columns this is a significant reduction in variance compared to the non-propanol experiments. We interpret this as adequate solvation of these stationary phases in highly aqueous environments, which was aided by a small amount of 1-propanol. The polar-embedded columns show no reduction in this variance because we believe they were adequately solvated in highly aqueous environments without any surfactant.

The last experiments for this project look at the correlations between gradient estimations and actual values of log k’_w for column classification. The results of these experiments are listed in table 3.5. We expected the Symmetry and Xterra columns to have less error for gradient determination of log k’_w than the other non-polar embedded columns. However, that does not seem to be true. These results show that methanol gradients give less than 10% error in most experiments and that acetonitrile gradients can

<table>
<thead>
<tr>
<th>Column</th>
<th>Total Variance</th>
<th># of Data Triplets</th>
<th>Average Variance/Triplet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zorbax</td>
<td>5</td>
<td>6</td>
<td>0.8</td>
</tr>
<tr>
<td>YMC</td>
<td>9</td>
<td>6</td>
<td>1.5</td>
</tr>
<tr>
<td>Symmetry</td>
<td>8</td>
<td>7</td>
<td>1.1</td>
</tr>
<tr>
<td>Xterra</td>
<td>6</td>
<td>5</td>
<td>1.2</td>
</tr>
</tbody>
</table>
give much larger error (>20%). This increased error is due to the LSS model failing because this relationship is not linear with volume fraction of acetonitrile. However, these results are column independent so no correlation between column and gradient accuracy can be made. This is in contrast to our earlier experiments in isocratic RPLC. We have previously concluded (Chapter 2) that the polar-embedded columns (Symmetry and Xterra) were more accurate at extrapolating log $k'_w$ values than the conventional (Zorbax) and polar-endcapped (YMC) columns. These conflicting results can be interpreted by the nature of the gradient conditions. Gradient RPLC is a constantly changing environment and there may not be enough residence time of a solute in purely (or highly) aqueous conditions to see the same trends that are seen in isocratic conditions. Re-packing the silica from 15 cm to 4 or 5 cm columns should not have any adverse affects to these pure water experiments since retention factor is independent of column length. Also the re-packed columns were tested prior to experimentation and had approximately the same plates per meter as their 15 cm versions.

### Conclusions

We investigated the use of gradient RPLC for accurate determination of the log $k'_w$ hydrophobicity parameter and whether newer bonded phases can more accurately determine these values. Small variances in the data are seen, but it is difficult to relate

<table>
<thead>
<tr>
<th>Zorbax</th>
<th>YMC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene</td>
<td>expt</td>
</tr>
<tr>
<td></td>
<td>1.75</td>
</tr>
<tr>
<td>Toluene</td>
<td>2.35</td>
</tr>
<tr>
<td>Ethyl Benzene</td>
<td>2.93</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>XMC</th>
<th>Symmetry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene</td>
<td>expt</td>
</tr>
<tr>
<td></td>
<td>1.66</td>
</tr>
<tr>
<td>Toluene</td>
<td>2.22</td>
</tr>
<tr>
<td>Ethyl Benzene</td>
<td>2.75</td>
</tr>
</tbody>
</table>

| Benzene  | expt     | MeOH | ACN  |
| Toluene  | 1.95     | 2.09 | 1.99 |
| Ethyl Benzene | 2.44  | 2.58 | 2.32 |
this to any real cause-effect relationship. Differences in log $k'_w$ values with changes in starting concentration of organic modifier in the gradient are examined. Those determined not to be random error are correlated with type of column. Many drifts are observed (and highlighted) and most of these are at values lower than 2.0 or greater than 2.7. Since these drifts cannot be directly correlated to column type, but rather absolute retention value, they are eliminated from the column evaluation portion. The remaining data is judged on a per column basis. These “Average Variance / Triplet” values show that there is less fluctuation in the data obtained on polar-embedded columns. This is consistent with our data from isocratic RPLC experiments.

Repeating these gradient experiments with the addition of 3% 1-propanol to the mobile phase reveals further information. This data show that the addition of the propanol has more effects on the Zorbax and YMC (non-polar embedded) columns. The “Average Variance / Triplet” values have decreased significantly in these columns whereas minimal affect to the Symmetry and Xterra (polar-embedded) columns are observed. This is interpreted as adequate solvation of the non-polar embedded stationary phases in highly aqueous environments when there is a small amount of 1-propanol added. On the other hand, the polar-embedded stationary phases are already stable at highly aqueous conditions and addition of 3% 1-propanol does not affect them.

The comparison of gradient extrapolated and actual chromatographic log $k'_w$ values did not give the results we expected. We anticipated seeing less error in the extrapolated data from the polar-embedded columns since our previous (isocratic) data showed that trend. Instead, the data showed that any column studied could give accurate values ($<10\%$ error) from gradient extrapolation when using methanol as the modifier. We attribute this discrepancy to the constantly changing environment of gradient elution RPLC, where there may not be enough residence time of a solute in pure (or highly) aqueous conditions to see the nuances of the stationary phase.
INTRODUCTION TO THIN LAYER CHROMATOGRAPHY AND PLANAR ELECTROCHROMATOGRAPHY

The Future of Planar Chromatography

The heyday of thin layer chromatography (or planar chromatography) has been over for some twenty-five years now. It has been replaced by high-performance liquid chromatography (HPLC), due to faster analysis time, easier quantitative analysis, cost reduction, and minimal training of users. The replacement of thin layer chromatography (TLC) was not only due to these tangible qualities that industries needed but also the chromatographic theory, which allows for higher peak capacities, efficiencies, and other chromatographic figures of merit. It has been stated by Poole, “At the turn of the century modern TLC faces an uncertain future, while conventional TLC is likely to survive as a general laboratory tool in the same mold as precipitation, crystallization and distillation, having survived as indispensable, low cost and low technology operations” [70].

Conventional TLC requiring only a solvent reservoir, TLC plate, and UV light source is still widely used for fast, cheap analyses. These applications include screening a large number of samples qualitatively before investing more time and money for instrumental analysis. Since there is no bulky instrumentation that requires large amounts of electricity, samples can be screened in the field and only relevant samples need be transported. Also, a simpler device could not replace the “quick and dirty” TLC method of separation, as to see how many products (and reactants) are present after organic synthesis.

High-performance TLC (HPTLC), utilizing smaller particles (5-µm) compared to conventional TLC (10-µm), allows for faster analysis times since the separation does not have to develop as far as its conventional counterpart to acquire the same separation
efficiency or resolution. Unfortunately, the use of smaller particles does not increase the zone capacity of the separation, which is limited to 10-14 zones. HPTLC has become an instrumentalized technique requiring expensive hardware and automated components, now being referred to as “Modern TLC”. This further separated TLC from the HPLC dominated industry for the routine analyses of compounds, since new instrumentation requires more money, training and validation. Modern TLC has survived, although severely neglected, due to a few attributes that no other separation technique can as easily provide. These include the use of post-chromatographic reactions or derivatizations for non-UV absorbing chemicals that cannot be easily detected by HPLC. Since the TLC plates are disposable, sample cleanup can be reduced or eliminated when there are matrix components that may irreversibly bind to the stationary phase, which would ruin an HPLC column. Also, multiple sample screening can now be quantitative as well as qualitative due to the instrumental advances. As an example of the quantitative capabilities of modern TLC, tablets known to contain 0.5 mg norgesterol and 2.0 mg ethynodiol diacetate per tablet, were analyzed by TLC using densitometric detection and were found to contain 0.509±0.008 mg norgesterol and 2.00±0.03 mg ethynodiol diacetate per tablet (n=10) [71]. The accuracy and precision of these results are comparable to other chromatographic techniques. Although the above example shows that TLC can be as useful as other separation techniques, there are fundamental limitations due to the capillary forces that drive the mobile phase which limit the separating power of modern TLC.

**Fundamental Limitations**

Migration in TLC is driven by capillary forces, which are weak forces due to the decrease in free energy of the mobile phase as it travels through the porous layer. These weak forces are not strong enough to create adequate mobile phase velocities so molecular diffusion becomes the limiting factor for the zone capacity of the system. Also, there is a quadratic decrease of mobile phase velocity with solvent front migration distance (and time) given by the equation:
where $Z_f$ is the zone front migration distance, $K$ is the velocity constant, and $t$ is time.

The velocity constant $K$ is dependant on the following parameters

$$K = 2K_o d_p (\gamma / \eta) \cos \phi$$

where $K_o$ is the permeability constant of the layer, $d_p$ is the average particle diameter of the substrate, $\gamma$ is the mobile phase surface tension, $\eta$ is the mobile phase viscosity, and $\phi$ is the contact angle between the chromatographic substrate and the mobile phase. This translates into higher velocities using a mobile phase that completely wets the layer ($\phi=0$), has high surface tension, low viscosity and using a layer with larger particles. The optimum velocity for a HPTLC plate is around 0.05 cm/s [72] and regardless of how these variables are manipulated common solvents can at best deliver a mobile phase velocity of 0.02 cm/s at the beginning and the rate decreases as a function of distance (See figure 4.1A, from ref. [73]). This only becomes worse when using reversed-phase layers since aqueous-organic solvents do not completely wet the hydrophobic layer.

Figure 4.1. A) Dependence of mobile phase velocity on solvent front migration distance for silica gel plates (TLC and HPTLC) under capillary and forced flow conditions. The solvent is hexane. B) Dependence of plate height as a function of solvent front migration distance for TLC and HPTLC layers, using capillary and forced flow conditions.
Since there is inadequate flow of mobile phase, zone broadening is primarily due to molecular diffusion, limiting the useful migration distances for separation. HPTLC layers will produce more compact zones than conventional TLC layers, as long as the migration doesn’t exceed 5-6 cm. After this distance, the mobile phase velocity becomes so low that zone broadening surpasses zone migration as can be seen in the relationship of plate height vs. solvent front migration (see figure 4.1B, from ref [70]). If longer migration distances are required then larger particles must be used to allow for better range of mobile phase velocities, but the efficiency of larger particles is lower. This dichotomy between the layers limits the actual zone capacity in any capillary TLC to 10-14 regardless of chromatographic substrate. This also limits the efficiency of the technique to a maximum of about 5000 plates, and this is dependent on the migration distance (since velocity is not constant) so that these efficiencies have to be reported as average values. In comparing TLC layers with HPLC columns, investigation of the Knox equation (equation 4.3) shows the contributions to diffusion and their correlations to columns (table 4.1, adapted from ref. [74]).

