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**A study of the retention mechanism and selectivity of cyano
and amino normal bonded-phase high performance liquid
chromatography**

Tang, Yonghua, Ph.D.

City University of New York, 1989

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**A STUDY OF THE RETENTION MECHANISM AND SELECTIVITY
OF CYANO AND AMINO NORMAL BONDED-PHASE
HIGH PERFORMANCE LIQUID CHROMATOGRAPHY**

BY

YONGHUA TANG

A Dissertation submitted to the Graduate Faculty in
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1989

This manuscript has been read and accepted for the Graduate Faculty in Chemistry in satisfaction of the dissertation requirement of Doctor of Philosophy.

14 March 1989

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Abstract

STUDY OF THE RETENTION MECHANISM AND SELECTIVITY OF CYANO AND AMINO NORMAL BONDED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

by

Yonghua Tang

Advisor: Professor David C. Locke

Normal bonded-phase chromatography has been studied less extensively than reversed bonded-phase liquid chromatography, which accounts in part for its being less used analytically than reversed bonded-phase chromatography. To improve the fundamental understanding of normal bonded-phase chromatography, we studied the retention of a wide variety of solutes in diverse mobile phases on cyano and amino stationary phases. The data allow development of a new model of

retention in normal bonded-phase chromatography.

Initially, the effect of strong adsorption sites, i.e. residual silanols, on retention using the cyano phase was isolated. Columns containing these silanol groups were compared with end-capped columns. An on-column silanization end-capping procedure was developed. Approximately 53% of the residual silanol groups were converted to non-adsorptive trimethylsilyl derivatives. Chromatographic behavior of phenols, anilines and other hydrogen-bonding solutes were completely changed. The retention interaction on the end-capped column involves only the bonded cyano groups.

The retention behavior of various solutes on cyanopropyl and aminopropyl columns with binary solvent mobile phases shows significant changes in the retention of solutes as the % polar solvent modifier is varied. For high % polar solvents, retention is changed from one involving specific adsorptive molecular interactions with the functional group of the stationary phase to a liquid-liquid partitioning of solutes between a surface-adsorbed liquid layer and a bulk-liquid mobile phase.

To account for this behavior, a new retention model for normal bonded-phase liquid chromatography was proposed. Equations are derived that expand upon the Snyder displacement model, which is useful at low

concentration of polar modifier, and describe retention data over the entire range of mobile phase compositions. The new model is based on the balancing of two comparable processes, the Snyder displacement adsorption interaction and the partitioning effect. The dominant process is determined by the mobile phase polarity and solute structure. The new model is consistent with our experimental data for all solutes and mobile phases studied.

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CHAPTER I

INTRODUCTION

I-1. Overview

High-performance liquid chromatography (HPLC) finds widespread use as one of the most important separation techniques and analytical methods in chemistry, biology, biochemistry, as well as in pharmaceutical, medicinal and food science. In recent years, the various HPLC techniques have progressed rapidly and new developments in instrumentation and column packings are evidenced by the vast growth in published scientific papers.

Chromatography is the term generally applied to a wide variety of separation techniques based on the sample partitioning between a mobile phase, which can be a gas or a liquid and a stationary phase, which can be a solid or a liquid. The discovery of chromatography is attributed to Tswett [1] in 1903, who obtained discrete colored bands of the pigments in green leaves on a chromatographic column of CaCO_3 washed with isooctane. He coined the term "chromatographic" to describe the colored zones which moved down the column. Thin-layer

chromatography (TLC) was demonstrated by Izmailov and Schraiber in 1938, who studied pharmaceutical tinctures by applying a thin layer of alumina adsorbent on a glass plate on which the samples were spotted, resulting circular chromatograms [2]. In 1949, Meinhard and Hall also obtained circular thin layer chromatograms on microscope slides coated with a starch binder containing a mixture of celite and alumina [3]. In the middle 1950s, the standardized commercially available adsorbents were developed and promoted the widespread use of TLC [4]. TLC, however, suffers from a number of disadvantages.

The remarkable work of Martin and Synge [5,6] revolutionized liquid - liquid chromatography and led to the development of paper chromatography and ultimately to gas chromatography [7]. The first paper on gas chromatography was published by Martin and James [8] in 1952. High - performance liquid chromatography, HPLC, arose in the late 1960s as a complementary technique to gas chromatography. The major theory came from the work of Van Deemter [9] and Giddings [10], which had been derived for GC. One of the first practical HPLC systems was made by Horvath and Lipsky [11-12] in 1967. The establishment of modern HPLC depended upon innovations

in the development of packing materials, column packing techniques, pump design and detection methods. In the past decade, HPLC has found great development and substantial applications in all fields of separations and chemical analysis.

In comparison with gas chromatography (GC), HPLC is the method of choice for those compounds which are less volatile, thermally unstable, ionic, polymeric, etc. The non-destructive detectors commonly used in HPLC offer the advantages of sample recovery and spectrophotometric analysis following the separation. In comparison with traditional column LC, HPLC has advantages such as reusable columns, faster analysis speed, higher resolution, sampling automation, sensitive detection, and automatic data handling.

In general, HPLC can be carried out in four different modes according to the stationary phase: normal phase including conventional liquid-solid chromatographic (LSC) adsorbents and polar bonded-phases, reversed-phase chromatography (RPC), ion-exchange chromatography (IEC) and size-exclusion chromatography (SEC). Chemically bonded-phase chromatography (BPC), including both normal and reversed phase, however, is the most popular and powerful in

HPLC. Bonded phases were originally developed by Halasz and Sebastian [13] to eliminate the operational problems in classical liquid-liquid chromatography (LLC) systems, such as the loss of mechanically-held stationary phase by the carrier solvent, the incompatibility with gradient elution due to the change of stationary solvent solubilities with change in eluent composition, and other operational difficulties. The advantages of BPC over liquid -liquid chromatography has been reviewed by Snyder [14].

Bonded-phase chromatography is usually classified into the following three groups according to the nature of the chemically bonded stationary phase [15]:

1. Non-polar hydrocarbon groups, such as octadecyl (C_{18}) C_1 , C_2 , C_4 and C_8 ; and silica support particles coated (or bonded) with alkyl polymers or cross - linking polymers.
2. Polar groups such as cyano-, aminopropyl, diamino, diol, ethers and glycols; and polymeric packings such as polymethyl cyanopropyl (PMSC₁) and polyvinyl pyrrolidone (PVP).
3. Ion-exchange groups such as sulphonic acid, amino and quaternary ammonium, poly (2-hydroxy, 3-N-ethyl-enediamino) butadiene (PHEB) and copolymer of butadiene

and maleic anhydride (PBDMA).

The preparation of the chemically bonded stationary phase involves surface derivatization of silanol groups on silica supports. This will be discussed in Chapter II.

In general, there are two different separation modes involved in bonded-phase HPLC, normal bonded-phase and reverse bonded-phase. The normal-phase mode, by the original definition, refers the fact that the stationary phase is more polar than the mobile phase in much the same manner as the LSC packing of a polar adsorbent such as silica. Samples with strong polarity or moderate polarity are normally well separated on the normal phase columns. In contrast, the reversed bonded-phase involves a non-polar stationary phase with a polar mobile phase to separate a wide variety of less polar samples. With the further study of the separation performance of bonded-phase chromatography in the past several years, "normal" and "reversed phase" columns have become more and more powerful in HPLC.

In the past ten years, considerable interest has been focused on the bonded reversed-phase mode. More than 70% of separations are estimated [16] to be carried out with hydrophobic column packings (C_8 or C_{18}) and

binary water-organic mobile phases; i.e. the reversed-phase mode. The major advantages of reversed-phase chromatography have been summarized by Karger and Giese [17].

Although reversed phase chromatographic applications currently outnumber normal phase applications, there is increasing interest in the use of various polar bonded-phase columns such as cyano-silica and amino-silica columns. These normal-phase packings provide selectivity different from that in reversed-phase and employ mobile phases that more readily dissolve organic compounds. A general review of the major advantages of normal bonded-phase chromatography has been given by Abbot [18].

As mentioned above, both liquid-solid chromatography based on silica and alumina packings and the polar bonded-phase chromatography are the normal-phase modes. However, the separation behavior and retention mechanism of these two methods are significantly different. A comprehensive and detailed model for LSC retention was developed by Snyder [19] in 1968. Soczewinski [20], a few years later, further developed a special case of this model. It has been shown that the Snyder and Soczewinski models are essentially equivalent [21]. The

well-known displacement model of LSC retention is referred to here as the Snyder model. The Snyder model assumes that retention of a solute molecule occurs by the competitive displacement of previously adsorbed mobile phase molecules from the active sites on the column. Practically, the original Snyder model [19] has been used in the optimization of mobile phase solvent strength in LSC [22-27]. The Snyder model, also provides an accurate theoretical basis for understanding the retention mechanism in conventional LSC.

Little is known regarding the basic retention mechanisms of chemically bonded polar stationary phases. De Ligny and his co-workers [28-30] have shown that the Snyder model can be used to interpret retention behavior in normal bonded-phase liquid chromatography. Hurtubise et al.[31] considered the Snyder LSC model for a u-Bondapak amino-bonded stationary phase. They showed that linear relationships were obtained from the logarithm of capacity factors (k') vs. logarithm of mole fraction (N_B) of the strong solvent in binary mobile phase. Hussain and co-workers [32] studied n-heptane / 2-propanol solvent composition effects with a cyano-bonded stationary phase in normal-phase liquid chromatography of alkylphenols and naphthols. Again,

plots of $\log k'$ vs. $\log N_B$ of strong solvent in the binary mobile phase were linear relationships.

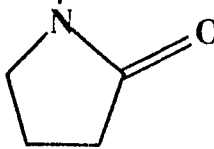
In recent years, a number of chromatographic researchers [33-39] have studied retention mechanisms on cyano-silica and amino-silica columns; however, none gave an exact model for the basic retention mechanism. Cooper and co-workers [40-42] derived the selectivity matrix from Snyder adsorption-displacement model for quantifying localization and secondary solvent effects on retention. Snyder et al. [35] studied the retention of various polar solutes on a cyano-propyl-silica column as a function of mobile phase composition. They concluded that retention data could be correlated quantitatively in terms of the general displacement model of LSC. It should be noted that all these studies of retention mechanisms on polar bonded-phases were based on binary mobile phases containing only a small amount of the polar solvent. On the basis of our experimental data, retention can in fact be interpreted in terms of the Snyder displacement model only when N_B is small. With a higher % polar mobile phases modifier, significant retention differences are observed and the simple Snyder displacement model no longer accounts for retention on the polar bonded phases.

