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FACTORS AFFECTING THE COMPOSITION OF THE BONDED STATIONARY PHASE IN LIQUID CHROMATOGRAPHY

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FACTORS AFFECTING THE COMPOSITION OF THE BONDED STATIONARY PHASE IN LIQUID CHROMATOGRAPHY

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To Patti, who through her patience, support, and love made this work her own.

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TABLE OF CONTENTS

		Page
	LIST OF ILLUSTRATIONS	vii
	LIST OF TABLES	ix
	ABSTRACT	x
1.	INTRODUCTION	1
	Chemical Modification of Surfaces	2
	Limitations of Chemically Modified Surfaces.	5
	The Solvophobic Theory	7
	Active Role of the Bonded Phase	8
	Factors Affecting the Formation of the Effective Stationary Phase	10
	Direction of Research	17
	Carbon Substrate for Bonded Phase Formation	18
	Carbon -13 NMR Studies of Bonded Phases	23
	Composition of the Stationary Phase	26
2.	CARBON SUBSTRATE FOR BONDED PHASE FORMATION	27
	Introduction	27
	Experimental	28
	Preparation of the Carbon	28
	Chromatographic Procedures	31
	In Situ Column Modification	32
	Grignard Modification	32
	ESCA	33
	Elemental Determinations	34
	Results and Discussion	34
	Reactivity of the Carbon Surface Groups	34
	Influences of the Carbon Substrate on Retention	43
	Conclusions	50
3.	CARBON - 13 NMR STUDIES OF SILICA-BASED BONDED PHASES	52
	Introduction	52
	Experimental	53
	Carbon-13 NMR Spectra	53

TABLE OF CONTENTS--Continued

Page

Silica-based Bonded Phases..... 53
Chromatographic Measurements..... 55
Results and Discussion..... 56
General Characteristics of the Spectra..... 56
Response of the Bonded Hydrocarbon to Chromatographic Conditions..... 63
Chromatographically Significant Temperature Effects..... 74
Description of the Stationary Phase..... 82

4. QUANTITATIVE MEASUREMENTS OF STATIONARY PHASE COMPOSITION..... 86

Introduction..... 86
Experimental..... 89
Measurements of t_o..... 89
Gas Chromatographic Measurement of Stationary Phase Composition..... 90
Results and Discussion..... 92
C_{18} Bonded Phase..... 92
Compositional Effects on Selectivity.... 103
Effect of Chain Length on Composition... 107
Effect of Temperature on Composition.... 107
Conclusions..... 111

5. CONCLUSIONS AND SUGGESTIONS FOR FURTHER WORK..... 112

APPENDIX: CHROMATOGRAPHIC THEORY..... 117

REFERENCES..... 127

LIST OF ILLUSTRATIONS

Figure		Page
1.	Depictions of Stationary phase structure under varied conditions.....	16
2.	Glass reaction vessel for production of Kel-F-derived carbon.....	30
3.	ESCA spectra of the C_{1s} region for carbonaceous materials.....	36
4.	ESCA spectra of the Cl_{2p} region for KFD carbon in stages of Grignard modification.....	40
5.	ESCA spectra of the C_{1s} region for KFD carbon in stages of Grignard modification.....	41
6.	Variation of ln k' with alkyl carbon number for n-alkylbenzenes on different columns.....	44
7.	Separation of PAH on KFD carbon.....	46
8.	Comparison of bonded C_{18} CMR spectrum with solution octadecane spectrum.....	57
9.	CMR spectra of ODS-2 in the absence of solvent.	60
10.	CMR spectrum of octadecane-coated silica in 1:1 MeOH:D_2O at 25°C.....	61
11.	CMR spectrum of octadecane-coated silica in 1:1 MeOH:D_2O at 40°C.....	62
12.	CMR spectra of octadecane-coated silica in the absence of solvent.....	64
13.	CMR spectra of RP-8 in tetrahydrofuran.....	69
14.	Van't Hoff plots for aniline probes on RP-8....	77
15.	Van't Hoff plots for aniline probes on RP-18...	78

LIST OF ILLUSTRATIONS--Continued

Figure		Page
16.	Van't Hoff plots for aniline probes on Spherisorb ODS	79
17.	Van't Hoff plots for aniline probes on ODS-2	80
18.	Intracolumn volumes for RP-18 as a function of mobile phase composition	93
19.	Stationary phase composition in methanol mobile phases	98
20.	Stationary phase composition in acetonitrile mobile phases	99
21.	Stationary phase composition in tetrahydrofuran mobile phases	100
22.	Selectivity for n-alkanols as a function of mobile phase solubility parameter	105
23.	Selectivity for n-alkanols as a function of organic component in the stationary phase	106
24.	Stationary phase composition in methanol mobile phases for RP-8	108
25.	Measurement of the asymmetry factor	126

LIST OF TABLES

Table		Page
I	Retention of Selected Probes on Unmodified Carbon.....	35
II	Effect of Silane Modification on Relative Retentions.....	38
III	Effect of Grignard Modification on Relative Retentions.....	43
IV	Enthalpies of Interaction (kcal/mole) on C_8 Packings.....	48
V	Commercial Packing Materials Used in This Study.	54
VI	Bulk CH_2 Peak Widths (ppm) in Neat Solvents.....	66
VII	Bulk CH_2 Peak Widths (ppm) as a Function of Methanol Concentrations in Aqueous Solutions	70
VIII	Bulk CH_2 Peak Widths (ppm) as a Function of Temperature in 50/50 Acetonitrile/D_2O.....	73
IX	Enthalpies of Transfer for Aniline Probes.....	76
X	Calculated Stationary Phase Thicknesses.....	95
XI	Measured Stationary Phase Compositions--RP-18...	97
XII	Enrichment of Modifier in the Mobile Phase Components Associated with the Stationary Phase for RP-18.....	102
XIII	Selectivities and Mobile Phase Parameters.....	104
XIV	Measured Stationary Phase Compositions--RP-8....	109
XV	Stationary Phase Compositions as a Function of Temperature.....	110

ABSTRACT

The stationary phase on chemically modified supports for liquid chromatography is described as a mixture of the surface-bonded species, the active unmodified surface, and associated mobile phase components. Each of these three factors in stationary phase formation is examined and improved qualitative and quantitative descriptions of the stationary phase are provided.

The role of the active unmodified surface was examined by synthesizing a carbon support and chemically modifying it with octyl groups. The modified carbon had a greater affinity for lipophilic probes than an octyl silica. The lipophilicity of the octyl carbon was attenuated relative to the unmodified carbon.

The physical state of the bonded species in C_{18} and C_8 packings was studied using carbon-13 NMR. Peak widths of 2-7 ppm indicated a liquid-like nature but with restricted movement. Only the 7 to 10 carbons in a C_{18} chain farthest from the surface were sufficiently motile to produce a signal. The C_8 packing showed more rigid chains with only the top 3 or 4 carbons responding. The

liquid-like nature of a C_{18} chain increased with the lipophilic character of the solvent, indicating that solvation of the bonded species was directly related to the mobile phase composition. Changes in temperature had little effect on the physical state of the bonded species, but chromatographic enthalpy measurements showed that changes in stationary phase composition could be induced by warming the column and held by subsequent cooling.

Quantitative measurements of stationary phase compositions revealed linear distribution isotherms for the organic modifiers methanol, acetonitrile, and tetrahydrofuran. Chromatographic selectivities for homologous n-alkanols correlated linearly with organic modifier concentrations in the stationary phases. The stationary phase volumes, which increased with increasing modifier concentrations, are interpreted as constituting filling of the pores in the support with a gradient of modifier enrichment toward the surface.

CHAPTER ONE

INTRODUCTION

Over the past decade modern liquid chromatography (LC, or high performance liquid chromatography, HPLC) has been developed into one of the most powerful analytical techniques available. The utility and widespread acceptance of LC have followed closely the improvements in related technology. The most significant of these developments has proven to be the introduction of the bonded phase, the stationary phase chemically bonded to the surface of the support. The chemically modified support is in many respects an improvement over its predecessors, the bare and coated supports. The bonded phase is more stable and efficient than coated stationary phases, and it has a higher capacity, a greater reproducibility, and a more controllable selectivity than the bare or coated support. The ability of the bonded phase to impart different and high degrees of selectivity to the chromatographic process has resulted in a proliferation of bonded phases ranging from hydrocarbons for general use (1) to highly specific chelating and chiral moieties (2, 3).

Liquid chromatography does not exclusively accrue the benefits of bonded phases. In fact, LC borrowed the concept of the bonded phase from gas chromatography (GC), for which stability and selectivity of the stationary phases were again the goals of the surface modifications (4). Supports for GC are routinely treated with silanizing reagents to contain the number of strong adsorption sites which can contribute to poor column performance. In other surface phenomena where there is a desire to control the selectivity or reproducibility of a process, chemical modification has proven to be an effective regulatory method. Chemically modified electrodes ranging from metals (5, 6) to semiconductors (7-12) and carbon (13-15) represent the diversity in the application of bonded phases to the study of electron transfer. Other physicochemical surface processes for which improvements have been achieved via surface modification include catalysis (16, 17) and flowing stream analytical methods using antibodies (18).

Chemical Modification of Surfaces

The synthesis of bonded phases via organosilane reagents is one of the most widely used routes to surface modification. Organosilanes are highly reactive and yield stable products through well-characterized reaction mechanisms and conditions. The reagents are readily available in high purity and span a broad range of organic moieties

from the chromatographically ubiquitous hydrocarbons to amine, cyano, and nitro functional groups supported by short hydrocarbon chains (109). Most popular are the chloro- and alkoxyorganosilanes, which react readily with active hydrogen on surface functional groups to form Si-O linkages to the surface:

$$\equiv\!\text{OH} + \text{R}_{(4-n)}\ \text{SiCl}_n \rightarrow \equiv\!\text{O-Si-R}_{(4-n)} + n\text{HCl} \qquad \text{eqn 1-1}$$

$$\equiv\!\text{OH} + \text{R}_{(4-n)}\ \text{Si (OR')}_n \rightarrow \equiv\!\text{O-Si R}_{(4-n)} + n\text{R'OH} \qquad \text{eqn 1-2}$$

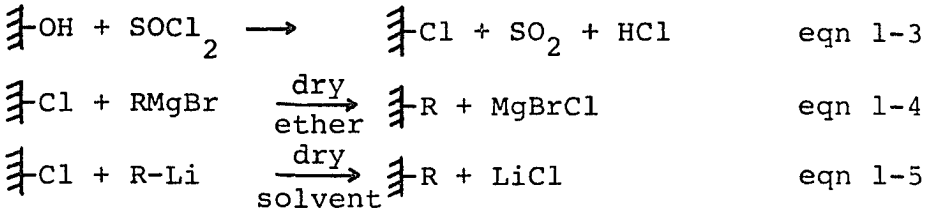
where R' = an alkyl group (usually methyl or ethyl) and R = organic moieties, not necessarily identical on a given silane. Both types of silane reagents yield equivalent products. When n = 2, then silane does not necessarily react with two neighboring groups on the surface, and it cannot react with more than two groups when n = 3 (19). Some of the remaining chloro or alkoxy groups will react with surface water to form silanols (Si-OH). The silanols themselves can condense with neighboring bonded silanes or with additional reagent to form a polymeric silicone (20, 21). Monofunctional silanes (n = 1) are thought to form a bonded phase resembling the bristles on a brush.

For chromatographic bonded phases in LC silica is used almost without exception as the support of choice for chemical modification via organosilanes. The silica surface is reactive to the reagents and is well characterized as an adsorbent (22). The bulk silica is reasonably inert to

chemical attack and mechanically stable, an important quality since high pressures are routine in LC. Furthermore, silica is reproducibly manufactured in the desirable microparticles at a reasonable cost. Alumina shares many chromatographically desirable properties with silica and has been used as a support for LC bonded phases in a few laboratory applications (23-25), but it is not commercially available in modified form as is silica.

Silane modification procedures are important for other metal oxides which are used as electrodes. Chemical modification is used to impart selectivity to the surface electrochemical reactions and has been applied to SnO_2 (9-11), RuO_2 (5), In_2O_3 (10), and TiO_2 (12). Electroactive derivatizations of these surfaces have also been synthesized using cyanuryl chloride as a linking agent to the surface (26).

Modification of the silica surface has been accomplished through a variety of synthetic routes in addition to organosilane chemistry, but a chromatographically acceptable product has not been synthesized. Direct reaction of the silanols with alcohols or amines can be performed (27), but the Si-O-C or Si-N-C linkage is not stable to the water and alcohols commonly used in mobile phases. Grignard reagents and organolithium reagents have also been applied to silica modification, following the chlorination of the surface (27-29):



eqn 1-3

eqn 1-4

eqn 1-5

The surface coverages obtained through organometallic reagents have been very low (30). Grignard reagents probably experience steric hindrance due to the bulk of the complexing ether molecules. Hindrance should not be as great for organolithium reagents, but halogen -lithium exchanges could interfere with the reaction. The most significant interference for both classes of organometallic reagents is certainly the water which is strongly and inevitably associated with the surface despite efforts to dry the silica (31). Silane reagents have the advantage of being able to react with the surface water and subsequently "cure" to high coverage and permanent attachment (19).

Limitations of Chemically Modified Surfaces

Despite the widespread application of bonded phases and surface-modifying chemistry and the demonstrated successes of the approach, relatively little is known about the structure of the bonded phase and its precise role in the surface phenomena it creates or modifies. In LC, these limitations have severely restricted the elucidation of a quantifiable retention mechanism. (See the Appendix for a brief review of chromatographic theory). The lack of this fundamental aspect of LC theory restricts progress in the

application of LC in three important areas. First, prediction of the conditions necessary to achieve a given separation cannot be made easily and accurately, but only empirically and approximately. Second, LC cannot be used to measure or verify other equilibrium processes in solution as GC is used for vapor phase measurements (32). Third, the design and production of improved bonded phases are also restricted to empiricism.

The greatest obstacle to a quantitative retention mechanism appears to be the lack of a comprehensive qualitative description of the factors which contribute to the formation and function of the stationary phase. Through the short history of bonded-phase LC the appearance, acceptance, and repudiation of a proposed mechanism have closely followed a similar pattern regarding the description of the role of the stationary phase. Initially, the bonded phase was thought of as an anchored liquid layer into which a solute could partition from the mobile phase (33, 23). It soon became apparent that an anchored monolayer could not behave as a true liquid because its motions were too restricted. The bonded phase was subsequently viewed as a non-polar adsorptive surface. Adsorption retention mechanisms were proposed which relegated the bonded phase to the role of inert passive receptor for solute molecules whose retention was governed by the properties of the mobile phase.

Locke (34) suggested that solubility of the solute in the mobile phase was the basis for retention. Horvath, et al. (35) thoroughly examined the role of the mobile phase (usually a polar mixture of water and organic modifier in what is known as reversed phase LC) and concluded that entropy dominated solvophobic repulsion of a solute from the mobile phase onto the bonded phase was the cause of retention and the regulator of selectivity. The solvophobic retention mechanism enjoys widespread acceptance at this time; however, recent studies indicate that the bonded stationary phase has a more active role in retention and selectivity than is accounted for by solvophobic theory.

The Solvophobic Theory

The fundamental premise of the solvophobic theory is that the interaction between solute, S, and stationary phase hydrocarbon chain, H, is a dispersive association to form a complex, SH, according to the equilibrium expression: $[S] + [H] = [SH]$. The formation of the complex is said to be governed by the entropy-dominated expulsion of solute from the mobile phase into dispersive association with the stationary phase and is characterized by an equilibrium constant, K, according to equation 1-6:

$$K = \frac{[SH]}{[S][H]} \qquad \text{eqn 1-6}$$

The value for K. which is the same equilibrium constant to be used in the fundamental retention equation (Appendix, eqn. A-1), can be evaluated, according to the solvophobic theory, from measured or measurable properties of the solute and the mobile phase. The most important of those properties for solvophobic chromatography are the molecular dimensions of the solute, both free and in the complex, the dipole moment of the solute and its polarizability, and the surface tension of the mobile phase.

According to the solvophobic theory, selectivity or relative retention is primarily a function of mobile phase composition since the solute-solvent interactions are the most important. The solute-hydrocarbon chain interactions are recognized as influencing absolute retention in a manner similar to column dimensions; retention increases with both hydrocarbon chain length and surface coverage presumably because both are directly related to [H] the hydrocarbon concentration. The manner in which a bonded phase of given chain length and coverage is prepared should not have an effect on the chromatography. Thus, equivalent monomeric and polymeric C_{18} phases should perform identically (36).

Active Role of the Bonded Phase

While the solvophobic theory elegantly accounts for the solute-mobile phase interactions in LC, it soon became

apparent that the role of the stationary phase is more complex than is assumed by the solvophobic theory. Discrepancies have been found in such fundamental considerations as the basic equilibrium expression, the effects of the stationary phase on selectivity, and the structure and composition of the bonded phase.

Soon after the original publication advancing the solvophobic theory appeared (35), Scott and Kucera reported the results of experiments which tested the formation of the solute-stationary phase complex that is central to the solvophobic theory (37). Under typical mobile phase conditions, the bonded phase should be a repository for the organic modifier which itself would be subject to entropic expulsion from the mobile phase. The introduction of a retained solute should then displace the modifier in order to form its own complex with the hydrocarbonaceous chain. Scott and Kucera measured the uptake of modifier into a bonded phase, then monitored the release of the modifier upon introduction of a retained solute. They found that no modifier was released by the interaction of solute with the stationary phase, and so no complex with the hydrocarbon chain was formed directly. Their work could not reveal the nature of the solute-stationary phase interactions which were responsible for retention, but it did demonstrate that retantion was a function of the <u>combination</u> of bonded species and associated modifier.

The effective stationary phase thus formed has been shown to have a dramatic effect on the retention and the selectivity of the chromatographic process. Blevins, et al. (38, 39) have demonstrated that reversals in the retention orders of certain peptide hormones can be achieved by using different manufacturers' columns with the same mobile phase. Although the columns used differed in surface coverage and synthetic precursors, these factors should not have contributed to changes in relative retentions which, according to the solvophobic theory, are controlled by the mobile phase composition. Since bonded phases cannot often be accurately reproduced even by a single manufacturer from lot to lot, it is apparent that ignoring the activity of the effective stationary phase removes a fundamental parameter from a description of the retention mechanism.

Factors Affecting the Formation of the Effective Stationary Phase

A revision of the qualitative description of the stationary phase has begun to emerge only recently as workers have identified those factors which apparently control the formation and activity of the effective stationary phase. Those factors include: type and concentration of organic modifier (and, concurrently, the presence of water in the stationary phase); length of the bonded hydrocarbon chain; surface coverage by the bonded species; method of anchoring

the bonded species (monomeric vs. polymeric); temperature; and the substrate to which the bonded species is attached. Evidence for the inclusion of each of these factors in the bonded-phase description will be discussed.

The type and concentration of organic modifier used in the mobile phase is perhaps the most studied of the variables in LC. The inclusion of the modifier in the stationary phase has been established to occur roughly in proportion to the eluotropic strength of the modifier and its concentration in the mobile phase (30, 37, 40-42). Two different interactions are responsible for the incorporation of the modifier in the stationary phase. The first is the ability of the modifier to solvate the bonded species. For the common modifiers--methanol, acetonitrile, and tetrahydrofuran--this solvating ability follows their eluotropic strengths (30). Cationic and anionic surfactants are often added to the mobile phase in dilute concentrations to increase the retention of counter-ionic solutes through ion pairing. These ion pair reagents have been shown to concentrate into the stationary phase according to their solvating abilities as given by their own hydrocarbon chain lengths (43, 44). Hydrogen bonding is the second important interaction, since the modified silica surface invariably retains some unreacted silanol groups (20) especially if a di- or trifunctional silane has been used in its preparation. Those silanol groups will be covered with water molecules

under usual mobile phase conditions (41, 45-47)), so a modifier can enhance its incorporation into the stationary phase by interacting with the ubiquitous water also.

The influence of the hydrated silanols has manifested itself in poor chromatographic performance such as the tailing of peaks for polar solutes (48). Recent studies (49, 50) have demonstrated that under certain conditions the silica surface can provide the dominant interactions for retention even with nearly maximal coverage of the surface by the bonded species. It had been assumed that the surface provides discrete adsorption sites, i.e. unreacted silanols, which form 1:1 complexes with solutes of appropriate polarity in accordance with the theory of adsorption chromatography advanced by Snyder (22). However, the comprehensive study by Scott, et al. (51-54) of the interactions of solute and solvent at the silica surface presented conclusive evidence that solutes generally interact with the several monolayers of solvent which blanket the surface. For the organoaqueous mixtures usually used with bonded phases, the blanket most likely would consist of at least three molecular layers of strongly adsorbed water topped with several molecular layers of water mixed with organic modifiers which, as previously stated, interact with water through hydrogen bonding.

Experimental evidence has been reported which demonstrates the similar retentive behavior of the "naked" and

chemically modified silica surface for certain solutes (50). The bonded species serves to reduce the effective concentration of the surface layer sorption sites, since it occupies a large number of those sites, without substantially changing the nature of the interactions with the silica surface layer. The presence of the bonded species on the silica surface will influence the composition of the surface layer, however, since the bonded species is one component of that layer and can itself interact with mobile phase constituents.

The incorporation of organic modifier into the stationary phase will also depend on the hydrocarbon chain length of the bonded species, not only because the effective concentration of nonpolar interaction sites is a function of the chain length, but more importantly because the structure of the bonded phase changes with this parameter. In studies of monomeric bonded phases ranging in length from one to twenty-two carbon atoms, Berendsen et al. calculated that the space between chains into which the mobile phase can penetrate increased with increasing chain length (55), and so did the dimensions of the resulting stationary phase (56). However, the chromatographic benefit from longer chains, i.e. greater retention and selectivity, leveled off between six and ten carbon atoms with longer chains showing insignificant increases in retention (57-59). These findings suggest that only a portion of the bonded moiety is

responsible for retention, and that too short a moiety restricts the activity of and/or access to that portion.

Access to the stationary phase has long been recognized to depend on the type of surface linkage--monomeric or polymeric--provided by the silane reagents. Mass transfer into the stationary phase is a measurably less efficient process for polymeric phases than for monomeric phases (23). Polymeric phases have a greater sorptive capacity than the monomeric (37, 60), but can require much longer equilibration times with organic solvents than would be expected from their greater capacities (61), while the equilibration of polymeric phases with water is more rapid than that of monomeric phases (47). These facts suggest that the structure of a polymeric bonded phase is more rigid than that of its monomeric counterpart (47), with a greater separation of the hydrocarbon chains providing freer access to the residual silanols. Additionally, the polymeric bonded layer has a greater depth than the monomeric layer. Since the structure of the polymeric layer is potentially anisotropic in three dimensions, it can be expected to influence the structure of the effective stationary phase in an anisotropic manner also.

The structure of the effective stationary phase has been found to be dependent, not surprisingly, on the solvating ability of the organic modifier and its concentration. Tanaka et al. (62) discovered that increasing the

concentration of methanol or acetonitrile in the mobile phase had effects on the retention of selected solutes that were similar to increasing the length of the bonded chain. Several authors have reported that the hold-up time (also called the dead time) of a given column is a function of both the concentrations (41, 44, 56, 57, 63) and the type (40, 41, 44) of the organic modifier. These reports indicate a modifier-dependent swelling of the bonded phase which contributes to the formation of the effective stationary phase. Relative phase ratios measured in several solvents (64) support the concept of an expanding stationary phase. Studies on the effect of temperature with model systems (65-67) suggest that this swelling can be enhanced by operation of the column at elevated temperatures.