Table 4.1. Chromatographic properties of pre-coated silica gel layers and columns. Data for layers determined by over-pressure layer chromatography.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Layers</th>
<th>Columns</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High Performance</td>
<td>Conventional</td>
</tr>
<tr>
<td>Porosity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0.65 - 0.70</td>
<td>0.65 - 0.75</td>
</tr>
<tr>
<td>Interparticle</td>
<td>0.35 - 0.45</td>
<td>0.35 - 0.45</td>
</tr>
<tr>
<td>Intraparticle</td>
<td>0.28</td>
<td>0.28</td>
</tr>
<tr>
<td>Minimum Plate Height (µm)</td>
<td>22 - 25</td>
<td>35 - 45</td>
</tr>
<tr>
<td>Optimum Velocity (mm/s)</td>
<td>0.3 - 0.5</td>
<td>0.2 - 0.5</td>
</tr>
<tr>
<td>Min. Reduced Plate Height</td>
<td>3.5 - 4.5</td>
<td>3.5 - 4.5</td>
</tr>
<tr>
<td>Optimum Reduced Velocity</td>
<td>0.7 - 1.0</td>
<td>0.6 - 1.2</td>
</tr>
<tr>
<td>Knox Equation Coefficients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flow Anisotropy (A)</td>
<td>0.4 - 0.8</td>
<td>1.7 - 2.8</td>
</tr>
<tr>
<td>Longitudinal Diffusion (B)</td>
<td>1.2 - 1.6</td>
<td>1.2 - 2.0</td>
</tr>
<tr>
<td>Resistance Mass Transfer (C)</td>
<td>1.4 - 2.4</td>
<td>0.70 - 0.85</td>
</tr>
<tr>
<td>Separation Impedance</td>
<td>10,000 - 20,000</td>
<td>11,000 - 13,000</td>
</tr>
</tbody>
</table>
Here, $h$ is reduced plate height, $\nu$ is reduced velocity, and $A$, $B$ and $C$ are fitting coefficients. The $A$-term characterizes eddies through the substrate (flow anisotropy) and is a function of the packing homogeneity. For HPTLC plates the $A$-term is almost the same as HPLC columns, representing adequate packing uniformity. Conventional TLC plates have a much larger $A$-term due to the wider distribution of particles (a typical HPTLC layer has an average particle diameter of 4.5 $\mu$m with 80% of the particles lying between 3.0-5.0 $\mu$m, whereas a conventional TLC plate has an average particle size of 10 $\mu$m with 80% of the particles lying between 5.5-12.0 $\mu$m [75]). The $B$-term portrays molecular diffusion (longitudinal diffusion) and has the same effect on either column or layer since the diffusion characteristics governed by the Einstein equation ($\sigma^2 = 2Dt$) should be the same. The $C$-term represents the resistance to mass transfer between the mobile and stationary phases. The values of the $C$-term for HPTLC plates are 30-50 times larger than those for HPLC columns. This is thought to be the result of the binder used to hold the chromatographic stationary phase to the backing plate. This binder is usually some salt of poly(acrylic acid) or gypsum, which give the layer its rigidity. It has been found that most of the binder resides in the pores of the silica [76]. This binder is believed to influence mass transfer by either restricting diffusion within the pores or by some complex adsorption mechanism [76,77]. Although the binder cannot be removed, it may be improved upon with further research into its role in the chromatographic retention process. Regardless, under capillary controlled flow the mobile phase velocity will never be fast enough, so the $C$-term is of negligible effects. Aside from this, the present layer technology doesn’t leave much room for improvement when capillary action is the driving force, as seen from the Knox equation.

Methods for improvement of planar chromatography utilizing capillary action involve “multiple development” techniques. In these techniques, a plate is developed, allowed to dry and then developed again. This process is repeated until the desired separation is achieved. There are many ways this can be employed effectively. In “multiple chromatography”, a plate can be developed with the same mobile phase, for the
same distance multiple times, or can be re-developed with the same mobile phase
allowing an incremented solvent entry position (some fixed position behind the slowest
zone) so the mobile phase does not have to travel through unused chromatographic phase
that just slows down the migration velocity. Another way this can be employed is using
“incremental multiple development” where the development distance (or time) is
increased each time the plate is developed, where the first development is the shortest.
“Automated multiple development” utilizes changing the mobile phase strength between
developments, and can also increase either development distance (or time) or solvent
entry position with each successive development. The major success of these multiple
development techniques is the zone refocusing mechanism. During the first few
developments, a zone will broaden, but after this the zone will start to refocus into a line,
this is the effect of the solvent front focusing the zone each time the solvent passes
through it. The mobile phase progresses molecules at the bottom of the zone before the
molecules at the top of the zone, so that there is compression of the zone when the mobile
phase moves across it. After the mobile phase has traversed the zone, the zone will again
start to diffuse in the expected way. With practice, one can move a zone a considerable
distance with little broadening. The drawback to all these multiple development
techniques is time; it is absurdly time consuming to allow weak capillary forces to
continually redevelop a plate and allow drying of the plate in between each development.
Therefore if modern TLC is to have a future, the elimination of capillary action as the
mobile phase driving force is a must.

Forced Flow Planar Chromatography (FFPC)

The future of modern TLC depends on some kind of external driving force of the
mobile phase so that the system can be chromatographically optimized. To date, there
are three ways known to force the mobile phase through the layer. The first is called
“rotation planar chromatography” (RPC) and is based on centrifugal forces. In this
system samples are placed on a TLC plate in a circle at a constant radius from the center,
then the plate is spun at high revolutions (100-1500 rpm) and the mobile phase dripped
onto the center of the plate and is drawn into the layer by capillary action and pulled
outward by the centrifugal forces in combination with the capillary forces [78]. This type of FFPC is the oldest and typically used only for screening large numbers of samples since up to 72 samples can be simultaneously screened [79].

The second type of FFPC uses a common HPLC pump to force mobile phase through a plate, called “over-pressured layer chromatography” or “optimum pressure laminar chromatography” (OPLC). In order to force the mobile phase through the chromatographic layer, a polymeric membrane is placed over the TLC plate and is forced into intimate contact with the layer. This is held in place by applying pressure with a water bag above the membrane. This instrument can handle up to 50 bar of pressure. For high pressure applications, a metal plate is used as the cover with a hydraulic press, which can handle up to 500 bar [80-82]. The sides of the plate also have to be sealed as to keep the flow in one direction only. Although this technique does give a constant mobile phase velocity and optimum chromatographic parameters are attainable, the apparatus is quite expensive and often leaks. In addition, the number of replicate simultaneous separations decreases because of the laminar flow profile.

The third type of FFPC is called “planar electrochromatography” (PEC). In this technique electrodes are attached to each end of a wet TLC plate and a DC potential is applied, creating electroosmotic flow (EOF). The EOF for a channel is given by the following equation (with the assumption that the size of the double layer is small compared to the channel size)

$$u_{eo} = \frac{\varepsilon_r \varepsilon_0 \zeta E}{\eta}$$  \hspace{1cm} (4.4)

where $u_{eo}$ is electroosmotic flow, $\varepsilon_r$ is dielectric constant, $\varepsilon_0$ is permittivity of vacuum, $\zeta$ is the zeta potential, $E$ is the applied electric field and $\eta$ is the viscosity of the mobile phase. The zeta potential has the following relation:

$$\zeta = \sigma \sqrt{\frac{RT}{2\varepsilon_r \varepsilon_0 c F^2}}$$  \hspace{1cm} (4.5)
where $\sigma$ is the charge density at the shear surface, $R$ is the ideal gas constant, $T$ is absolute temperature, $c$ is the molar concentration of buffer, and $F$ is Faraday’s constant. These equations serve more for fundamental relationships rather than for computation and are rarely used to predict EOF through a channel. Although these equations were developed for capillary systems, PEC is nothing more than a wide capillary electrochromatography separation technique.

**Planar Electrochromatography**

The idea of using electroosmosis as a mechanism for development of a planar medium can be traced back to A.J.P. Martin [83] and R.L.M. Synge [84]. However, it wasn’t until 1974 when Pretorius introduced his version of PEC using TLC plates [85], not gels. This paper has been called “one of the most frustrating papers in modern chromatography insofar as the lack of detail and the experimental methodology given makes repeating the work almost impossible” [82]. Since then, there have been few scientists that have indulged their interest in TLC using applied voltage (not including gel electrophoresis or other related fields), possibly due to the inability to replicate Pretorius’ experiments. Of these researchers, some are investigating the use of direct current (DC) electric fields on dry layers with the electric field either parallel or perpendicular to that of the mobile phase flow [86-90]. In these instances capillary action is still the driving force of the mobile phase, not electroosmotic flow. However, there are electrophoretic effects due to the applied electric field that are quantitative. Another useful approach employing electric fields is termed “planar dielectrochromatography” (PDEC) [91-93]. This research illustrates the use of alternating current (AC) applied fields perpendicular to the direction of chromatography, giving rise to polarization effects that help move the mobile phase across the plate at a faster rate, leading to improved separations.

The current literature using electric fields to induce EOF as the means of moving mobile phase through a TLC plate is limited, and much remains to be done in this field before this technique will gain any real recognition. The research so far has used glass-backed silica-based TLC plates, either modified ($C_{18}$) or not, using organic-buffer mobile phases [94-101]. These stationary phases contain enough silanols to create the electrical
double layer on the solid surface, and these mobile phases have enough ions to sustain EOF. There has been some initial work on effects of buffer concentration, pH of mobile phase, organic modifier concentration and applied field [98,100], but this work is qualitative, no quantitative accuracy can be guaranteed due to instrumental limitations that include uncontrollable solvent evaporation, inhomogeneous applied fields and Joule heating. Since initial investigation of PEC in 1998, instrumentation has advanced significantly, and many of the initial instrumental obstacles have been addressed and corrected. Recently the first review on history and techniques of PEC was published [102], recognizing these advancements and the growing interest in this field.
CHAPTER 5

INITIAL INSTRUMENTATION AND RESULTS FOR PLANAR ELECTROCHROMATOGRAPHY

Introduction

In 1998, David Nurok’s group published the first work (since Pretorius’ vague paper [85]) on what is now called PEC describing the performance of reversed-phase planar chromatography using electroosmotic flow [94]. This work contains only two references, Pretorius’ work and a short paper by Poole and Wilson (explaining the theoretical advantages of planar chromatography using electroosmosis) [82]. They describe a rudimentary experimental system that results in faster separations with better peak shape than conventional TLC, proving the concept can work. By 2000, the

Figure 5.1. Schematic of initial PEC apparatus. From ref. [94].
experimental setup had been revised and is illustrated in figure 5.1. In this figure the TLC plate is in the center of the apparatus with an electrode placed on the top of the TLC plate and the bottom of the plate submersed in a reservoir with the other electrode. The rest of the device is simply holding the TLC plate in place. This work [97] reported a three-fold increase in theoretical plate count with a two-fold decrease in separation time with the use of an applied field (3600 plates in 18 min. vs. 1300 plates in 37 min.) using 4.5 cm migration distance for the least retained solute. They concluded that electroosmotic flow enhanced the separation but did note flaws in the instrumentation such as solvent evaporation and solvent streaking. Solvent streaking is due to the gravitational forces on the mobile phase. In this apparatus, there was a cover plate placed about one millimeter above the surface of the chromatographic phase to reduce solvent evaporation (small headspace above chromatographic layer) but solvent can be driven out of the stationary phase and will then become trapped between the chromatographic layer and the glass cover and gravity will pull the solvent bead back down, hindering the separation. Since there is less electrical resistance through the solvent bead this allows the current to pass through the bead and not through the chromatographic phase, also changing the separation. In these experiments they also show that the solvent velocity is still dependent upon migration distance, which would not be the case if the mechanism of fluid transport were solely due to electroosmosis. This proves that solvent evaporation produces some amount of capillary action and therefore solvent velocity gradients.