To summarize our experimental observations and theoretical studies, the simple Snyder displacement model cannot predict and describe the retention behavior on normal bonded cyano and amino stationary phases when a wide range of polar solvent compositions is used. A new model derived from experimental and theoretical determinations successfully describes the retention process over the entire range of polar mobile phase compositions. It is assumed that two factors contribute to the total sample retention: Snyder displacement molecular interactions and a partition phenomenon arising from the distribution of solute between the surface - adsorbed liquid layer and bulk mobile phase. Discussion of the new model is given in Chapter III.

I-2. Advantages of Normal Bonded - Phase Liquid Chromatography

Of the various commercially available chemically bonded phases, non-polar columns, especially C_{18} have been used the most. However, polar bonded-phase columns (e.g. CN and NH_2) have also become important because of their wider range of selectivity which is different from that in the reversed-phase mode. A variety of stationary phase systems for normal bonded-phase liquid chromatography is commercially available for different separation purposes. In the ordinary normal phase systems, the stationary phase is the bonded layer on the silica support surface, which contains polar functional groups that undergo the selective molecular interactions with solutes. The common commercially available columns are listed in Table I-1. However, polymeric packings have become popular and many were introduced only recently [43-44]. Polymeric packings are usually consist of silica-coating using variety of prepolymers or the immobilized cross-linked polymers with different polar functional groups. Schomburg [45] has reviewed the methods of chemical modification by polymer coating and summarized the advantages of this method.

Table I-1. Polar Normal Bonded-Phase Structures

Functional Group	Structure
1. Cyanopropyl	$-(\text{CH}_2)_3\text{CN}$
2. Aminopropyl	$-(\text{CH}_2)_3\text{NH}_2$
3. Diol	$-(\text{CH}_2)_3\text{OCH}_2\overset{\text{HO}}{\underset{ }{\text{CH}}}\overset{\text{OH}}{\underset{ }{\text{CH}}}_2$
4. Diamino	$-(\text{CH}_2)_3\text{NH}(\text{CH}_2)_2\text{NH}_2$
5. Dimethylamino	$-(\text{CH}_2)_3\text{N}(\text{CH}_3)_2$
6. Polymethyl cyanopropyl (PMSC)	$\left[\begin{array}{cc} \text{CH}_3 & \text{CH}_3 \\ & \\ \text{---Si---O---Si---O---} \\ & \\ \text{CH}_3 & \text{CH}_2\text{CH}_2\text{CN} \end{array} \right]_n$
7. Polyvinyl pyrrolidone (PVP)	$\left[\text{CH} \text{---} \text{CH}_2 \right]_n$ 

Abbott [18] has discussed the advantages of normal bonded-phase chromatography. These include:

1. The ability to separate highly hydrophilic compounds which are not retained on reversed-phase columns.
2. The ability to do class separations, such as lipid separations and the separation of polynuclear aromatic hydrocarbons based on the number of condensed rings.
3. The unique ability to separate isomers, especially using the aminopropyl bonded phase.
4. Lower viscosity of organic mobile phases, resulting in lower operating pressures.
5. Greater compatibility of the eluent with on-line LC-MS because of the higher volatility of organic solvents compared with the aqueous solvents used in the reversed-phase mode.
6. The compatibility with pure organic mobile phase for the compounds having low solubility in aqueous phases.

In addition, normal bonded-phase packings can behave in a multi-model fashion. Namely they can play different roles depending on the selection of the mobile phase [46-47]; they function as both normal and reversed phase materials, and as size exclusion media.

I-3. Rationale of This Research

The applications of polar bonded-phase columns such as aminopropyl and cyanopropyl in normal-phase HPLC continue to grow. However, the mechanism involved in sample separation and retention on those columns is still incompletely understood. Until recently, as mentioned above, the few studies made concerning sample retention on amino and cyano bonded-phase showed that the Snyder displacement mode could be used to predict retention characteristics for normal bonded-phase systems. On the basis of our investigation, however, the Snyder model generally cannot describe the aminopropyl and cyanopropyl normal bonded-phase systems with higher polar solvent compositions. The retention mechanism on polar bonded-phase columns is evidently more complicated because of (i) the presence of residual silanols; (ii) change of the principal type of interaction by changing the polarity of the mobile phase; and (iii) solute molecules having different molecular structures have different types of molecular interactions on the polar bonded-phases.

In order to develop further the technique of normal bonded-phase chromatography and to expand its

applications, the aims of this research are the following:

(1) To explore the potential of normal bonded-phase liquid chromatography.

(2) To interpret the retention mechanism and chromatographic performance of amino and cyano bonded-phase chromatography.

(3) To modify the Snyder displacement model and further to establish a new model which can accurately characterize the retention behavior of polar bonded-phase columns over a wide polarity range of mobile phase.

(4) To characterize the column selectivity and solvent selectivity in commercially available cyano and amino bonded-phase columns.

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CHAPTER II

THE EFFECT OF FREE SILANOL GROUPS ON RETENTION BEHAVIOR OF PROPYLCYANO BONDED STATIONARY PHASE

II-I. Preparation and Properties of Chemically Bonded- Phase Columns

II-I-1. Introduction

The normal bonded-phase column packings studied in our research are surface-reacted (permanently bonded) propylcyano and propylamino stationary phases on porous silica supports. To investigate the retention mechanism and separation behavior of solutes on these polar bonded groups, all residual silanol groups must be eliminated. These exposed residual silanols complicate the interpretation of the retention mechanism. The usual method of eliminating residual silanol groups is to silylate them with a small derivatizing group such as trimethylsilyl. Various reagents are available for this reaction. The silylation of residual silanols is

referred to as end capping or capping.

The on-column derivatization (silylation) reaction described below is a simple, rapid and inexpensive method for eliminating residual silanol groups. BSA (N, O-bis(trimethylsilyl)acetamide) was used as the silylation reagent. This procedure generally involves pumping a solution of BSA at the desired concentration through the non-capped propylcyano column to cover the exposed silanols and thereby produce an end-capped column. The success of the on-column silanization reaction and the column performance before and after removing the residual silanols is discussed below.

II-I-2. Structure of Silica Surface

Porous microparticulate silica is the most widely used solid support for HPLC chemically bonded phase packing materials. To understand the adsorption behavior and the chemical reactivity of silica used in chromatography, a basic knowledge of the surface structure of silica is necessary.

The silica surface consists of a plane of exposed silicon atoms to which hydroxyl groups are covalently bonded. However, the hydroxyl groups attached to these

silicon atoms are not exactly equidistant from each other and are not in same geometrical arrangement [1]. According to Iler [1], Snyder [2,3] and Gilpin [4], there are five postulated types of hydroxyl groups on the surface of silica [Fig.II-1]. The relative amount of each surface type depends on the structural considerations, the preparative method and condition of the silica gel. In general, it is believed that the surface of silica is covered with a monolayer of silanol groups with an irregular distribution.

The concentration of surface hydroxyl groups, $\bar{a}_{OH}$, has been measured in various ways, such as chemically or spectroscopically. There are about 4.6 OH groups per nm^2 of surface on the silica gel, corresponding to 8 umoles per m^2 , as reported by De Boer and Vleeskens [5]. This value has been accepted and used elsewhere [6]. The calculated mean molecular cross-sectional area (A_m) of hydroxyl group is 0.217 nm^2 , and the mean distance between two adjacent hydroxyl group is about 0.5 nm [5]. Although the fully hydroxylated silica surface contains about 8 umoles of silanol groups per m^2 , only about 50%, that is 4.5 umoles of silanols per m^2 , can be reacted [6]. This is probably caused by the steric hindrance of the bulky reagent molecule, which is unable to react

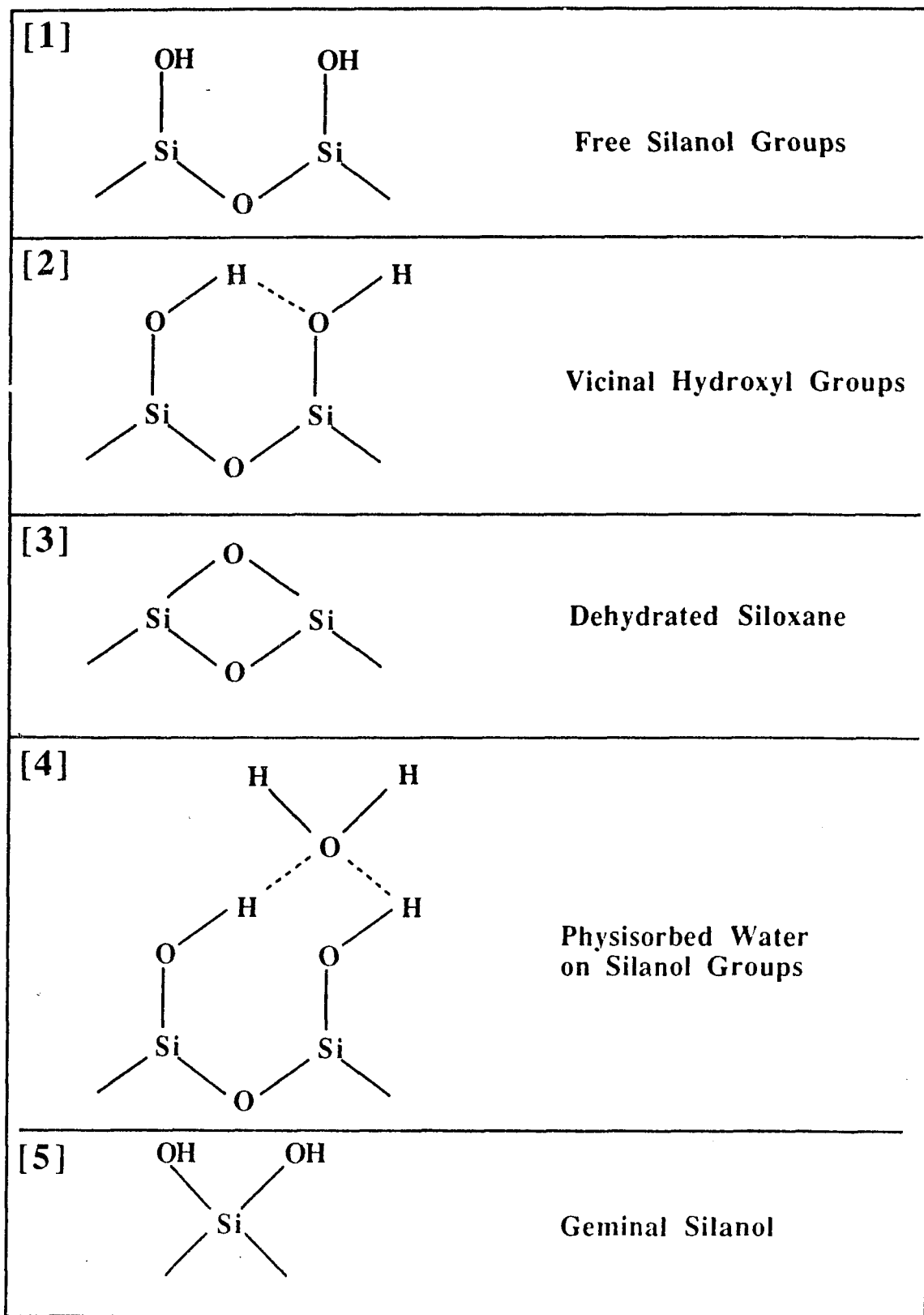


Figure II-1. Surface of the Silica Gel before Bonding

with the less accessible silanol groups located on the surface or in the fine pores of the silica. It is reported by Unger [7] that the maximum value of surface coverage $\bar{a}_{\text{exp}}$ (mole/m²) is about 4.7 umoles/m² for the smallest modifier groups (trimethylsilyl,) and is about 1.5 umole/m² for tribenzylsilyl . The remaining hydroxyl groups are referred as residual silanols. Their effect on chemically bonded-phase chromatography will be discussed below.