The many influences on the formation of the stationary phase outlined in the previous paragraphs can be collated into qualitative depictions of the stationary phase under different donditions. Figure 1a provides a generalized picture of the primary components of the effective stationary phase: the solvated bonded species, the solvated surface, and the associated, non-solvating mobile phase components. At very low modifier concentrations and relatively low temperatures (25^{o}C) Figure 1b is probably valid. The bonded chains engage in a high degree of interchain association; as a result, the depth of the stationary phase

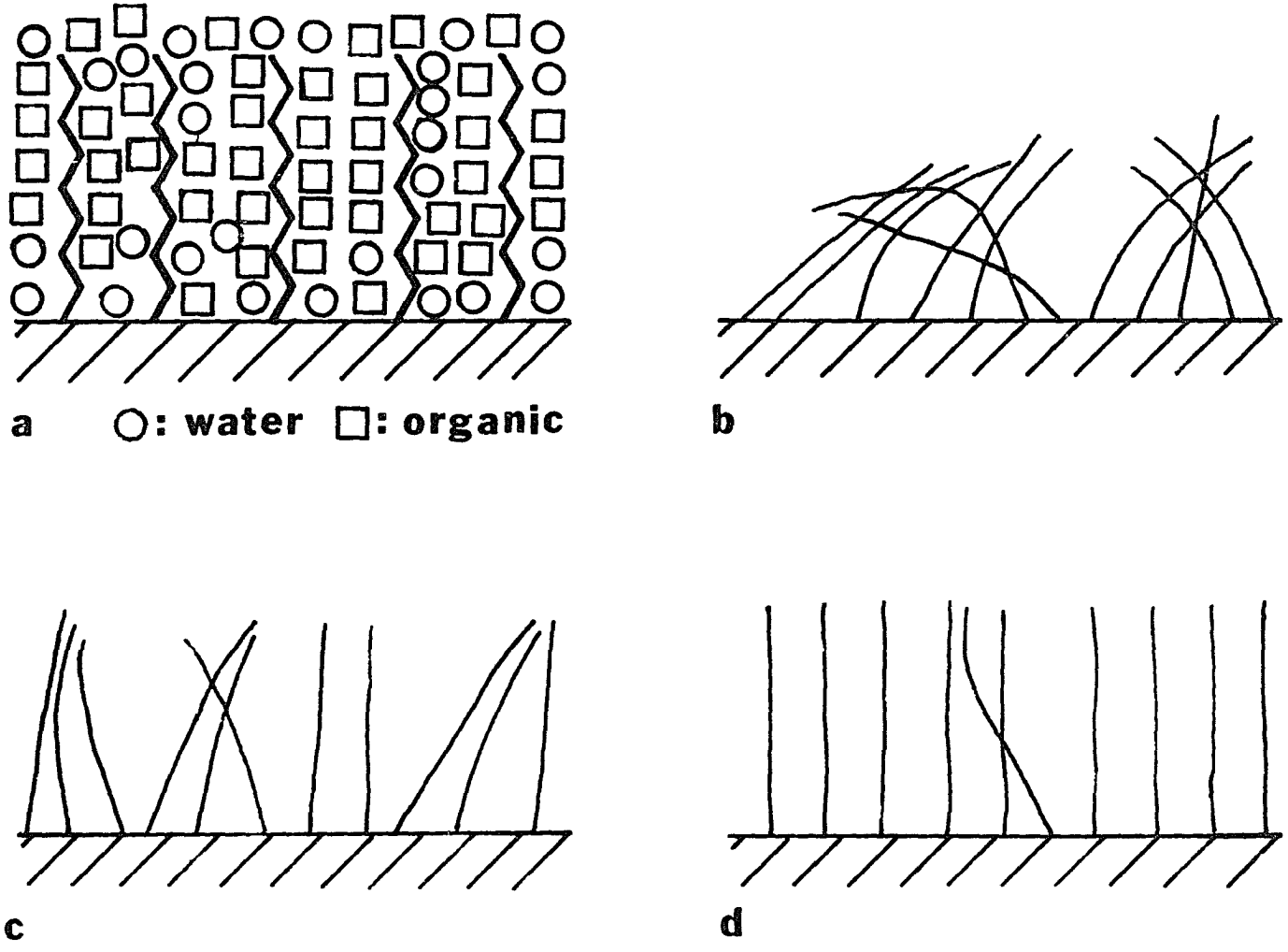


Figure 1. Depictions of stationary phase structure under varied conditions.

is minimal and the structure, closed and tight, may present an adsorbent surface for interacting long-chain moieties. For shorter chains, with less interchain association, a solvent-modified deactivated silica surface is presented. Increasing either the modifier concentration, the chain length, or the temperature opens up the structure of the stationary phase (Figure 1c) through solvation, spatial resolution, or increased molecular motion respectively. Finally, at still higher modifier concentrations or the substitution of a better solvating modifier, or at still higher temperatures, the stationary phase has swollen to maximum dimensions (Figure 1d).

Direction of Research

These descriptions of the stationary phase are admittedly unrefined. Many questions remain unanswered, particularly regarding the interdependence of the factors responsible for the formation of the stationary phase. In this work, the results from two projects designed to examine some of the influences on the formation of the stationary phase will be presented in order to clarify the picture. The first of these projects involved the study of the role of the substrate material in the formation of the stationary phase using a specially synthesized carbon material for bonding. The second project probed the stationary phase on commercially available C_{18} silicas by the independent

technique of carbon-13 nuclear magnetic resonance spectrometry as a means of investigating on the molecular level the nature of the interactions between solvent and bonded species. Chromatographic measurements are used extensively in both projects to provide information about the nature of the interactions of probe molecules with the stationary phase under investigation, and in the determination of the composition of the stationary phases.

Carbon Substrate for Bonded Phase Formation

The current understanding of the bonded stationary phase in LC has been derived by varying one parameter at a time and observing the effects on the chromatography. This experimental method has been applied to the study of the role of mobile phase composition (38-40, 68, 69), the bonded species (55, 57, 70), the structure of the solute (36, 71), and the temperature (67, 72). Since the substrate providing the surface for bonding has been identified as an influence on the composition of the stationary phase also, it became necessary to introduce the substrate as a variable in the study of the effective stationary phase.

A suitable variant substrate would possess many of the properties of silica such as a potentially reactive surface and mechanical stability in small particle size while exhibiting substantially different adsorptive

properties in order to clearly reveal that contribution to stationary phase formation. Of the many substrates already mentioned as modifiable, carbon proved to possess the best combination of desired properties.

Carbon in its various forms (graphite, carbon black, and activated carbon) is a unique adsorbent. Its catenated structure enhances the dispersive interactions with adsorbates so carbon is both a strong and nonspecific adsorbent (22, 73, 74). Simultaneously, depending on the type of carbon used, strong specific interactions occur due to the conjugated unsaturated nature of much of the structure and also to the polar oxygenated surface groups. The former specific interactions cause the well-known adsorption of aromatic compounds, and the latter permit the wetting of the surface with water and the adsorption of polar molecules. This combination of specific and nonspecific adsorption properties have made carbon indispensable in industrial filtering applications, particularly the removal of organics from aqueous process streams and drinking water (75). The adsorptive properties of carbons have been exploited in chromatography for many years. The nonspecific nonpolar interactions have been most important in GC to the exclusion of polar interactions (76, 77). In classical (low pressure) column chromatography, carbon has been used primarily for its polar nature (22).

The surface of unpyrolyzed carbon retains a polar nature due to the presence of oxygenated surface groups (78). Oxygen chemisorbs to the surface atoms of a conjugated structure (79, 80) to form reactive oxides. The chemical nature of the oxides has been a subject for debate and study for some time. The most consistent evidence supports carboxyl and hydroxyl functional groups (78, 81, 82). The surface groups are reactive in a variety of derivatizing schemes including esterification and ether formation (78, 81, 83), silanization (83), chlorination (78), and Grignard alkylation (84). These derivatizations were used for the determination of the surface oxygen and for the formation of bonded phases on carbon electrodes (13, 14, 26, 80). The surface groups are also the sites of attachment of polymers grafted onto carbons (85, 86).

Carbon has only recently been used in LC following the development of special forms of microparticles which can withstand the fluid pressures normally encountered. Colin and coworkers initially reported success with carbon blacks coated with the graphitized products of benzene pyrolysis (87, 88). While incompressible, those carbon particles were difficult to pack into columns because of their agglomeration. A more successful carbon packing was made by coating silica microparticles with the same products of benzene

pyrolysis without graphitization (89, 90). Most recently Unger et al. have made chemically and thermally treated cokes and active carbons of proper hardness and size for use in LC (91).

Those adaptations of more familiar forms of carbon for LC produced adsorbents with the highly desired properties of carbon. However, the transformation to high-strength forms had two deleterious effects on the use of those materials as substrates for bonded phase formation: The surface areas were reduced by factors of up to 1000 from the precursors, thus severely limiting capacity, while the heat treatments removed most of the surface oxides and with them the anchoring sites for the bonded phase.

Fortunately another route to the production of a high-strength carbon preserves the surface area and the surface oxides (92). Produced through the reduction of Fluoropolymers by lithium amalgam at a low temperature (20^{o}-100^{o}C) in vacuo, this carbon is a highly conjugated and unsaturated linear array of carbon atoms (93, 94) which chemisorb greater than 10% by weight of oxygen upon contact with air or water (95). Its surface is demonstrably polar, yet the material exhibits the strong dispersive interaction characteristic of elemental carbon (96). The inventors of this carbon have sought to remove the polarity of the oxygenated surface through thermal treatment (97) in an

attempt to produce a more chromatographically homogeneous surface. In this work, the surface oxides will be preserved as sites for the attachment of bonded species and the formation of the effective stationary phase.

The first portion of the work with the carbon will establish the reactivity of the surface with respect to modification through a variety of qualitative and quantitative methods. The composition of the surface will be qualitatively determined using electron spectroscopy for chemical analysis (ESCA); ESCA will be used also to follow the progress of silane and Grignard modification procedures. Complimentary quantitative data will be obtained, albeit from the bulk of the material and not just the surface, through elemental carbon determinations. Finally, the significance of the modifications will be established through chromatographic measurements compared on the unmodified and modified carbons.

Having demonstrated the success and significance of the surface modification, the effect of the carbon substrate on the formation of the stationary phase will be measured. Again, chromatographic measurements will be employed, particularly the retentions of probes with demonstrable sensitivity to the presence of carbon vs. silica. Both chromatographic and bulk measurements of the adsorptive capacities of carbons and silicas for probes and mobile

phase components will be used to quantify the effect of the carbon substrate on stationary phase activity and composition.

Carbon - 13 NMR Studies of Bonded Phases

The bonded species has been shown to play an active role in the stationary phase through its interactions with the mobile phase components; these interactions apparently are dependent on the type of bonded layer (monomeric vs. polymeric), the chain length, surface coverage, and of course the organic modifier. These factors have been varied systematically to study their contributions to overall chromatographic behavior. However, due to the interdependence of these variables, that approach to studying the effective stationary phase perturbs the system under study and thus can reveal only general trends. The nature of the interactions responsible for this interdependence can be investigated only by employing techniques which provide surface-specific molecular information under realistic, unperturbed mobile/stationary phase conditions.

Carbon-13 nuclear magnetic resonance spectometry (CMR) is one of the few techniques which can satisfy those rigid criteria. CMR is inherently sensitive to the kinds of interactions among solvent, bonded moiety, and surface which are of interest, since these interactions comprise the predominant relaxation mechanisms. Therefore subtle differences

in these interactions due to the type of bonded support or the particular organic modifier can potentially be detected. CMR instrumentation is now sufficiently sensitive to probe commercially available hydrocarbonaceous bonded supports at natural isotopic abundance. By virtue of the ^{13}C chemical shifts and the resolution of the spectrometer, interferences from the commonly used organic modifiers such as methanol and acetonitrile are minimal. These qualities give CMR distinct advantages over the few alternate techniques which are applicable within the limitations of the problem. For example, photoacoustic spectroscopy has been used to study the solvent-bonded species interactions (98), but it was necessary to synthesize bonded phases with chromophores which absorbed in the visible region of the spectrum in order to obtain a signal. Infrared spectrometry (60) also requires chromophores on the bonded moiety to avoid the interferences from C-H bands in the organic modifiers.

The CMR experiment provides information about the structure of the species under investigation by the chemical shift of the signals in the spectrum relative to a reference. The chemical shift is also a minor measure of the chemical environment of a nucleus according to its value in a range of observed values. Because of the breadth of the peaks that have been reported for bonded moieties (30), the

chemical shift information will be limited to identifying those portions of the bonded species which are being studied.

The width of the CMR signal, measured at half the signal height, is a measure of the effectiveness of the radiationless relaxation processes through which the nuclei excited by the absorption of energy at their characteristic, or Larmor, radio frequencies under the influence of the static magnetic field, return to their equilibrium distribution of ground and excited states. Two principal relaxation pathways are available: the first involves the release of energy through the thermal motions of the molecules to their surroundings, usually called spin-lattice relaxation. The second process, called spin-spin relaxation, involves the release of energy through inter- and intramolecular dipolar interactions. In liquids, the rapid tumbling of molecules does not allow sufficiently long-lived dipoles on the NMR time scale, and spin-lattice relaxation with its time constant T_1 predominates. In solids and liquids with restricted motions (such as the anchored moiety in bonded phases) dipole interactions exist for sufficient time to permit spin-spin relaxation, characterized by T_2 ($T_2 \leq T_1$). Spin-lattice relaxation also is made more efficient in cases of restricted motion.

The width of the peak is inversely proportional to the relaxation time constants. A tumbling molecule in a liquid will release its energy slowly so that the nuclei

cannot be re-excited by the same radio-frequency pulse. In solids and slowly reorienting liquids, the nuclei can relax through dipolar interaction, reorient slightly, and re-absorb energy to add to the peak width at a slightly different chemical shift (99), thus the peak width will be a function of T_2 primarily. The contributions from other possible sources such as T_1, magnetic field inhomogeneity, and structural chemical shift anistropy are considered minor in such cases.

The peak widths in CMR of bonded phases will thus be a measure of the relative liquid state of the bonded species under given conditions. Peak shapes as a function of organic modifier and its concentration, bonded phase type, surface coverage, and temperature are shown to reflect the contributions from these factors to the structure of the effective stationary phase. The independent measurements obtained through CMR are combined with complementary chromatographic data to provide an improved qualitative description of the stationary phase.

Composition of the Stationary Phase

Finally, quantitative measurements of the composition of the stationary phase will be reported. The relationships among components will be discussed, and the influence of stationary phase composition on the chromatographic process will be demonstrated.

CHAPTER TWO

CARBON SUBSTRATE FOR BONDED PHASE FORMATION

Introduction

In the current practice of high performance liquid chromatography (HPLC) the majority of column packings consist of bonded-phase adsorbents. Among these, the most common are the silicas whose surfaces have been chemically modified with a hydrocarbon (alkyl or phenyl), amine, cyano, or nitro moiety. The chemically modified silica surface provides an effective stationary phase which is not only highly reproducible but which has the advantages of greater capacity and a more controllable selectivity than the unmodified silica. The chromatographic properties of the effective stationary phase have been described recently as being determined by the combination of solvated bonded species and solvated substrate which is controlled by the mobile phase composition (30). Thus, the solvated bonded species contributes to, but does not comprise, the stationary phase, and the substrate must also be considered.

Investigations into the nature of the retention mechanism on bonded-phase materials have concentrated on the

effects of changing either the mobile phase composition or the bonded species. Since the substrate is considered active in the formation of the effective stationary phase, it was necessary to introduce the substrate as a variable in stationary phase formation. Carbon was chosen for this purpose because of the truly different adsorptive properties compared with alternatives like alumina or tin oxide, and because of the reactivity of the oxygenated surface groups found on all carbons (74, 78).

The investigation using the fluoropolymer-derived carbon consisted of two parts: first, the determination of the success and significance of chemically modifying the surface, and second, the demonstration of the effect of a new substrate on the chromatographic behavior of the stationary phase.

Experimental

Preparation of the Carbon

The carbon was prepared through the reduction of Kel-F 300 LD, 30-40 mesh, (Analabs, Inc., North Haven, CT) by lithium amalgam. The amalgam, approximately 0.04% Li by weight, was prepared slowly, in an inert atmosphere, using triply-distilled Hg which had been drawn through a sintered-glass funnel of medium porosity and then dried for several hours at 100^{o}-150^{o} under vacuum. A chambered reusable reaction vessel, based on the disposable vessel described by

Jansta, et al. (104), was constructed by the Chemistry Department Glass Shop at the University of Arizona (Figure 2). The Kel-F and amalgam were mixed in the reaction vessel under vacuum and were maintained at 120° under vacuum for one week with daily manual and ultrasonic agitation. Other fluorocarbon polymers, such as Fluoropak 80, 20-80 mesh (The Fluorocarbon Co., Fullerton, CA), Teflon 6 (DuPont, Parkersburg, WV), and thin strips of methanol-washed thread sealant (Ribbon Dope-P-412, Permacel, New Brunswick, NJ) were also used but gave low yields of carbon due to poor wetting of the surface by the amalgam. Batches of the Kel-F-derived (KFD) carbon were washed with water (batches 1012 and 1023) or THF (batch 104B) and subjected to ultrasonic agitation to suspend the finer particles, which were collected on a filter (type LS, 5.0 μm pore size, Millipore Corp., Bedford, MA). The general refining procedure included the refluxing of the carbon in 2M HCL, washing with hot water until no precipitate with $AgNO_3$ solution was formed by the filtrate, and drying at 150°C. Some batches were subjected to gravitational sedimentation in 0.05M sodium oxalate, which demonstrated that most of the carbon particles were smaller than 20 μm equivalent Stokes' diameter.

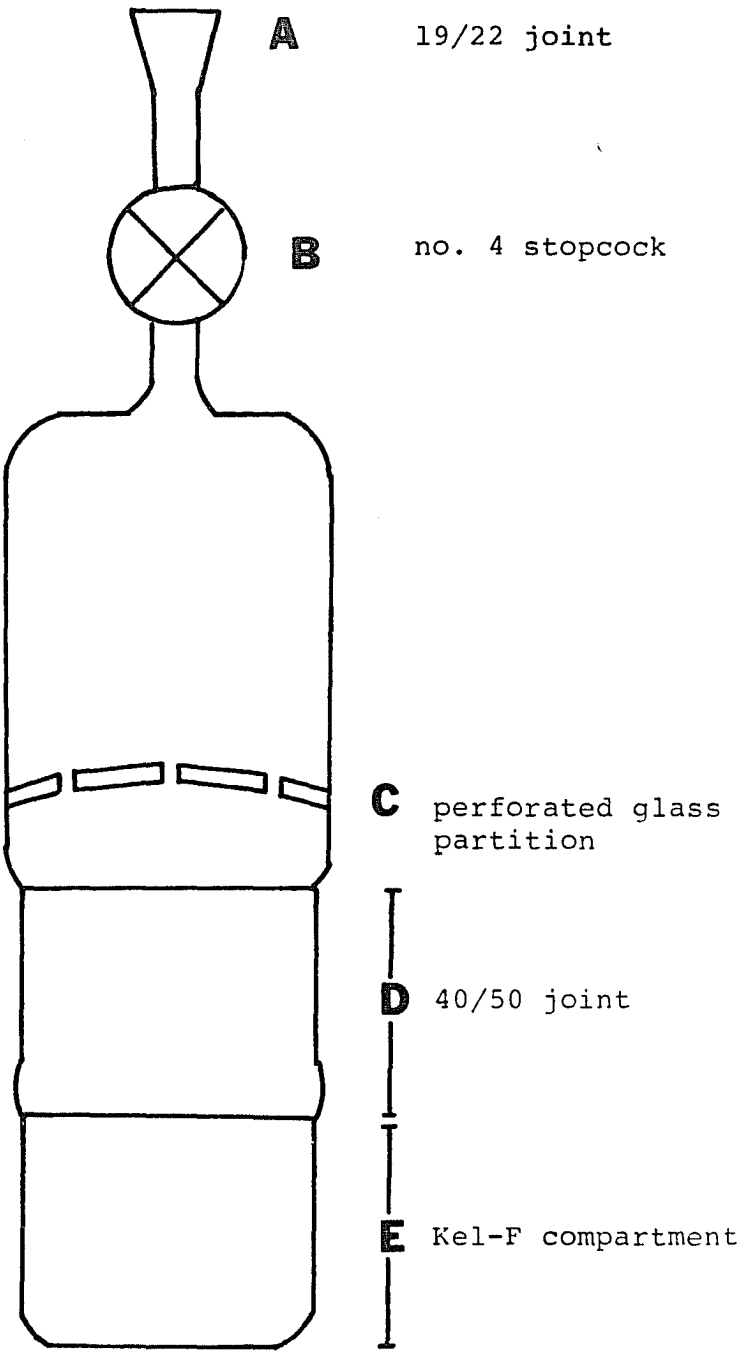


Figure 2. Glass reaction vessel for production of Kel-F-derived carbon.

Approximately 2/3 actual size

Chromatographic Procedures

Batches of the carbon were dried in air at 150° prior to packing into columns, using the balanced density slurry technique. Batch 1012 was slurried in carbon tetracholride and forced into a 140 x 2.1 mm stainless steel column using hexane. The 1012 column was subsequently washed with chloroform followed by methanol. Batches 1023, 104B and 104BC8 were slurried in 23:2 iodomethane: methanol and packed into a 70 x 2.1 mm column (1023) or 40 x 2.1 mm columns with a methanol stream. Chromatographic measurements were made on either a Spectra-Physics (Santa Clara, CA) model 3500 B or an Altex (Berkeley, CA) model 332, both gradient liquid chromatographs with loop injectors and photometric detectors (254 nm). The Schoeffel model 970 fluorescence detector was used on loan from Klane Scientific and Engineering (Tustin, CA). Retention times (t_R) were measured with a Spectra-Physics System I computing integrator. All retention data are reported as capacity factors, k', where $k' = (t_R - t_o)/t_o$, or as separation factors α, where $\alpha = k'_2/k'_1$. The column dead time (t_o) was taken as the retention time of either a dilute $NaNO_2$ solution or the solvent spike from the sample. All solvents used as mobile phases were filtered through a 0.45 μm filter (Millipore) and vacuum degassed prior to use. Methanol was either reagent grade (Fisher Scientific Co., Fair Lawn, NJ) or

"distilled-in-glass"(Burdick and Jackson Laboratories, Muskegon, MI). Acetonitrile (reagent grade, Mallinckrodt, St. Louis, MO) was distilled prior to use. Tetrahydrofuran (THF, Fisher reagent grade) was stored over KOH for 24 hours, then distilled from KOH before use. Toluene and chloroform (Fisher reagent grade) and hexanes (Burdick and Jackson) were used as received. House-distilled water was re-distilled from alkaline permanganate. Sample components were obtained from various manufacturers and typically were dissolved in the mobile phase at the 0.02% (w/v and v/v) level.

In Situ Column Modification

Trimethylchlorosilane (TMCS, 98%, Alfa Div., Ventron Corp., Danvers, MA) was used to modify the batch 1012 column according to the method of Gilpin, et al. (105). The post-modification wash with water-saturated toluene was eliminated, since water would degrade the labile Si-O-C bond. For this same reason, the acetonitrile was dried before using on the modified columns by refluxing and distilling twice from calcium hydride (4-40 mesh, MCB, Norwood, OH) and storing over calcium hydride and under nitrogen.

Grignard Modification

A portion of batch 104B was refluxed for 48 hours in thionyl chloride (97%, J. T. Baker Chemical Co.,

Phillipsburg, NJ). The chlorinated carbon was collected on a filter pad and washed with THF (dried by distillation from CaH_2) until the filtrate produced no precipitate with dilute $AgNO_3$ solution. The carbon was then dried in air at 150°C. The Grignard reagent was prepared from 1-bromooctane (Eastman Organic Chemicals, Rochester, NY, dried over 3A molecular sieves) in dry THF. The chlorinated carbon was added as a slurry in dry THF and the mixture was kept under reflux for 4 days. The octyl-modified carbon was collected on a filter pad and washed with 0.5M NH_4Cl in water, with THF, and then with water until no precipitate with $AgNO_3$ was formed by the filtrate. After a final wash with THF the modified carbon was dried at 150° in air.

ESCA

All spectra were obtained on a GCA-McPherson ESCA 36 electron spectrometer. A magnesium anode was used in the X-ray source for Kα radiation at 1254 eV. Samples were prepared as either thin layers pressed onto KBr pellets or as smears on double-sided cellophane tape mounted on aluminum planchettes. Ten scans were added to produce each spectrum, and binding energies were determined relative to the O_{1s} peak at 532.0 eV. Carbosieve-B, a carbon black molecular sieve, was obtained from Supelco (Bellefonte, PA). Graphitic oxide was produced by the oxidation of graphite in $KMnO_4/H_2SO_4$ and was donated by Ralph Kamin at the University of Arizona.

Elemental Determinations

C, H, and N determinations were performed by the University Analytical Center (University of Arizona, Tucson, AZ).

Results and Discussions

Reactivity of the Carbon Surface Groups

A series of experiments were performed first to establish the activity of the carbon surface with respect to modification. Activated carbons are considered polar adsorbents due to the presence of chemisorbed oxygen as carboxyl, carbonyl, or hydroxyl groups (74). The existence of similar surface groups on the Kel-F derived (KFD) carbon was presumed based on the oxygen content. From Table I it is seen that the surface of the KFD carbon was sufficiently polar to retain polar probes such as nitrobenzene and phenol with THF as a mobile phase, while the alkylbenzenes were not retained.

Additional qualitative evidence for the proposed reactive surface groups was obtained through ESCA. In Figure 3a the C_{1s} scan for the KFD carbon exhibits the shoulder on the high binding energy side that is typical of the C-O bond. The width of the shoulder suggests the presence of more than one type of C-O bond. This shoulder is not the result of residual C-F or C-Cl bonds from the Kel-F based on the position of the shoulder; scans of the F_{1s} and the

TABLE I. Retention of Selected Probes on Unmodified Carbon

Column: Carbon 1012
Mobile Phase: THF, 0.5 ml/min.