The next step in improving these instruments was the incorporation of horizontal TLC chambers as the base for the PEC apparatus. A.G. Howard et al. first reported this in 1999 when they detailed a normal-phase separation using electroosmosis [95]. They showed an order of magnitude decrease in separation time (1.5 min. vs. 18 min.) for the electroosmotic separation compared to the conventional TLC separation. They also noted that there were effects of both evaporative migration (due to capillary action) as well as electroosmosis. By 2002 Nurok’s group also had incorporated horizontal TLC chambers for their PEC instrument [99]. Since their work is based on reversed-phase media, the solvent system is much more applicable to electroosmotic flows. These horizontal systems eliminated solvent streaking back down the TLC plate by gravity. These systems also saturated a finite space below the chromatographic layer with solvent to reduce
evaporation (see figure 5.2). This simple setup has two solvent reservoirs at each end of the TLC plate and solvent is carried to the chromatographic layer by capillary action through the paper wick. This system is sealed as to reduce evaporative effects as much as possible. Unfortunately, this design has the chromatographic phase in a “face-down” configuration. This is not ideal because gravity can still pull solvent away from the chromatographic phase and create beading. Although these solvent beads no longer streak, they still create electrical shorts. A sealed chamber saturated with solvent vapors does not eliminate evaporation because the applied field can induce Joule heating and evaporate solvents off the chromatographic layer. The incorporation of a horizontal chamber did further the knowledge of instrumentation in this field, but no routinely consistent apparatus was directly derived from these studies.

One issue that had yet to receive attention is how voltage crossed a TLC plate. In a capillary, solvent is confined and has such a small cross-sectional area that flow moves homogeneously. On a TLC plate this cannot be assumed. The cross-sectional area is much larger. According to theory a linear voltage drop is expected and should be the same voltage drop at each point across the width of a TLC plate (see figure 5.3). If the voltage drop is greater at one location compared to another there will exist flow inhomogenieties. For example, if the left side of a TLC plate has a greater voltage drop than the right side, then solvent on the right side will migrate at an angle over to the left. If there are localized zones with greater voltage drop then there will be complex solvent flow. In order to have an apparatus that is consistent and efficient this needs to be
understood.

Our design idea was to continue the horizontal format but place the TLC plate “face-up” so any effects of gravity would pull the solvent into the stationary phase. Also the design of a cover plate that laid directly on top of the chromatographic layer would leave no headspace and therefore be the best design for reduction / elimination of solvent evaporation. Platinum “reader” electrodes placed into the cover plate could monitor the potential at discrete positions as to gain insight into how voltage traversed a plate. With these instrumental improvements the only solvent flow should be through the chromatographic phase and be due only to electroosmosis, not by capillary action induced by solvent evaporation.

**Experimental**

![Figure 5.3. Expected voltage profile for PEC experiment. The TLC plate should have the same linear voltage drop at any parallel line going between the two reservoirs. There should also be no difference in potential at any points perpendicular to the applied potential at the same distance from the reservoir.](image)
The base plate for our initial design was machined from acrylic plastic and contained two screws in the front and a pivot at the center in the rear. This allowed leveling of the apparatus for best results. On the acrylic are two Teflon solvent reservoirs that allow for up to 20 cm TLC plates to be placed upon them. The reservoirs are adjustable between the 10 and 20 cm distances. This enabled the use of this instrument with any plate width (up to 20 cm) and plate lengths of either 10 cm or 20 cm. These Teflon reservoirs (figure 5.4) have a recess machined half the depth of the material; this is where solvent was held. Attached to the end of each reservoir is a banana clip that connects to the power supply leads. Platinum wire (0.051 cm or 0.020 inch) is soldered to the banana clip and run along the bottom of the reservoir at the edge closest to the TLC plate. This ensures the same potential across the top of a TLC plate. Preliminary experiments with an electrode not run through the reservoir, but rather just dipped into the reservoir showed potential gradients across the top of a TLC plate. Also milled into

Figure 5.4. Picture of PEC base, the Teflon solvent reservoir is shown in detail with the glass strip engaged in the vertical position. The Tygon tubing was replaced with black rubber for ease of sight. A 10 cm X 10 cm TLC plate is lying face up in the apparatus.
the Teflon block is a notch that holds a length of Tygon tubing securely. This was necessary for the chromatographic phase “face-up” design. Again, initial experiments showed that when a TLC plate was placed face up, solvent would weep under the glass backing of the TLC plate and would cause an electrical short when solvent wept across the whole plate. Incorporation of Tygon tubing blocked solvent from weeping under the bottom of the TLC plate. When a TLC plate was placed across the two solvent filled reservoirs, solvent would access the TLC plate by the vertical glass strip. One edge of this glass strip would lie in the bottom of the reservoir next to the electrode, and rest at an angle (\(\sim 45^\circ\)) against the back wall of the reservoir. When the glass strip was pushed into the vertical position (hinged at the bottom) it would enable capillary action between the glass and the Teflon reservoir wall (see figure 5.4). Solvent would then rise up the wall of the reservoir, be blocked by the Tygon tubing and proceed up to the chromatographic phase.

The cover plate (figure 5.5) was machined from 10 cm X 10 cm Teflon (1 cm

Figure 5.5. Picture of the Teflon cover grid. Inserted are the 35 platinum reader electrodes in a 5X7 array. These allow monitoring of the applied potential.
thick) with 35 reader electrodes (0.020 in. platinum) positioned in a five by seven grid. The five columns (seven reader electrodes in the direction of voltage drop per column) were positioned at 1.0, 3.0, 5.0, 7.0, and 9.0 cm across the width of the Teflon, and the seven readers per column were positioned at 0.5, 2.0, 3.5, 5.0, 6.5, 8.0 and 9.5 cm down each column (symmetrical from center position). Reader electrodes were mounted into the Teflon so that they are flush with the bottom surface of the cover grid. This ensures that there is no disturbing the chromatographic phase.

All chemicals were supplied by Fisher (Fisher Scientific, Fair lawn, NJ, USA) and water was purified with a Barnstead NANOPure II system (Barnstead International, Dubuque, IA, USA). Acetate buffer was prepared at 100 mM by dissolving the correct amount of sodium acetate into purified water and adjusted to pH 4.5 with acetic acid using a Orion SA520 pH meter (Orion Research Inc., Beverly, MA, USA). This solution was then diluted to 10 mM without change in pH. This diluted buffer was then mixed with methanol to be 75/25 (methanol/buffer) by volume.

The TLC plates were Analtech HPTLC-RPS plates (Analtech, Newark, DE, USA). These are a reversed-phase impregnated silica (coated, not bound) with a large chain hydrocarbon around C<sub>30</sub> in size (actual structure is proprietary) that can be readily used in any proportion of methanol / water solvent system without any appreciable loss of the hydrocarbon [103]. Prior to use in the apparatus, these plates would be soaked in the run buffer for 30 minutes to pre-equilibrate the plate with the buffer ions.

Experimental setup required placing the wet TLC plate into position (face-up) across the two reservoirs and placing the Teflon cover grid on top of the chromatographic phase. Run buffer would then be placed in each of the two reservoirs and the vertical glass strips engaged to allow solvent flow to the TLC plate. The apparatus would be left for about five minutes to allow any evaporated run buffer to be replaced by capillary action. Once the plate was re-saturated with run buffer, voltage would be applied and data recorded. The power was applied via a Glassman EH10P10.0 high voltage power supply (Glassman High Voltage, Whitehouse Station, NJ, USA). This power supply can deliver a 10 KV maximum potential and up to 10 mA maximum current. Voltage was monitored through the reader electrodes by a B&amp;K Toolkit 2703A Voltmeter (B&amp;K Precision, Yorba Linda, CA, USA).
Results and Discussion

The first experiments performed mapped all 35 reader electrodes and enabled visualization of the voltage profile as it traversed a TLC plate. The apparatus was assembled as previously mentioned with a 10 cm X 10 cm TLC plate. A 1000 VDC potential was applied when the system was ready. Two dimensional intensity maps such as figure 5.6 were generated by MatLab to visualize the data. The potential at each reader is numerically listed on top of the intensity map. This plot interpolates between data points with a linear function and does not extrapolate beyond the borders of the outermost voltage readers. Therefore the voltage function in the area between the edges of the TLC plate and outermost reader electrode was not extrapolated. These plots illustrate a couple of important and informative pieces of information. First, although the voltage drop does move in the correct fashion, from positive source electrode to negative source electrode, there does exist perturbations in the voltage drop for differing columns of reader electrodes (figure 5.7). These differences in potential drop will create heterogeneous flows due to differences in voltage drops at an angle to the applied field. It is expected that for 1000 VDC applied across 10 cm plate there should be a constant 100 V/cm drop from positive source electrode to negative source electrode. This should be consistent for any column of reader electrodes. Second, the two outermost columns of reader electrodes illustrate a different environment than the middle three columns. It was later found that solvent would weep outside the TLC plate and create a path of fluid outside the chromatographic layer. Fluid residing outside the chromatographic layer provides a shorter path to ground in the same manner as a solvent bead on top of the chromatographic layer. Using a Rhodamine B marker and watching its migration enabled visualization of fluid flow. The marker would migrate not in the direction of applied field, but rather at an angle from where the solute was placed toward the edge and then proceed to the anode through the solvent lying on the edge of the TLC plate. This explains why voltage profiles at the outermost columns of reader electrodes were different than the middle ones.
The above experimental data could not be taken simultaneously because it took some finite amount of time to manually place the voltmeter at each position and get a stable reading. On average it took about three or four minutes to read and record all 35 reader electrode potentials. Consecutive measurements of all 35 reader electrodes.

Figure 5.6. Two dimensional intensity plot of voltage across a TLC plate. 1000V applied across a 10 cm X 10 cm HPTLC plate.

Figure 5.7. Voltage drop as a function of distance (reader 1 through reader 7) for each of the five columns. According to theory, each of these trends should be linear.
showed that there was some dependence on time. To further understand this, another set of experiments was performed and showed how the potential at one reader electrode changed as a function of time. These experiments monitored one reader electrode position for one hour while under an applied field. Each experiment was performed on the same TLC plate during consecutive days and pre-equilibrated each day in the same fashion as described earlier, then run with the same solvent system and applied field. Data shown in figure 5.8 represents three independent experiments monitoring three different reader electrodes positioned within the center column of reader electrodes (0.5, 5.0 and 8.0 cm away from positive reservoir). This data clearly shows a dependence of voltage with time, and in fact, is believed to be an equilibration trend throughout the TLC plate.