II-1-3. Preparation of Bonded - Phase Packing

As indicated in Figure II-1, the surface of silica is covered with a layer of silanol groups (Si-OH), and all synthetic reactions involve introducing organic groups onto the reactive silanol surface. The preparation of chemically bonded phase packing in HPLC has been developed by a number of researchers [9-16]. The basic reactions used to prepare bonded-phase packings are summarized in Figure II-2. These are:

1. Esterification of silanol groups with an alcohol to give an alkoxy silane phase [9-10]: This was the first method reported for liquid chromatography use. The bonded phase silica is prepared by direct

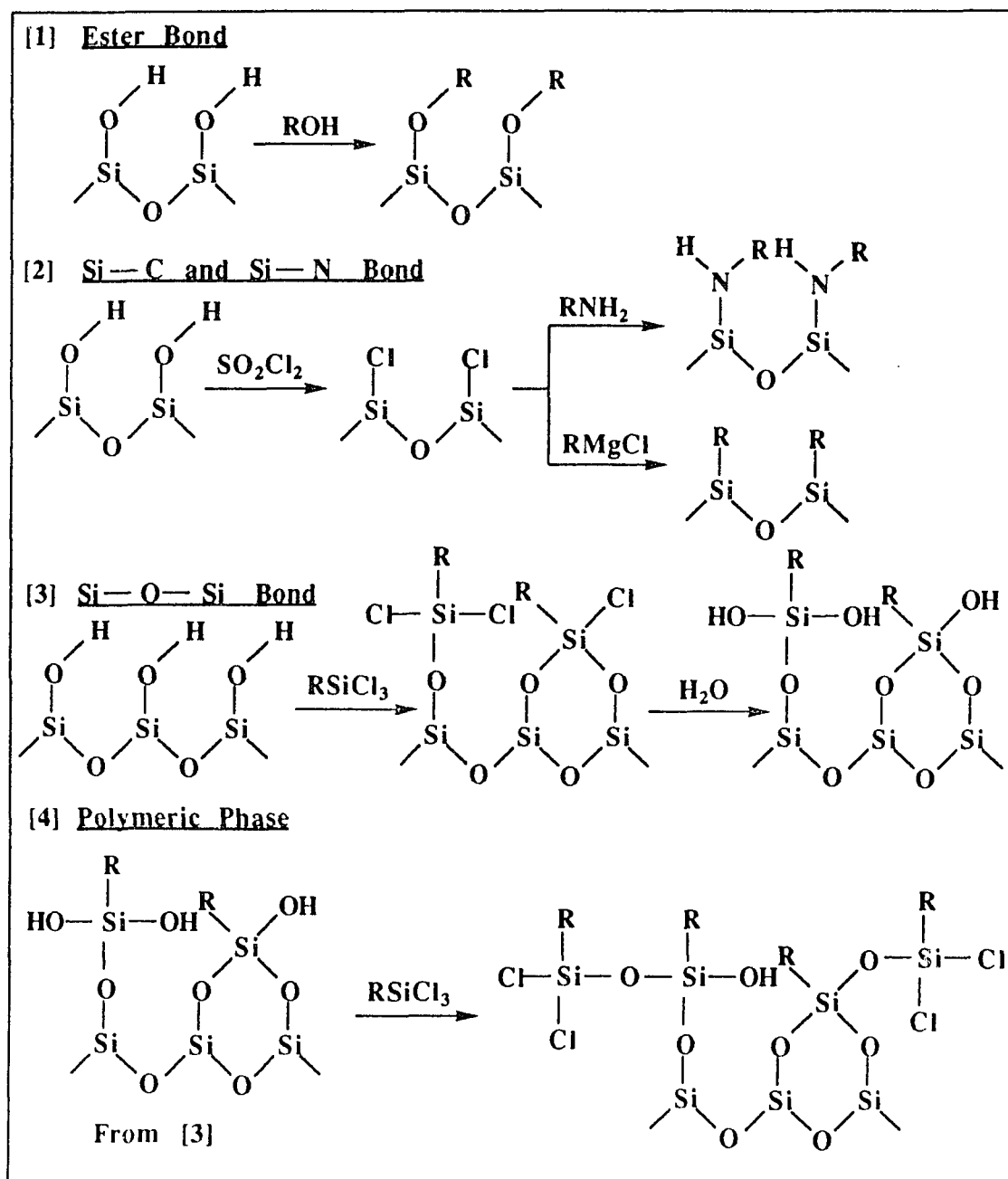


Figure II-2. Surface Reaction for Preparing Bonded-Phase Packing

esterification of silanol (Si-OH) with an alcohol. The ester bonds, unfortunately, are subject to hydrolytic attack and are also thermally unstable. This type of bonded-phase packing is restricted to non-aqueous and non-alcoholic solvents in the mobile phase.

2. Reactions involving chlorination of the silanol groups using thionyl chloride, followed by the formation of covalent Si-C or Si-N bonds using a Grignard reagent [11-12]: The Si-C and Si-N bonds are more stable than silicate esters and possess good hydrolytic and thermal stability. However, the Grignard reagent may produce a low concentration of surface organic groups and leave inorganic residues on the surface [15].

3. Reaction of silanol groups with organochlorosilane or organoalkoxysilane reagents [13-16]: The most useful and widely available bonded-phase materials are those derived from these reactions, which produce a siloxane bond (Si-O-Si). Bonded-phase packings of this reaction are hydrolytically stable over the pH range 2-8.

4. Preparation of polymeric stationary phases: Although monomeric phase packings are the most widely used, polymeric phases are also useful. The preparation of polymeric adsorbent phases can generally be classified

into two different groups: (1) the polymeric stationary phase is synthesized in situ on the silica support surface. The silanol groups are reacted with organodi- or tri-chlorosilane first, and then the unreacted chlorine atoms are hydrolysed to reproduce silanols and a further reaction is performed (see Fig.II-2); (2) prepolymers and immobilized cross-linked polymers synthesized externally are bonded (or coated) onto the silica support. A number of commercially polymeric bonded phases are available. Their nature and properties have been discussed elsewhere [8, 17 - 22].

II-1-4. Properties of Bonded-Phase Packing

The presence of polar residual silanol groups renders undesirable chromatographic properties to bonded-phase packing. The free residual silanols on the surface of the chemically bonded stationary phase act as strong adsorption sites and thus affect the retention, selectivity, and separation mechanism for polar solutes. This confuses the interpretation of chromatographic data and the prediction of chromatographic performance of such bonded-phase columns. Therefore, to study the function and exact retention mechanism of the bonded

functional groups alone, it is necessary to eliminate the adsorption effects of residual silanols. This can be done most simply by treating the residual silanol groups with trimethylchlorosilane (TMS), hexamethyldisilane (HMDS) or other silylating reagents. This procedure is referred to as end-capping [6,15,23].

II-2. On-Column Silylation of A Cyano Bonded-phase

Column for Elimination of Residual Silanol Groups

II-2-1. Introduction

The unique separation capabilities of normal phase HPLC have been used for chromatographic analyses of a variety of compounds. However, the retention characteristics and separation mechanism of normal bonded-phases, as mentioned above are still controversial. No good, comprehensive model for normal phase separations is available in the literature. A new model incorporating elements of Snyder's displacement model and a liquid partition model has been developed (refer to Section III-5-3 below).

In the presence of residual silanols, the interaction mechanism of bonded-phases cannot easily be allocated between the SiOH groups and the Si-R. To demonstrate such a dual retention mechanism for cyano bonded-phase column, trimethyl silylated (end-capped) columns must be compared with non-capped columns. Using an on-column silanization reaction on a propylcyano column, the adsorption effect of residual silanols is removed from the silica surface to provide the

comparison column.

The basic synthetic scheme for the silylation reaction on the normal bonded cyano column involves the reaction of residual silanol groups with the silylating reagent BSA (N,O-bis (trimethylsilyl) acetamide). The reaction is shown in Figure II-3. A discussion of the on-column end-capping silanization reaction and a comparison of column performance before and after end-capping is given in the following pages.

II-2-2. Experimental Section

(1) Equipment

The HPLC instrument used in this research is a Perkin-Elmer Series 400 Liquid Chromatograph, consisting of a dual reciprocating pump, a Rheodyne model 7125 sample valve, a LC 90 variable wavelength UV detector and LCI-100 computing integrator. All measurements were obtained at a wavelength of 254 nm. The column temperature was controlled by a SSI series H-6805014 thermostator (Scientific Systems Inc., State University, PA). The HPLC column used for on-column silylation reaction was a 250 x 4.6 mm I.D. stainless-steel

ON COLUMN END-CAPPING REACTION

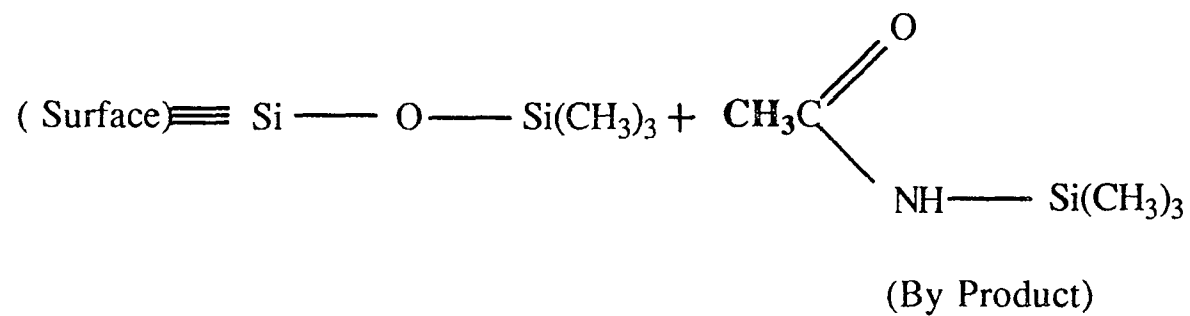
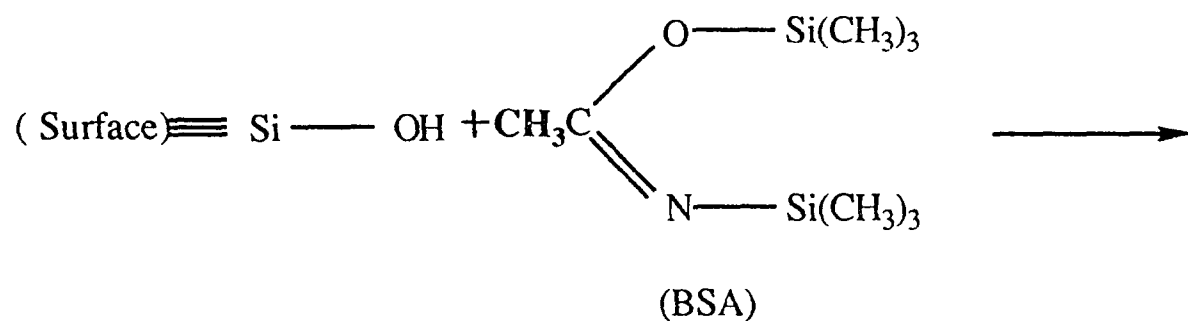


Figure II-3. Synthetic Scheme for On-Column Silylation Reaction

column packed with Ultrasphere-CN of particle size 5 μm .