Probe	k'
Benzene	0
Toluene	0
Ethyl Benzene	0
Phenol	0.43
Nitrobenzene	2.39

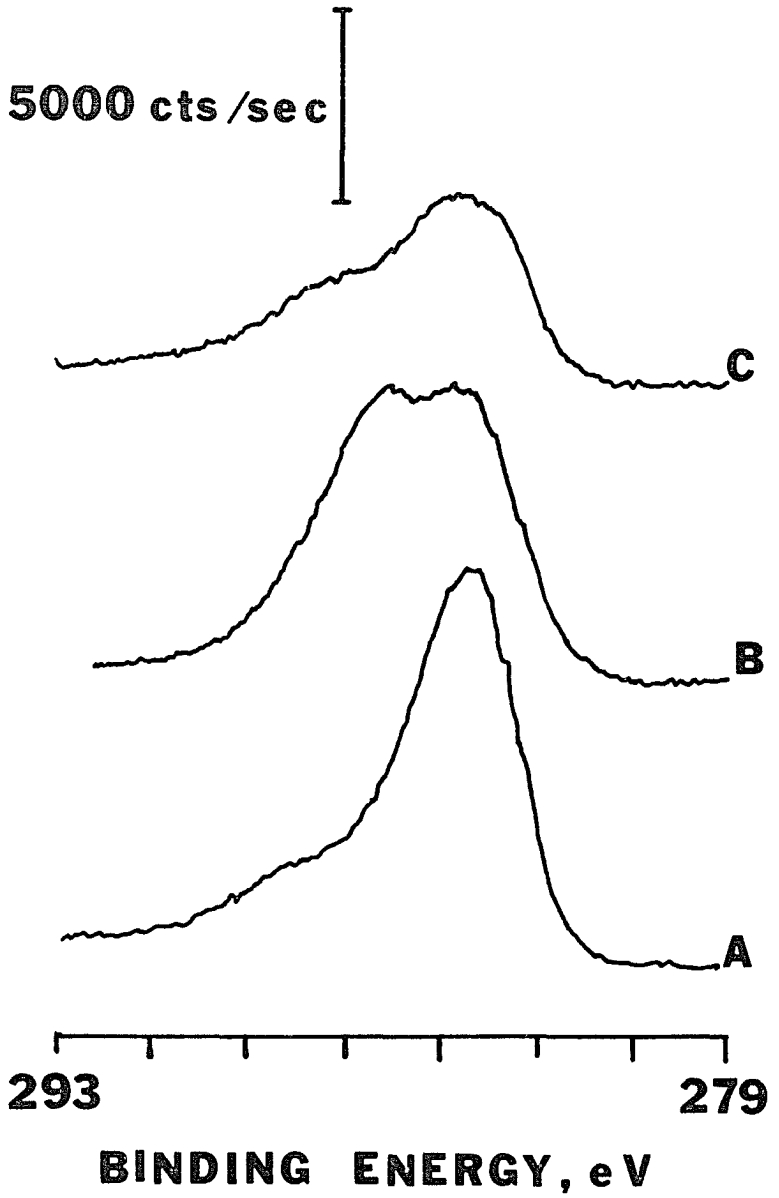


Figure 3: ESCA spectra of the C_{1s} region for carbonaceous materials.

A: KFD carbon; B: Graphitic oxide;
C: Carbosieve-B.

Cl_{2p} regions showed the absence of these elements in this sample, while the O_{1s} scans produced intense broad peaks supportive of several forms of surface oxygen. The C-O shoulder is more well-defined in the scans for graphitic oxide and Carbosieve-B (Figure 3b and c, respectively) which are two carbonaceous materials chosen to represent the relative high and low oxygen limits for carbons. The graphitic oxide spectrum shows the pronounced C_{1s} chemical shift which has been attributed to the enolic network structure of this material (106). That network may model the structure of the KFD carbon. The high binding energy shoulder of the KFD carbon more closely resembles that of the Carbosieve-B, suggesting a mixture of oxygenated surface groups. These ESCA studies also indicate the presence of reactive sites such as hydroxyl and carboxyl groups, and therefore the KFD carbon should be suited for a variety of surface chemical reactions.

The first attempt at modifying the carbon surface consisted of an <u>in situ</u> reaction with TMCS (Equations 2-1 and 2-2).

$$R\text{-}OH + Cl\text{-}Si(CH_3)_3 \longrightarrow R\text{-}O\text{-}Si(CH_3)_3 + HCl \qquad \text{eqn 2-1}$$

$$R\text{-}\overset{\overset{O}{\|}}{C}\text{-}OH + Cl\text{-}Si(CH_3)_3 \longrightarrow R\text{-}\overset{\overset{O}{\|}}{C}\text{-}O\text{-}Si(CH_3)_3 + HCl \qquad \text{eqn 2-2}$$

This method has been shown to be just as effective as batch reactions (105). While a successful reaction was indicated according to the retention data presented in Table II, only a deactivation of the surface could be claimed. This was especially evident for those probes such as nitrobenzene and phenol which were likely to have interacted strongly with the unreacted surface groups. For TMCS a ternary stationary phase was not likely to have been formed, since the works Stetzenbach (30) and Berendsen and De Galan (57) have indicated a minimum hydrocarbon chain of six carbon atoms for this phenomenon. The silyl ethers and esters formed by the TMCS were hydrolytically unstable, and the column performance degraded rapidly as the dried solvents absorbed water from the atmosphere. The silane modification was therefore not practical for any extended experiments, but it served to demonstrate the reactivity of the surface.

TABLE II. Effect of Silane Modification on Relative Retentions

Mobile Phase: Acetonitrile, 0.5 ml/min
u = unmodified Carbon 1012
m = modified Carbon 1012

Probe	α_u	α_m
Benzene	1.0	1.0
Ethyl Benzene	1.1	1.0
Phenol	4.5	3.6
Nitrobenzene	6.4	5.3

A stable modification was developed through a Grignard reaction and the resulting C-C bond (Equations 2-3 through 2-6).

$$R\text{-}OH + SOCl_2 \longrightarrow R\text{-}Cl + HCl + SO_2 \qquad \text{eqn 2-3}$$

$$R\text{-}\overset{\overset{O}{\|}}{C}\text{-}OH + SOCl_2 \longrightarrow R\text{-}\overset{\overset{O}{\|}}{C}\text{-}Cl + HCl + SO_2 \qquad \text{eqn 2-4}$$

$$R\text{-}Cl + C_8H_{17}MgBr \longrightarrow R\text{-}C_8H_{17} + MgBrCl \qquad \text{eqn 2-5}$$

$$R\text{-}\overset{\overset{O}{\|}}{C}\text{-}Cl + C_8H_{17}MgBr \longrightarrow R\text{-}\overset{\overset{O}{\|}}{C}\text{-}C_8H_{17} + MgBrCl \qquad \text{eqn 2-6}$$

This modification sequence, beginning with chlorination of the surface was followed with ESCA and elemental determinations to gauge the significance of the modification. In Figure 4 the Cl_{2p} scans illustrate the progress of the reaction. Scan A shows the background chlorine signal due to traces of unreacted Kel-F in the batch. The intensity of the signal doubled after chlorination, as shown in scan B. The Gringard reagent removed some of the chlorine, with the balance presumably hydrolyzed in subsequent washing, for the absence of signal depicted in scan C. A similar corroborative sequence of spectra was obtained in the C_{1s} region. After chlorination, a distinct peak appeared (Figure 5b) that was indicative of the C-Cl bond. Finally, with the addition of the C_8 groups the C-Cl peak diminished at least to background

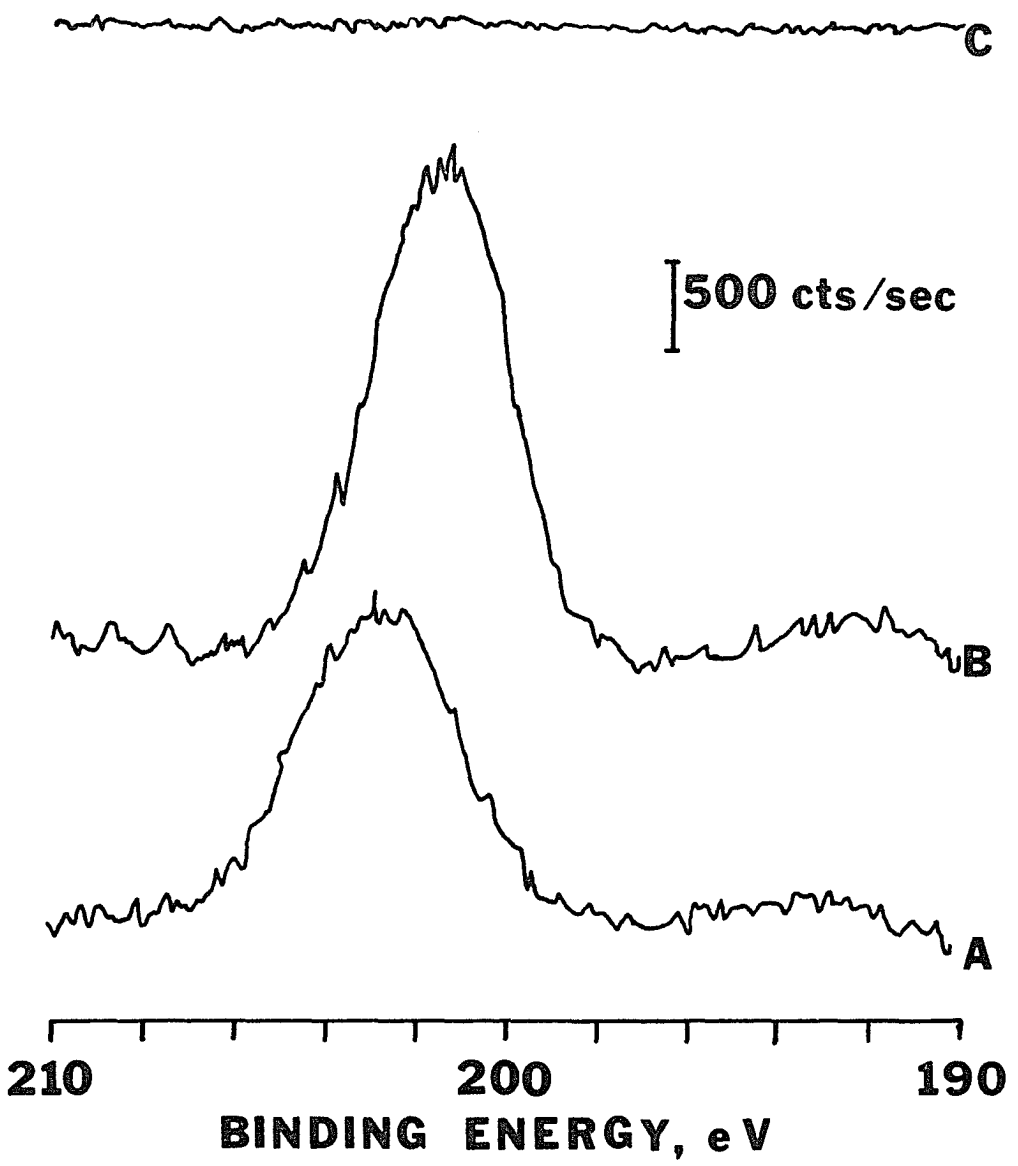


Figure 4: ESCA spectra of the Cl_{2p} region for KFD carbon in stages of Grignard modification.

A: background signal prior to reactions;
B: after chlorination;
C: after alkylation with Grignard reagent.

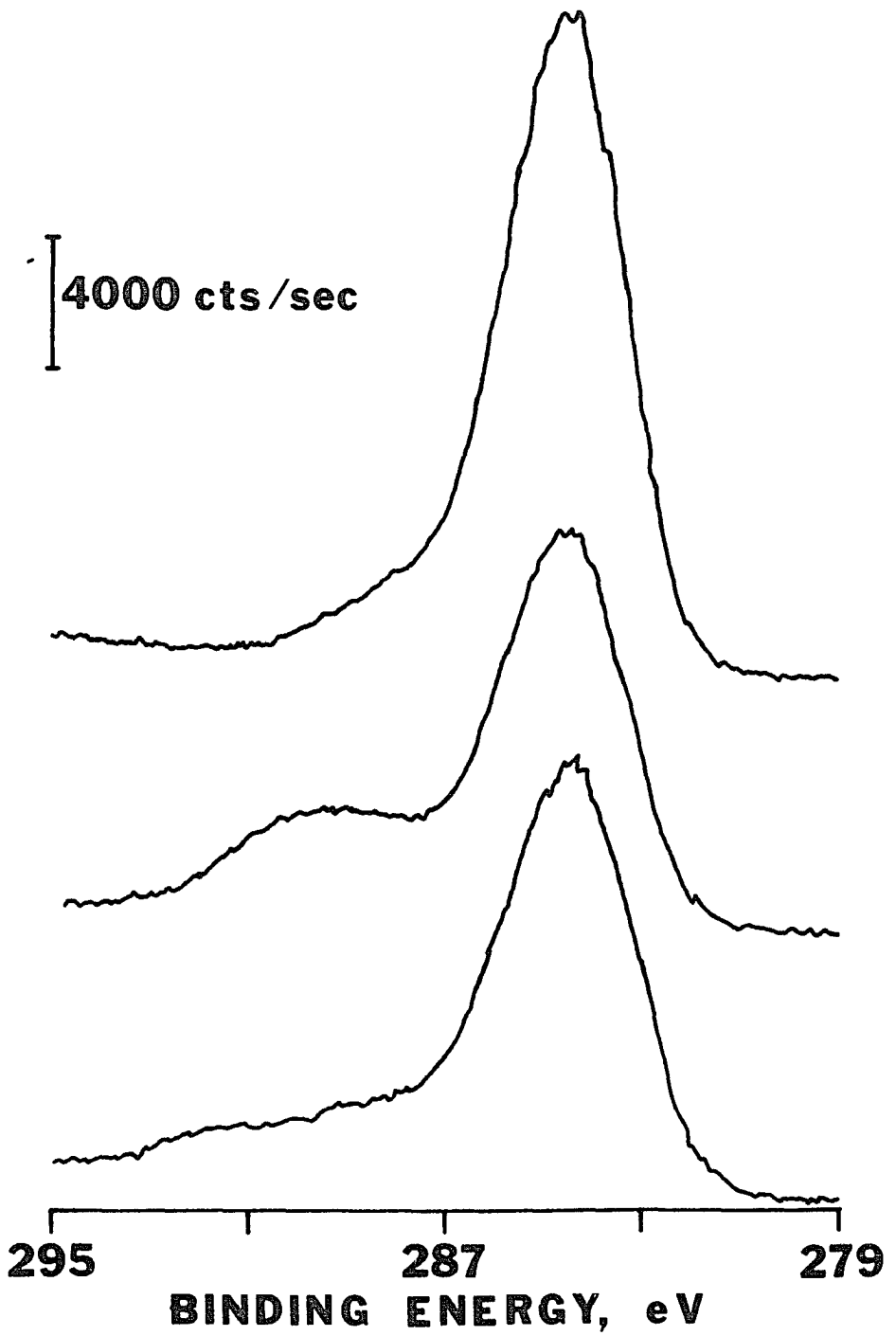


Figure 5: C_{1s} region for KFD carbon

while the intensity of the main peak doubled.

The success of the Grignard modification was clearly demonstrated through ESCA, but the significance of the reaction could be gauged quantitatively only through elemental determinations. The highly desired C-C or Si-C bond has been sought through Grignard reactions for many years, but the modification has not been competitive with organosilane reactions. Locke et al. (28) reported the Grignard modification of low surface area silicas with the addition of about 4% carbon. Using high surface area silicas, Stetzenbach (30) was able to add only 0.5 - 1% carbon, presumably due to the steric hindrance of the bulky Grignard reagent. The KFD carbon, a high surface area material, was able to accommodate approximately 5% additional carbon through the Grignard reaction, perhaps because of a more well-solvated surface. The carbon addition amounted to 2.1 mmoles C_8 per gram of material, which compares favorably with the commercially available C_8 silica, RP-8 (E. Merck, Darmstadt, GFR), with 1.5 mmoles C_8 per gram claimed by the manufacturer.

The number of C_8 groups that were added to the carbon surface indicated that a significant modification had been achieved. This was also demonstrated through the chromatographic behavior of the modified carbon 104BC8 compared to that of the unmodified carbon 104B, as shown in Table III.

As with the silane modification, the greatest effect on relative retention was for those polar probes most likely to interact strongly with the reactive sites. In contrast to the silane modification, the larger differences in relative retentions demonstrate that a substantial change in the stationary phase has been made through the Grignard modification. Furthermore, peak asymmetry on 104BC8 was generally reduced by a factor of two from 104B, also indicative of an altered stationary phase.

TABLE III. Effect of Grignard Modification on Relative Retentions

Mobile Phase: Methanol, 0.5 ml/min.

Probe	104B	104BC8
Benzene	1.0	1.0
Ethyl Benzene	1.5	1.3
Nitrobenzene	10.5	6.1
Aniline	13.3	8.0

Influence of the Carbon Substrate on Retention

This new modified surface proved to be a reverse phase material of intermediate character when compared to unmodified carbon and both modified and bare silica. Figure 6 shows the variation of ln k' with alkyl carbon number for the homologous n-alkylbenzenes in methanol on

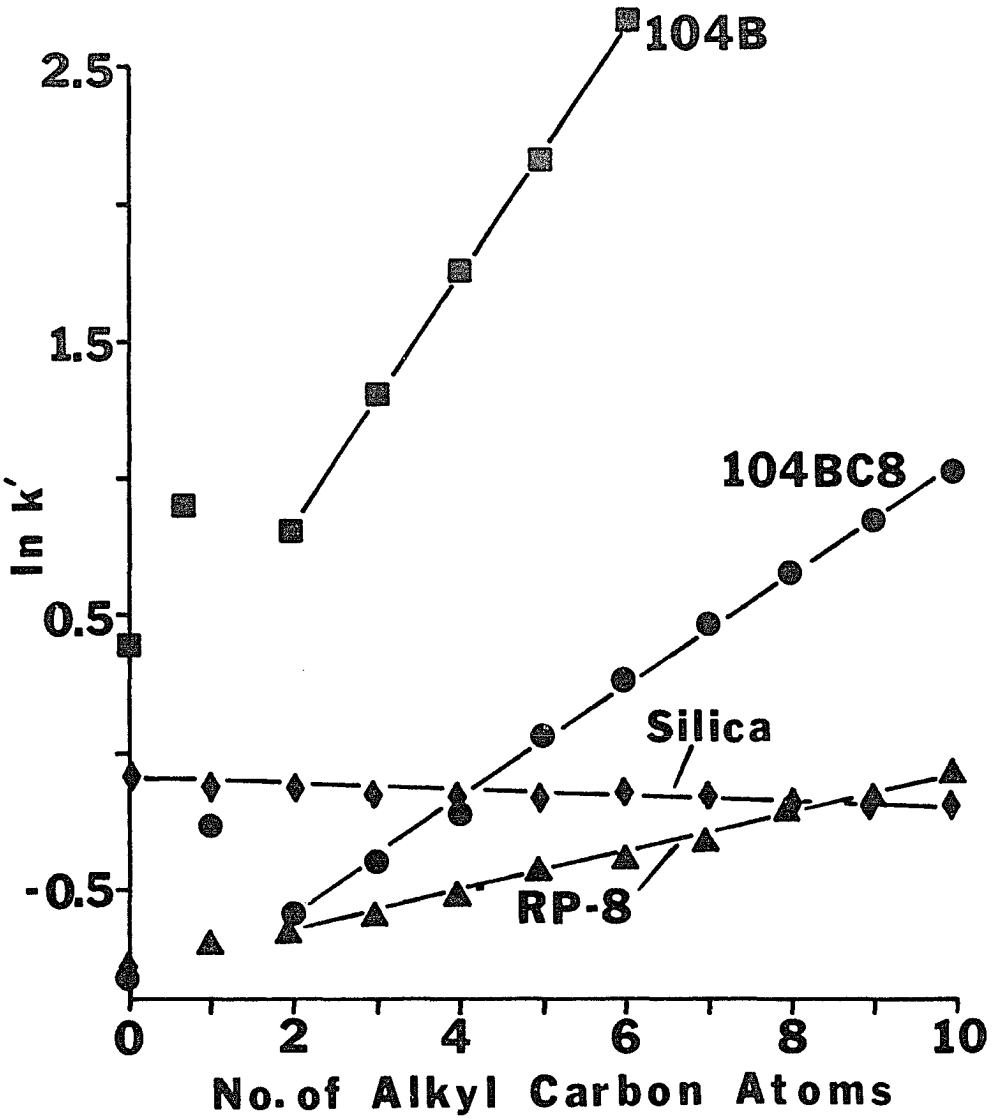


Figure 6: Variation of ln k' with alkyl carbon number for n-alkyl-benzenes on different columns.

Mobile phase: methanol, 0.5 ml/min; 104B: KFD carbon; 104BC8: octyl-modified KFD carbon.

the various stationary phases. The slope of each line is proportional to the incremental free energy of interaction per methylene group. The isolated contribution of the bonded, solvated C_8 groups was demonstrated by the retention behavior of RP-8, since silica was solvated to saturation and therefore completely deactivated under the conditions used; there was subsequently no selectivity between homologues on the Spherisorb silica ($\alpha = 1.0$). The octyl groups on the RP-8 provided separation of the homologues, with an α of 1.1 and a slope of 0.07. If the C_8 groups on the modified carbon were solely responsible for retention of these probes, values for α and the slope would have been similar to those for RP-8. Instead, a methylene group selectivity of 1.2 and a slope of 0.21 were obtained. The stronger interactions on the modified carbon can be attributed to the substitution into the stationary phase of the carbon substrate, the activity of which ($\alpha = 1.6$, slope = 0.45) was moderated by the relatively weak contribution of the octyl groups per se.

The most striking evidence for the overt activity of the carbon substrate in this new bonded stationary phase was found in the selectivity for aromatic rings. Polynuclear aromatic hydrocarbons (PAH) were so strongly adsorbed on the unmodified carbon (Figure 7) that a straight toluene mobile phase was required for reasonable though long elution times.

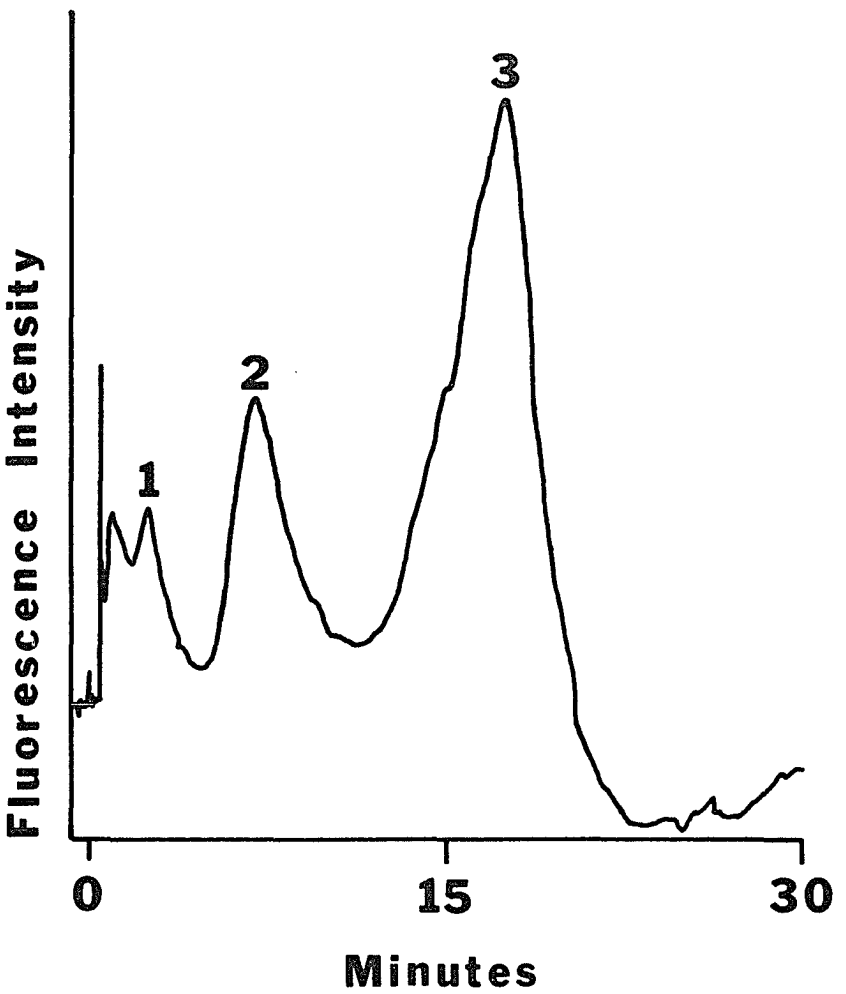


Figure 7: Separation of PAH on KFD carbon.

Column: carbon 1023; Mobile phase: toluene, 0.5 ml/min; excitation : 285 nm; emission cutoff: 389 nm filter;
1: anthracene, 2: phenanthrene
3: pyrene.

This type of interaction was still present on the modified carbon, and was investigated by comparing the selectivity factors, α, for 2-naphthol and phenol from column to column with 100% methanol as the mobile phase. Silica did not separate these probes, so the α for RP-8 of 1.1 can be attributed again to the isolated effect of the bonded, solvated octyl groups. Since the α for methylene groups was also 1.1 on RP-8, the dispersive interactions provided by the octyl groups alone could not differentiate between aromatic and aliphatic hydrocarbons. The unmodified carbon, on the other hand, irreversibly adsorbed the 2-naphthol. After the C_8 modification, the selectivity factor became measureable at 48 but was still more than 40 times the on the RP-8. The activity of the carbon substrate was therefore responsible for the ring selectivity of the modified carbon.