During the first day, we monitored the position 0.5 cm from the positive electrode and the voltage function initially decreases for about two minutes, then increases rapidly over the next few minutes followed by a slow increase in voltage then stabilizes after about 25-30 minutes and remains fairly stable for the rest of the experiment. On the

![TLC Plate Equilibration](image)

Figure 5.8. Graph of voltage vs. time for three reader electrodes in the center column of a 10cm X 10cm HPTLC plate. The positions of the reader electrodes are distance from the positive reservoir. 1000 V DC applied, 75/25 Methanol/Acetate Buffer (pH 4.5, 10mM).
second day, the 5.0 cm reader electrode was monitored and again there is an initial decrease in voltage over the first two minutes, followed by a period of fairly stable, slow increase in potential followed by a rapid increase in potential at about 15 minutes. Again the voltage vs. time function becomes stable around 25-30 minutes. On day three, position 8.0 cm from the positive was monitored. The voltage again shows an initial decrease over the first two minutes. This is followed by a long slowly increasing period then a short rapid increase in potential at around 20 minutes. Again by about 25-30 minutes the voltage vs. time function stabilizes. This data is believed to show the equilibration profile of a TLC plate. All three experiments have an initial drop in potential over the first two minutes. This could be from the changing resistance of the TLC plate when the field is first applied, or establishment of EOF up the vertical section of reservoir (between reservoir wall and vertical glass plate) before electroosmosis starts passing through the chromatographic phase. Each curve in figure 5.8 shows a point at which there is a rapid increase in potential over a short time. These points are indicative of full electroosmotic flow establishment. There is a slow EOF establishment between the reservoirs. The 0.5 cm reader electrode starts to see the onset of full EOF front at around two minutes, the 5.0 cm reader electrode observes the onset of full EOF front at ~14 minutes, and the 8.0 cm reader electrode observes the onset of full EOF at ~19 minutes. This is not to state there is no EOF before these inflections occur, but rather some reduced EOF due to initial non-equilibration conditions of the TLC plate. In this apparatus there is a significant volume of excess solvent. This may create a delay in electroosmotic migration of solvent. There may also be a minor buffer concentration gradient. Although the plate was pre-equilibrated in run buffer, some solvent evaporates before placed into the apparatus and fresh run buffer is then replenished by capillary action prior to applying the field. When the plate was rewetted buffer ions may migrate slower than methanol, inducing a buffer gradient. There may also be effects of chemicals used in manufacture that are also responsible for the observed equilibration. After the electroosmotic front has passed through the whole TLC plate (25-30 min) there seems to be little to no fluctuation in voltage with further time, up to one hour. There are no observable effects of Joule heating in these experiments, but there may have been some minor temperature fluctuations.
These assumptions of the observed inflections in the voltage vs. time graphs are compared with those calculated by the Smoluchowski equation

\[
\frac{u_{eo}}{\eta} = \frac{\varepsilon_e \varepsilon_0 \zeta E}{\eta} = \frac{\left(8.85 \times 10^{-12} \text{ C}^2 \cdot \text{N}^{-1} \cdot \text{m}^{-2}\right) \times (45) \times (0.030V) \times \left(10,000 \text{V} \cdot \text{m}^{-1}\right)}{0.0017 \text{kg} \cdot \text{m}^{-1} \cdot \text{s}^{-1}} \tag{4.5}
\]

where \(u_{eo}\) is electroosmotic flow, \(\varepsilon_0\) is permittivity of vacuum, \(\varepsilon_e\) is dielectric constant, \(\zeta\) is the zeta potential, \(E\) is the applied electric field and \(\eta\) is the viscosity of the mobile phase. The permittivity of a vacuum is a constant and can be found in any physics textbook, the dielectric constant of our mobile phase system is estimated from Suresh’s work [104]. A zeta potential had to be assumed to fit our data since they cannot be readily calculated, and the one chosen falls within the limits (~20-200 mV) accepted in the literature [105,106]. The applied field is expressed in volts per meter, and the viscosity taken from Thermo-Hypersil-Keystone product literature (1.7 cpoise) transferred to the correct units. This equation yields a flow of about 0.4 cm/min. Comparison of this flow rate with the inflections seen in figure 5.8 gives credit to the assumption that these inflections do mark the EOF.

<table>
<thead>
<tr>
<th>Reader Electrode Position (cm from positive reservoir)</th>
<th>Calculated EOF Onset Time (min)</th>
<th>Experimental EOF Onset Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 cm</td>
<td>1 min</td>
<td>2 min</td>
</tr>
<tr>
<td>5.0 cm</td>
<td>13 min</td>
<td>14 min</td>
</tr>
<tr>
<td>8.0 cm</td>
<td>20 min</td>
<td>19 min</td>
</tr>
<tr>
<td>10.0 cm</td>
<td>25 min</td>
<td>28 min</td>
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</tbody>
</table>

These initial experiments also included trying another type of TLC plate. Analtech HPTLC-RP18 plates were used and found not applicable for PEC use. The main difference between the two types of plates is in reversed-phase design. HPTLC-RPS plates (all previous data) are a reversed-phase impregnated stationary phase where a long chain hydrocarbon (~C\(_{30}\)) is coated on the silica (not bonded). This allows the use of completely aqueous solvent systems. The HPTLC-RP18 plates are a C\(_{18}\) ligand.
conventionally bonded to the silica. These stationary phases cannot be readily used in purely aqueous solvent systems due to the inability of the solvent to wet the stationary phase. The problem with using these plates was not due to stationary phase type, but rather the manufacturers choice of binder. The RPS plates use an inorganic binder and the RP18 plates use an organic binder. Under the experimental conditions used the organic binder in the RP18 plates was dissolving and allowing negatively charged silica particles to migrate off the glass backing and into the positive reservoir.

Figure 5.9. Pictures illustrating the dissolution of organic binder and electro-migration of silica particles off the TLC plate. Plates were positioned so the left side was toward the positive reservoir

Conclusions

We have introduced a new apparatus for planar electrochromatography (PEC) trying to improve the knowledge in this field. This apparatus addresses the problems with other instruments in the literature. These include solvent evaporation, solvent beading on top of the chromatographic phase as well as uninvestigated complex voltage profiles. This apparatus incorporates a chromatographic phase “face-up” design. Other apparatus’ using horizontal chambers use a “face-down” design allowing gravity to pull
on the solvent creating solvent beads outside the chromatographic layer. Lower resistance in solvent beads (compared to the chromatographic phase) creates electrical shorts and inhomogeneous fields. Our “face-up” design in combination with the cover plate does not allow excess solvent to reside outside the chromatographic layer. This configuration also reduces solvent evaporation because there is no headspace above the chromatographic layer for evaporation to occur. Voltage reader electrodes in the cover plate allow evidence of how applied field traverses a TLC plate. Data obtained while monitoring applied field allowed visualization of voltage traversing a 10 cm X 10 cm TLC plate. This initial voltage data showed the need to seal the sides of the TLC plates in order to compensate for solvent wicking out of the chromatographic sandwich and creating electrical shorts to ground, and inhomogeneous fields. A plate equilibration time was also discovered. The data presented in this chapter also showed the importance of plate selection. Many instrumental improvements became obvious from this initial work. These include more applied pressure on top of the chromatographic phase. This will reduce the amount of excess solvent and should reduce equilibration time. Sealing the sides of a plate will force the solvent to stay within the chromatographic phase allowing only one path to ground, through the chromatographic phase. Voltages greater than 1000 V DC could not be used because of Joule heating. Reducing plate width should help increase resistance therefore reducing the current required to produce the same potential. Experimental observations also showed the cover plate could move slightly during voltage monitoring changing the voltage pattern seen. Other instrumental inconsistencies included placement of the vertical glass strip. When the strip was moved into the vertical position (allowing capillary action to the chromatographic phase) it was important to watch how the vertical glass met the Teflon reservoir wall and TLC glass backing plate. If an air bubble were trapped between the Teflon reservoir, TLC glass backing and Tygon tubing, this would change solvent delivery and ultimately change the voltage profile. A more hands free apparatus would reduce these inconsistencies. Also to better understand the voltage profiles, computerized data acquisition would enable simultaneous monitoring of all reader electrodes as well as provide rapid sampling frequencies not attainable by hand.
CHAPTER 6

LOW PRESSURE PLANAR ELECTROCHROMATOGRAPHY
INSTRUMENTATION AND RESULTS

Introduction

The last chapter described the initial apparatus design and results for planar electrochromatography. This improved upon other PEC devices by constructing a chromatographic phase “face-up” design and cover plate that reduced solvent evaporation and beading, which damage the electroosmotic flow profile. The cover plate was designed with platinum electrodes for monitoring potential drop between the source electrodes. Results of this work showed there is an equilibration time for a TLC plate under an applied field, there was not a linear voltage drop between source electrodes and there were potential differences in the axis perpendicular to the applied field. According to theory there should be a linear voltage drop in the direction of applied field and no change in potential perpendicular to the applied field. These inconsistencies are attributed to inadequate instrument design. Instrumental improvements needed include sealing the sides of a TLC plate in order to trap the solvent inside the chromatographic layer, applying pressure to the cover plate creating a down-force on the chromatographic layer effectively squeezing out excess solvent. Design of a more stable system would eliminate shifting/sliding of the cover plate and inconsistencies from engaging the vertical glass strip. Prior results also showed the need for increased data acquisition. Acquiring data manually with a voltmeter does not allow measurement of a whole plate simultaneously. In order to obtain a better understanding of how potential moves through a TLC plate these reader electrodes have to be measured simultaneously at a much faster rate than manually capable. This chapter describes a newer version PEC instrument that tries to correct for the shortcomings of our initial apparatus.
Experimental

All chemicals were supplied by Fisher (Fisher Scientific, Fair lawn, NJ, USA) and water was purified with a Barnstead NANOPure II system (Barnstead International, Dubuque, IA, USA). Buffer was prepared at 100 mM by dissolving the correct amount of sodium acetate into purified water and adjusted to pH 4.7 with acetic acid using a Orion SA520 pH meter (Orion Research Inc., Beverly, MA, USA). This solution was then diluted to 10 mM without a change in pH (4.7). The buffer was then mixed with methanol in a 50/50 proportion, by volume.

The instrument used was designed and built in-house. There are three main sections (figure 6.1), the first is the instrument base containing the power supply (source) electrodes and solvent reservoirs; the second is the cover grid containing the voltage reading (reader) electrodes, and the third section is the voltage divider box that allows transfer of information to the computer. The formica covered wood beneath, aligns the

Figure 6.1. Picture of planar electrochromatographic instrument. 1=instrument base and solvent reservoirs, 2=cover grid, and 3=voltage divider box.
sections in the correct positions and distances.