(2) Materials

BSA (N,O-bis(trimethylsilyl)acetamide), the silylation reagent, was obtained from Pierce Chemical Co. (Rockford, IL, USA). HPLC-grade mobile phase solvents, hexane, heptane, cyclohexane, 2-propanol, ethanol, acetonitrile, dichloromethane and chloroform were used as received from J.T. Baker (Phillipsburg, NJ, USA). The standard solutes were obtained from Aldrich (Milwaukee, WI, USA) and were used without further purification.

(3) Silylation Procedure

The end-capping silylation reaction of the CN column was carried out at 55°C; the column temperature was controlled by the thermostated jacket. Before the reaction, the column was flushed sequentially with 50 ml H_2O , 50 ml acetonitrile and 50 ml hexane, at a flow rate of 2.0 ml/min to wash out any organic residues. The silylation reagent, 10% (v/v) BSA in hexane (0.42 M), was pumped through the column at a flow rate of 0.5

ml/min for 10 minutes. The pump was stopped, and the column was allowed to stand for 20 minutes at 55°C to complete the reaction. After this, an additional 60 ml BSA solution was pumped through the column at 0.5 ml/min.

Following silanization, the column was flushed with 30 ml hexane, 30 ml acetonitrile and 30 ml 2 - propanol at flow rate 2.0 ml/min to remove the excess BSA. The end-capped cyano column was then ready for chromatography.

II-2-3. Determination of Silanization Coverage

To measure quantitatively the extent of silanization coverage, effluent fractions were collected as the reaction proceeded. The fraction of unreacted BSA was determined using HPLC with a UV detector. BSA itself is not, however, a UV-detectable compound. To form a UV-absorbing compound, benzyl alcohol was added to each fraction to react quantitatively with the excess BSA to produce $C_6H_5-CH_2O-Si(CH_3)_3$ (see Figure II-4). The peak areas of the product as the function of on-column silylating reaction time is shown in Figure II-5. By integrating the area under the curve in Figure II-5, the

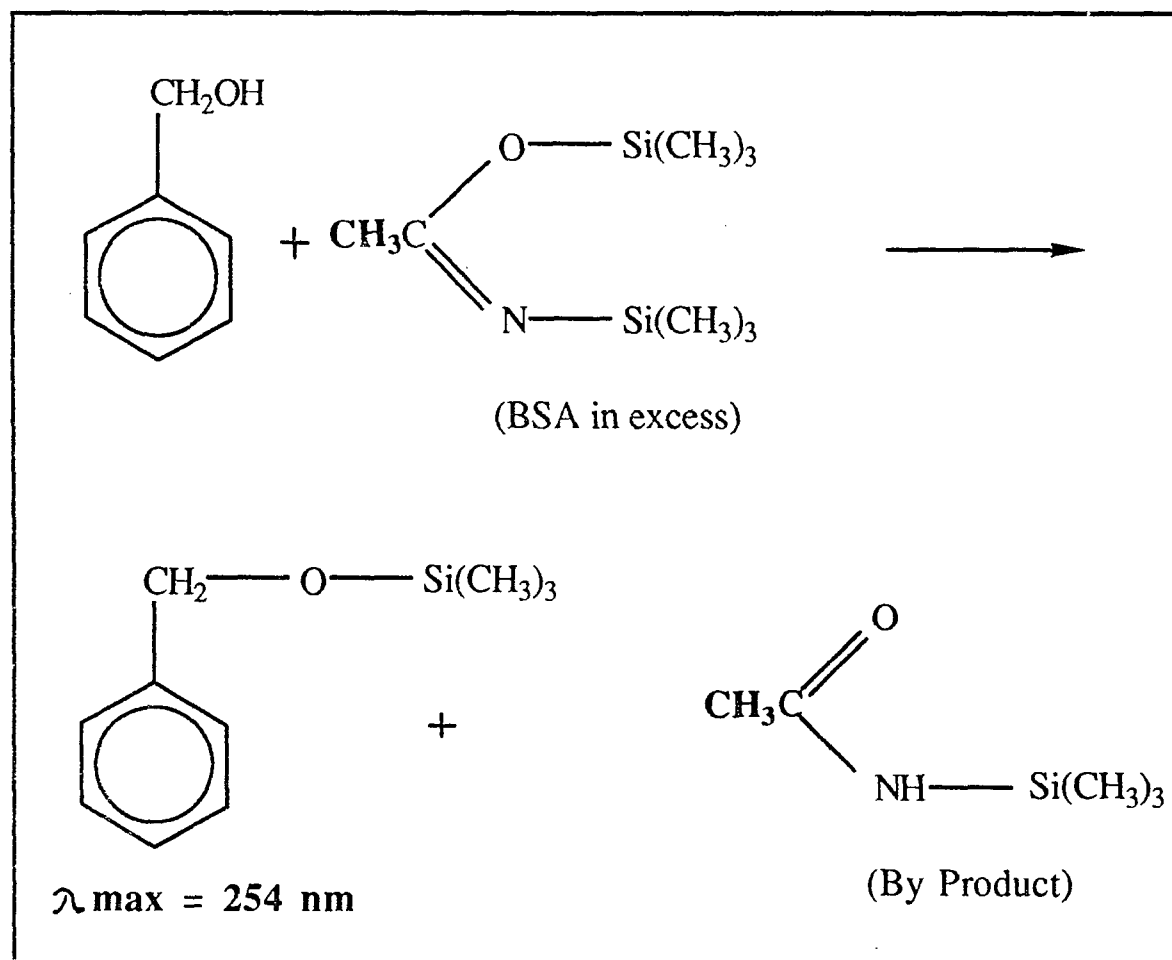


Figure II-4. Preparation of UV-Absorbing Derivative

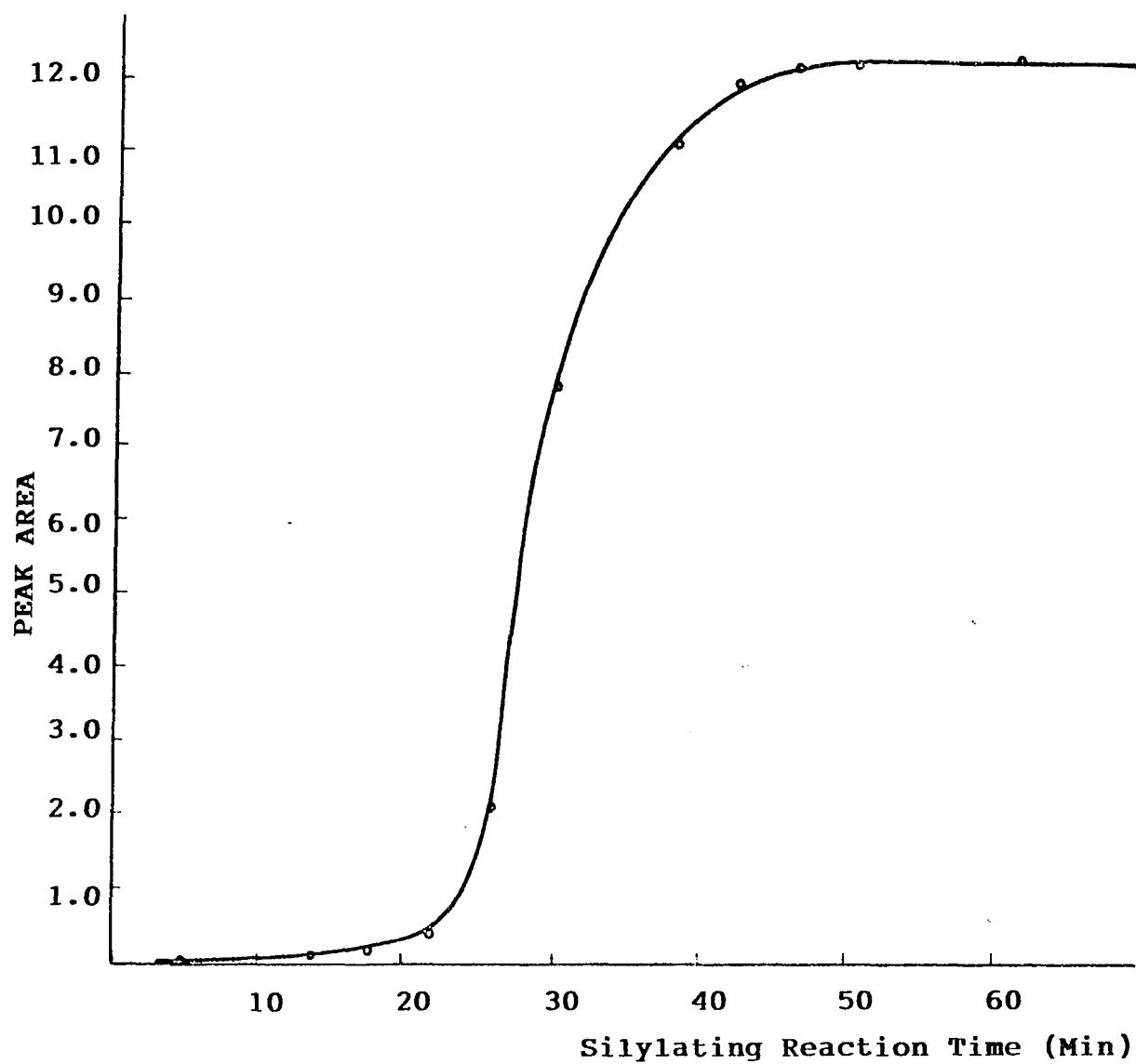


Figure II-5. Reaction Profile of Peak Area of UV - Absorbing Product (Benzyl-TMS Ether) Vs. On-Column Silylation Time.