A measure of the extent to which the carbon substrate influenced retention more subtly, through stationary phase composition, is found in the partial molar enthalpies of interaction (ΔH) for selected probes listed in Table IV. In order to achieve ΔH values on the RP-8 that are comparable to those on the modified carbon, a partially aqueous mobile phase must be used. The stationary phase becomes relatively more non-polar than the mobile phase, and ΔH decreases with the addition of water. Since 100% methanol was the mobile

TABLE IV. Enthalpies of Interaction (kcal/mole) on C_8 Packings

a--100% methanol
b--50% methanol/water
c--25% methanol/water
d--values from ref. 30

Probe	104BC8[a]	RP-8[d]
Benzene	-3.7	-3.9[b]
Phenol	-3.9	-1.9[c]

phase with 104BC8, the additional non-polar character of the stationary phase must arise from the combination of the carbon substrate and octyl groups which was apparently more highly solvated by the methanol than the silica-octyl combination. The importance of solvation in determining the composition and thus the relative polarity of the stationary phase was further demonstrated by the use of aqueous acetonitrile mobile phases with the modified carbon and modified silica. The composition of the mobile phases were adjusted to normalize the retention of 1-phenylpentane to a k' of 1.0. The 104BC8 column required 83% (v/v) acetonitrile in water, while the RP-8 required 92% acetonitrile. Apparently the modified carbon is not solvated by the acetonitrile as

well as the RP-8, therefore the relative polarity of the mobile phase must be increased to compensate.

The modified carbon exhibited another solvation effect unlike anything heretofore observed with modified silica. At a given flow rate, the unmodified carbon required a pressure (relative to methanol) that was proportional to the viscosity of the solvent (acetonitrile, THF, or hexane). The same regular behavior was observed for the modified carbon with all solvents except THF. Neat THF or THF-water mixtures would quickly increase the pressure to the maximum limit. Slow introduction of a miscible solvent would eliminate the "plugging" with no adverse effects on column performance. This reversible plugging can be attributed to the unique combination of octyl groups and oxygenated carbon surface, each of which can be highly solvated by THF. The carbon surface is visualized as providing sites for the anchoring of the oxygen of the THF molecules, while the hydrocarbon portion of the THF solvates the octyl groups, to approach solubilization of the carbon framework of the microparticles which agglomerate to form a single carbon macroparticle. The net effect is a swelling which blocks the flow channels. Equilibration with a miscible solvent eventually removes the THF and the swelling.

Conclusions

The results of the substitution of carbon for silica as a substrate for bonded-phase formation have demonstrated the activity of the substrate in bonded-phase separations. This activity manifested itself overtly, through strong solute-substrate interactions, and covertly, through the composition of the effective stationary phase bonded upon solvation effects. The overt activity of the carbon substrate was observed because of the strong adsorptive properties of the KFD carbon under the solvent conditions used. The same mobile phases rendered silica inactive, thus its role in bonded-phase separations has been largely neglected, except for recognition of peak tailing attributed to unreacted silanols (69).

The modified carbon has been used in this study as a tool for the identification of the role of the substrate in bonded-phase separations. The results of this work also portend the benefits of chemically modified carbons as packing materials for liquid chromatography. The demonstrated moderation of the strong adsorptive properties of carbon through bonded-phase formation addresses the characteristically inefficient mass transfer from carbon. Moreover, the selectivity advantages of the carbon, while favorably attenuated, are retained. Thus carbon-based bonded-phase

packings may provide the efficient sorptive interactions for the separation of similarly structured compounds and for preparative liquid chromatography.

This work adds to the growing body of literature (37-39, 47, 51, 57, 59, 101) which supports the conclusion of Kikta and Grushka (107) that the retention mechanism on bonded-phase materials is more complex than can be described by solvophobic theory or solubility alone. The retention mechanism remains elusive, but it is now apparent that its description will have to include a variable stationary phase whose composition is a function of the bonded species, the mobile phase composition, and the substrate.

CHAPTER THREE

CARBON-13 NMR STUDIES OF SILICA-BASED BONDED PHASES

Introduction

A modified surface is neither true liquid nor true solid. The C_8-KFD carbon work suggests that the apparent physical state of the bonded moiety can be affected by the mobile phase with a concomitant change in the structure of the stationary phase as a whole. The problem of obtaining a substantial description of the interactions among stationary phase components was discussed in Chapter One. Carbon-13 NMR (CMR) was established as the technique of choice for studying the stationary phase. In this chapter, the CMR spectra from several commercial packings are discussed. Peak shapes as a function of organic modifier and its concentration, bonded phase type, surface coverage, and temperature are shown to reflect the contributions from these factors to the structure of the effective stationary phase. The independent measurements obtained through CMR are combined with complementary chromatographic data to provide an improved description of the stationary phase.

Experimental

Carbon-13 NMR Spectra

CMR spectra were obtained at 62.9 MHz and 25° on a Bruker WM-250 spectrometer. Each spectrum was the average of 40,000 transients except where noted. The radio-frequency pulse width was 20.0 microseconds with a flip angle of 45°. The pulse was followed by a 25 microsecond pre-delay and the 0.2048 sec acquisition time, with no post-delay before the next pulse. Field/frequency stabilization was performed prior to data acquisition for each sample, except for the elevated temperature spectra which had an internal deuterium lock. The absence of an internal lock with the superconducting solenoid magnet did not add any measureable broadening. All spectra were broadband decoupled. Peak widths at half height were measured manually with an estimated error of 0.3 ppm.

Silica-based Bonded Phases

The hydrocarbon-modified silicas were all commercially available 10 μm particle size packing materials. These are listed in Table V along with their pertinent characteristics. For CMR spectra the packings were first prepared as slurries in the appropriate 0.5 μm-filtered organic solvent; doubly-distilled, 0.5 μm-filtered water was added slowly, with stirring, to make the desired mixture.

TABLE V. Commercial Packing Materials Used in This Study

All are 10 μm particle size

Identity (%C)	Batch No.	Surface Area (m^2/g)	Calculated Surface Coverage	Manufacturer
Spherisorb ODS (5%)	17/154	200	5.4%	Phase-Sep
ODS-2 (15%)	100290 100355	400	23.1%	Whatman
RP-18 (20%)	VV1282	150	73.6%	E. Merck
RP-8 (12%)	VV1417	250	54.2%	E. Merck
SI-60	EH8	500	-----	E. Merck

The slurry was poured into a 15 mm O.D. tube (Wilmad Glass Co., Buena, NJ) and briefly ultrasonicated to remove trapped air. The slurry was allowed to settle overnight, then the supernatant was removed and a vortex plug inserted. Approximately 3 g of packing material were required to fill the tube to a depth of 4 cm and sufficiently overlap the receiving coils. After each use the packings were washed in five 25 ml portions of filtered methanol and collected on a Millipore filter. The washed materials were dried overnight at 40°-70°C at about 260 mm Hg pressure.

Chromatographic Measurements

Chromatographic measurements were obtained with an Altex model 332 gradient liquid chromatograph (Altex, Inc., Berkeley, CA) operating in an isocratic mode. Retention times were recorded by a Spectra-Physics (Santa Clara, CA) System I computing integrator to the nearest 0.1 sec. The columns were 4.6 x 100 mm and were packed in house using conventional slurry-packing technique. The mobile phase was 50/50 redistilled acetonitrile in doubly-distilled water; both solvents were filtered separately, then were mixed and briefly degassed under vacuum. The flow rate was 1.0 ml/min with a measured precision of 1%. Temperature control was accomplished with a water jacket for the column and a Haake (Saddle Brook, NJ) model FE circulating water bath which maintained the temperature to within 0.25°C. When required

the water was cooled by circulation through an ice bath. A one meter coil of thin-walled stainless steel tubing connected between the pump and the injection valve was immersed in the water bath for mobile phase pre-equilibration. Aniline and N-methylaniline were purified by distillation and dissolved in the mobile phase. Column dead volumes were measured as the elution times of D_2O from an injection of 50/50 acetonitrile/D_2O.

Results and Discussion

General Characteristics of the Spectra

A typical CMR spectrum of a C_{18} bonded phase is compared in Figure 8 with the solution spectrum of an equivalent amount of octadecane. Chemical shift assignments for the bonded C_{18} are made by referencing the solution spectrum. The free end methyl carbons appear in the peak at approximately 14 ppm, the β-CH_2 carbons at about 23 ppm, and the remaining detectable carbons in the intense peak at 29 ppm. The width of this bulk CH_2 peak prevents resolution of the γ-CH_2 signal which occurs at 32 ppm for octadecane in solution.

The octadecane solution spectrum was obtained from a slurry of octadecane in methanol/chloroform (1/1) with Lichrosorb SI60 silica so that the beads' contribution to peak width from magnetic field inhomogeneities could be evaluated. In Figure 8 the octadecane solution peak widths

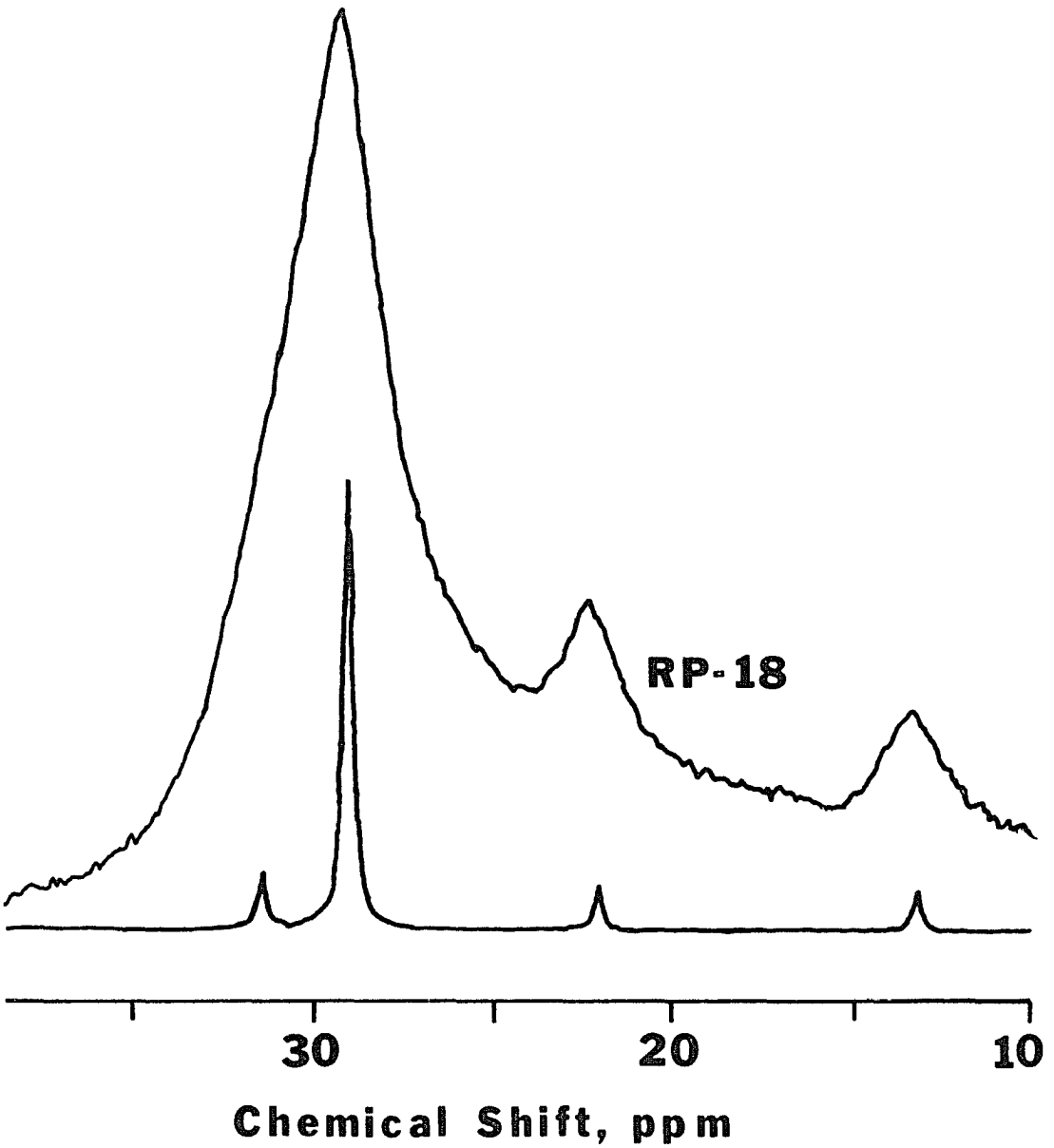


Figure 8: Comparison of bonded C_{18} CMR spectrum with solution octadecane spectrum.

are an insignificant fraction of the widths of the bonded C_{18} peaks, which are 100-400 Hz (2-5 ppm) wide at half-height. Peak widths of that magnitude are usually encountered in cases of restricted molecular motion such as occurs in polymers, but are narrower by a factor of 10^2-10^3 than the peak widths for solids (103). It is apparent that the bonded hydrocarbon can engage in restricted molecular motion but not as a true liquid. The observed peak widths contain contributions from chemical shift anisotropy due to the variety of local environments in which the slowly reorienting chains exist. The chemical shift ranges produce the broad envelope of the bulk CH_2 signals with an exponentially decaying baseline on the high field side of the peak. The peaks are broadened further by the inter- and intramolecular dipolar interactions which are not averaged on the CMR time scale. The enhanced dipolar interactions make spin-spin relaxation more effective for the bonded C_{18} than for dissolved octadecane so that the full width at half-height of a peak ($w_{\frac{1}{2}}$) will be inversely proportional to the spin-spin relaxation time, T_2 (49).

The $w_{\frac{1}{2}}$ of a peak is thus a measure of the combined effects of molecular motion, interchain interactions, and solvent-chain interactions. Relative values for $w_{\frac{1}{2}}$ can reveal the effects of a variation in solvent composition, temperature, or bonded species on these interactions. The conditions which result in narrower peaks are assumed to

render the bonded phase more liquid-like. The middle portion of the bonded moiety provided the most information about the liquid state of the bonded phase. For example, the spectra of ODS-2 in the absence of solvent at 25°C and 45°C are shown in Figure 9. The signals from the CH_3 and β-CH_2 carbons at the free end of the chains were not significantly affected by the increase in temperature. The bulk CH_2 peak visibly narrowed to reflect an increase in molecular motion induced by the increase in temperature.

In addition to being independent of the influence of temperature, the α-CH_3 and β-CH_2 peak widths for C_{18} bonded phases were not influenced by solvent, surface coverage, or the absence of lock. It is concluded that the free-end carbon atoms of the bonded chain are restricted in their motions only by virtue of being part of an anchored chain. The freedom of motion at the chain's end can be observed in unbound octadecane coated onto SI60 at the level of 13% carbon (w/w) by evaporation from chloroform solution. At 25°C in 1:1 methanol:D_2O the four peaks that are characteristic of the solution spectrum of octadecane are observed at typical solution $w_{\frac{1}{2}}$s of 8 x 10^{-3}Hz to 1.3 x 10^{-2}Hz (Figure 10). When cooled to -40°C (Figure 11) the peaks for the α, β, and γ carbon atoms are still visible and narrow (1.3 x 10^{-2}Hz), while the bulk CH_2 peak disappears completely. At -40°C the middle portion of the octadecane molecule behaves

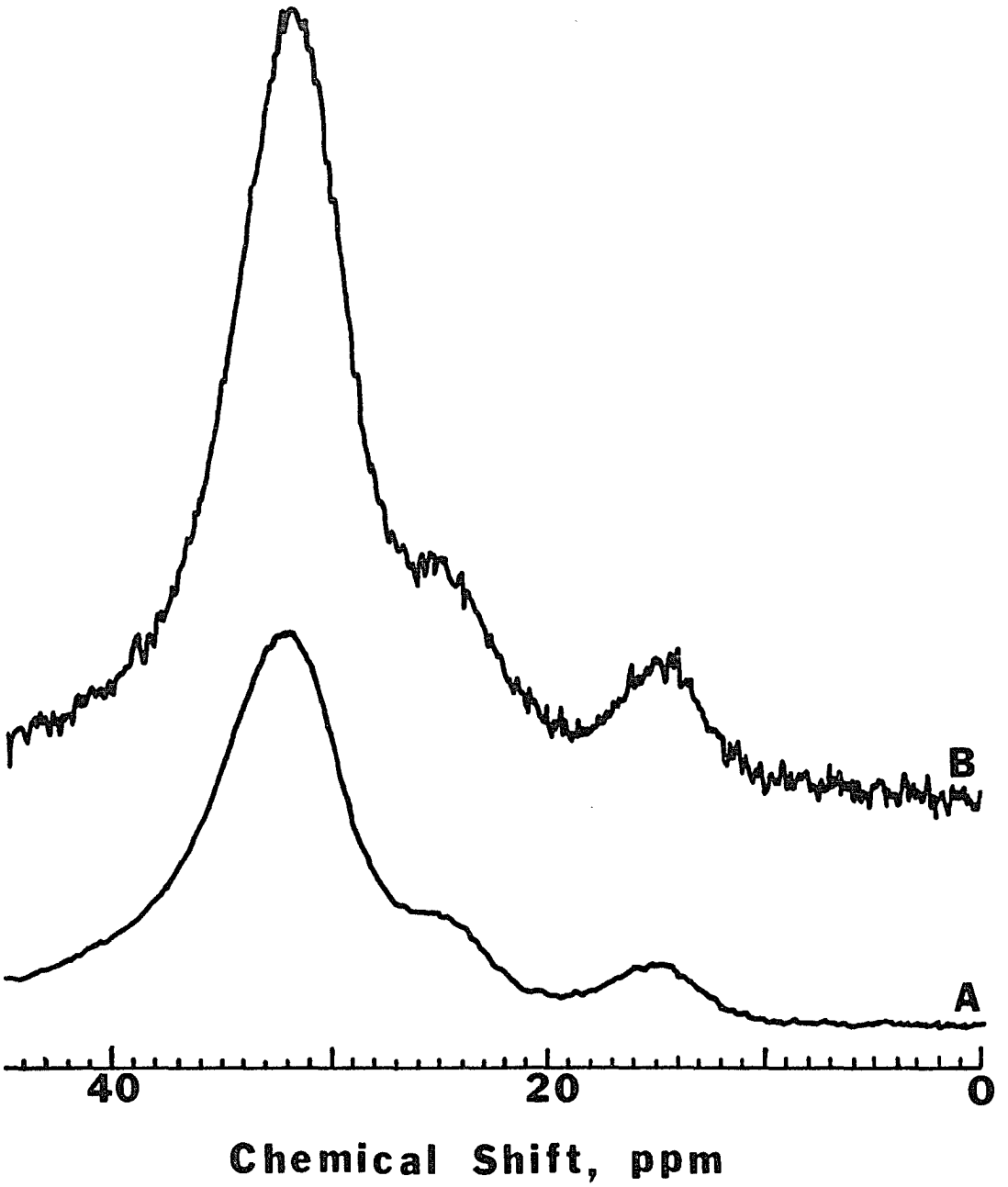


Figure 9. CMR spectra of ODS-2 in the absence of solvent.

A: 25°C; B: 45°C

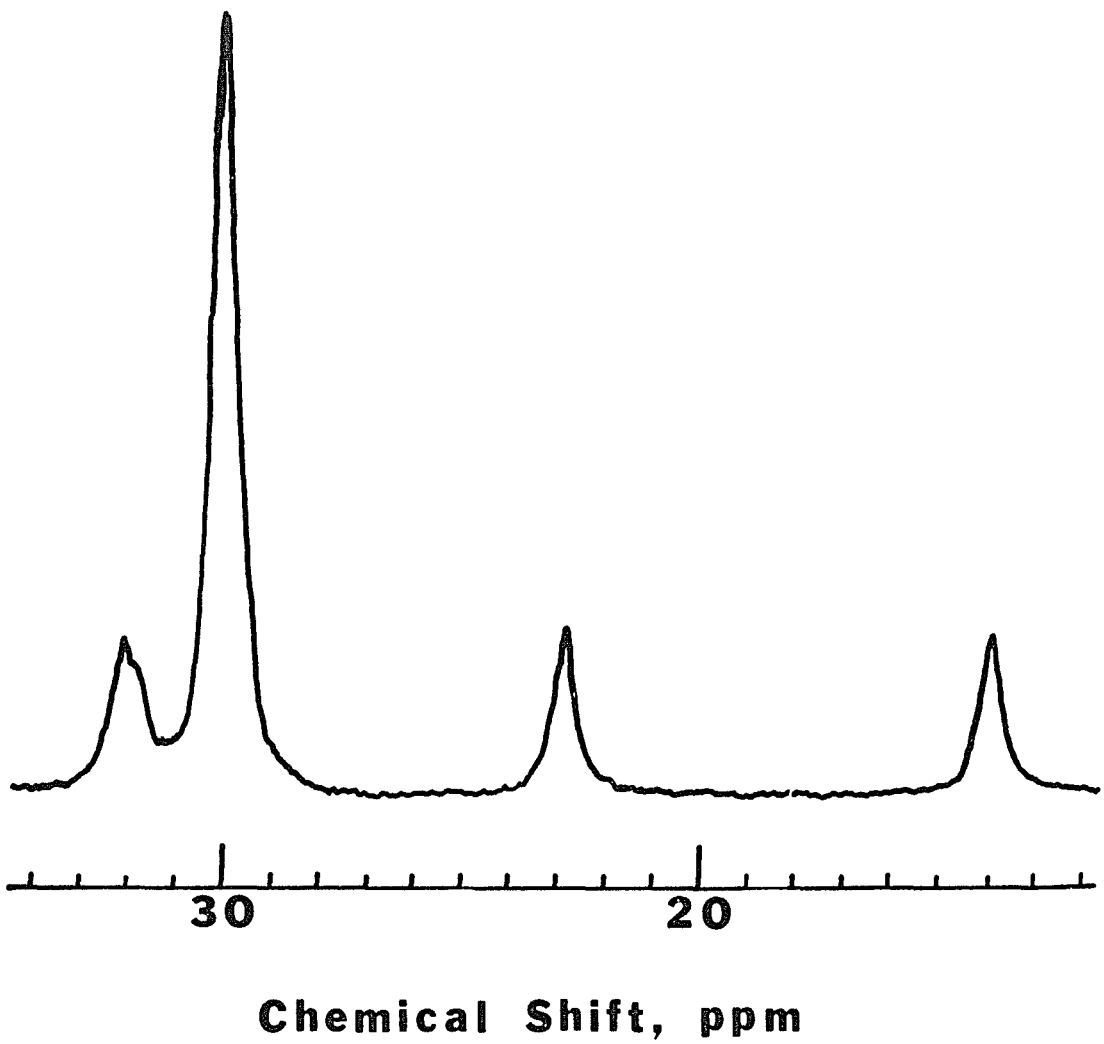


Figure 10. CMR spectrum of octadecane-coated silica in 1:1 MeOH: D_2O at 25°C.

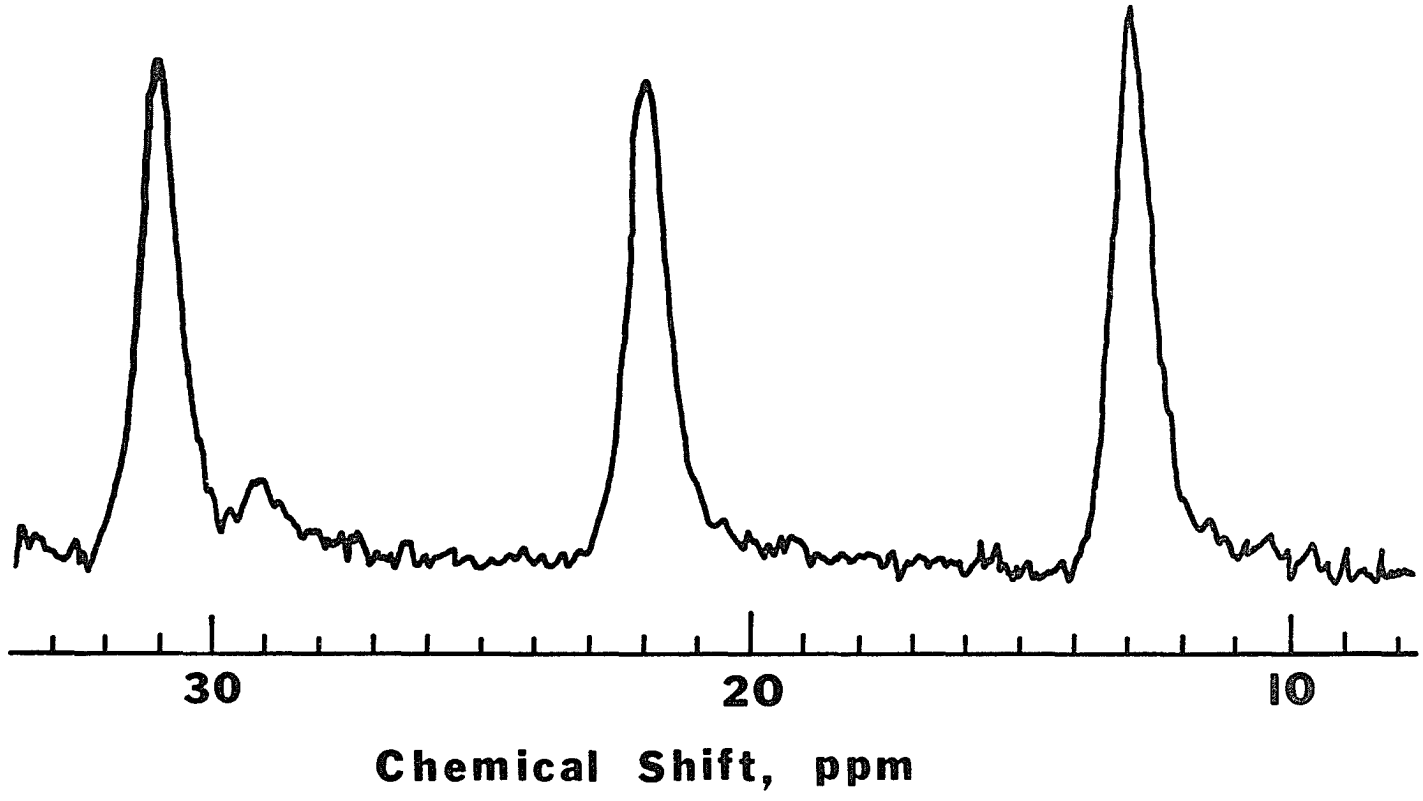


Figure 11. CMR spectrum of octadecane-coated silica in 1:1 MeOH:D_2O at -40°C.

like a solid while the ends are scarcely affected. It is important to note the role of the solvent in maintaining the molecular motion of the chain ends. The spectrum of the same octadecane coated silica taken in the absence of any solvent (Figure 12) shows broader peaks of 1.8 ppm for the end carbon atoms. These values fall within the range of 1.6 - 2.1 ppm for $w_{\frac{1}{2}}$s measured for bound C_{18} of various types under many conditions. At -40°C, however, the chains solidify completely and the spectrum is the broad, featureless hump characteristic of solids.