The instrument base can be seen in closer view in figure 6.2. The base plate (1) is an 18 X 23 X 1 cm piece of acrylic plastic with three aluminum level adjusters (2). Located on that are two 18 X 4 X 1 Teflon blocks (3) with solvent reservoirs milled 10 mm wide and 8 mm deep, 5 mm from the inside edge. Placed in the solvent recess is a 0.051 cm (0.020 inch) platinum wire (4) attached at both ends to female banana clip receptacles (5). This is the “source” electrode. Source electrodes are connected directly to a Glassman EH10P10.0 high voltage power supply (Glassman High Voltage, Whitehouse Station, NJ, USA). This power supply can deliver a 10 KV maximum potential and up to 10 mA maximum current. Between the solvent trough and the inside

Figure 6.2. Detailed picture of PEC base and solvent reservoirs. 1=base plate, 2=levelers, 3=solvent reservoirs, 4=source electrode, 5=electrode plugs, 6=Tygon seal, 7=reservoir screws, 8=glass slide, 9=vertical glass plate, 10=PVC covered aluminum chiller base.
edge of the reservoir is a length of 0.318 cm (0.125 inch) Tygon tubing (6) recessed into the Teflon, which provides a seal when the TLC plate is placed into position. Without this seal, solvent would weep under the TLC plate and short the system. On the back side of the solvent trough are nylon screws that hold the reservoirs in position (7).

Horizontally on top of the Teflon reservoir lays a glass slide (8) and its two PVC hold-downs. When pushed inward, this allows the vertical glass plate (9) to start the solvent flow. This horizontal plate also acts as a solvent cover to reduce evaporation. Between the two reservoirs is a PVC covered aluminum chiller base (10), here, used only as a support for the weight placed on top of the TLC plate. It can also be seen that the left reservoir is stationary while the right is adjustable. This is because most 10 cm TLC plates are actually between 9.9 and 10.1 cm and the moveable reservoir corrects for these fluctuations.

The cover grid is shown in detail in figure 6.3. This picture shows the bottom side of the cover grid where the TLC plate is attached. This cover grid was designed to fit up to 10 X 10 cm TLC plates, and shown is a 2.5 cm plate attached to the cover grid such that the second column of reader electrodes is centered on the TLC plate. The cover grid is made from a 10 X 10 cm square of Kel-F that is 2.85 cm (1.125 in.) thick. Inserted into it are thirty-two 0.051 cm (0.020 inch) platinum reader electrodes in a four column, eight row matrix. The columns are 2.0 cm from the edges and each other, and the reader electrodes within a column are 1.1 cm from the edges and each other (the center spacing is 1.2 cm for symmetry). Each reader electrode is flush mounted to the surface of the Kel-F on the bottom (shown) and the other end side is soldered to a high voltage (HV) wire and routed to the voltage divider box (figure 6.1). The wiring is run through channels in the cover grid so the applied weight will lay flat on top (see figure 6.4) and routed through the wire guides to the right of the cover grid.

The voltage divider box (figure 6.1) is needed because the voltage from the reader electrodes is too high to route to a computer data acquisition card (DAQ). This is the acrylic box with vents and a fan. The dividers are mounted inside on a phenolic plate far enough apart to dissipate heat and have no arcing. A high voltage wire from the reader electrode to the high side of the voltage divider is a 15 KV rated 22 AWG (7/30) SPC, 0.030 HV-Silicone wire (PN: 39X2215-9A) from Dearborn CDT, Wheeling, IL, USA.
The voltage dividers are 10 KV rated 1000:1 dividers from Caddock Electronics Inc., Roseburg, OR, USA (PN: THV10-A100M-1.0-25). The low side of the divider is connected to the DAQ via common 37 pin ribbon cable. The DAQ is an Omega OME-PISO-813 32 channel (single ended) input card (Omega Engineering Inc., Stamford, CT, USA). The software used for monitoring and recording the data is LabView 7.0 (National Instruments, Austin, TX, USA). Data was evaluated with Microsoft Excel (Microsoft, Redmond, WA, USA).

Figure 6.4 shows the instrument set-up for use. This illustrates how the cover grid lies on the instrument base and the weight is applied to the top. The pressure of the applied weight is distributed evenly across the TLC plate due to the PVC coated chiller block underneath it. A clear view of the chromatographic layer sandwiched between the glass backing and the Kel-F cover grid can be seen in figure 6.5. Also visible is the means by which the solvent is carried to the plate. The solvent in the reservoir will travel
between the vertical glass plate and the wall of the reservoir by capillary action to the TLC plate. Once the plate is completely wet and the reservoirs are filled, sliding the vertical glass plates against the wall of the reservoir completes the electrical circuit that will drive the EOF.

The TLC plates used were Analtech HPTLC-RPS plates (Analtech, Newark, DE, USA). These are a reversed-phase impregnated silica (coated, not bound) with a large chain hydrocarbon around C_{30} in size (actual structure is proprietary) that can be readily used in any proportion of methanol / water solvent system without any appreciable loss of the hydrocarbon [103]. Prior to experimentation, the 2.5 cm TLC plate was sealed to the cover grid by the sides with an RTV Silicone sealant (Permatex Inc., Solon, OH, USA) and was given at least 24 hours to dry. Sealing the sides ensures that the solvent can only

Figure 6.4. Picture of PEC instrument ready for use. Here the cover grid (and TLC plate) is placed into the base and an external force is applied (brass weight).
flow in one direction. Previous experiments on unsealed plates showed that the solvent (and voltage) will run off the sides, resulting in poor flow characteristics.

Once the sealed plate was dry it was trimmed of any excess sealant and placed onto the base. The TLC plate would lay directly on top of the PVC covered aluminum block, and aligned square with the two solvent reservoirs. After it was assured the plate was in correct position, external weight (~27 kg) would be placed on top of the grid. This weight created an applied pressure of ~1.0 bar (~15 psi) inside the sealed chromatographic area and completed the assembly prior to experimentation. At this point buffer was placed into the positive reservoir and the vertical glass strip engaged, allowing buffer to be drawn into the TLC plate by capillary action. After complete wetting of the layer, as noticed by the translucence of wet vs. dry silica, buffer was placed into the negative reservoir and that vertical glass strip would be engaged. Then the power supply could be turned on and data recorded.

![Figure 6.5](image_url)

**Figure 6.5.** A close up of how the TLC plate is mated to the instrument. The TLC plate is mounted with the chromatographic layer “face up”, so the glass backing is shown underneath the layer and the Kel-F is above the layer. The vertical glass plate lies in the bottom of the reservoir and stands vertically against the inside wall of the reservoir and contacts the TLC plate and Kel-F cover grid. The cover glass is lying horizontally on the outside of the vertical glass strip, to reduce solvent evaporation.
Once setup was complete, data would be obtained in the following sequence. The power supply was turned on for 60 minutes (equilibration run) then turned off for one to two hours. During this time the solvent was not disconnected from the TLC plate, allowing the plate to stay completely saturated. In fact, the apparatus was not touched at all. After the allotted time, power was restored for 15 minutes, and then turned off again for another one to two hours. Again power would be restored for another 15 minutes, and then turned off and at this point the apparatus would be disassembled and cleaned. The TLC plate would remain attached to the cover grid until it was deemed no longer useful (after the third experimentation day). Removing the plate could disturb the chromatographic bed, altering the flow characteristics for the next day’s experiments. These periods of time where voltage was not applied served two purposes. This enabled the ability to see “wet memory” effects, that is, how well the plate would restore (remember) the flow and voltage from the previous equilibration run. This also assured that no Joule heating was affecting equilibration of the TLC plate. If there were Joule heating, the memory runs would not have the same stable voltage characteristics as the end of the equilibration run. After the apparatus was cleaned, it would sit for one to two days, allowing the TLC plate to completely dry, then this procedure would be repeated for a second and third experiment day.

**Results and Discussion**

The first experiment was to investigate the reusability of a single plate. Although TLC plates are not designed for multiple uses due to the hindrance of solute elution off a plate under capillary mediated flow, a forced flow technique does allow for full elution and therefore could possibly be used for multiple analyses. For these experiments a 2.5 cm X 10 cm TLC plate was attached to the instrument, completely wetted with the run buffer and 1000VDC was applied. It was previously determined that 60 minutes was sufficient time to see voltage equilibration across a plate. It should be noted that “plate equilibration” here refers to the time it takes for a TLC plate to exhibit constant voltage at all reader electrode positions. This is not to be confused with the term used by other authors to describe “plate pre-equilibration” or “conditioning” of a TLC plate by thermal
pre-treatment and/or pre-soaking the plate in the run buffer prior to PEC [95,98,100,102,107].

The day-to-day results of a single plate can be seen in figure 6.6. On the first day

![Graphs showing daily equilibration effects with 1000VDC applied. Each data series corresponds to an individual reader electrode and are in sequential order, where the top series is 1.1 cm from the positive reservoir all the way to the bottom series representing 8.9 cm from the positive reservoir. Top graph = Day 1, Middle graph = Day 2 and Bottom graph = Day 3.](image-url)
of experiments there are nice smooth “S” shaped curves for each reader electrode positioned on the TLC plate. The top data set represents the reader electrode closest to the positive reservoir (1.1 cm from positive) and follows in order to the bottom curve representing the reader closest to the negative reservoir (8.9 cm from positive). There is an equilibration time for each distance, the shortest equilibration time closest to positive reservoir (~10 minutes) and longest furthest from positive reservoir (~ 40 minutes). As the distance from positive increases so does the time before the first inflection of the “S” curvature, as well as decreasing slope of the curve. As with previous data shown in the last chapter, we believe this represents slow establishment of an EOF front. As solvent is allowed to initially wet the plate by capillary action, buffer ions move slower than the solvent front and there may be a buffer gradient along the TLC plate. When a field is applied the buffer starts to move and eventually enough buffer reaches the whole plate. This buffer movement would also bring with it a greater EOF velocity that could justify these inflection points. Also, in PEC there is a much greater solvent volume that could cause a slow response or lag time much like a steam locomotive trying to start with a heavy load where the steel wheels spin on the track before the train moves properly. Due to the large surface area and solvent volume, the first moment that voltage is applied to a plate buffer ions may take time to locate charged sites on the silica (and binder) to maintain a constant electrical double layer (and EOF) at that point. In reality the observed phenomena is most likely a combination of all the above effects (the last being small compared to the others). Therefore the first point of inflection on each “S” curve may show the establishment of correct EOF at each position across the plate. This is not to say there is no EOF until this inflection is reached, but it may be the onset of a greater EOF velocity. The data also shows a small spike at around 35 minutes. Over the course of these experiments this was seen many times and has been found that hitting the benchtop can cause small perturbations in the instrument, most likely causing small movements in the vertical glass strips. Also if air bubbles form at the right (90°) angle between the vertical glass strip and TLC plate, hindering the intake of solvent, this can also cause small voltage perturbations.

The 60 minute equilibration run for the second day of experiments illustrates a slightly different trend than seen in day one. The beginning looks the same for all readers
and the top three data sets look alike for both days, but the bottom five do not level off as they did in day one. There is more noise in each trend and some slight fluctuations not seen before. This is believed to be from the residual buffer from day one experiments. After day one was completed the plate was removed from the base but remained attached to the cover grid by the silicon sealant. In this configuration the plate could not be removed for rinsing, and any internal rinsing by capillary action would have created another buffer gradient. The third day of experiments shows even more inconsistencies. All the curves have even more noise and greater fluctuations. Again this is attributed to the additional buffer from the prior experiments. Voltage spikes are common on this day and are believed to be caused by buffer precipitation, not instrumental shifting. These trends seen during the three experimentation days were reproducible from plate to plate. It is apparent that the first day gives the best results and each subsequent day provides less reliable information. Therefore in this configuration the third experiment day was not used for further investigation. However if a different configuration was adopted with the means of adequate rinsing, there might be a longer lifetime for each plate.