total amount of BSA left in each collection was obtained using the calibration curve shown in Figure II-6. The amount of BSA reacting with residual silanol groups can be determined by difference and was about 1.6×10^{-3} moles. The end-capping coverage is defined as moles of reacted silylation reagent per square meter of silica surface. The total surface area of silica particles can be calculated from the volume of silica particles in the column (V_m), silica density (d) and specific silica surface area (S). The volume of silica particles can be estimated from the geometrical volume of the column times the fractional volume, f , occupied by the particles, i.e. $\pi r^2 L f$ where r and L are the column radius and length and $f \cong 0.5$. Thus,

$$\begin{aligned} \text{Total Surface Area} &= S \times (d V_m) \\ &= 250 \text{ m}^2/\text{g} \times (0.9 \text{ g/cm}^3 \times 2.1 \text{ cm}^3) \\ &= 473 \text{ m}^2 \end{aligned}$$

The end-capping coverage is calculated from:

$$\begin{aligned} \text{end-Capping Coverage} &= \frac{\text{moles of reacted reagent}}{\text{total surface area}} \\ &= \frac{1.6 \times 10^{-3} \text{ moles}}{473 \text{ m}^2} \end{aligned}$$

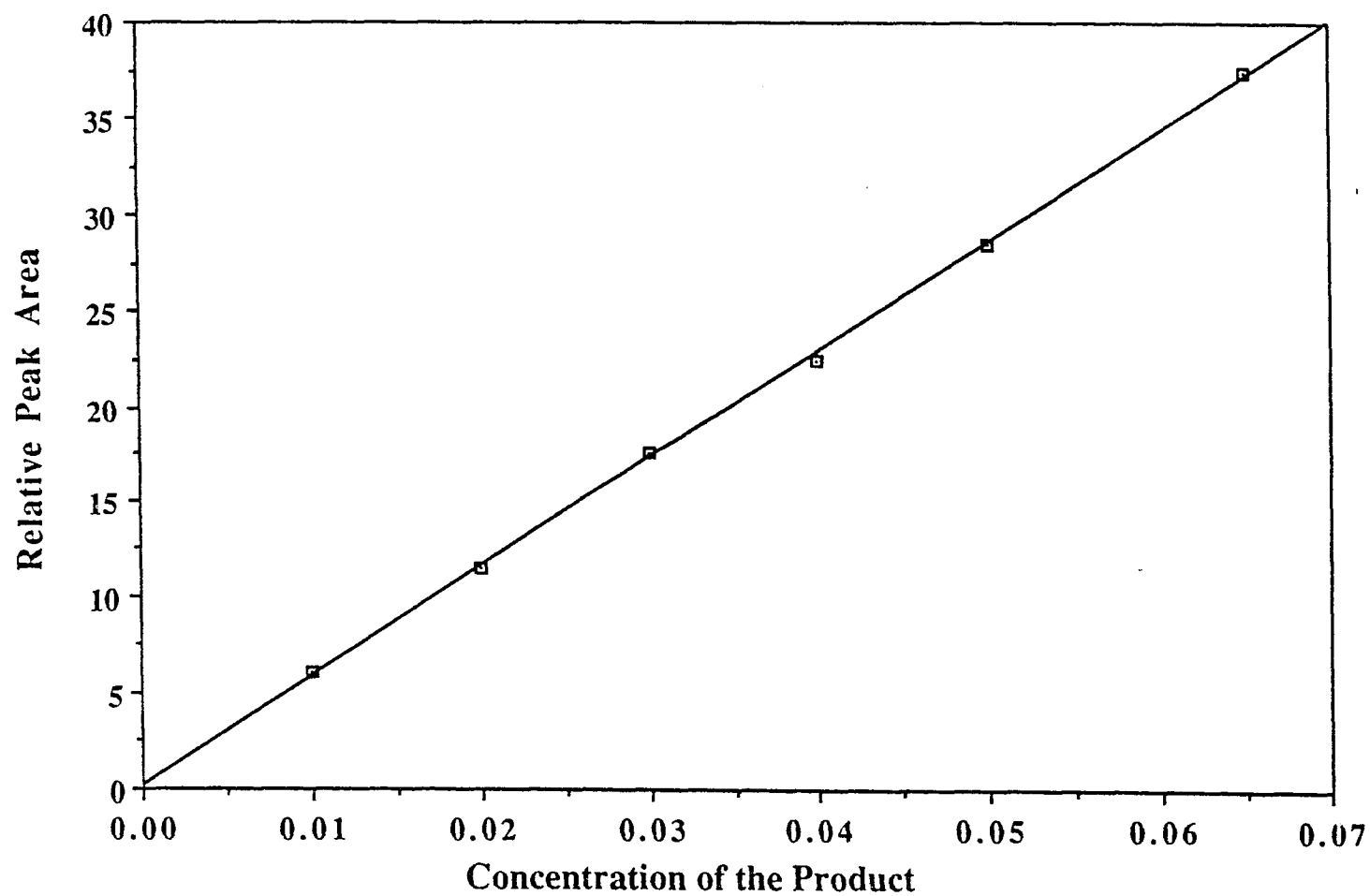


Figure II-6. Calibration Curve For UV - Absorbing Product
(Benzyl - TMS Ether)

$$= 3.3 \times 10^{-3} \text{ umoles/m}^2$$

The fully hydrolyzed silica would contain about 8-9 umoles/m². At best, because of steric hindrance, a maximum of about 4.5 umoles/m² can be reacted with the propyl cyano groups, leaving about 50% of the accessible silanol groups. If we assume that the non-capped Ultrasphere-CN column has about 50% of the silanols not reacted, there should be about 4 umoles/m² of free residual silanol groups. Thus, about 73% of these residual silanols have been removed by the on-column silylation reaction.

II-2-4. Comparison of Column Performance of End-Capped and Non-Capped Cyano Column

A comparison of column performance characteristics and retention behavior before and after end-capping on the same Ultrasphere-CN column was made by a series chromatographic retention measurements for a variety of compounds.

(1) Retentivity

Previous work [15-16] has reported that cyano-bonded silica is a weaker adsorbent than silica, but provides similar retention times for the same solute and mobile phase. On the non-capped cyano column the residual silanol groups lengthen the retention time. One would expect for polar solutes, longer retention times on the non-capped cyano column than on the end-capped cyano column. In Tables II-1 --- II-5, capacity factors are given for several groups of compounds on both the non-capped and end-capped cyano columns. Various compositions of hexane/2-propanol mobile phase are shown for a flow rate of 2.0 ml/min. Apparently, the capacity factors, k' , of these compounds are generally reduced by end-capping the column. On the cyano column, the chromatographic functional group is the electron-rich nitrile group, a Lewis base, an electron pair donor. For the non-capped column, in addition to the nitrile groups, residual silanols contribute to solute retention. It is demonstrated in Table II-1 that the k' values of anilines are considerably reduced after eliminating the acidic silanols, with which the electron rich amines interact

more strongly than do the cyano groups. The retention times were, therefore, dramatically reduced after the column was end-capped. The effect of the residual silanol groups on retention is most apparent for compounds which strongly interact with the silanols. As demonstrated in Tables II-1 --- II-5, compounds behave differently depending on the strength of interaction with the residual silanol groups.

Table II-1. k' Values of Substituted Anilines on In-Situ End-capped (#1) and Non-capped (#2) Ultrasphere - CN Columns Using Hexane/Isopropanol Eluent

<u>Compound</u>	<u>Column</u>	<u>Capacity Factor k' (HEX/IPA)</u>				
		<u>70/30</u>	<u>80/20</u>	<u>90/10</u>	<u>94/6</u>	<u>99/1</u>
4-Nitro aniline	#1	0.45	0.86	1.92	2.79	6.97
	#2	1.08	1.57	3.22	5.43	13.74
4-Trifluoro methylaniline	#1	0.17	0.26	0.44	0.52	0.96
	#2	0.63	0.83	1.43	1.89	3.92
4-Chloro- aniline	#1	0.16	0.24	0.38	0.46	0.79
	#2	0.60	0.72	1.38	2.68	3.70
Aniline	#1	0.16	0.21	0.30	0.35	0.50
	#2	0.49	0.66	0.98	1.49	2.13
4-t-Butyl- aniline	#1	0.16	0.21	0.28	0.32	0.47
	#2	0.34	0.35	0.48	0.54	0.75

Table II-2. k' Values of Substituted Phenols on In-Situ End-capped (#1) and Non-capped (#2) Ultrasphere - CN Columns Using Hexane/Isopropanol Eluent

<u>Compound</u>	<u>Column</u>	Capacity Factor k' (HEX/IPA)				
		<u>70/30</u>	<u>80/20</u>	<u>90/10</u>	<u>94/6</u>	<u>99/1</u>
4-Nitro phenol	#1	0.20	0.36	0.75	1.09	5.40
	#2	0.30	0.42	0.91	1.65	10.37
4-Cyano phenol	#1	0.19	0.27	0.38	0.91	3.27
	#2	0.28	0.30	0.79	1.18	6.45
4-Chloro phenol	#1	0.08	0.15	0.26	0.35	1.36
	#2	0.26	0.31	0.56	0.86	2.92
Phenol	#1	0.08	0.15	0.26	0.35	1.36
	#2	0.09	0.21	0.38	0.60	2.08
4-t-Butyl phenol	#1	0.04	0.08	0.16	0.22	0.88
	#2	0.08	0.15	0.28	0.48	0.75

Table II-3. k' Values of Nitrobenzenes on In-Situ
End-capped (#1) and Non-capped (#2)
Ultrasphere - CN Columns Using Hexane/
Isopropanol Eluent

<u>Compound</u>	<u>Column</u>	Capacity Factor k' (HEX/IPA)				
		<u>70/30</u>	<u>80/20</u>	<u>90/10</u>	<u>95/5</u>	<u>99/1</u>
o-Dinitro benzene	#1	0.41	0.58	0.92	1.15	1.46
	#2	0.66	0.97	1.55	2.08	3.29
m-Dinitro benzene	#1	0.24	0.31	0.35	0.41	0.51
	#2	0.33	0.46	0.75	0.97	1.26
p-Dinitro benzene	#1	0.21	0.26	0.35	0.41	0.51
	#2	0.16	0.31	0.42	0.50	0.70
Nitro- benzene	#1	0.13	0.16	0.19	0.21	0.24
	#2	0.31	0.43	0.61	0.77	0.89

Table II-4. k' Values of Electron-donor Compounds on In-Situ End-capped (#1) and Non-capped (#2) Ultrasphere-CN Columns Using Hexane/Isopropanol Eluent