Response of the Bonded Hydrocarbon to Chromatographic Conditions

Neat Organic Modifiers. The widths at half-height of the bulk CH_2 peaks from commercially available bonded-hydrocarbon packings (C_8 and C_{18}) were measured under conditions of solvent and temperature commonly encountered in practice of HPLC. The observed trends in the changes in $w_{\frac{1}{2}}$s reflected the influences upon the bonded moiety as the parameters which contribute to the formation of the effective stationary phase were varied. The parameters investigated included organic modifier and its concentration in aqueous solution, surface coverage, bonded chain length, and temperature.

The organic modifier type and concentration are the variables most easily manipulated in the optimization of

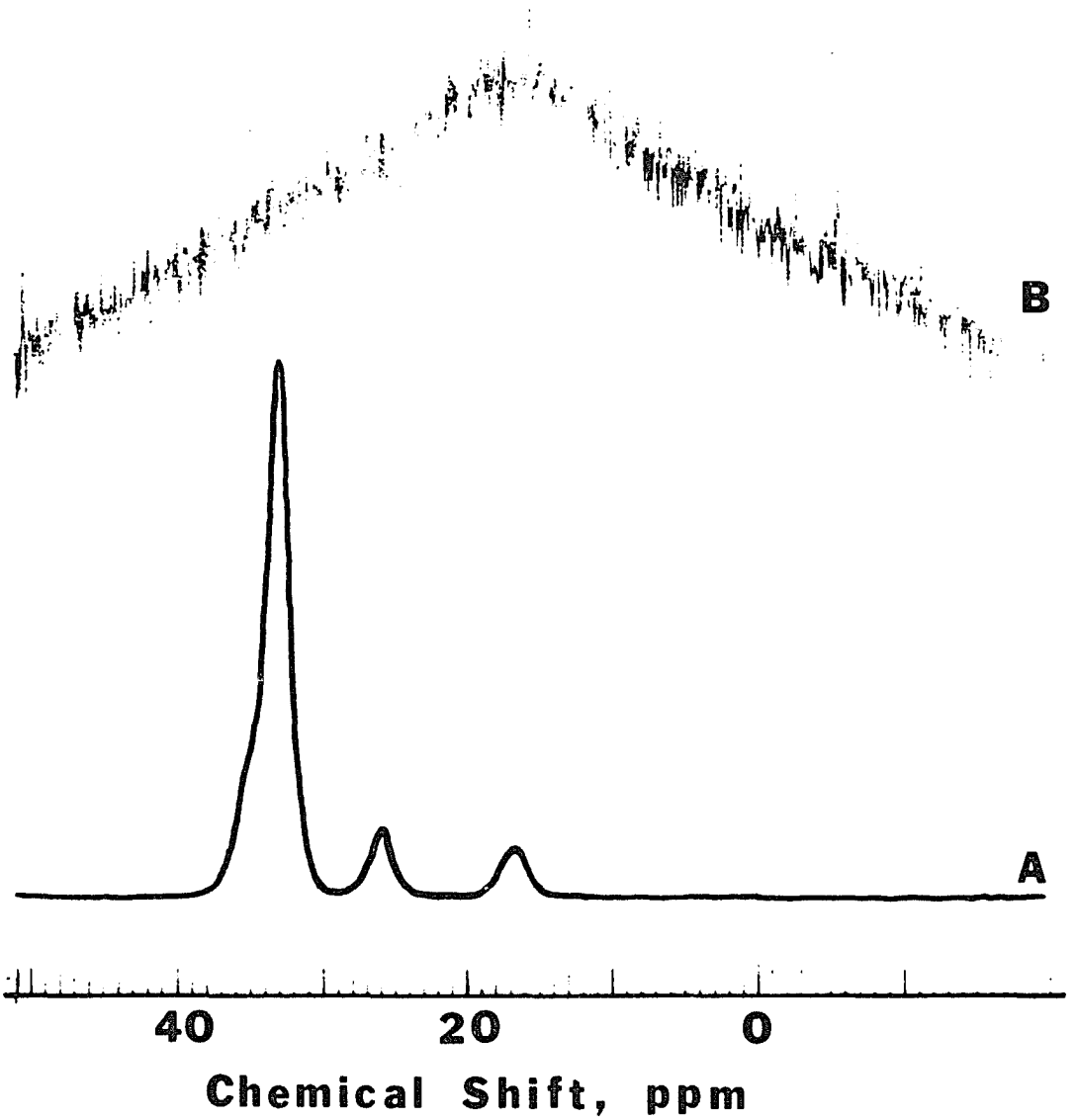


Figure 12. CMR spectra of octadecane-coated silica in the absence of solvent.

A: 25°C; B: -40°C.

a separation. They are also the most powerful in their effects on a separation. Consequently the nature of the interactions between modifier and bonded moiety is fundamental to the understanding of bonded phase separations. It is apparent from the $w_{\frac{1}{2}}$s in Table VI that significant differences in the relative liquid state of a given bonded hydrocarbon can be attributed to the organic modifier. Previous work (30) indicated that the liquid-like nature of the bonded chain increased with the eluotropic strength of the water-miscible organic modifier from methanol to acetonitrile to dioxane. The greater resolution of the spectrometer used in this study reveals anomalous behavior for acetonitrile when compared with methanol. If eluotropic strength, which follows the solubilizing ability of the modifier for the hydrocarbon, was solely responsible for the liquid state of the bonded chain, then $w_{\frac{1}{2}}$s in acetonitrile should have been measureably lower than in methanol. A slight but significant increase in $w_{\frac{1}{2}}$, signalling a decreased liquid nature, was obtained for RP-18 and Spherisorb ODS in acetonitrile compared to methanol.

TABLE VI. Bulk CH_2 Peak Widths (ppm) in Neat Solvents

	Spherisorb ODS	ODS-2	RP-18	RP-8
Methanol	3.90	4.27	4.05	9.60
Acetonitrile	4.35	4.57	4.35	9.90
Dioxane	3.37	5.85	3.90	9.60

The behavior of acetonitrile can be explained by the two-site solvation model previously proposed for hydrocarbon-modified carbon. According to this model methanol has superior solvating properties for bonded hydrocarbon compared to acetonitrile by virtue of its hydroxyl group which can interact with hydrated silanols via hydrogen bonding. This difference is reflected in the ε^{o} scale of Snyder (22) for adsorptive energy on silica. By the ε^{o} scale acetonitrile has a value of 0.50 which is about 33% below methanol's value of 0.73. The second site for solvation is provided by the hydrocarbon portion (CH_3-) of the solvent, which is identical for methanol and acetonitrile. The silica interaction can be concluded to contribute to the difference in the ability of a solvent to effectively solvate the bonded hydrocarbon, which ability is reflected in the CMR peak widths.

Dioxane has the potential for interaction with hydrated silanols that is equivalent to acetonitrile's (ε^{o} dioxane = 0.49). Due to the greater hydrocarbon-solvating contribution of the four CH_2 groups dioxane is a demonstrably better solvent for C_{18} than either acetonitrile or methanol. This characteristic should appear in narrower $w_{\frac{1}{2}}$s for the bonded phases and can be observed for Spherisorb ODS. There is no significant difference in peak widths for RP-18 between methanol and dioxane, presumably because the nearly complete surface coverage in RP-18 prevents pervasive solvation by the dioxane within the bonded phase. The increase in $w_{\frac{1}{2}}$ for ODS-2 can be attributed to the rigid, more open structure of the polymeric bonded phase (47). Dioxane molecules are capable of penetrating to the silica surface and establishing a gel-like network with the surface and the hydrocarbon as previously reported for the modified carbon.

The peak widths for RP-8 in dioxane, methanol and acetonitrile are indistinguishable from each other and are much wider than the $w_{\frac{1}{2}}$s for the C_{18} packings. A very rigid effective stationary phase in which the octyl groups resemble the bristles on a brush is envisioned for the RP-8. Initially it appears that the rigidity of the short chain stationary phase is independent of solvent. However the standard 40,000 scans for the spectra did not produce a signal for RP-8 in dioxane. The peak width reported in Table VI was

measured on a 280,000 scan spectrum (Figure 13). The number of nuclei giving rise to the signal at 40,000 scans were therefore reduced by a factor of at least 2.6, or $\sqrt{7}$, since the signal is proportional to the square root of the number of transients. Since a CMR signal disappears as a result of the broadening associated with solids, the RP-8/dioxane combination must provide a solid-like environment for the majority of the nuclei compared to the other solvents. A similar behavior for carbon-based C_8 bonded phase with both tetrahydrofuran and dioxane resulting in the plugging of the column has been observed (Chapter 2). Apparently the rigidity of a C_8 stationary phase is synergistically enhanced by the surface and hydrocarbon solvating abilities of cyclic ethers. Furthermore, access to the surface for this effect is easier for the short chain hydrocarbon as evidenced by the more polar chromatographic behavior of the short chains compared to the C_{18} analogues. Thus the RP-8 provides evidence for the effective stationary phase of substantial differences in structure, dimensions, and composition compared to C_{18}.

Aqueous Binary Mixtures. Not unexpectedly, the peak widths increased as the modifier concentration decreased in aqueous solution as given in Table VII. Studies with methanol showed differences from packing to packing. The smallest overall increase in $w_{\frac{1}{2}}$ of 1.35 ppm occurred with RP-18 as the methanol concentration was decreased from neat to 20/80. The greatest increase in peak widths was observed for

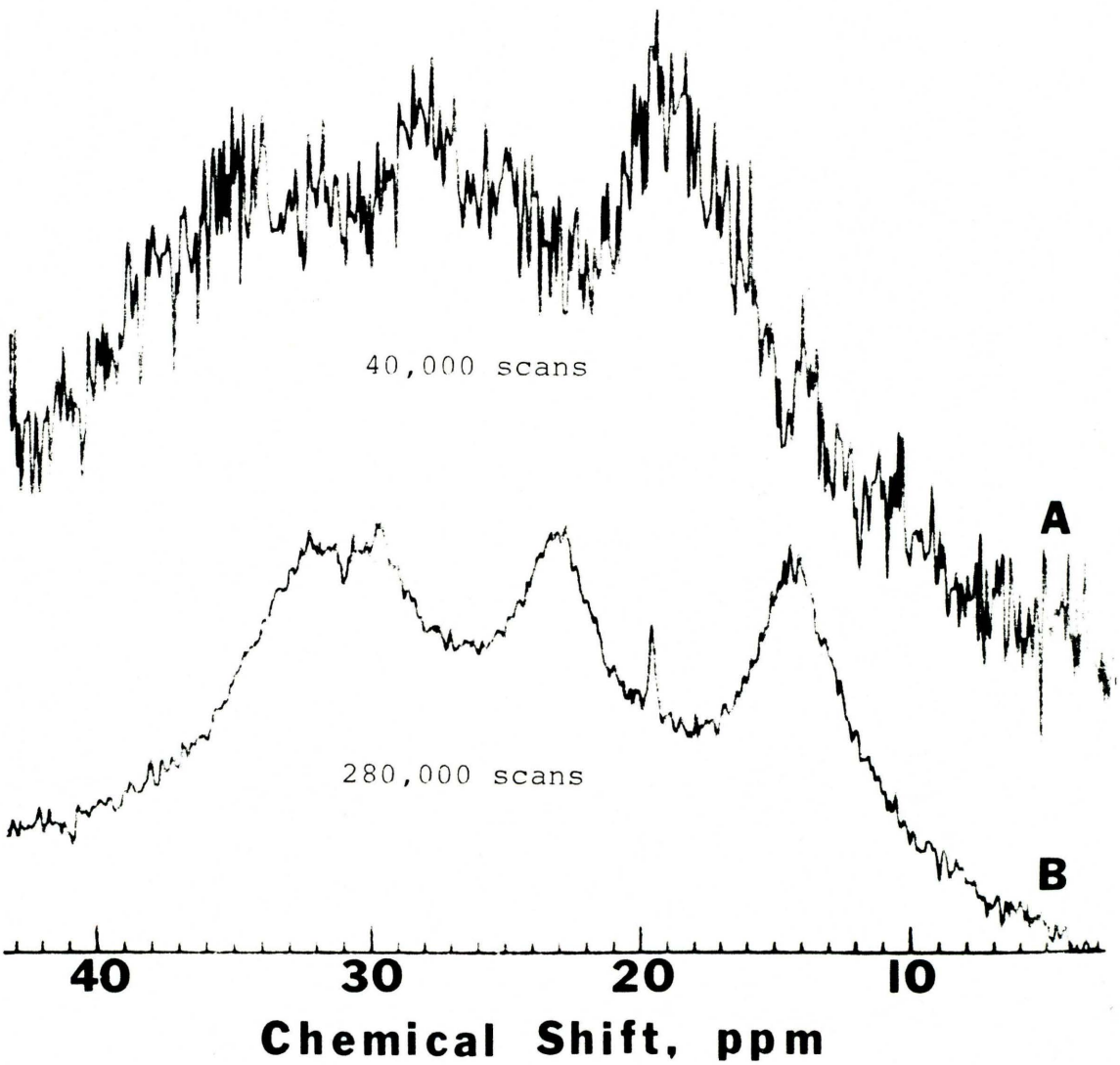


Figure 13. CMR spectra of RP-8 in tetrahydrofuran.

TABLE VII. Bulk CH_2 Peak Widths (ppm) as a Function of Methanol Concentration in Aqueous Solution

% Methanol	Spherisorb ODS	ODS-2	RP-18
100	3.90	4.27	4.05
80	4.43	4.80	4.05
60	4.88	5.55	4.58
40	5.85	6.00	4.87
20	5.77	4.95	5.40

Spherisorb ODS (1.95 ppm) and ODS-2 was intermediate (1.73 ppm). Since these trends follow the per cent carbon by weight bonded to the surfaces, they suggest that the ability to maintain a liquid-like state for the bonded hydrocarbon is a function of the positioning of the moieties on the surfaces. Assuming uniform distributions of the bonded phases, the trends in peak widths follow the resulting restrictions by the bonded species of access to the surface by water. As the water concentration increases, the hydrocarbon becomes less well solvated; the less hydrocarbon that is present improves access to the surface by the water and hastens the desolvation process.

Both ODS-2 and Spherisorb ODS reach the same maximum peak width of about 6 ppm at 40/60 methanol/water corresponding to the viscosity maximum of the mixture. This indicates a penetration by the bulk solvent into the bonded phase to an extent of about half the chain length. RP-18 does not exhibit a maximum because its structure is too closely packed to be influenced by the bulk solvent. The peak widths in 20/80 methanol/water suggest that the interchain interactions in RP-18 help maintain the liquid nature of that bonded phase vis-a-vis Spherisorb ODS. The greater affinity of the polymer ODS-2 for water ultimately results in better overall solvation of the bonded phase by the solvent.

Effect of Temperature. CMR spectra of the bonded phases were obtained in 50/50 acetonitrile/D_2O to facilitate locking as the temperature was changed. The bulk CH_2 peak widths measured separately at 25°C are listed in Table VIII with those measured sequentially by heating the sample to 55°C and cooling to 25°C. The sequential spectra were collected at 10°C intervals after at least 30 minutes equilibration time. It is apparent from the peak widths that the RP-18 is the only bonded phase that is significantly affected by temperature. Not only are the bonded chains in RP-18 rendered more liquid-like on warming to 55°C, but the chains retain the increased liquid character on cooling back to 25°C. This singular response to temperature for the RP-18 can be explained as the thermal disruption of the intermolecular interactions among the closely packed chains. Once disrupted the dispersive interactions are prevented from reforming by the increased solvation of the chains by the acetonitrile component of the solvent. The other bonded phases do not show a similar measureable response to temperature due to their less-dense arrangements of the bonded chains and concomitant decrease in interchain interactions. The RP-8, by the equivalence of its response to temperature with that to solvent, exhibits the expected behavior of rigid, bristle-like chains.

TABLE VIII. Bulk CH_2 Peak Widths (ppm) as a Function of Temperature in 50/50 Acetonitrile/D_2O.

Temperature	Spherisorb ODS	ODS-2	RP-18	RP-8
25°	4.50	4.95	5.25	9.14
55°	5.03	5.25	3.87	9.76
45°	4.80	5.25	3.87	9.60
35°	4.88	5.10	4.07	9.24
25°	4.95	5.10	4.40	9.60

The temperature-induced changes in $w_{\frac{1}{2}}$s for RP-18 are measureable by CMR but nonetheless are not large enough to be thought of as representing a gross structural change in the effective stationary phase. Indeed, Gilpin (67) has reported that temperatures above 60°C are required to significantly alter the physical state of bound (polymeric) C_{18} in pure water solvent. It is presumed that the presence of acetonitrile at the levels and temperatures reported here is sufficient to cause that same change in physical state, which amounts to replacing interchain dispersive forces with those of solvation. Under ordinary mobile phase conditions temperature must have a subtle influence on the C_{18}-water-modifier network which comprises the stationary phase since

that influence probably affects the dispersive interactions among chains. Thermally induced motion of the bonded chains can still influence the stationary phase independently of interactions among chains, since that motion can, in general, cause the bonded chains to stand away from each other. An increase in the effective "concentration" of the solvated surface interaction sites, while not observed by CMR, can be probed chromatographically.

Chromatographically Significant Temperature Effects

Recent studies have confirmed that certain classes of compounds, such as amines and crown ethers, which are hydrogen bond acceptors are particularly sensitive to the influence of the residual silanols in the stationary phase (49, 50). Under constant mobile phase composition, a change in the stationary phase will be reflected in the Van't Hoff plot of the ln k' for the probe molecule versus 1/T as either a discontinuity or as an overall change in the slope of the straight line. The slope is equal to $-\Delta H/R$, or the enthalpy of transfer for the probe between the mobile phase and the stationary phase divided by the gas constant.

The enthalpies of transfer for the probes aniline and N-methylaniline between 50/50 acetonitrile/water and the various stationary phases are listed in Table IX. The enthalpies were calculated from the Van't Hoff plots (Figures 14-17) first as the temperature was increased from

25°C to 55°C, and again as the temperature was decreased back to 25°C. The constant composition of the stationary phase for RP-8 is reflected in the identical values for the enthalpies upon heating and subsequent cooling. For aniline on RP-18 an insignificant difference is enthalpy between heating and cooling was measured. However, for N-methylaniline on RP-18 and for both probes on Spherisorb ODS and ODS-2, the enthalpies of transfer are up to 1 kcal/mole greater for the cooling sequence. The less-negative enthalpies indicate that the probes transfer into a stationary phase whose composition, on cooling from 55°C, more closely resembles the composition of the mobile phase than does the stationary phase on the initial warming sequence. Since the Van't Hoff plots are linear and without discontinuities (correlation coefficients > 0.99) the dynamics of the formation of the "new" stationary phase on warming and of its being fixed on cooling are not associated with any particular temperature, nor do they cause a discrete change in the physical state of the bonded hydrocarbon. Indeed, the interpretation of these data must be tempered with the realization that the significant differences in calculated enthalpies, taken as they are over the entire temperature cycle, undermine the assumption that the enthalpies are constant with temperature. Clearly they cannot be

TABLE IX. Enthalpies of Transfer For Aniline Probes

Mobile phase (50/50 acetonitrile/H_2O) with different temperature profiles

	ΔH(kcal/mole) 25° 50°		ΔH(kcal/mole) 50° 25°	
	aniline	CH_3aniline	aniline	CH_3aniline
Spherisorb ODS	-3.63	-3.47	-2.66	-2.72
ODS-2	-3.31	-3.16	-2.24	-2.15
RP-18	-2.80	-3.13	-2.64	-2.49
RP-8	-5.36	-4.80	-5.33	-4.77

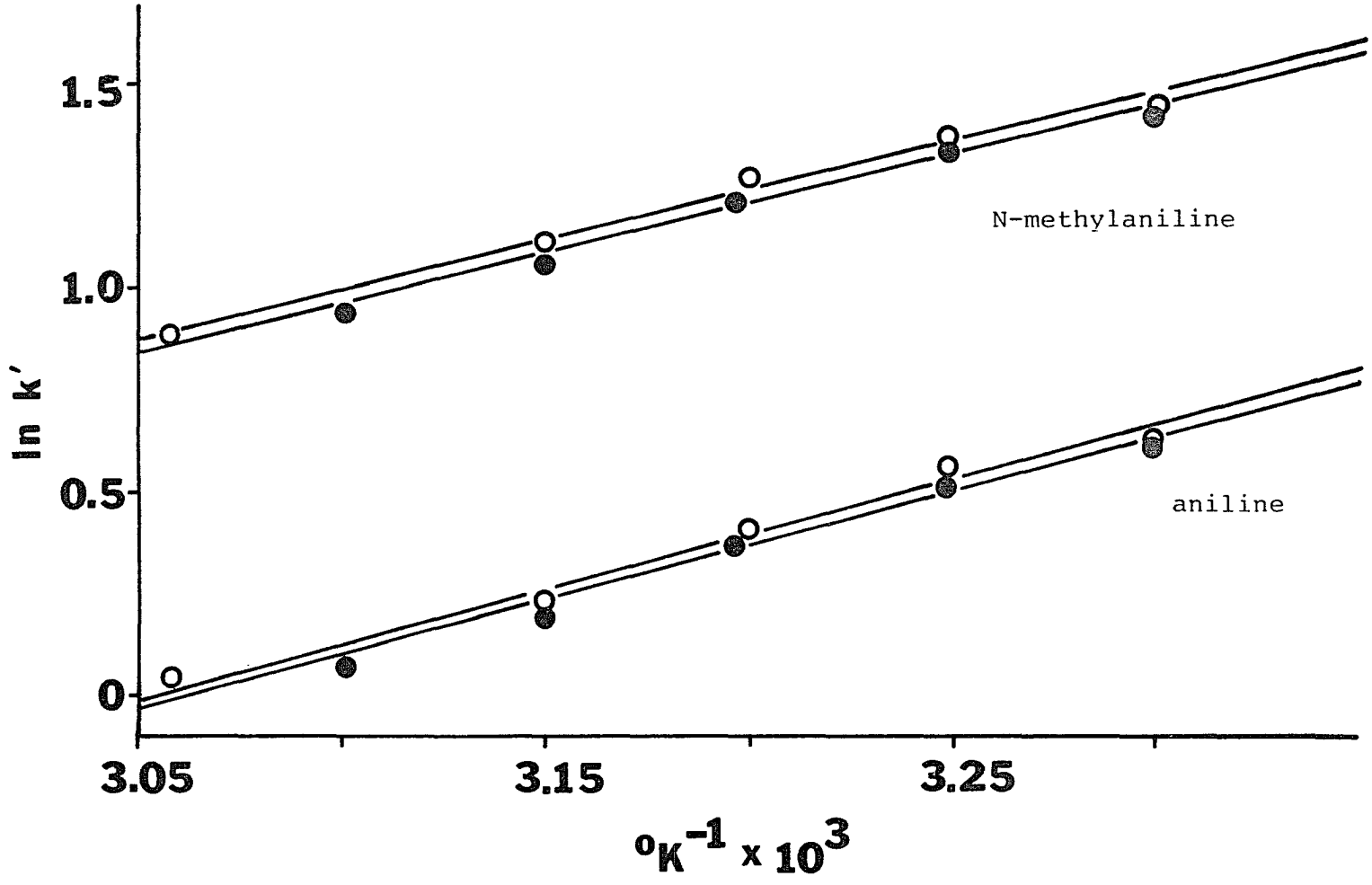


Figure 14. Van't Hoff plots for aniline probes on RP-8.

Open circles: increasing temperatures; closed circles: decreasing temperatures.