**Voltage Drop**

Theory provides for a linear relationship between voltage and distance, such that 1000 V applied over 10 cm should have a 100 V/cm slope. The data shown in figure 6.7 shows a different trend. Instead of a constant 100 V/cm drop we see a curve that changes with time, at least during the equilibration period. The beginning of the equilibration run shows concave quadratic curvature, changing to sigmoidal during the middle and as plate equilibration finishes turns into a convex quadratic curve. During equilibration the voltage drop can be localized because a buffer gradient exists, therefore there are more volt carriers at the positive (top) side of the plate and this changes over time, until equilibration and a constant EOF has been established across the whole plate. Once there is equilibrium across the plate there should exist the linear voltage drop provided by theory. It stands to reason that instrumental inadequacy has caused the observed curvature. There are not many PEC instruments being tested and developed, so it may still be some time before theory and experiment coincide. Nurok’s group has recently achieved constant EOF (and presumably voltage drop) with their pressurized planar
electrochromatographic instrument [101]. Their instrument was run at pressures greater than 50 atmospheres, which are not achievable with our current design. Consequently, higher pressures may be required for a better voltage profile.

To look at this data in a different dimension a linear regression equation was fit to voltage profiles at every 100 seconds and the data was graphed (figure 6.8). These graphs illustrate the linearity of the system and it is obvious that there is an ever-changing

![Graph](image)

**Figure 6.7** Plots of voltage drop during the 60 minute equilibration runs. Distance is measured from the positive reservoir to the reader electrode. These illustrate a nonlinear changing behavior over time, ending in a convex quadratic curve after plate equilibration.
environment. It is also noticeable how this data compares to ideal values. Overall the data does come close but further instrumental development is needed to assure a truly linear voltage drop where the regression, slope and intercept are representative of the applied voltage.

Re-equilibration

On day one, after the 60 min equilibration run and sufficient time with no applied potential, the instrument was turned on again to see how the EOF would be re-

Figure 6.8. Graphs of linear regression variables vs. time for three days of experiments. Top represents voltage drop (slope) vs. time and bottom represents $R^2$ vs. time. For these experiments the ideal voltage drop is 100 V/cm.
established. These experiments can be compared with those that “pre-equilibrate” or “condition” a TLC plate prior to PEC as mentioned earlier, since any excess ions from manufacture and / or incomplete solvation of the stationary phase would have been eliminated in the prior 60 minute runtime and subsequent standby time.

These results (figure 6.9) show a much more constant voltage vs. time relationship at each reader electrode, which was expected since the plate was previously

![Graph A](image1)
![Graph B](image2)

Figure 6.9. Post equilibration voltage trends. Graph A represents the first post equilibration run and B the second. The data series in each graph are in sequential order, where the top series is 1.1 cm from the positive reservoir all the way to the bottom series representing 8.9 cm from the positive reservoir. Also apparent are the inflections showing the EOF profile.
equilibrated. Also, the plots of voltage vs. reader electrode distance (figure 6.10) holds the same convex curvature seen after equilibration during the 60 minute run. What were interesting to see were the small “S” shape curves still present in each reader (figure 6.9).

This further reinforced the belief that this phenomenon mimicked the EOF. If the distance at which the reader electrode was positioned was plotted against the time of the

![Graph showing voltage drop over time](image)

Figure 6.10. Post equilibration trends in voltage drop. The top graph is the first post equilibration run and the bottom is the second. Distance is measured from the positive reservoir to the reader electrode. This illustrates the mostly constant concave quadratic curvature in voltage drop for up to four hours after equilibration.
onset of “S” curvature (from figure 6.9), the velocity of this front can be calculated. The graph of this (figure 6.11) shows a clear quadratic dependence ($R^2 = 0.9986$), but for the sake of calculation we are going to assume a bi-linear function, such that the first four and last four data points represent two individual linear functions (although it would be more accurate to take a tangent at every data point the meaning and purpose become lost). The linear correlation coefficients ($R^2 = 0.9986$ and 0.9964) do credit this assumption. Assuming linear functions, velocities can be calculated (represented by the slope of the line) and are 1.1 cm/min through the top half of the plate and 2.0 cm/min through the bottom half of the plate.

For independent confirmation that this could be the correct EOF velocity, data after the front passed the whole plate (14 minutes) was used to find the voltage drop across the plate and used to solve eqn. 4.4 for EOF velocity. As with the previous graph, there was a clear quadratic curvature in the relationship of voltage drop vs. distance (figure 6.12) and again we assumed a bi-linear function. The top half of the plate shows a voltage drop of ~50 V/cm and the bottom half shows a drop of ~150 V/cm. Plugging these numbers into eqn. 4.4 (assuming $\zeta = 0.070$ V$^1$) gives 0.7 cm/min at the top half of

![Graph of distance (from positive reservoir) against time the inflection occurs (from fig. 9) at each reader along the plate (Day 1). Using a bi-linear fitting function, the two slopes (1.1 and 2.0 cm/min) represent the changing EOF velocity as it moves down the TLC plate.](image)

Figure 6.11. Graph of distance (from positive reservoir) against time the inflection occurs (from fig. 9) at each reader along the plate (Day 1). Using a bi-linear fitting function, the two slopes (1.1 and 2.0 cm/min) represent the changing EOF velocity as it moves down the TLC plate.

\[^1\] Although the zeta potential is arbitrarily chosen it does fall within the limited range of accepted values noted earlier, which helps justify the assumption that EOF is mimicked by these inflections.
the plate and 2.0 cm/min for the bottom half of the plate (see table 6.1). These post-equilibration experiments were performed on day two and the same quadratic curves for determining velocity by inflection and voltage drop were seen. Using the bi-linear assumption, these results are also listed in table 6.1. The zeta potential ($\zeta$) for Day 2 was assumed to be 0.100 V and this change can be correlated to the increase in buffer concentration on the second day.

Table 6.1 EOF velocities calculated by different means

<table>
<thead>
<tr>
<th>Day</th>
<th>Top Half of Plate</th>
<th>Bottom Half of Plate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Velocity by Voltage Drop</td>
<td>0.7 cm/min</td>
</tr>
<tr>
<td></td>
<td>Velocity by Inflection</td>
<td>1.1 cm/min</td>
</tr>
<tr>
<td>Day 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Velocity by Voltage Drop</td>
<td>0.8 cm/min</td>
</tr>
<tr>
<td></td>
<td>Velocity by Inflection</td>
<td>1.0 cm/min</td>
</tr>
</tbody>
</table>

By calculating velocities of flow in two independent ways, one based on the time inflections were seen, the other based on voltage drop across the plate at a fixed time lends proof that these inflections seen in the data are EOF markers as it progresses across

Figure 6.12. Graph of voltage vs. distance (from positive reservoir) used to find the voltage drop across the plate (Day 1). Again a bi-linear function was used to obtain two slopes (50 and 150 V/cm) and these values were subsequently used to calculate the EOF velocity (0.7 and 2.0 cm/min).
the plate. Note that in the post-equilibration runs these inflections are mostly marking the EOF not establishing it, as it was in the 60 minute equilibration runs. The difference is seen in the voltage vs. time graphs. In the 60 minute runs the inflection point marks the beginning of a great change in the voltage, significantly changing the EOF at that point. In the post-equilibration runs there are only minor voltage changes for each reader that alter the EOF slightly, but rather these small inflections are markers of EOF velocity as it crosses the plate.

**Larger Plate Widths**

One of the greatest attributes of TLC is its ability to perform simultaneous separations. In order to keep that attribute we are investigating the use of larger plate widths for use in PEC. As plate width increases, electrical resistance across it decreases requiring larger current to hold the same potential. As a result the current on a 5.0 cm TLC plate ranged from 2-4 mA on average, where a 2.5 cm plate averaged 1-2 mA for the same 1000 VDC potential. As current is increased, Joule heating can become a problem. Using a 5.0 cm plate with 1000 VDC applied; if the current surpassed ~4 mA the plate would become warm (or hot) to touch. This heating changes the properties of the binary solvent (viscosity, dielectric constant) therefore changing the EOF. The current normally did not break this threshold on the first day of experiments, but by the third day it could become a problem (buffer accumulation).

Using the same instrumental conditions previously described we attached a 5.0 cm TLC plate to the cover grid. Here the plate was attached to the center of the grid having two columns of reader electrodes available for data acquisition. Reader electrodes were positioned 1.0 cm from the center of the TLC plate on each side and were 1.5 cm from the edges. In this configuration 50 kg of weight was added on top of the cover grid keeping the same pressure on the plate (~1.0 bar). During the equilibration hour the two columns of reader electrodes displayed very similar curves for most data sets (figure 6.13). There were some discrepancies in the reader positioned 5.6 cm from positive and looks incorrect only on the left column. This may have been caused by non-homogenous amounts of binder or residual ions in the chromatographic phase from manufacture or
mishandling of the plate prior to use. Regardless, after the plate was equilibrated this anomaly disappeared and does not diminish the quality of data.

This hour equilibration resembles those presented earlier with the general increase and leveling of the voltage curves. The post-equilibration runs also are similar to the 2.5 cm plate data with very steady voltage at each reader and the inflections within each set of data (figure 6.14). These inflections appear earlier in the data sets than with 2.5 cm plates but the 5.0 cm plates were more difficult to reproduce and it is not clear whether the flow was faster or if Joule heating affected these profiles. Trends between left and right columns are superimposable assuring that there is only a voltage gradient between

![Graphs showing voltage profiles for a 5.0 cm wide HPTLC plate.](image)

**Figure 6.13.** Data shown for a 5.0 cm wide HPTLC plate. The top graph shows the voltage profiles for the right column of reader electrodes and the bottom shows the left. Note the similarities in most data series, except the third from the bottom (reader 6.7 cm from positive reservoir). Again, the data series are in sequential order, where the top series is 1.1 cm from the positive reservoir all the way to the bottom series representing 8.9 cm from the positive reservoir.
buffer reservoirs, and not between the sides of the plate. Unwanted voltage gradients would induce localized flow in the direction of greatest voltage drop, and solutes would not migrate in a straight line. The use of larger plate widths would also benefit from a higher-pressure apparatus by enabling larger applied fields without solvent boiling and reducing the amount of solvent in the chromatographic layer.