<u>Compound</u>	<u>Column</u>	Capacity Factor k' (HEX/IPA)				
		<u>80/20</u>	<u>90/10</u>	<u>96/4</u>	<u>98/2</u>	<u>99/1</u>
Pyridine	#1	0.48	0.74	0.91	1.41	1.52
	#2	1.35	2.17	2.86	4.75	10.31
Quinoline	#1	0.23	0.33	0.39	0.55	0.62
	#2	0.81	1.26	1.62	2.03	4.69
7,8-benzo-quinoline	#1	0.10	0.12	0.13	0.14	0.16
	#2	0.24	0.24	0.34	0.44	0.58
Acetone	#1	0.17	0.29	0.41	0.50	0.58
	#2	0.41	0.54	0.64	0.88	0.94
Cyclohexanone	#1	0.12	0.16	0.31	0.42	0.48
	#2	0.28	0.39	0.48	0.70	0.83
Acetophenone	#1	0.10	0.17	0.19	0.26	0.31
	#2	0.22	0.32	0.47	0.47	0.54
Benzophenone	#1	0.09	0.15	0.20	0.24	0.27
	#2	0.15	0.22	0.25	0.30	0.36

Table II-5. k' Values of poly Aromatic Hydrocarbons (PAHs) on In-Situ End-capped (#1) and Non-capped (#2) Ultrasphere - CN Columns Using Hexane/Isopropanol Eluent

<u>Compound</u>	<u>Column</u>	<u>Capacity Factor k' (HEX/IPA)</u>				
		<u>60/40</u>	<u>80/20</u>	<u>90/10</u>	<u>95/5</u>	<u>100/0</u>
Benzene	#1	0.01	0.00	0.01	0.03	0.06
	#2	0.01	0.00	0.01	0.02	0.10
Naphthalene	#1	0.02	0.02	0.03	0.06	0.08
	#2	0.02	0.02	0.02	0.03	0.20
Anthracene	#1	0.03	0.04	0.06	0.08	0.10
	#2	0.03	0.03	0.07	0.10	0.33
Pyrene	#1	0.05	0.06	0.08	0.10	0.12
	#2	0.05	0.05	0.10	0.16	0.46
Chrysene	#1	0.07	0.09	0.10	0.10	0.14
	#2	0.06	0.12	0.16	0.23	0.57
Triphenylene	#1	0.07	0.09	0.10	0.12	0.15
	#2	0.07	0.13	0.27	0.31	0.72
Benzo-[a]pyrene	#1	0.08	0.10	0.11	0.14	0.16
	#2	0.10	0.20	0.30	0.35	0.81

To illustrate the effect of free residual silanols on chromatographic retention, the capacity factor data for 4-chloroaniline are plotted in Figure II-7. In Figure II-8, the chromatograms for pyridine, quinoline and 7,8-benzoquinoline on the two columns are shown. The elution order on both columns, as expected, is the order of increasing lone-pair electron density (i.e. basicity) on the nitrogen atoms of these compounds, that is 7,8- benzoquinoline < quinoline ($pK_b = 9.1$) < pyridine ($pK_b = 8.75$). The elution time for all compounds, however, is shorter on the end-capped column.

One should also note that the peak shape of the most retained compound, pyridine, is much better on the end-capped column. The peak asymmetry factors (A_s) of pyridine were calculated from $A_s = CB/AC$, where CB and AC are the distances from the central axis of the peak at 10% of peak height to the left and right sides of the peak respectively. A_s is 4.6 on the non-capped and 1.4 on the end-capped column. The asymmetry factors are one measure of the relative column efficiency for asymmetric bands [15]. It is obvious that the significantly tailing of the pyridine peak on the non-capped bonded phase packing arises from adsorption on the free residual silanol groups.

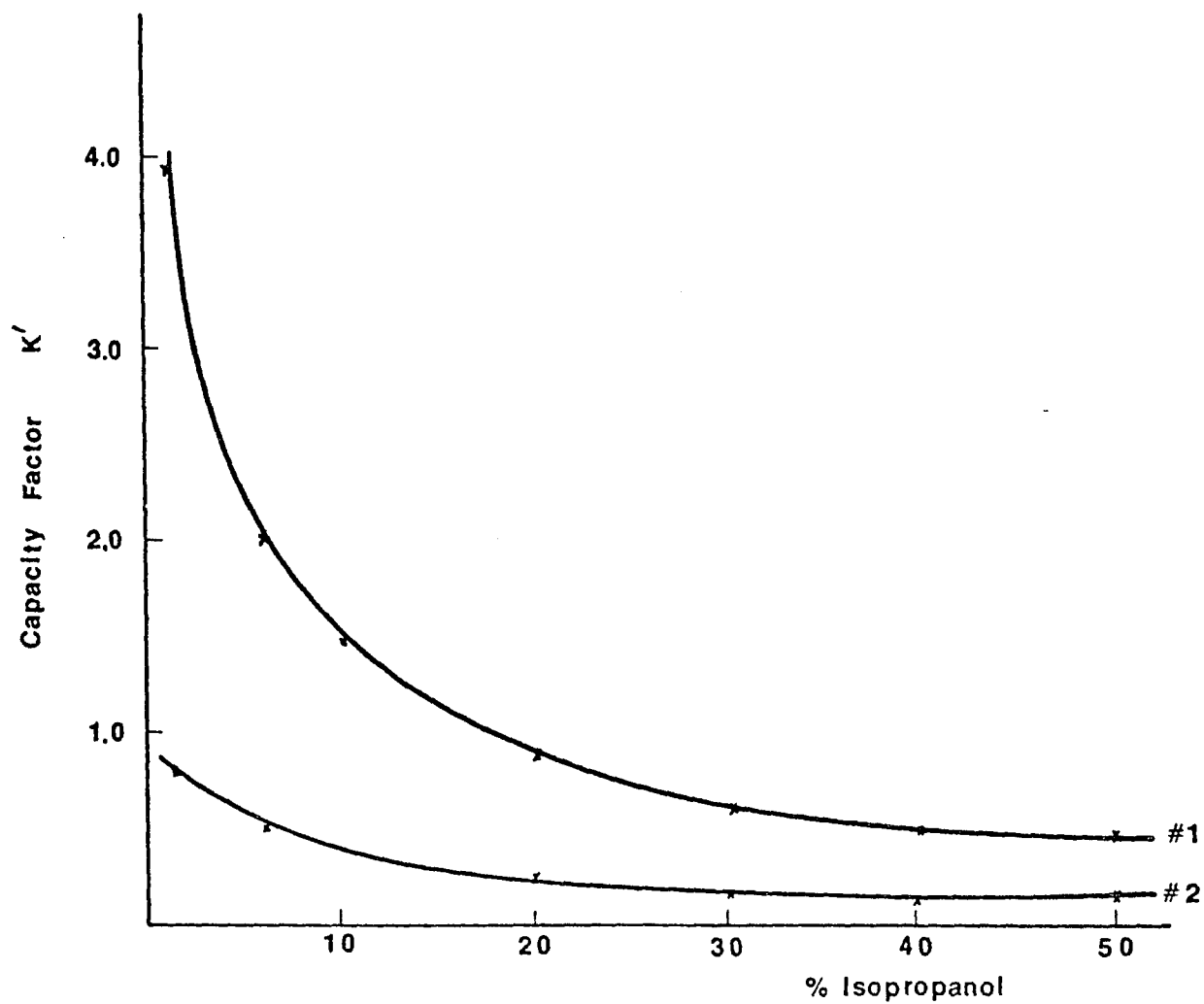


Figure II-7. Retention Of 4-Chloroaniline On Non - Capped (#1) And End - Capped (#2) Cyano Columns With Hexane / 2-Propanol Eluent.

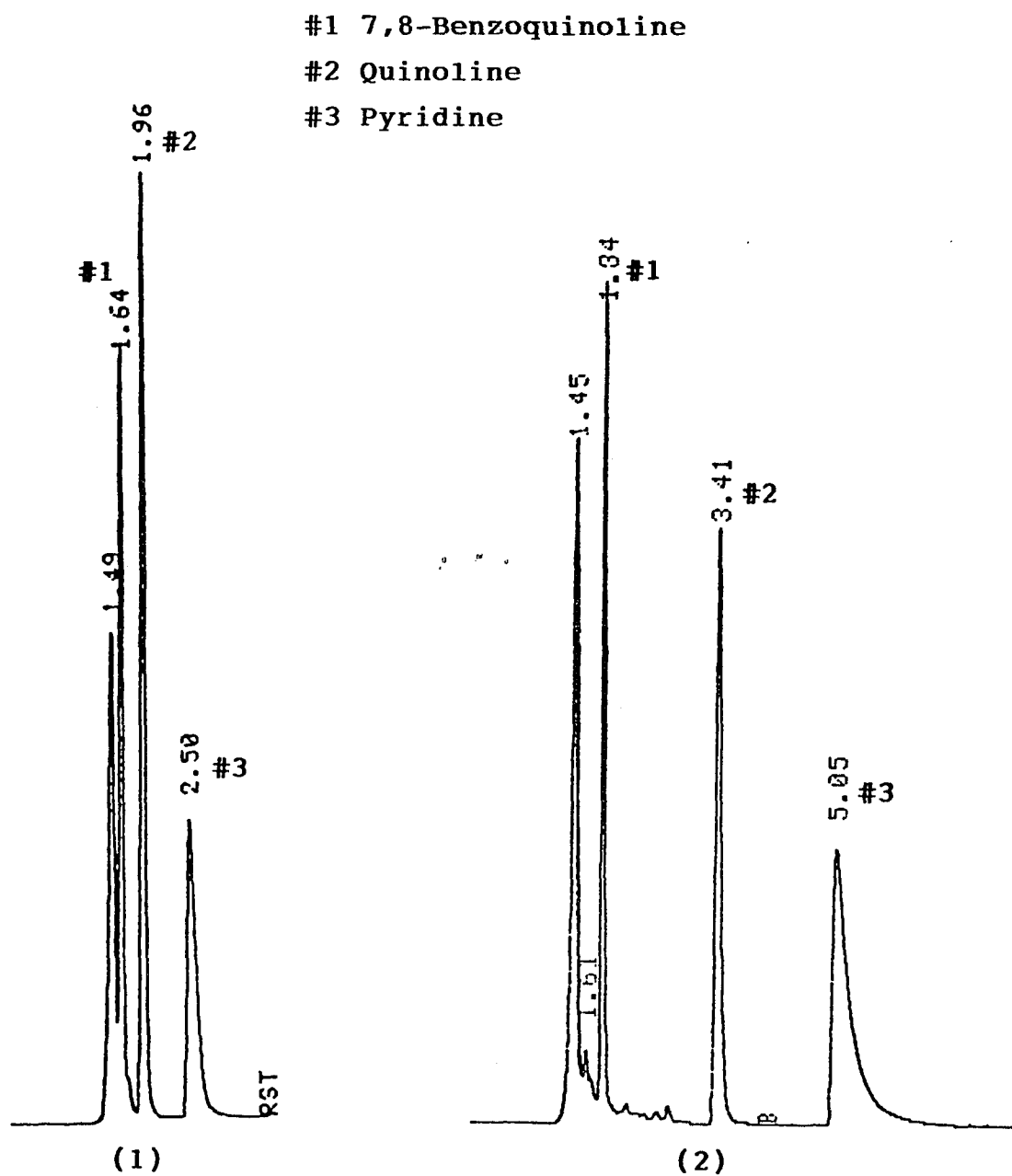


Figure II-8. Chromatograms of Pyridine, Quinoline and 7,8-Benzoquinoline on End-Capped (1) and Non-Capped (2) Cyano Column With Hexane / 2-Propanol Eluent (90/10).