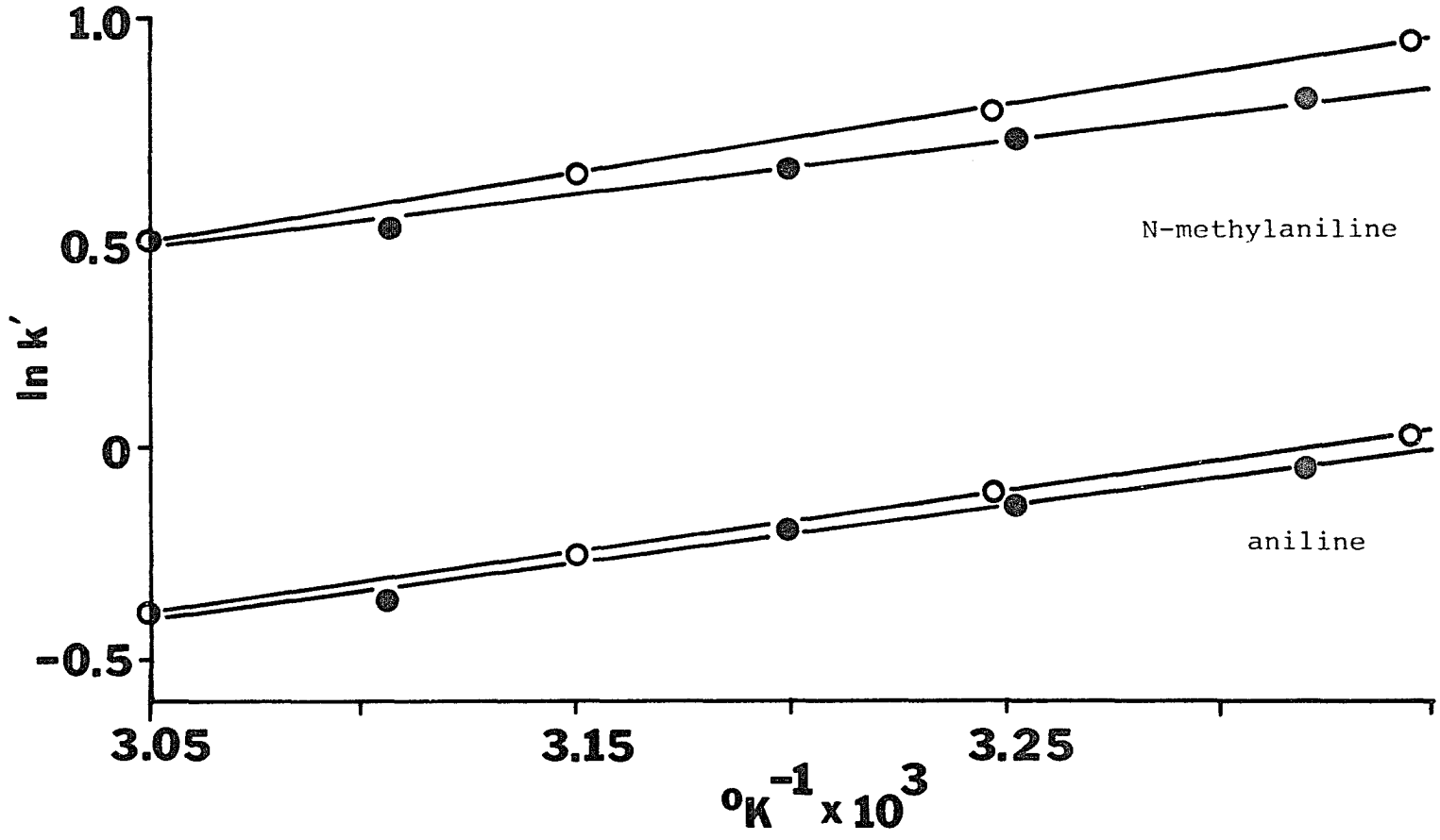


Figure 15. Van't Hoff plots for aniline probes on RP-18

Open circles: increasing temperature;
closed circles: decreasing temperatures.

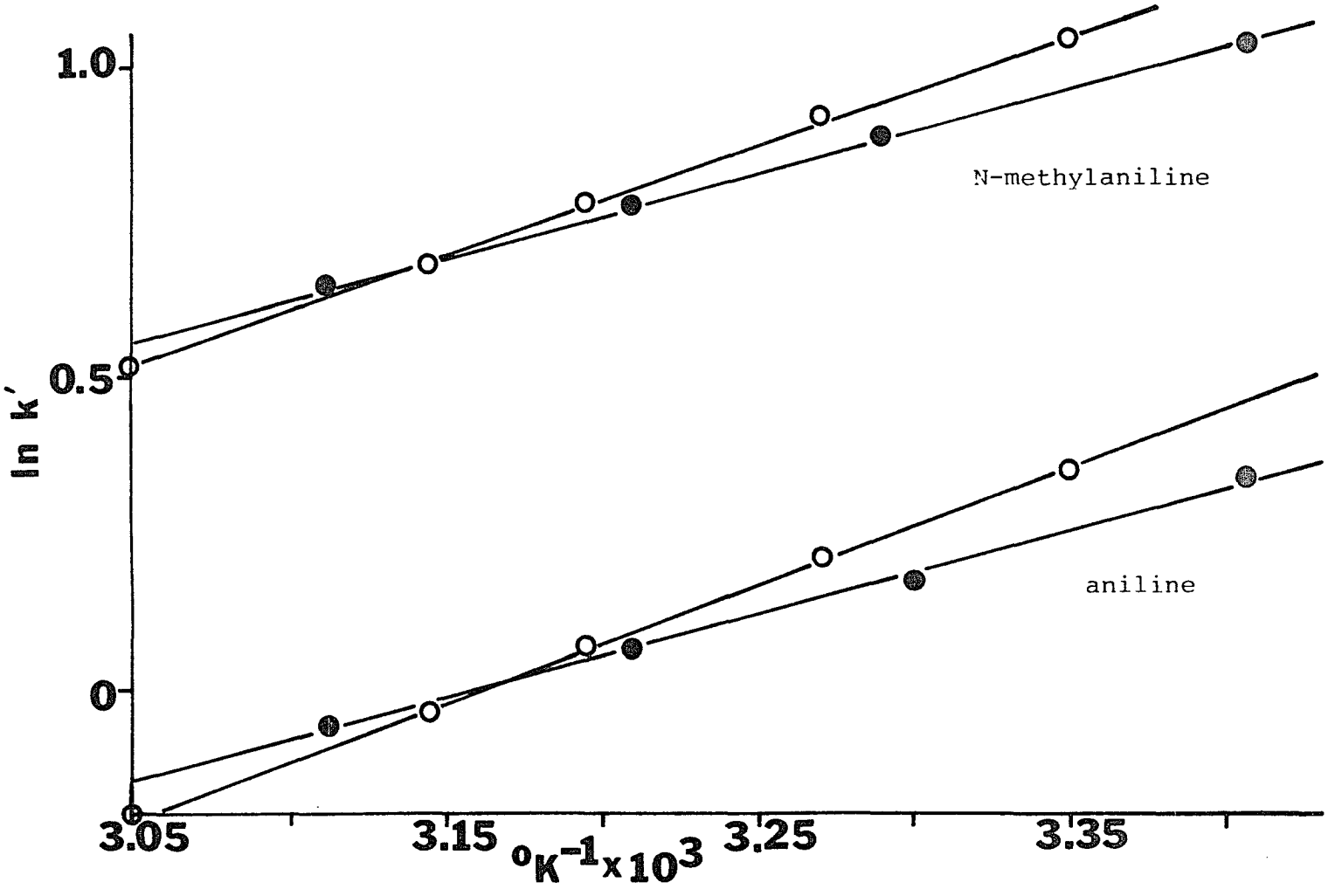


Figure 16. Van't Hoff plots for aniline probes on Spherisorb ODS

Open circles: increasing temperatures; closed circles: decreasing temperatures.

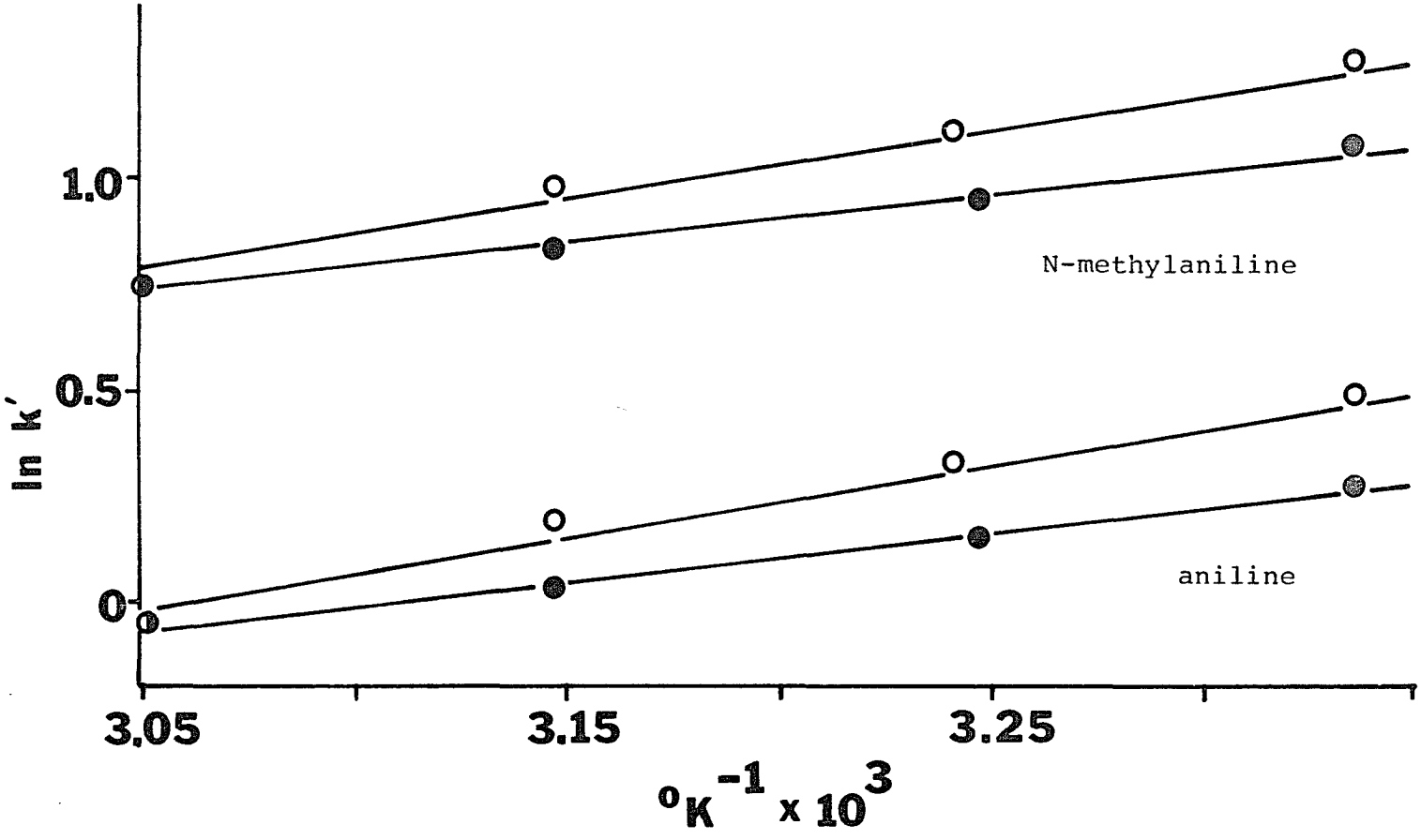


Figure 17. Van't Hoff plots for aniline probes on ODS-2

Open circles: increasing temperatures;
closed circles: decreasing temperatures.

constant, and the effect of an altered ΔH is not significant at a given temperature, but the manner in which ΔH is measured can reveal the change in enthalpy.

The calculated enthalpies suggest a gradual change in the composition of the effective stationary phase as the temperature is increased. The compositional changes were achieved slowly at temperatures below 35°C as indicated by the time necessary to obtain constant retention times for the probes following a change in temperature. These equilibration times ranged from 0.5 to about 6 hours in the order ODS-2 < Spherisorb ODS < RP-18 < RP-8. Because of the nature of the probes' interactions with the stationary phases, these observations are an approximation of the time required for equilibration of the stationary phase with water from the mobile phase. The shorter equilibration time for ODS-2 compared with RP-18 was in accord with published observations (47). The longest time observed for RP-8 is consistent with the static nature of the stationary phase through the temperature cycle and with the rigidness of the C_8 bristles demonstrated through CMR. Most significantly, the enthalpies of transfer for RP-8 indicate a stationary phase whose composition is significantly more non-polar than the mobile phase. Thus, while access to the polar sites in the stationary phase is expected to be greater for RP-8 than for the C_{18}s, exchange with the mobile phase is actually a slower

process because short, rigid hydrocarbon chains are less apt to give up dispersive interactions between chains and solvent than motile long chains. The result is effectively a frozen hydrocarbonaceous surface layer which severely restricts access to the silica surface. Among the C_{18}s, equilibration roughly follows the restriction of access to the surface by the bonding characteristics of the C_{18}. At temperatures above about 35°C equilibration times became roughly equal and less than 30 minutes. Thus access to and exchange with the surface improves with increasing temperature; for the C_{18}s the increase in temperature is most likely accompanied by a reduction in interchain association as shown in the CMR for RP-18. Upon cooling, ODS-2 and Spherisorb ODS remain sheathed in new solvation media which more closely resemble the mobile phase; RP-18 experiences partial restoration of the interchain associations, as evidenced by the smallest differences in calculated enthalpies and by the CMR peak widths.

Description of the Stationary Phase

The observed responses of the bonded hydrocarbons to changes in mobile phase and temperature can be used to construct a picture of the physical state of the bonded phase and its likely impact on the chemical composition. This picture begins with consideration of the portion of the bonded hydrocarbon which is sufficiently mobile to be

observed in the CMR experiment. Assuming that the areas of the α-CH_3 and β-CH_2 peak each represent one carbon atom, the areas of the bulk CH_2 peaks indicate that the next 7 to 10 carbons down the chain (carbons 7 to 16 away from the surface) represent the variably liquid portion of the C_{18} chain. The remaining carbons are too solid-like to be observed, except as broad baseline rises observed near $\delta = 0$ for Spherisorb ODS. The environmentally independent motion of the α-CH_3 and β-CH_2 carbons has already been discussed but should be restated: The free end of the C_{18} chain stands far enough away from the surface to remain unaffected by changes in solvent and temperature.

A similar examination of the C_8 chain shows that the α, β, and γ carbons produce the three CMR peaks for RP-8. However, these carbons are considerably more solid-like than the analogous portion of the C_{18} chain and are not measureably affected by changes in solvent and temperature.

These observations clearly show that the physical nature of the bonded hydrocarbon is stratified much as the hydrocarbon chain of n-octanol micelles are physically stratified (108). The enthalpy experiments indicate that the forces which act upon the physical state of the bonded hydrocarbon can also influence the effective composition of the stationary phase. The static nature of the RP-8 stationary phase suggests a near-surface region of the

effective stationary phase which is primarily influenced by proximity to the silica substrate. The length of the bonded chain should not affect the polar nature of the near-surface layer, only the access of a solute to the layer as demonstrated by the equilibration times and enthalpies for the C_{18} phases. The mobilities of the near-surface carbon atoms are observed not to depend on the chain length. The behavior of the C_{18} materials on temperature cycling indicate that temperature has little effect on the solvation and subsequent liquid nature of the mobile portion of the chain.

The evidence does indicate that an increase in temperature facilitates the equilibrium exchange of mutual components between mobile and stationary phases by disrupting solvent-chain and interchain interactions. Thus it is easier for water to equilibrate with a stationary phase at elevated temperature because the solvated chains produce less of a lipophilic barrier. A subsequent decrease in temperature apparently can fix the composition of the stationary phase nearer to that of the mobile phase without a great effect on the liquid character of the bonded chain.

The CMR experiments have demonstrated the quasi-liquid nature of the bonded hydrocarbon and the influences on this liquid nature. The use of high resolution techniques such as magic angle spinning and cross polarization would not provide this information, since they effectively

eliminate the hydrocarbon-solvent interactions which are observed through conventional pulsed NMR. Those interactions were responsible for the peak widths and their changes.

These experiments have demonstrated in a general sense that the liquid nature of the bonded hydrocarbon varies with distance from the substrate. A more thorough mapping of the physical state of the bonded hydrocarbon could be accomplished with more specific structural probes. For example, a double bond or a phenyl ring could be moved along the chain in specially synthesized bonded phases in order to more clearly define the regions of liquid character and the influences on those regions. Isotopic (^{13}C) labeling would be more useful since relaxation times could be measured and the movement of the bonded moiety more clearly defined.

CHAPTER FOUR

QUANTITATIVE MEASUREMENTS OF STATIONARY PHASE COMPOSITION

Introduction

A comprehensive qualitative description of the bonded stationary phase is a necessary but intermediate step toward the ultimate goal of quantitatively accounting for the factors which determine liquid chromatographic separations. The importance of the qualitative description in this historically iterative accounting process cannot be overestimated. Consider the fundamental chromatographic retention expression,

$$V_R = V_M + KV_S \qquad \text{eqn 4-1}$$

V_R is the elution volume of a chromatographically retained species; V_M is the dead volume of the column, or the elution volume of an unretained species; K is the distribution coefficient for the retained species between mobile and stationary phases; and V_S is the volume of the stationary phase. In eqn. 4-1, V_R can be reliably determined given the mobile phase flow rate and the elution time of the species. The other terms V_M, V_S, and K are ultimately dependent on an

accurate description of the stationary phase for their proper measurement. The distribution coefficient K, for example, could be measured independently in a batch extraction experiment if a proper compositional model for the stationary phase was available. The apparently complex role of the surface in stationary phase formation likely precludes any non-chromatographic measurement. K could be determined chromatographically given reliable values for V_M and V_S; however, the dynamics of stationary phase formation as previously described introduce new considerations for the measurements of V_M and V_S. The choice of a truly unretained species for the measurement of V_M precludes the general and traditional use of the mobile phase components, since they actively participate in stationary phase formation. Similarly, a value for V_S will depend on the extent for the incorporation of mobile phase components into the stationary phase.

These measurement criteria have been studied by several workers in the field but often in different contexts. In a report comparing the different approaches to measuring V_M, Berendsen, et al. (56) concluded that a determination of V_M based on the linearization of the retentions of members of a homologous series was more generally applicable than the injection of salts or mobile phase components. The latter methods, while reasonably accurate for mobile phase of about

50% by volume of organic modifier, were found to experience interferences from size exclusion and chromatographic retention, respectively, over a wide range of mobile phase compositions. The linearizations method is based on a plot of the retention times of the N + 1 homologs (N = carbon number) versus the retention times of the N homologs. From the fundamental definitions of $k' = (t_R - t_o)/t_o$ and $\alpha = (k'_2/k'_1)$ the following equation can be derived:

$$t_{R(N+1)} = \alpha t_{R(N)} - t_o(\alpha - 1) \qquad \text{eqn 4-2}$$

where t_R and t_o are retention time and column dead time, respectively, measured at constant flow rate. In this approach t_o is calculated as the retention time of a hypothetical homolog of zero carbon number. Conceptually, such a homolog should be free from retentive and steric exclusion interferences, and so the method is judged to provide the best current measurement.

Tilly-Mellin et al. (42) and McCormick and Karger (40), measured the amounts of organic modifier incorporated into the stationary phase of a C_8 bonded phases by flushing the columns with dimethylformamide or dioxane. The amounts of modifier in the flushes were measured by gas chromatography. The corrections for V_M were based upon salt or mobile phase components injections, and the amounts of water in the stationary phases were not determined, so these

measurements were not necessarily representative of V_S. Nonetheless, the approach demonstrated the feasibility of quantitative investigations into stationary phase formation.

In this chapter the amounts of organic modifier and water in the effective stationary phase are studied as a function of bonded chain length, modifier type and concentration, and temperature. The results provide a quantitative description of the stationary phase composition which confirm the proposed refined description of the effective stationary phase.

Experimental

Measurements of t_o

The measurements of t_o were made by the method of linearization of n-alkanols as described by Berendsen, et al. (56). The liquid chromatographic system was described in the previous chapter; for this work the detector was a Waters (Milford, Mass.) Model 403 refractive index detector which was connected to the column's thermostating bath for temperature control. The flow rate was measured for each mobile phase by means of a buret and timer; a precision of $\pm$ 1% and an accuracy of $\pm$ 2% was exhibited at the nominal flow rate of 1.0 ml/min.

The n-alkanols (C_1-C_{10}, C_{12}, C_{14}, C_{16}, C_{18}) were obtained from various suppliers and were used as received. The higher the organic modifier concentration in the mobile

phase, the longer the chain lengths of the n-alkanols used. Linerization plots were constructed from the retentions of at least 3 alkanols. At least 5 replicate injections of the n-alkanol (20 µl of a 2 mg/ml solution in the mobile phase) were averaged to give the retention time.

The mobile phases were prepared from 0.45 µm filtered solvents: methanol (MeOH, Fisher Scientific); acetonitrile (MeCN, Mallinkrodt, redistilled); tetrahydrofuran (THF, Fisher Scientific, redistilled from potassium hydroxide); and water (house-distilled, redistilled from alkaline permanganate). The appropriate volumes were mixed to make a total volume of about 1 liter, which was ultrasonically degassed for 15 minutes. At least 200 ml of mobile phase were passed through the column before retention times were measured.

The columns were 4.6 mm x 100 mm stainless steel packed at 6000 psi by conventional slurry-packing technique.

Gas Chromatographic Measurement of Stationary Phase Composition

After the retention times were measured and t_o calculated, the total amounts of mobile phase components were flushed from the column and connecting tubing between the injection valve and the detector. About 48.75 ml of dioxane (purified by distillation from KOH) were passed through the

column and tubing into a 50.0 ml volumetric flask containing 1.00 ml of internal standard (isopropanol for MeOH and THF mobile phases; MeOH for MeCN mobile phases).

The volumes of mobile phase components that were flushed out of the column and tubing were measured by gas chromatography. The organic components were measured on a Varian Model 1700 chromatograph with a flame ionization detector. The column was a 50/50 (wt/wt) mixture of Porapak Q and R in a 6' x ¼" copper column. Column temperature was 185°C with detector and injector temperatures of 200°C. Nitrogen was the carrier gas at 125 ml/min. The water was determined using a Gow-Mac model 550 chromatograph with a thermal conductivity detector. A 4' x ¼" copper column filled with Porapak R was used at 140°C with helium as the carrier gas at 25 ml/min. The detector bridge current was 200 mA at 200°C, and the injector temperature was 180°C.

Calibration curves were prepared from the injections of 3 µl of solutions of known volumes of mobile phase component plus 1.00 ml of internal standard in 50.0 ml of dioxane solution. Peak areas were measured with a Spectra-Physics Autolab Minigrator. At least 3 area ratios of component to internal standard were averaged to prepare a calibration curve. Component blanks were also chromatoraphed. The dioxane flush solutions were chromatographed as 3 µl injections at least three times, and the component

volumes were calculated from the average area ratios using the calibration curves.

After the component volumes were determined, corrections were applied for the volume of component due to the tubing and for the volume of component due to the LC chromatographic dead volume, V_M ($V_M = t_o$ x flow rate). The corrected volume was taken as the volume of the component in the stationary phase. Three replicate measurements including t_o determination and flushing at 80% MeOH/RP-18 varied by no more than 30 µl, which value was taken as the error in the volumes reported.

Results and Discussion

The fundamental observation ensuing from the measurements of t_o is that the stationary phase increases in volume as the concentration of organic modifier in the mobile phase increases. This observation has been reported previously (40) and has been attributed to the incorporation of mobile phase components into the bonded stationary phase. The purpose of this work is to describe quantitatively the compositional and dimensional changes in the stationary phase as functions of the mobile phase composition.