Conclusions

This work furthers understanding of the relationship between voltage and solvent

![Figure 6.14. Post-equilibration run for 5.0 cm plate. The top graph is the right column of reader electrodes and the bottom graph is the left. Again, the data series are in sequential order, where the top series is 1.1 cm from the positive reservoir all the way to the bottom series representing 8.9 cm from the positive reservoir. This illustrates the ability of a plate to hold the same voltage profile regardless of path down the plate.](image-url)
flow in planar electrochromatographic experiments. The cover grid measures detailed information about voltage profiles and characteristics of a TLC plate under electroosmotic forces. Improved PEC instrumentation enabled confirmation of our previous results (see Chapter 5) that there is an equilibration time for TLC plates in PEC. This may be caused by both the buffer gradient established when initially wetting the chromatographic phase and the large volume of solvent needed to be moved (locomotive effect). Also observed was the voltage drop across the plate where the initial application of potential caused a concave quadratic decrease evolving into a sigmoidal curve and once equilibrated, sustaining a convex quadratic decrease. It was deduced that instrumental inadequacy was the cause of the non-linearity. Following equilibration, the plate was shown to be robust in re-establishment of the voltage drop and EOF, for at least four hours after equilibration. Once a plate was dried (overnight) and re-wet, the hour-long equilibration would need to be performed again. After two days of experimentation the plate could not be used further because of buffer build up. A different experimental configuration may allow more replicates, but is not a disadvantage since most TLC analyses are not performed in an elution mode.

During post equilibration experiments an empirical relationship between EOF velocity and inflection point was determined and correlated to the theoretical EOF velocity by means of the Smoluchowski equation. Trends in this data are clearly quadratic ($R^2 > 0.99$) and unfortunately cannot be solved as such. Theory requires a linear voltage drop to solve this equation and a linear relationship is necessary for distance vs. time (giving a slope of velocity). Assuming a bi-linear relationship where the top and bottom halves of the TLC plate are individual linear functions, the equations can be solved. Although the data sets are small ($n=4$ for each) the results of these calculations show the intended relationship quite well. The calculated EOF velocity from the voltage drop (0.7 & 2.0 cm/min) was relatively the same as the observed velocity by means of inflection points (1.1 & 2.0 cm/min) [first number in parentheses is top half velocity, second is bottom half velocity]. These inflections signify some front starting at the top of the plate when the power is turned on, like a wave riding the EOF.

Larger plate widths were briefly investigated here and determined that there is very realistic chance of attaining simultaneous separations on large width plates (10 cm
and higher). The data presented illustrated two different voltage reader columns and, once plate equilibrium was established these data were virtually identical. This assures that the same EOF will be seen on either side of a plate as movement goes from top to bottom. Any voltage gradient from side to side would cause localized flow perturbations and send an ongoing separation off its straight-line path causing a chromatographic crash.

All data presented here gives useful information about solvent and voltage flow characteristics, as well as information on further design and capabilities of this type of instrument. Better understanding of instrument performance allows fixing those shortcomings and inadequacies. Some of the data presented here not only allowed us to see flow characteristics, but also see the instrumental flaws, that could not have been seen without the cover grid and reader electrodes mapping the pathway. We believe higher external pressure is the key to an instrument that will perform with more linear characteristics. Nurok has shown that higher pressures allow higher voltages [101], and hence better separations and our ability to see inside the separation will allow further information for designing an adequate instrument.
CHAPTER 7

HIGH PRESSURE PLANAR ELECTROCHROMATOGRAPHY
INSTRUMENTATION AND RESULTS

Introduction

Planar electrochromatographic instrumentation has continued to evolve as evidenced in chapter six. Sealing the sides of a TLC plate and applying external pressure produces a much more homogeneous flow through a TLC plate. This was visualized by incorporating digital data acquisition to the reader electrodes. Increased sampling rates also allowed more in-depth interpretation of voltage vs. time curves, proving the existence of a plate equilibration time as well as flow markers of an equilibrated TLC plate. These small variations in potential would not have been seen with manual data sampling discussed in chapter five. Data in chapter six also showed there was still not a linear voltage drop between source electrodes. The instrument was rebuilt on the basis that higher external pressures could show increased linearity in voltage drop, therefore giving constant EOF across a TLC plate. This was based on Nurok’s initial results with his pressurized PEC instrument [101]. They reported one minute separations at 7 KV and 870 psi (59 atm). This separation had nine simultaneous separations on one 2.3 cm X 12

\[\text{Figure 7.1. Simultaneous separation of 4-cholesten-3-one, 17-}\alpha\text{-acetoxy-progesterone, 2'-acetonaphthone, benzanilide, o-}\text{nitroaniline by high pressure PEC. Nine replicate separations in one minute, using 7000 VDC, 59 atm pressure. Solvent is 55/45 ACN/Acetate (pH 4.5, 25 mM). From ref [101].}\]
cm (effective size) TLC plate (figure 7.1). The reproducibility of each separation infers there must be a near constant EOF throughout the plate. This chapter will address instrument performance by using higher applied pressure to the PEC instrument and greater applied fields for linear voltage profiles. Experimental methodology for separations will also be addressed here.

**Experimental**

There are only two major changes to instrumentation for these experiments. First is inclusion of a laboratory press capable of high pressure. The laboratory press is a Wabash Hydraulic Press (Wabash Metal Products Company, Wabash, IN, USA) with a new Norco/KYB model 76412G 12 ton hydraulic jack with gauge port (Norco Industries, Compton, CA, USA). The jack is equipped with an Enerpac G2517L 0-6000 psi (0-400 bar) hydraulic pressure gauge (Enerpac, Milwaukee, WI, USA). The instrument base,
TLC plate and cover grid are seen in the throat of the press (figure 7.2). The box of voltage dividers can be seen mounted level with the press’s throat because there is a limited length of high voltage wire between the electrodes and voltage dividers.

The second major renovation is elimination of the negative source electrode reservoir. In place of a reservoir, the platinum source electrode is soldered to a 2 cm X 3 cm piece of platinum foil 0.002 inch (0.051 mm) thick (see figure 7.3). This foil is placed in between the TLC plate and Kel-F cover grid as the ground electrode for the electrochromatographic system. As solvent electromigrates through the chromatographic phase, solvent is removed from the system by a piece of tissue paper that can wick excess solvent away so no accumulation occurs. This tissue paper helps evaporate eluent by increasing the surface area exposed to air. The reservoir is replaced with this foil because initial high pressure experiments showed significant heating of solvent after the distance where external pressure ceases. When potential was applied to the system, the part of the

Figure 7.3. Picture of high pressure base. 1 = black Delrin negative source electrode holder, 2 = Teflon solvent reservoir and positive source electrode, 3 = Kel-F reader electrode grid, 4 = wick paper for effluent, 5 = platinum foil source electrode
plate before external applied pressure (first 1 mm) and that part under external pressure remained at or close to room temperature, whereas the part of the plate after external pressure (last 1 mm) and joint into the negative reservoir became very hot. This is due more in part to the changing resistance at the joint than the applied pressure, but they are not independent. Placing the foil electrode inside the chromatographic system under pressure prevents this phenomenon and its resulting temperature increase.

In order to integrate the new negative source electrode and laboratory press, the base of the system needed to be modified. The base plate is PVC (14 cm X 21 cm) with the positive Teflon reservoir securely attached to one side. This reservoir does not have a recess for Tygon tubing like the earlier version. This design does not use a chromatographic “face-up” design since these plates will have much greater pressure than gravity placed upon them. This simplifies the solvent delivery mechanism and reduces the chance of fouling. The reservoir height was adjusted (with an acrylic spacer) so the top of the reservoir is the same height as the Kel-F reader electrode grid. In place of the negative source reservoir is a black Delrin frame that again was adjusted to match the height of the Kel-F grid. On the Delrin frame four spring clips hold the platinum foil and wick paper in place. These clips are made of acrylic plastic and held with nylon screws. The Teflon reservoir and Delrin frame are mounted just far enough apart to allow the reader electrode grid to fit snugly in-between. Everything was mounted so that the Kel-F grid is centered on the base. The reader electrode grid is placed “reader side up” onto the base (see figure 7.3). The Kel-F side touching the solvent reservoir is sealed with silicon sealant to prevent solvent weeping through the crack. This apparatus is placed into the press and the Kel-F reader grid is centered above the ram using a brass pin inserted into the PVC base plate that seats in the centering hole on the press. Other parts of this apparatus not detailed here are described in chapter six, and have not been altered.

All chemicals were supplied by Fisher (Fisher Scientific, Fair Lawn, NJ, USA) except Rhodamine B (Kodak, Rochester, NY, USA). Water was purified with a Barnstead NANOPure II system (Barnstead International, Dubuque, IA, USA). Buffer was prepared at 10 mM by dissolving the correct amount of sodium acetate into purified water and adjusted to pH 4.7 with acetic acid using a Orion SA520 pH meter (Orion Research Inc., Beverly, MA, USA). The buffer was then mixed with methanol to either
50/50 or 75/25 methanol(buffer, by volume. This work continues to use the Analtech HPTLC-RPS plates described previously (Analtech, Newark, DE, USA) sealed to the Kel-F reader grid by use of an RTV Silicone sealant (Permatex Inc., Solon, OH, USA).

**Results and Discussion**

**Plate Equilibration and Voltage Drop**

In order to be consistent with previous research, mobile phases consisting of 50/50 methanol/acetate buffer (pH 4.5, 10 mM) were used with 1000 VDC applied. The TLC plates were sealed to the Kel-F grid the day preceding experimentation and were not pre-equilibrated.

![Figure 7.4. Equilibration of a HPTLC-RPS plate under external pressure of 1000 psi. Plate is equilibrated in 20-25 minutes. The data series in each graph are in sequential order, where the top series is 1.1 cm from the positive reservoir all the way to the bottom series representing 8.9 cm from the positive reservoir.](image-url)
As seen in figure 7.4, equilibration trends for a TLC plate are similar to those seen with the low pressure version in that there is some finite time for establishment of full EOF at each reader electrode position and the time increases with distance from the positive reservoir. However, the time necessary to equilibrate a plate is much less. In low pressure PEC it took around 40 minutes to show stability at each reader electrode position, whereas high pressure PEC only takes around 20 minutes. This is correlated to the reduced volume of solvent present. In column chromatography, the term “column volume” is used to express the volume of mobile phase present within the chromatographic media and its borders (stainless steel column). When mobile phases in HPLC change, a number of column volumes need to pass through the column before it is equilibrated. In PEC this could be a parallel condition. Although the volume of solvent within the constraints is not known, there might need to be a critical solvent volume passed through for equilibration of the chromatographic phase under specified conditions. This volume would include that necessary to deliver enough buffer ions throughout the complete plate, as well as mobile phase / stationary phase interactions prevalent in RPLC such as chain swelling / shrinking. This reduction in equilibration time might also be due to more efficient flow by the reduction of “locomotive effect” described in chapter six.

![Figure 7.5](image)

**Figure 7.5.** Plot of voltage vs. distance for high pressure PEC plate equilibration. Within 20 minutes the TLC plate is equilibrated and stays so for the whole hour. 1000VDC applied.
By expelling excess solvent, the fraction of solvent near the double layer increases so electroosmotic forces may produce more work (solvent motion) in these systems.

Plotting this data in another dimension shows the voltage drop across the TLC plate (figure 7.5). It is clearly evident that within 20 minutes the TLC plate exhibits a linear voltage drop and maintains that linearity for the rest of the hour. This data proves that higher external pressures were needed to obtain a linear voltage drop across a TLC plate. Now that linearity exists between voltage and distance, reliable and reproducible PEC instrumentation is possible. This is further evidenced by the linear slope and correlation coefficient (figure 7.6); low pressure PEC never stabilizes illustrating an ever-changing environment, whereas high pressure PEC is not only stable over a long period of time, the values are near ideal for the experimental parameters.