(2) Column Efficiency

Column efficiency is evaluated by either the theoretical plate number (N) or the height equivalent to theoretical plate (HETP). The plate number is determined from experimental data from $N = 16(t_R/W)^2$, where t_R is retention time and W is peak width at the baseline, and $HETP = L/N$, where L is the length of the column. HETP is usually the preferred measure of column efficiency because it allows comparison of the column performance between columns of different lengths. Although the plate number N is widely used as the expression of column efficiency, it is only suitable for symmetrical bands. For tailing peak, it is generally simpler to calculate the peak asymmetry factor.

A comparison of column performance characteristics before and after the on-column silylation reaction is given in Table II-6. The results indicate that the apparent column efficiency for all solutes is generally improved following end-capping of residual silanols. This is especially true for the compounds which undergo strong acid-base interactions with residual silanols, such as pyridine, quinoline and aniline. From the

Table II-6. Column Efficiency of End-Capped (#1) and Non-Capped (#2) Ultrasphere-CN Columns With Hexane / Isopropanol Eluent(99/1)

<u>Compound</u>	<u>Column</u>	<u>k'</u>	<u>N</u>	<u>HETP</u>	<u>As</u>
pyridine*	#1	0.72	1276	0.20	1.4
	#2	2.48	1133	0.22	4.6
quinoline	#1	0.65	2304	0.11	1.3
	#2	4.70	762	0.33	4.5
aniline	#1	0.50	3379	0.07	1.0
	#2	2.14	1920	0.13	1.7
4-chloroaniline	#1	0.79	3906	0.06	1.0
	#2	3.70	3675	0.07	1.7
phenol	#1	1.10	4762	0.05	1.0
	#2	2.08	3745	0.07	1.2
o-dinitrobenzene	#1	1.46	5097	0.05	1.0
	#2	3.29	4807	0.06	1.0
benzophenone	#1	0.27	5717	0.05	1.0
	#2	0.38	4444	0.06	1.0
benzene	#1	0.05	3697	0.06	1.0
	#2	0.08	3259	0.07	1.0

* The retention data used for pyridine is obtained with hexane/isopropanol (90/10) mobile phase.

values of the peak asymmetry factor for various compounds in Table II-6, it is confirmed that the peak tailing (asymmetry) is largely diminished by reducing the amount of residual silanols. Again, the dramatic changes in peak shape indicates strong interaction with free silanols.

On the other hand, the apparent column efficiencies of phenol, dinitrobenzene, benzophenone and benzene show a little improvement with end-capping. This can be rationalized in terms of the specific molecular interactions between the exposed silanols and the solute molecules. As discussed earlier, the acid-base interaction between anilines, pyridine and other basic solutes and the acidic free silanols should be stronger than the interaction between phenol and silanols because phenol also has an acidic character. A dipole-dipole interaction and a π -electron transfer interaction is assumed to account for the retention of dinitrobenzene and benzene, respectively. These interactions are much weaker than acid-base interaction. Therefore, the retention time is reduced for all these compounds after the free silanols are eliminated but the column efficiency and the peak asymmetry factors are relatively

unchanged. This phenomenon can be further accounted for in terms of the relative capacity factors as follows.

Relative capacity factors, calculated from $(k'_{un} - k'_{end}) / k'_{end}$, are listed in Table II-7 for 99/1 Hexane/IPA. The relative capacity factor is a measure of the strength of interaction of a solute molecule with the silanol groups. The larger the value of the relative capacity factor, the greater the interaction with the solute. The values for pyridine, quinoline and anilines are larger while those values for ketones and PAHs are smaller. As expected, pyridine, quinoline and anilines exhibit stronger interactions with silanols, and PAHs weaker interactions. The relative capacity factor can be used as a parameter to estimate the peak tailing or asymmetry factor associated with the effect of free silanols. A larger value of the relative capacity factor corresponds to a larger peak asymmetry factor for the non-capped column.

(3) Column Selectivity

The selectivity of a column is a function of the thermodynamics of the retention process. For chemically bonded-phase columns, the selectivity usually depends on

Table II-7. Relative Capacity Factor On Un-Capped & End-Capped Cyano Columns (99% HEX / 1% IPA)

Compound	$(k'_{un} - k'_{end}) / k'_{end}^*$
Pyridine	5.78
Quinoline	6.23
4-Chloroaniline	3.68
Aniline	3.26
4-CF ₃ -aniline	3.08
4-Nitrophenol	0.92
Phenol	0.90
4-Cyanophenol	0.97
4-Chlorophenol	1.18
o-Dinitrobenzene	1.25
m-Dinitrobenzene	1.47
Acetone	0.62
Acetophenone	0.74
Cyclohexane	0.73
Benzene	0.33
Naphthalene	0.14
Anthracene	0.33

*: k'_{un} -- capacity factor on non-capped column

k'_{end} -- capacity factor on end-capped column

three factors: change of mobile phase solvent, change of stationary phase and change of temperature. Different types of stationary phase have different functional groups, which show different selectivities. Change of mobile phase can greatly change the column selectivity, especially for normal bonded-phase columns. Temperature is also an important parameter which can alter the column selectivity. These separation variables are discussed in detail in Chapter III. However, for a given propylcyano column with a given mobile phase system at constant room temperature, the presence of the exposed residual silanols is a major factor affecting the column selectivity.

To study the influence of residual silanols on column performance and selectivity, a series of phenols and anilines was chromatographed on both the end-capped and non-capped Ultrasphere-CN columns. The k' values are listed in Table II-8. The acidic phenols should interact with the lone-pair electrons on the nitrile group and, thus, elute later than anilines which interact less strongly via hydrogen bonding between an amine hydrogen and the lone-pair electrons on the cyano stationary phase. However, this is not observed for the non-capped cyano column. The retention times of all

Table II-8. k' Values of Phenols and Anilines on End-Capped and Non-Capped Ultrasphere-CN Column Using Hexane/Isopropanol Eluent (99/1).

Compound	Non-Capped	End-Capped
4-Methoxyphenol	4.69	1.63
4-Methoxyaniline	7.36	1.07
4-CF ₃ -Phenol	3.14	1.46
4-CF ₃ -Aniline	3.96	0.96
4-Chlorophenol	2.92	1.36
4-Chloroaniline	3.70	0.79
Phenol	2.08	1.10
Aniline	2.13	0.50
4-t-Butylphenol	0.41	0.75
4-t-Butylaniline	0.75	0.47

anilines on the non-capped column are greater than those of the corresponding phenols. Thus, for the non-capped column, the accessible residual silanols are the major functional group contributing to retention. Figure II-9 depicts the interactions {1} between the phenolic hydroxyl hydrogen and the lone-pair electrons on cyano nitrogen, which is stronger than the interaction {2} between the hydrogen on the amine group and the lone-pair electrons. In contrast, the interaction {3} of aniline with silanols is stronger than the interaction {4} of phenol with silanols. Therefore, both types of functional groups, cyano and residual silanol, contribute to the retentivity. The column selectivity is dependent on which factor predominates. Anilines elute later than phenols on non-capped cyano column because of the presence of residual silanols.

If the exposed residual silanols are eliminated by end-capping, the cyano groups become the major retention sites. The end-capped k' values in Table II-8 illustrate that all phenols elute after the corresponding anilines, as expected.

A reversal of separation of phenol and aniline between the non-capped (Figure II-10) and the end-capped cyano phase (Figure II-11) is observed. The mobile

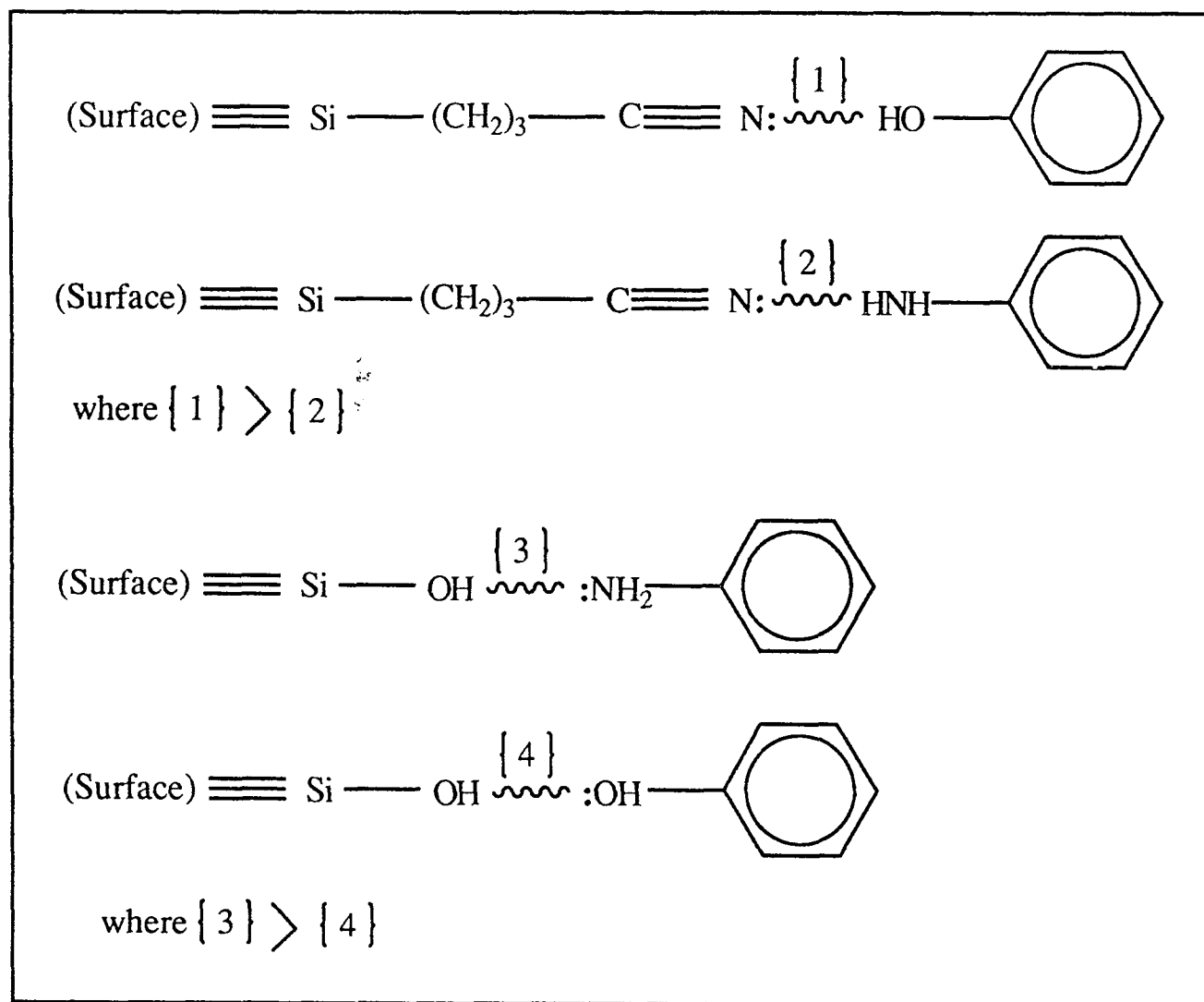


Figure II-9. Proposed molecular interactions between the Non-Capped Cyano Surface and Solutes