C_{18} Bonded Phase

The extent to which V_M decreases in RP-18 was found to be dependent on the solubilizing ability of the modifier for the bonded hydrocarbon (Figure 18). The largest change

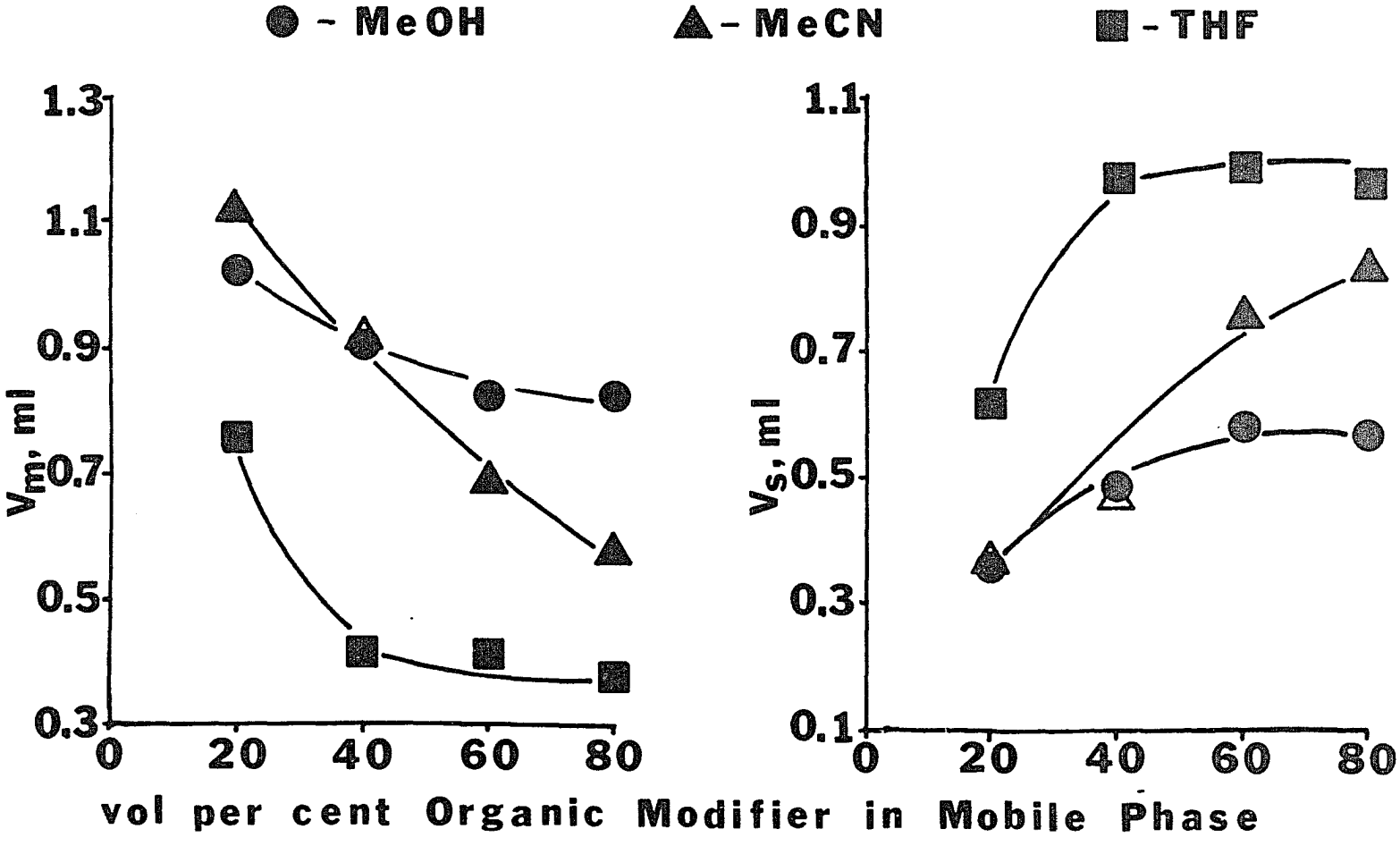


Figure 18. Intracolumn volumes for RP-18 as a function of mobile phase composition.

in V_M occurred with THF, the smallest with methanol; acetonitrile was intermediate, but this modifier was not as easily incorporated into the stationary phase at mobile phase concentrations below 50%. Figure 18 also displays minimum limiting values for V_M (maximum V_S) which are also dependent on the organic modifier. It is important to note that the composition of the stationary phase did continue to change after the minimum V_M was reached.

From Figure 18 it is readily apparent that no amount of methanol or acetonitrile can form a stationary phase with a volume equivalent to that at 20% THF (mobile phase). Since the maximum dimensions of a bonded C_{18} moiety are fixed, these data suggest the existence of an effective stationary phase which extends beyond the length of the hydrocarbon moiety. In Table X the stationary phase volumes are listed along with the calculated thicknesses of those stationary phases based on the manufacturer's surface area measurements. The thicknesses of the stationary phases range from the hydrocarbon chain lengths to 4 times the extended chain length. It is unlikely that the dispersive forces which are assumed to predominate between hydrocarbon and modifier, especially at high modifier concentrations, can structure the associated mobile phase components to such an extent above the surface. The stationary phase volumes therefore are not thought to cover effectively planar surfaces to

TABLE X. Calculated Stationary Phase Thicknesses

Fully extended C_{18} = 2.5 nm
RP-18 surface area: 150m^2/g
RP-18 in column: 0.8948g

% V/V Organic Modifier in Mobile Phase	RP-18/MeOH V_S ml	Thickness nm	RP-18/MeCN V_S ml	Thickness nm	RP-18/THF V_S ml	Thickness nm
20	0.36	2.7	0.37	2.8	0.62	4.6
40	0.50	3.7	0.48	3.6	0.98	7.3
60	0.58	4.4	0.76	5.6	1.00	7.4
80	0.57	4.3	0.84	6.2	0.97	7.2

the depths calculated. These volumes are concentrated inside the pores and the measured decreases in V_M occur as the stationary phase fills the pores. This description has been used to define a stagnant mobile phase contribution to V_M. However, such a stagnant volume would not be expected to change as a function either of modifier concentration or modifier type. Furthermore, the composition of the stagnant mobile phase would be the same as that of the flowing mobile phase. The composition of the stationary phase which occupies the pores was found to vary significantly from that of the flowing mobile phase.

The volume of the stationary phase, V_S, is the sum of the individual contributions from the bonded hydrocarbon, the associated organic modifier, and the associated water according to the ternary stationary phase model. These contributions to V_S are listed in Table XI and are plotted both as component volumes and per cent of V_S vs. the per cent modifier in the mobile phase in Figures 19-21. The volume of organic modifier in the stationary phase depends both on mobile phase concentration and on modifier type. The volume contribution of the C_{18} is a constant, of course, and was calculated for the RP-18 column to be 0.28 ml according to Berendsen, et al. (55). However, the extractive nature of chromatographic process dictates that concentrations be considered in evaluating the influences on stationary phase

TABLE XI. Measured Stationary Phase Compositions--RP-18

Organic Modifier % V/V Mobile Phase	Modifier in V_S, ml	H_2O in V_S, ml	Bonded C_{18}, ml	V_S, ml	V_M, ml	% V/V in V_S Modifier	H_2O	Bonded C_{18}
			RP-18/MeOH					
20	0.039	0.039	0.28	0.358	1.033	11	11	78
40	0.140	0.075	0.28	0.495	0.914	28	15	57
60	0.220	0.084	0.28	0.584	0.829	38	14	48
80	0.267	0.025	0.28	0.572	0.828	47	4	49
			RP-18/MeCN					
20	0.093	0	0.28	0.373	1.131	25	0	75
40	0.168	0.032	0.28	0.480	0.935	35	7	58
60	0.348	0.129	0.28	0.757	0.691	46	17	37
80	0.443	0.113	0.28	0.836	0.582	53	14	33
			RP-18/THF					
20	0.191	0.146	0.28	0.617	0.763	31	24	45
40	0.438	0.260	0.28	0.978	0.412	45	27	28
60	0.547	0.172	0.28	0.999	0.425	55	17	28
80	0.635	0.056	0.28	0.971	0.384	65	6	29

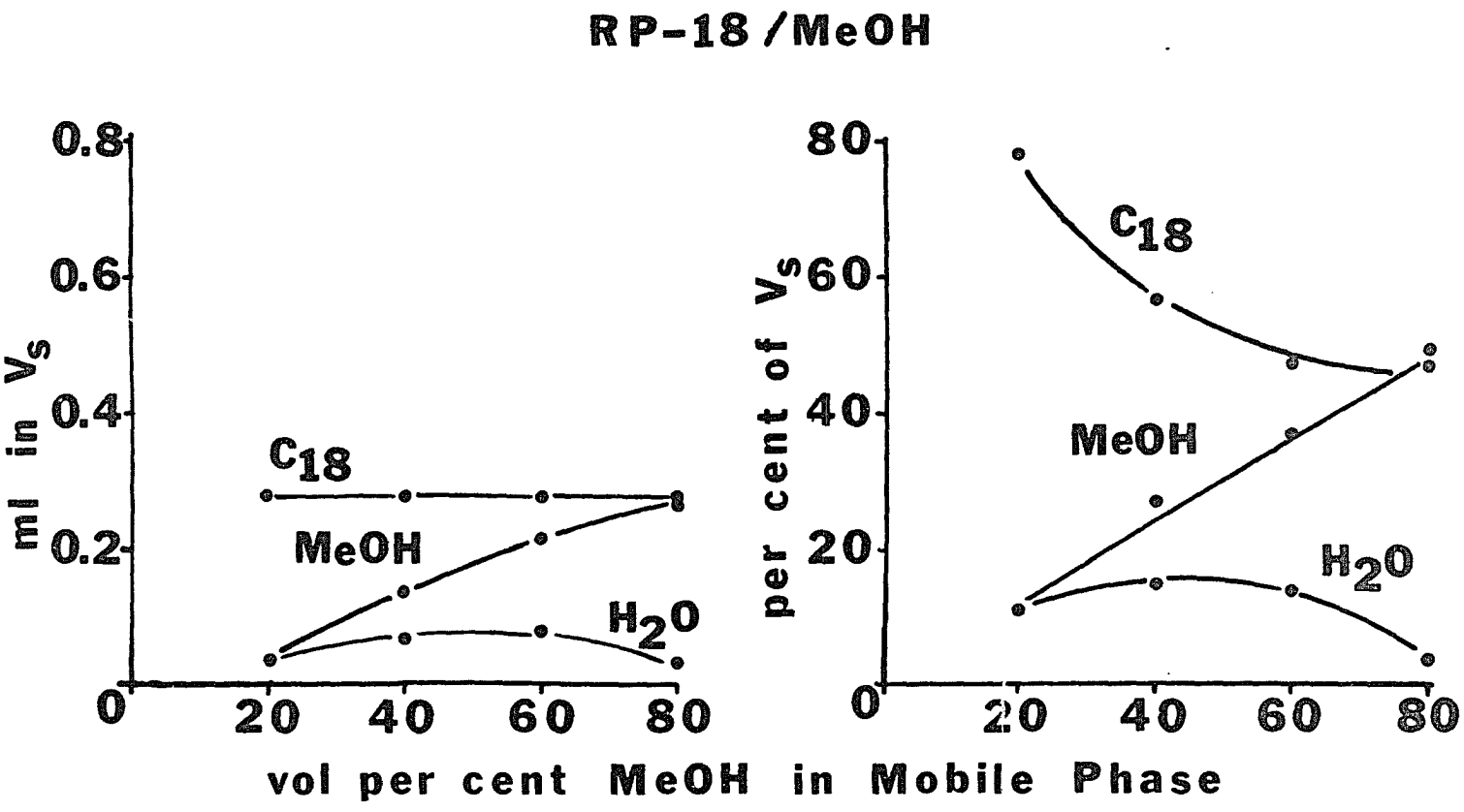


Figure 19. Stationary phase composition in methanol mobile phases.

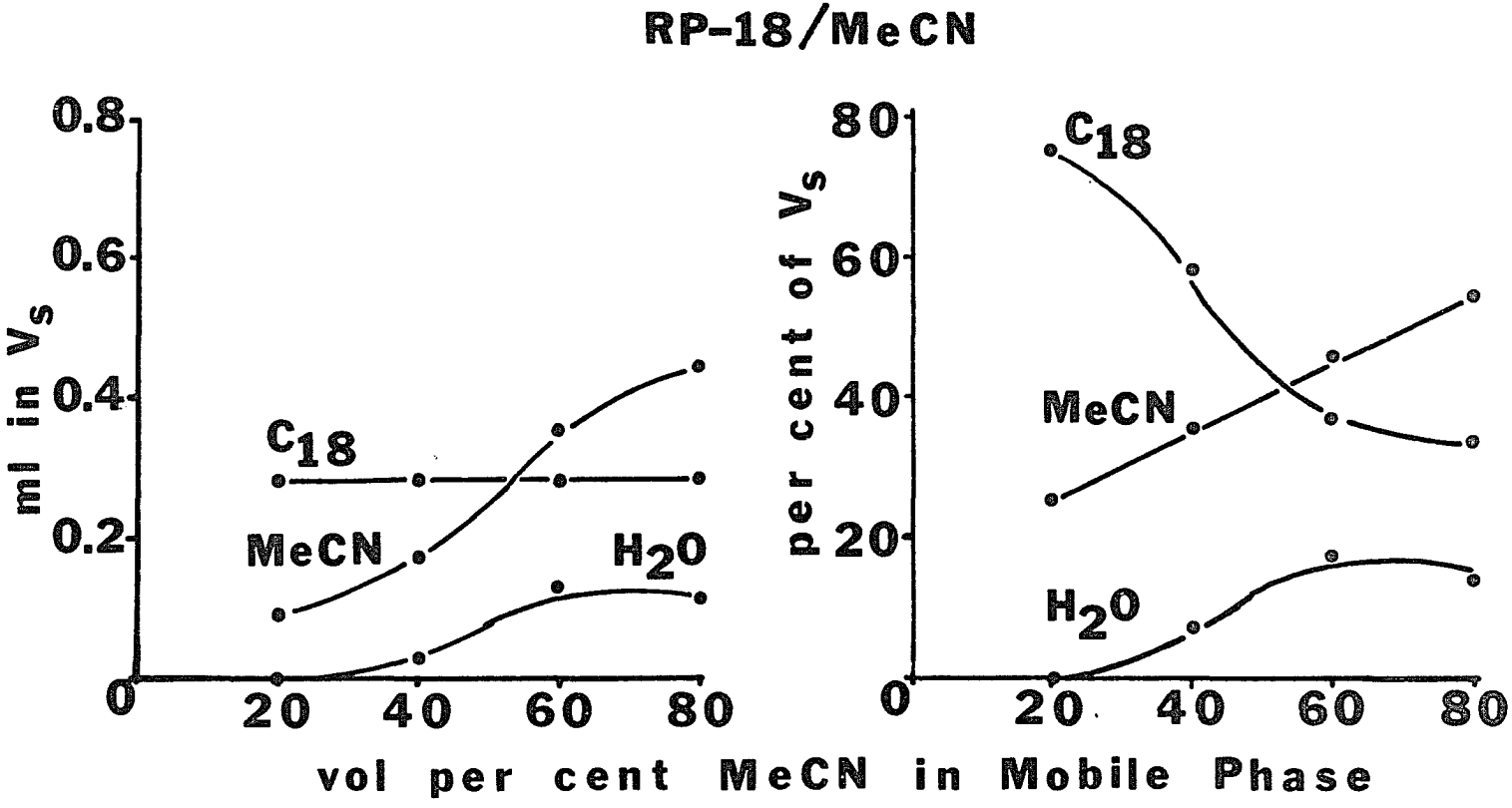


Figure 20. Stationary phase composition in acetonitrile mobile phases.

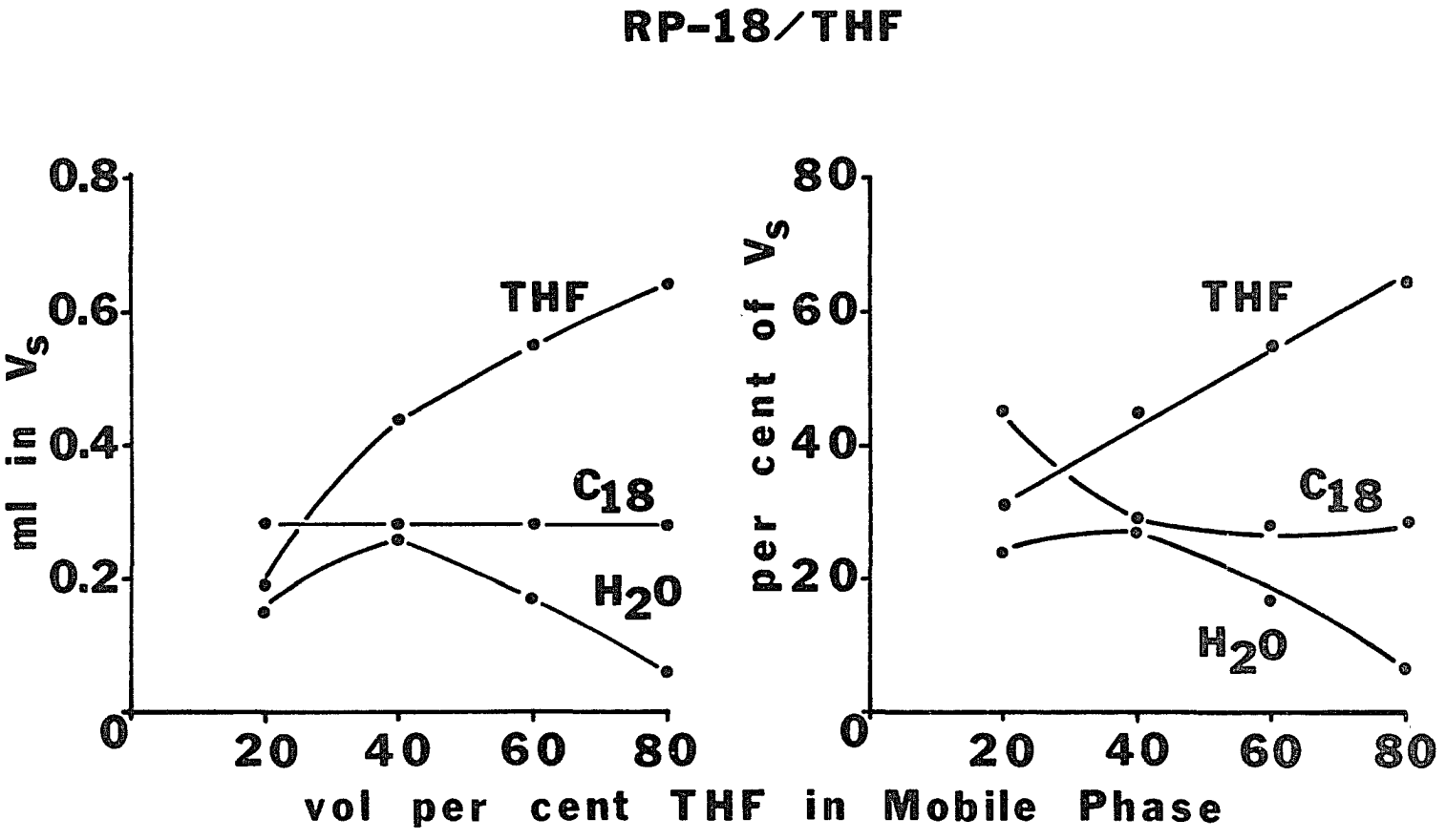


Figure 21. Stationary phase composition in tetrahydrofuran mobile phases.

formation, especially since the phase ratio V_S/V_M in general became nearly constant above 50% modifier (mobile phase) while the composition of the stationary phase continued to change.

Thus while the volume of the C_{18} is a constant, the concentration of C_{18} decreases in a non-linear manner as the modifier concentration in the stationary phase increases. The crossover point at which the modifier becomes the major component of the stationary phase decreases, with respect to mobile phase concentration, with the ability of the modifier to dissolve the C_{18}, i.e. THF > MeCN > MeOH.

The linear increase of modifier concentration in the stationary phase with respect to mobile phase concentration is important support for the ternary stationary phase model. The model assumes a pervasive association of mobile phase components with the modified surface as opposed to a one dimensional adsorption postulated by the solvophobic theory, with saturation at low mobile phase concentrations. Figures 19-21 present distribution isotherms for the modifiers. The mobile phase establishes stationary phase compositions at constant modifier concentration ratios.

The presence of the bonded hydrocarbon is expected to lead to an enrichment of the modifier in the stationary phase. The distribution isotherms in Figures 19-21 initially suggest a depletion of modifier in the stationary phase.

However, when the modifier concentration in the volume of mobile phase components associated with the stationary phase is compared with the mobile phase concentration (Table XII) the expected enrichment is observed.

TABLE XII. Enrichment of Modifier in the Mobile Phase Components Associated with the Stationary Phase for RP-18

% V/V Organic Modifier in Mobile Phase	% V/V Organic Modifier in Associated Mobile Phase Components		
	RP-18/MeOH	RP-18/MeCN	RP-18/THF
20	50	100	57
40	65	84	63
60	72	73	76
80	91	80	92

The concentration profile of water in the RP-18 stationary phases varies with organic modifier, but some general points can be ascertained. Since the water concentration does vary with mobile phase concentration, the water in the stationary phase must be associated with the organic modifier to a great extent. If the water were exclusively associated with surface silanols, a constant concentration of water in the stationary phase would have been observed.

Compositional Effects on Selectivity

Selectivity, α , is a measure of the discriminatory capability of the chromatographic system. The relationship between selectivity (or its components, k') and any of the chromatographic variables is not completely unambiguous; however selectivity is most often correlated with some measureable parameter of the mobile phase. Thus empirical relationships have been obtained such as

$$\alpha = A(\%\text{Organic}) + B \qquad \text{eqn 4-3}$$

or

$$\alpha = C(\delta\ ) + D \qquad \text{eqn 4-4}$$

where A, B, C, and D are constants and δ is the solubility parameter of the mobile phase ($\delta = \delta_{ORG} \times \%\ \text{organic} + \delta_{H_2O} \times \%\ H_2O$).

These equations can be applied to describe the selectivity between adjacent n-alkanols in this study, (Table XIII). The correlation coefficients for equations of form 4-3 are -0.997, -0.955, and -0.919 for MeOH, MeCN, and THF respectively. An equation of form 4-4, whose constants should not depend on modifier type, has a correlation coefficient of 0.916 (Figure 22). However, both forms ignore the possibility of a contribution from the stationary phase. An equation of the form

$$\alpha = E(\%\ \text{organic stationary phase}) + F \qquad \text{eqn 4-5}$$

was found to apply in this study with a correlation coefficient of -0.959 (Figure 23) and was independent of

TABLE XIII. Selectivities and Mobile Phase Parameters

% V/V Organic Modifier in Mobile Phase	RP-18/MeOH		RP-18/MeCN		RP-18/THF	
	α	δ	α	δ	α	δ
20	3.48	19.4	2.99	19.2	2.80	18.8
40	2.59	17.8	1.97	17.3	1.49	16.6
60	1.90	16.1	1.55	15.5	1.14	14.3
80	1.54	14.5	1.43	13.6	1.09	12.2

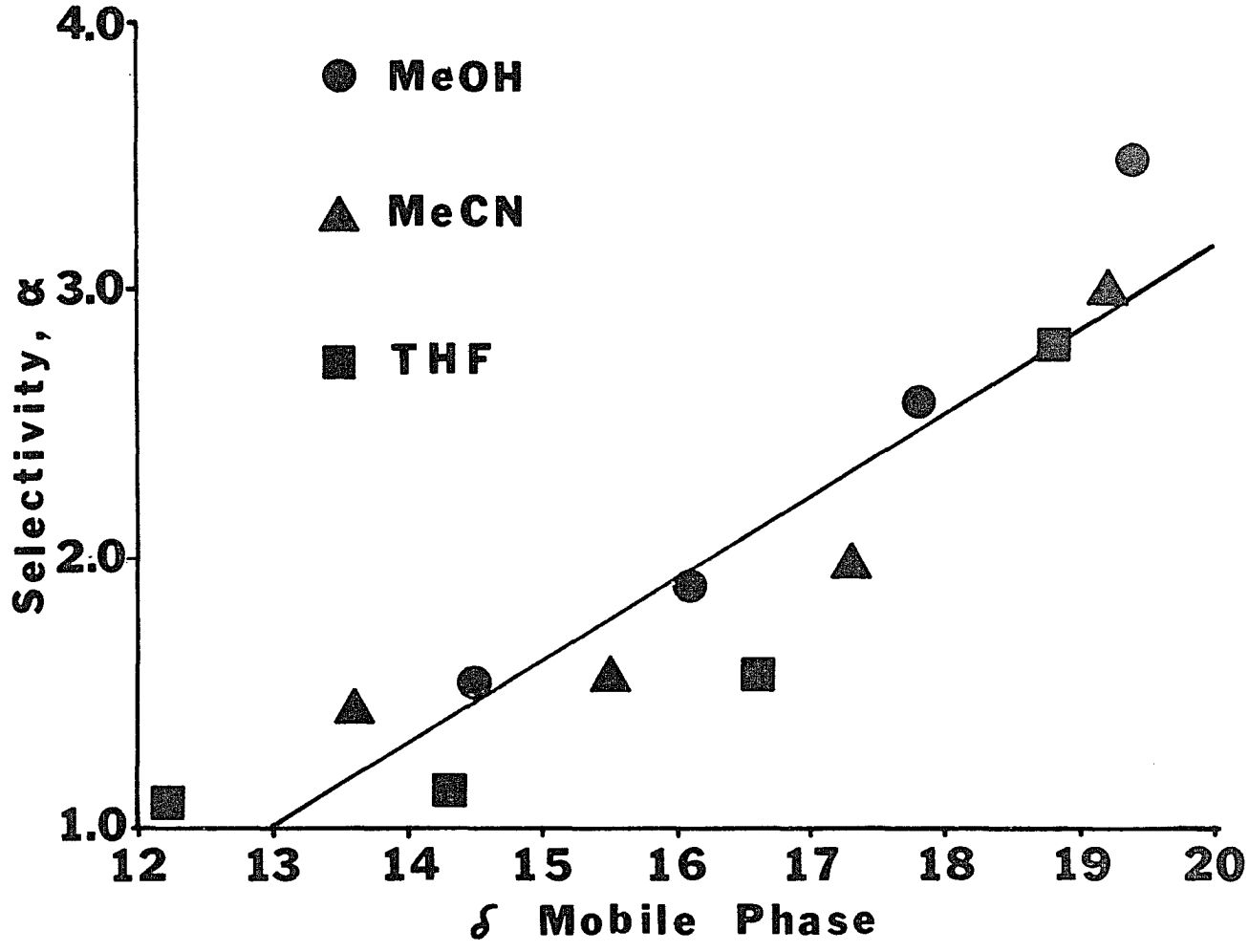


Figure 22. Selectivity of n-alkanols as a function of mobile phase solubility parameter.