Figure 7.6. Comparison of linearity for high and low pressure PEC instruments. Here the ideal values are indicated with a solid black line.
Increased Fields

In order to test the limits of the high pressure PEC instrument, greater applied fields were used to see if linear voltage drops could be maintained. For the 1000 VDC experiments there seemed to be very little Joule heating with the experimental conditions described. However, when using larger field strengths this quickly became problematic.

Table 7.1. Linearity of voltage drop with applied field

<table>
<thead>
<tr>
<th>Applied Field V or (V/cm)</th>
<th>Linear Equation</th>
<th>Correlation Coefficient</th>
<th>Duration of Linearity (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000 (100)</td>
<td>( y = -97.27 , x + 1027 )</td>
<td>0.9967</td>
<td>&gt;2400</td>
</tr>
<tr>
<td>2000 (200)</td>
<td>( y = -187.2 , x + 1874 )</td>
<td>0.9916</td>
<td>&gt;120</td>
</tr>
<tr>
<td>3000 (300)</td>
<td>( y = -292.9 , x + 2994 )</td>
<td>0.9879</td>
<td>~60</td>
</tr>
<tr>
<td>5000 (500)</td>
<td>( y = -493.1 , x + 4944 )</td>
<td>0.9966</td>
<td>~ 8</td>
</tr>
</tbody>
</table>

Table 7.1 illustrates the linear voltage drops seen prior to Joule heating corrupting EOF. The data used for the linear equation was after the initial plate equilibration and before the effects of Joule heating were seen. This table indicates that an efficient heat sink would allow the use of larger fields for a longer timeframe, thereby increasing separation capacity. The separation shown earlier (figure 7.1) was performed with an instrument equipped with a ceramic nitride electrical insulator / thermal conductor, which was presumably integral for the 7000 V separation achieved. These concurring data demonstrate that use of extremely large electric fields is possible if Joule heating can be efficiently dissipated.

Sample Application
For routine analysis, the most reliable, reproducible way to use PEC is by applying sample spots directly onto an equilibrated, wet plate. Introducing a sample to a plate that is already sealed on the apparatus is not ideal and always produced immense band spreading upon application. Sample spotting prior to sealing a plate on the apparatus does not allow use of an equilibrated, wet plate. In order to solve this problem a new liquid seal that can be readily attached and removed from the Kel-F was developed. Attaching a TLC plate to a sheet of Teflon (in the same manner as previously attached to the Kel-F block) and letting the sealant set overnight produces a seal that is permanently attached to the glass TLC plate, but can easily be peeled off the Teflon. This is illustrated in figure 7.7. Two different techniques were investigated. In the first technique the edges of a 2.5 cm wide TLC plate were scraped to produce a 2.0 cm wide chromatographic layer. These plates were sealed to a sheet of Teflon so the sealant was in the space provided by scraping. The first plates investigated this way did not have a spacer, so the sealant layer was at the same level as the chromatographic phase when removed from the Teflon sheet. This provided a lousy seal when placed in the apparatus. The next set of plates sealed had a spacer between the chromatographic phase and the Teflon (see figure 7.7). This resulted in better sealing when placed in the apparatus. When applying pressure, sealant under the glass was displaced in all directions and did

![Figure 7.7. Preparation and assembly of a TLC plate. Left side: process starts by scraping chromatographic phase off edges, followed by adding a spacer then temporary sealing this to a Teflon sheet. After sealant is dry, remove plate; discard spacer and place on Kel-F. Finally, applying pressure compacts the silicon creating a liquid seal and pressurizes the chromatographic phase properly. Right side: same as left except do not scrape chromatographic layer, prohibiting sealant under glass.](image-url)
disturb the chromatographic phase, and sometimes broke the plate due to uneven pressures. The second technique did not scrape the plates, eliminating uneven pressures produced by sealant thickness variations. These plates produce adequate sealing, are easier to produce and do not break at the edges under stress. These plates were examined with different spacer thicknesses (none, 0.005 inch and 0.010 inch) and the 0.010 inch spacer provided the most robust seal. When pressure is applied to these plates sealant is displaced away from the glass and does not disturb the chromatographic phase.

This new technique enables easy assembly and disassembly of a plate from PEC instrumentation and allows use of plate pre-equilibration techniques such as soaking the plate in buffer prior to PEC. The data shown in figure 7.8 A illustrate a plate equilibration with the new plate sealing technique and plate pre-equilibration in the run buffer for 30 minutes. In comparison to earlier experiments the voltage trends are the

![Figure 7.8](image)

Figure 7.8. Top: Graph of voltage vs. time, illustrating the new TLC plate sealing method does not change the flow profile or equilibration time. Bottom: Voltage profile after EOF equilibration, plate removal (simulate spotting) and replacement into apparatus.
same, showing a 20 minute equilibration time and voltage drop after equilibration is linear. This confirms there are no appreciable changes in flow profiles by using this new sealing technique. Although the voltage profile does not vary with pre-equilibration, the current required to drive the solvent is much lower (about half), allowing use of higher voltages. After equilibration the plate was removed from the apparatus simulating sample application. The plate was then re-inserted and electrical field was applied. Data in figure 7.8 B illustrate that plate removal and reinsertion do not ruin the linear voltage profile established during the equilibration run.

**Rhodamine B Mobility**

Monitoring voltage profiles and understanding how to interpret them is invaluable for designing an instrument that has reproducible, accurate flow characteristics, but this offers no visual satisfaction. Since the TLC plates used do not have an F254 fluorophore for easy UV detection a fluorescent marker is used to see migration in PEC. These experiments were performed at 3000 VDC, 1000 psi with a 75/25 methanol / acetate buffer (pH 4.5, 10 mM). Each plate was pre-equilibrated by soaking in run buffer for 30 minutes, prior to applying sample solution with a 10 µL GC syringe. With some small syringe modification and practice a reproducible sample spot can be delivered. During sample application solvent will evaporate out of the chromatographic phase, so they were momentarily resubmersed in run buffer. Before placing these spotted, solvent saturated

![Graph of Mobility of Rhodamine B marker on three separate TLC plates. Distances were measured to the center of the zone.](image)

Figure 7.9. Mobility of Rhodamine B marker on three separate TLC plates. Distances were measured to the center of the zone.
plates into the instrument, excess solvent was removed by patting with a Kimwipe. These were quickly placed into the instrument, external pressure applied, then solvent was allowed to contact the TLC layer, and power turned on. Enabling solvent to contact the layer before applying external pressure induced capillary action through the channel of air from the spacer, diffusing the sample spot. Applying pressure first removes this channel of air by compressing the sealant.

Figure 7.9 shows migration of Rhodamine B on three separate TLC plates. From these experiments the mobility of Rhodamine B was determined to be 0.29 cm/min \( (R^2 = 0.9996, N = 6) \). There are three data points for the first plate and they were taken by running PEC for a specified time, lowering the press enough to mark migration, reapply pressure then voltage, and repeating. Repeatedly releasing and applying pressure caused extensive zone broadening from solvent perturbations. Plate two was checked only at 5 minutes and at the end, significantly reducing diffusion (see figure 7.10), and plate three not checked during migration. Rhodamine B was spotted at the center of a 2.5 cm wide TLC plate and migration stayed in the direction of voltage drop and did not move off line. Figure 7.10 shows an 8.5 cm migration in 30 minutes where the spot stays in the center of the 2.5 cm wide plate with zone dispersion (zone width of 2.6 mm) only in the direction of voltage drop. Analysis of this spot yields about 14000 plates (or plate height of about 6 µm). This is the highest plate count in PEC reported to date.

![Figure 7.10](image)

Figure 7.10. Picture showing the 8.5 cm migration of Rhodamine B in 30 minutes. Notice how the spot stays in the center of the TLC plate.
Conclusions

The addition of high pressure to PEC instrumentation has improved the efficacy of this technique by producing homogenous flow profiles that are near theoretical predictions. Linear voltage profiles have finally been achieved, as well as reduced plate equilibration times. These improvements are attributed to the increased efficiency of the electroosmotic forces acting on the reduced solvent volume. The anode reservoir has also been replaced with a platinum foil electrode placed inside the pressurized TLC plate. This eliminated the heating phenomenon at the joint between the TLC plate and the vertical glass strip that enables solvent contact. The use of higher field strengths will also increase the usefulness of this technique. This should be easily implemented with an efficient heat sink to combat Joule heating.

A new method for introducing TLC plates onto the apparatus has also been described. This eliminates the need to seal a plate to the Kel-F grid 24 hours prior to experimentation, and is essential for sample application and ease of use. This method allows sealant to be attached to the glass sides of a TLC plate, while cured on a removable sheet of Teflon. The plate is then removed from the Teflon after 24 hours and could be inserted into the apparatus anytime afterwards. A 0.010 inch gap between the chromatographic surface and Teflon sheet (when curing) allows compression of the sealant, trapping solvent within the chromatographic phase when external pressure is applied. A plate can now be pre-equilibrated before use (soaking in run buffer) reducing the current required to drive EOF. Sample can then be applied to a plate and placed into the apparatus for PEC. Unfortunately the plates used do not contain a fluorescent indicator, and routine chromatographic analysis could not be detected. Use of Rhodamine B did allow visualization of solute movement in PEC. These results visually confirmed that migration is only in the direction of voltage drop, and band broadening is primarily in the same direction resulting in the highest plate counts reported for PEC at about 14000.

We have shown in these last few chapters that there is now a realistic chance that TLC plates can be used under electroosmotic forces to produce efficient chromatography.
This opens the door for many mixtures that cannot be readily separated by HPLC due to complex adsorption to the stationary phase, but contain too many analytes to previously be separated by TLC. Continued research into this new chromatographic technique will elucidate the relationship between variables such as solvent composition, buffer concentration, applied pressure and applied voltage, as well as further the instrumental development. We believe the future of planar chromatography will be better than most expect.
REFERENCES


BIOGRAPHICAL SKETCH

Peter was born on September 8, 1975 in Westerly, Rhode Island. He was raised there and graduated Westerly High School in 1993. He started the University of Rhode Island in the fall of 1993, only completing one semester before leaving. After his academic absence, he restarted school at the Community College of Rhode Island in spring of 1995 taking night classes. He transferred back to the University of Rhode Island in the fall of 1996 and started to work for Dr. Jim Smith as an undergraduate researcher until he received a Bachelors of Science in Chemistry in December 1999, and then stayed at URI through the spring as a graduate teaching assistant prior to leaving for Florida. Peter began the Doctor of Philosophy program at Florida State University in the fall of 2000 and joined the Dorsey research group in January 2001. He spent the summer of 2002 as an intern with Pfizer Global Research and Development, Analytical Research Division in Groton CT. under the direction of George Reid. He will receive a Ph.D. in Analytical Chemistry from FSU in April 2005.