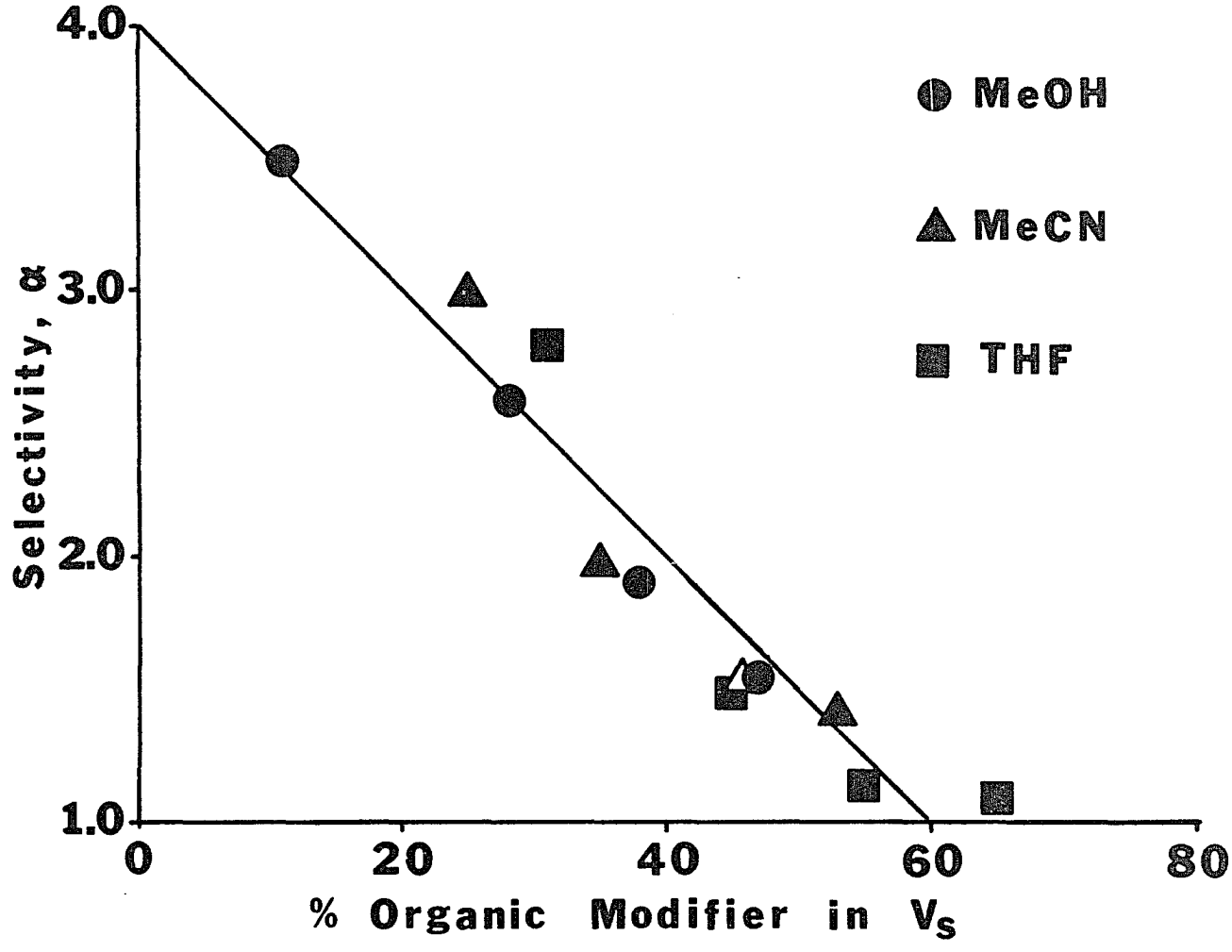


Figure 23. Selectivity for n-alkanols as a function of organic component in the stationary phase.

modifier type. Thus the influence of the stationary phase composition can be experimentally demonstrated with a degree of association between experimental parameters similar to other proposed relationships.

Effect of Chain Length of Composition

RP - 8 is the octyl analog of RP-18 in that a monomeric bonded phase of high surface coverage is formed. The shorter chain length contributes to a stationary phase which is well-documented as more polar than RP-18 by chromatographic measurements. The polarity is attributed to the surface silanols; the composition of the RP-8 stationary phase reflects this influence. The MeOH concentration increases beyond that of the hycrocarbon (Figure 24, Table XIV) and is significantly higher compared with RP-18/MeOH. The water concentration is not significantly higher than in RP-18, but it is invariant with mobile phase concentration. The water in the RP-8 stationary phase must be closely associated with the surface silanols to behave in this manner.

Effect of Temperature on Composition

Stationary phase concentrations were measured for RP-18 and RP-8 at 26°C and 45°C with a mobile phase of 50% MeCN/H_2O. Since the measured V_Ms did not change significantly with the increase in temperature (Table XV), no dimensional changes in the stationary phases were effected.

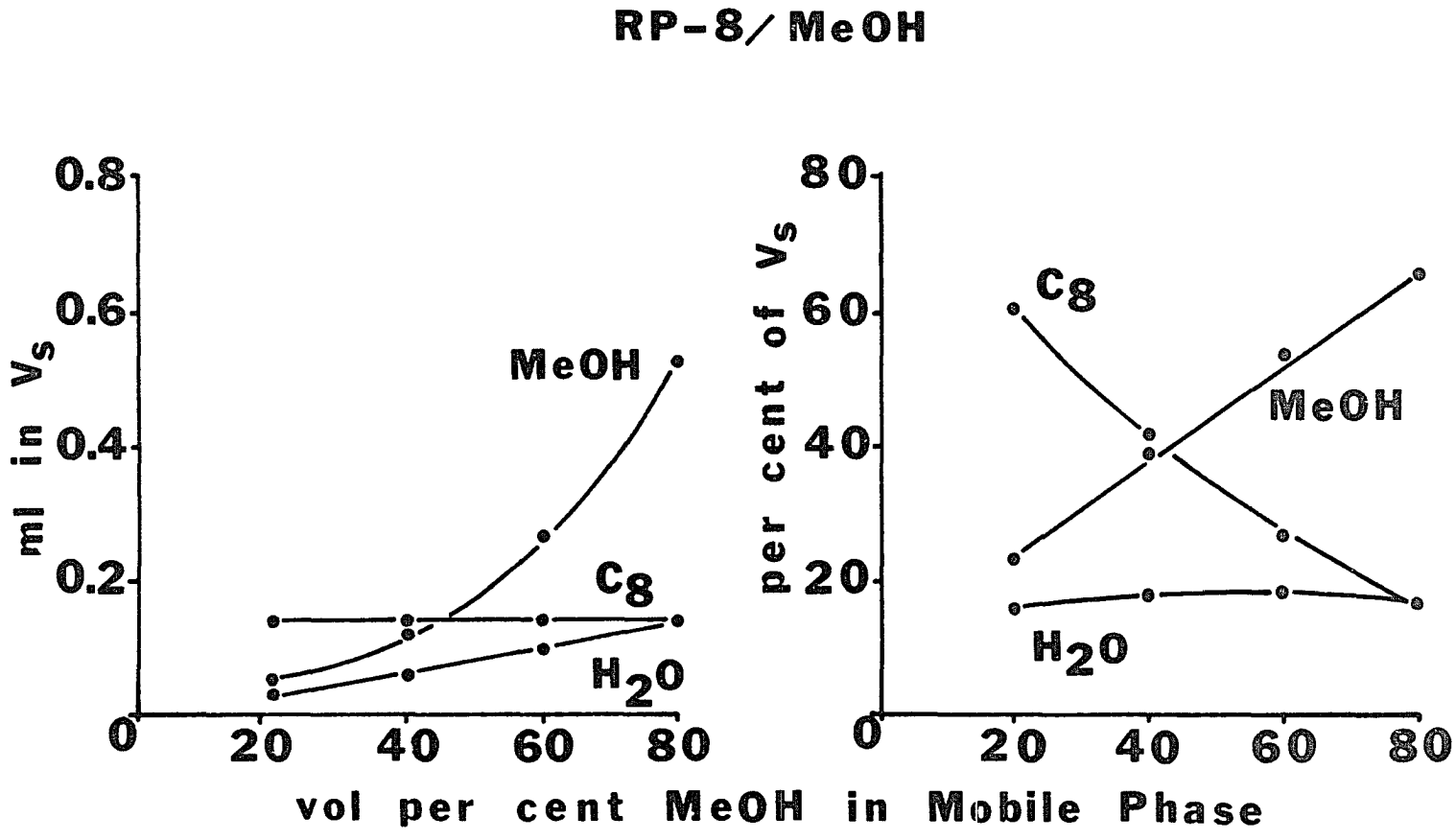


Figure 24. Stationary phase composition in methanol mobile phases for RP-8.

TABLE XIV. Measured Stationary Phase Compositions--RP-8

MeOH in Mobile Phase, % V/V	MeOH in V_S, ml	H_2O in V_S, ml	Bonded C_8, ml	V_S, ml	V_M, ml	% V/V in V_S Bonded MeOH	H_2O	C_8
			RP-8/MeOH					
20	0.053	0.036	0.14	0.229	1.132	23	16	61
40	0.132	0.060	0.14	0.332	1.044	40	18	42
60	0.273	0.097	0.14	0.510	0.955	54	19	27
80	0.530	0.139	0.14	0.809	0.755	66	17	17

The bonded hydrocarbon is therefore fully extended through solvation effects. The measured volumes of MeCN and H_2O did not change significantly with temperature in RP-8; in RP-18 small decreases in the volumes were measured, but these do not amount to a definitive compositional change. It can be concluded that an increase in temperature does not exert a significant influence on stationary phase composition.

TABLE XV. Stationary Phase Composition as a Function of Temperature

Mobile Phase 50% MeCN/H_2O

	V_M		MeCN in V_S, ml		H_2O in V_S, ml	
	26°	45°	26°	45°	26°	45°
RP-8	0.945	0.914	0.314	0.281	0.126	0.153
RP-18	0.835	0.804	0.356	0.304	0.106	0.060

Conclusions

These measurements confirm the association of mobile phase components with the stationary phase in accordance with the ternary phase model. This association is based on the affinity of the modifier for the hydrocarbon primarily, although a shorter bonded chain length permits a measureable contribution from the surface. The organic modifier concentration in the stationary phase depends on the concentration in the mobile phase according to a linear distribution isotherm. The stationary phase volume expands within the pores to maintain the distribution. A concentration zone is thus established rather than a physically distinct phase. The stationary phase composition has been shown to influence selectivity to a degree similar to other relationships between chromatographic variables and selectivity. Since the mobile phase determines the stationary phase characteristics, the versatility of the mobile phase in determining chromatographic separation is shown to extend beyond solvent strength.

CHAPTER FIVE

CONCLUSIONS AND SUGGESTIONS FOR FURTHER WORK

Each of the three major areas of experimentation in this work have contributed both to the confirmation of the ternary stationary phase model for LC and to a greater understanding of the chemically modified surface in general. The model describes the bonded phase under liquid chromatographic conditions as an operationally effective mixture of bonded species, active surface, and associated mobile phase (solvent) components. The bonded species and the surface are viewed as providing a framework around which the stationary phase is formed with mobile phase components. This concept was confirmed in several experiments.

The apparent swelling of the carbon-based stationary phase with THF, while no similar effect was observed for the unmodified surface, demonstrated that each of the three components can play an active role in stationary phase formation. Such dramatic synergism is not the rule, of course, and the chromatographic characterization of the octyl-modified carbon followed the general case in which one of the

stationary phase components predominates. The adsorptive strength of the carbon surface was found to be the major component in retention compared to the octyl-modified silica whose deactivated surface relinquished that role to the bound hydrocarbon. The experiments with carbon thus delineated the generality of the ternary phase model and the validity of the role of each component.

The ^{13}C NMR measurements on hydrocarbon-modified silicas revealed aspects of the structure of the stationary phase consistent with the model. The bonded hydrocarbon chain was found to be physically stratified with the more liquid-like character further away from the surface. The liquid-like nature of the chain was affected by type and concentration of organic modifier in the solvent according to trends expected of entities participating in stationary phase formation. Thus the solvation of the bonded hydrocarbon was enhanced by the ability of the modifier to interact both with the hydrocarbon and the surface. In the face of a desolvating force (increasing water concentration) the liquid-like nature of the hydrocarbon was enhanced by a higher surface coverage which maintained the solvating interactions. Changes in temperature had little effect on the CMR measurements. However the retentive behavior of chromatographic probes and the effect of temperature on the RP-18 CMR peak widths indicated that a higher temperature

can facilitate the association of mobile phase components with the surface by causing the stationary phase network to open up and improve access to its depths.

The ternary stationary phase model postulates an equilibrium distribution of organic modifier into a hydrocarbon-based stationary phase. The quantitative measurements of stationary phase composition clearly showed that such a distribution exists in the systems investigated. Furthermore, the consequences of that distribution - relative enrichment of modifier in the stationary phase, and enrichment according to lipophilic character of the modifier- were found to hold true.

In addition to confirming the ternary stationary phase model, the experiments described herein also provide an improved description of the stationary phase structure. The common assumption is maintained that the chemical modification takes place uniformly and almost entirely within the pores of a silica support. Even a small concentration of modifier in the mobile phase will cause the full extension of the bonded hydrocarbon as the compositional and CMR/temperature measurements indicate. Continued increases in modifier concentration will thus contribute to an extension of the stationary phase network which amounts to a filling of the pores by a modifier-enriched concentration zone. The distinction between phases is an energetic one, wherein the bulk mobile phase begins where the dispersion

and hydrogen-bonding forces ordered by the ternary phase can no longer maintain the modifier enrichment. Such a "phase boundary" must be gradual and constantly shifting, like the diffusion layer near a growing mercury-drop electrode.

Greater insight into the factors responsible for stationary phase formation can be had by continuing many of the experiments described in this work. The effect of chain length on the THF-hydrocarbon-carbon surface synergism could be investigated by preparing longer chain analogs. The structuring is expected to diminish beyond a ten or twelve carbon chain. The modified carbons thus synthesized could also be studied by CMR to aid in the definition of the chain length required to form the rigid near-surface layer. The CMR experiments could be used with specially modified silicas to probe the physical stratification described in this work. Structural markers, such as phenyl or olefinic groups, could be inserted at varying intervals along a chain to aid in defining the portions of the molecule most strongly affected by mobile phase composition. The results would provide a better definition of the compositional stratification of the stationary phase suggested by this work. It would be most useful for generalizing the ternary stationary phase model to determine the compositions of other bonded phases such as phenyl, cyanopropyl, and aminopropyl. Compositional differences directly related to structural ones, such as

more hydrogen-bonding components in the aminopropyl phase relative to cyanopropyl, would add more evidence to support the ternary stationary phase model. Such information about the dynamics of stationary phase formation would greatly improve the practicing chromatographer's ability to select a mobile phase appropriate for a given column, and the column appropriate for a given separation.

APPENDIX

CHROMATOGRAPHIC THEORY

Chromatographic separations are achieved by the differential migration of the components of a mixture (also referred to as solutes or samples in this discussion) which distribute themselves between a mobile phase and a stationary phase. The column is the heart of the chromatographic system; the column is a reservoir for the stationary phase, which is coated or bonded onto, or consists of the surface of, a suitable support material. Upstream from the column a pump is used to force the mobile phase through a sample introduction device and then through the column; the effluent from the column passes through a detector which is sensitive to some property of the solutes, such as refractive index or molar absorptivity, not shared with the mobile phase. The output of the detector as a function of time or eluent volume is a series of peaks, one for each separated solute, called a chromatogram.

The analytically useful information in a chromatogram arises from the position of the peaks and their shape, particularly their breadth. The position of a peak on a

chromatogram is determined primarily by thermodynamic parameters, such as the distribution coefficient of the solute between the mobile and stationary phases (and thus by the composition of those phases) and by the temperature of the system. The peak shape is primarily due to the kinetic aspects of the chromatographic process, such as the rates of mass transfer between the mobile and stationary phases, and diffusive and convective mass transport within the phases. The mathematical descriptions for generalized peak position and peak broadening are well known, but they will be briefly recounted here since they are central to this study and will be referred to frequently.

The fundamental retention equation (eqn A-1) describes the position of a peak in terms of volumes:

$$V_R = V_M + K\ V_S \qquad \text{eqn A-1}$$

V_R is the elution volume of the solute

V_M is the elution volume of an unretained species the void volume or dead volume

K is the thermodynamic distribution coefficient for the solute; K = (concentration in stationary phase)/(concentration in mobile phase)

V_S is the volume of the stationary phase

For a given column V_M and V_S are constant, and the chromatographically significant volume, the net elution volume V_E ($= V_R - V_M$), is proportional to K. Slight column-to-

column and instrument-to-instrument variations in V_M are accounted for by the capacity factor, k', for comparative purposes:

$$k' = \frac{V_E}{V_M} \qquad \text{eqn A-2}$$

From eqn A-1:

$$k' = K \frac{V_S}{V_M} \qquad \text{eqn A-3}$$

Thus for a given column under given conditions, $k' \propto K$, and the constant of proportionality is the phase ratio, sometimes designated $\emptyset$. The capacity factor is usually calculated in units of time since they are proportional to volumes at a constant flowrate, i.e. $k' = (t_R - t_M)/t_M$.

In LC, V_S is not well-defined, therefore K cannot be determined chromatographically. Furthermore, K cannot be applied from independent measurements to predict k', and thus the conditions for the separation. It can be concluded that knowledge of the stationary phase in LC limits the usefulness of thermodynamic measurements taken from or applied to chromatography.

The uncertainty in the magnitude of V_S can be eliminated through the use of the ratio of k' values for two solutes under a given set of conditions. This ratio, called the separation factor and designated α, is usually calculated to be greater than or equal to 1.0, i.e. $\alpha = k'_2/k'_1$. A comparison of values obtained on one column under different

mobile phase conditions reveals the combined effects on the separation of the changes in mobile phase interactions and stationary phase interactions. However, when identical mobile phases are used with different stationary phases, the separation factor can be used to compare the interactions between the solute and the stationary phases. In this study

is used as a sensitive indicator of changes in the stationary phase.

The well-known expression for the free energy change accompanying a process is:

$$\Delta G = -RT \ln K \qquad \text{eqn A-4}$$

From eqn. A-3

$$\Delta G = -RT \ln\left(k' \frac{V_M}{V_S}\right) = -RT \ln k' + RT \ln \emptyset \qquad \text{eqn A-5}$$

Thus from k' one can obtain a value that is proportional to the free energy change accompanying the chromatographic process, but the constant of proportionality contains the phase ratio. The term containing the phase ratio can be eliminated by calculating the difference between two free energy changes, or $\Delta\Delta G$. Of course, it must be assumed that the phase ratio remains constant for each ΔG determination.

One thermodynamic parameter that can be measured without correction for the phase ratio is ΔH, which in LC represents the enthalpy of transfer of solute from the mobile phase to the stationary phase. From the Gibbs-Helmholtz equation (eqn. A-6)

$$\frac{d\ (\ln K)}{d\ (1/T)} = \frac{-\Delta H}{R} \qquad \text{eqn A-6}$$

a relationship between K, and thus k', and ΔH is obtained. A plot of ln k' versus the reciprocal of absolute temperature (called a Van't Hoff plot) yields a straight line with a slope of $-\Delta H/R$. The phase ratio is relegated to the intercept value.

Finally, at a given temperature T, the change in entropy ΔS for the transfer from the mobile phase to the stationary phase can be calculated from eqn. A-7, but since the value

$$\Delta G = \Delta H - T\Delta S \qquad \text{eqn A-7}$$

used for the free energy change contains a term for the phase ratio, the value for ΔS will also. The usefulness of ΔS and $\Delta\Delta S$ so determined will be subject to the same limitations as ΔG and $\Delta\Delta G$. Subsequently, calculated entropy changes for conditions of highest interest in the study of the stationary phase, namely varied stationary phases or varied mobile phases, will also reflect the altered phase ratio arising from those variations.

In summary, the best pieces of information available from the peak position for the study of bonded stationary phases are the separation factor α and the enthalpy of transfer ΔH. The remaining thermodynamic parameters, ΔG, $\Delta\Delta G$, ΔS, and $\Delta\Delta S$ can be the sources of ambiguous information.

The shape of the chromatographic peak is generally (but rarely perfectly) gaussian or bell-shaped. Consequently, the variance of the peak, σ^2, can be used as a measure of the peak width. Many factors contribute to the variance and thus the width, and if these factors can also be expressed as variances, then their contributions to the total peak variance are additive. For example, the major sources of band broadening in the chromatograph are: the chromatographic process within the column; and laminar mixing within the injector, the detector, and the connecting tubing and fittings. The total peak variance can therefore be accounted for by equation A-8:

$$\sigma^2_{total} = \sigma^2_{column} + \sigma^2_{injector} + \sigma^2_{detector} + \sigma^2_{tubing} \qquad \text{eqn A-8}$$

Band broadening from the injector, detector, and tubing can be minimized through precise technique, zero dead volume fittings, and small lengths of narrow bore tubing; for a given instrument their contributions are constant. In studies of the chromatographic process and particularly the stationary phase, σ^2_{column} can be highly varied and is thus a source of information.

The variance due to the column has been dissected into its components by many chromatographic theoreticians, most notably Martin and Synge (109), van Deemter, Zuiderweg, and Klinkenberg (110), and Giddings (111). The contributions to the column variance are primarily the flow path, the

transfer of mass in the mobile phase, and the transfer of mass in the stationary phase. Knox (112) has assembled these factors for LC into an expression similar to and based upon the familiar van Deemter equation for gas chromatography (equation A-9). In this expression Knox has introduced

$$h = \frac{B}{v} + Av^{0.33} + Cv \qquad \text{eqn A-9}$$

reduced parameters which account for differences in particle size and diffusivity. In equation A-9, h is the reduced plate height, a dimensionless parameter which gives the number of particle diameters over which an equilibration stage, or plate, takes place (eqn. A-10). The value for h is calculated from the plate height, H, according to equation A-11; H represents the segment of column length over which an equilibration stage takes place and is ultimately related to the peak shape through N, the number of plates for a given peak measured directly from the chromatogram according to equation A-12.

$$h = \frac{H}{d_p} \qquad \text{eqn A-10}$$

where d_p is the average particle diameter of the packing

$$H = \frac{L}{N} \qquad \text{eqn A-11}$$

where L is the column length

$$N = 5.545\ (t_R/W_{\frac{1}{2}})^2 \qquad \text{eqn A-12}$$

where $W_{\frac{1}{2}}$ is the full width of the peak at one-half the peak height, and is measured in time units.

The other reduced parameter in equation A-9 is the reduced velocity, v, which is calculated according to equation A-13:

$$v = (u\ d_p/D_M) \qquad \text{eqn A-13}$$

In equation A-13, u is the linear velocity of the mobile phase through the column and is obtained by dividing the volume flowrate by the cross-sectional area of the column; D_M is the diffusion coefficient of the solute in the mobile phase, typically $10^{-9}m^2sec^{-1}$.

The constants A, B, and C in equation A-9 give measures of different aspects of column performance. The dispersion of the band due to axial diffusion within the column is given by the constant B. Scott (113) has recently shown that B is dependent on the k' of the test solute due to extra-column band broadening at low values of v. The constant A accounts for flow stream effects within the packed bed; a poorly packed, non-uniform bed will result in a large value for A. Most important to this study is the value of the constant C. This term represents the contribution to peak width provided by the rates of mass transfer in the mobile and stationary phases. The relative magnitudes of each component can be determined by holding one phase constant and varying the other.

One final measurement on the peak shape that can provide useful information regarding the stationary phase is the asymmetry factor, A_s. The asymmetry factor is

measured as depicted in Figure 25, and is used to determine the influence of secondary retention mechanisms, such as hydrogen bonding to surface polar groups in the bonded phase, on overall peak shape. These secondary retention mechanisms generally modify the ideally gaussian peak shape in an exponential manner, thus forming a tailing edge on the peak.

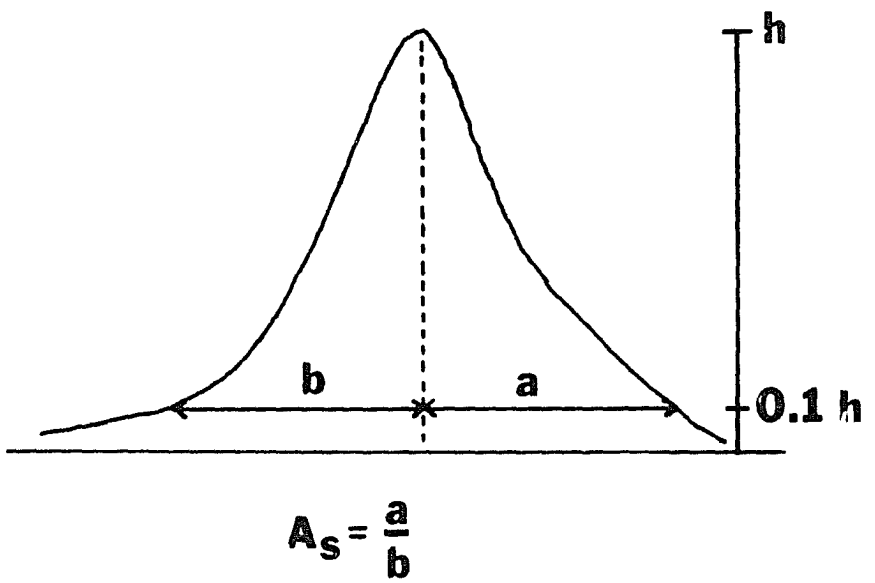


Figure 25. Measurement of the asymmetry factor.

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