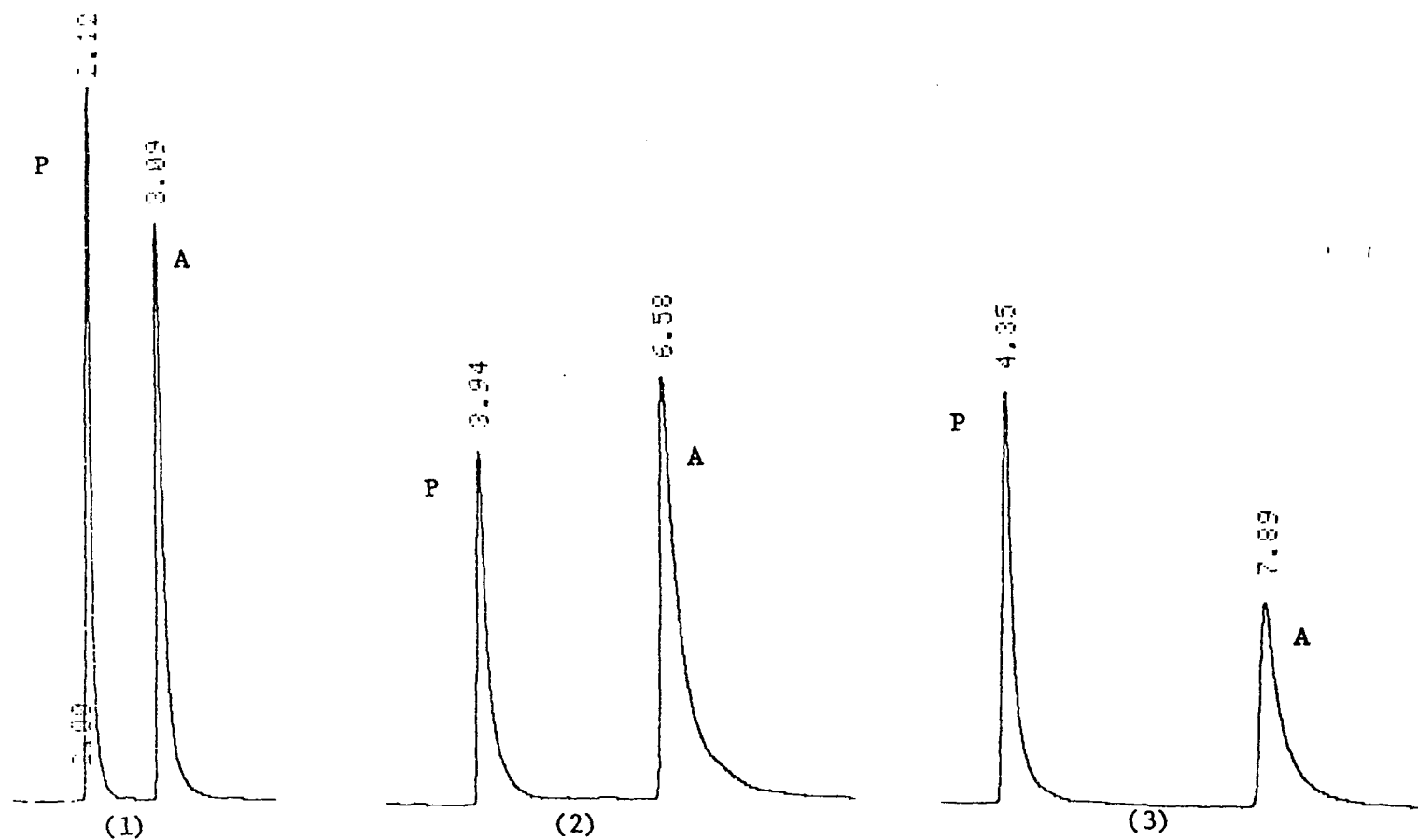


Figure II-10. Separations of Phenol (P) and Aniline (A) on Non-Capped Cyano Column With Hexane/Ether Eluent: (1) 70/30, (2) 88/12 and (3) 90/10.

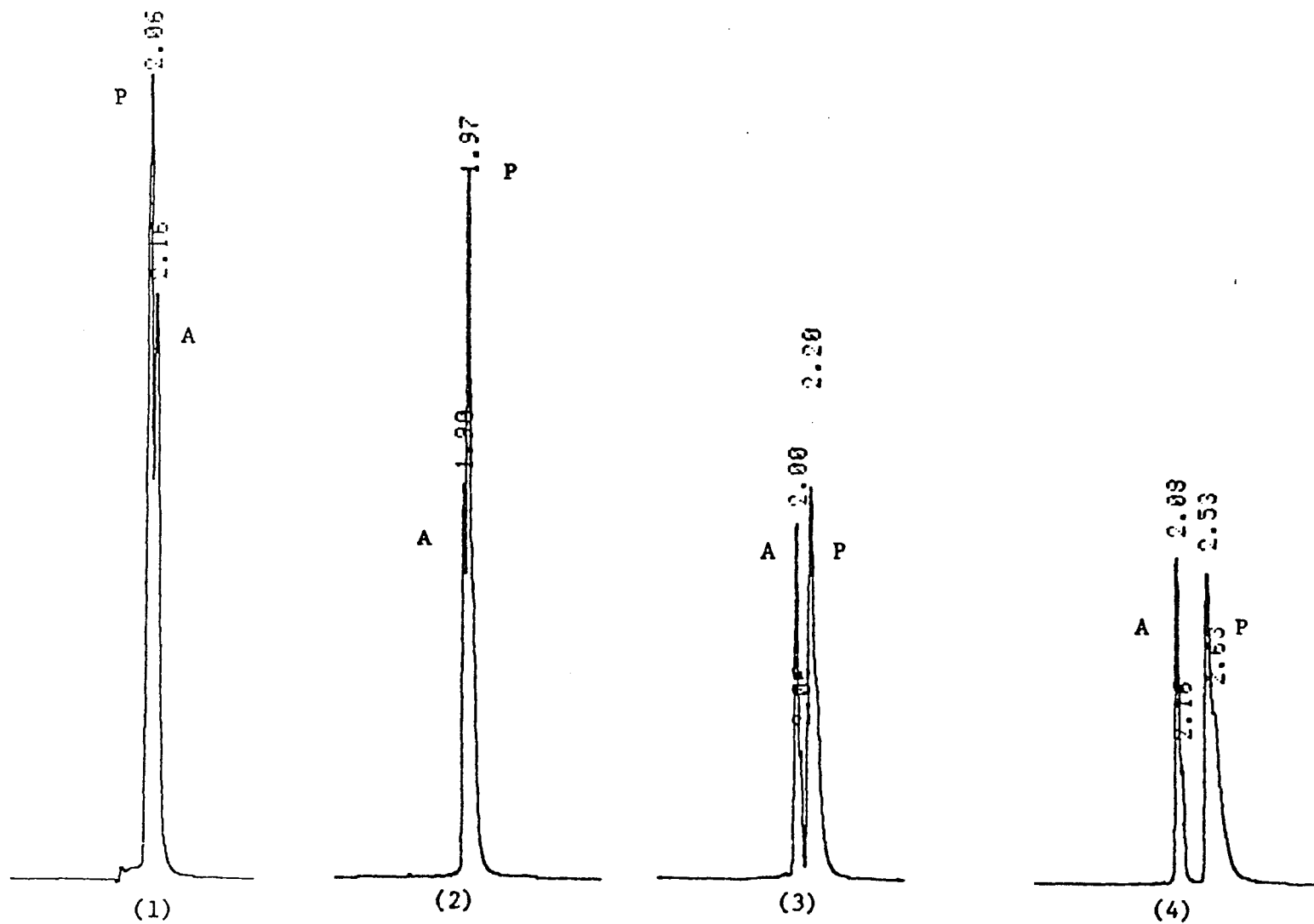


Figure II-11. Separations of Phenol (P) and Aniline (A) on End-Capped Cyano Column With Hexane/Ether Eluent (1) 60/40, (2) 88/12, (3) 93/7 and (4) 95/5.

phase used for the separation is hexane/diethylether of various compositions, 70/30, 88/12 and 90/10. The selection of ether as the polar mobile phase is to show the secondary effect of solvent on retention. As a polar solvent modifier, ether has a greater interaction with silanols. On the non-capped cyano column, as seen in Figure II-10, aniline is retained longer than phenol at any mobile phase composition. Furthermore, the phenol and aniline peaks get closer as the ether composition increases. This is the secondary solvent effect: ether at sufficiently high concentrations can largely tie up the residual silanols, and greatly decrease the retention of aniline compared with phenol.

The chromatograms in Figure II-11 show the separation of phenol and aniline on the end-capped cyano column with the mobile phase solvent of hexane/ether at the compositions 60/40, 70/30, 93/7 and 95/5. Reversal of retention is again obtained. At low ether concentrations, aniline is less retained than phenol. Apparently, this is caused by the weaker interaction of aniline than phenol with cyano groups. At ether concentrations greater than 30%, the retention of aniline becomes longer. This is unexpected based on the above discussion. In fact, at higher concentrations of

ether there is another factor involved in retention. That is, the mobile phase acid-base interactions of ether and phenol make phenol elute sooner, so aniline is retained relatively longer.

To compare the result with the commercially end-capped cyano column of Keystone Hypersil-CN, Figure II-12 shows the separation of phenol and aniline on the end-capped Hypersil-CN column with the same hexane/ether solvent. The result is similar to that obtained from our laboratory - end-capped cyano column. Further discussion of column performance and retention behavior on Keystone Hypersil-cyano and Hypersil-amino columns is given in Chapter III.

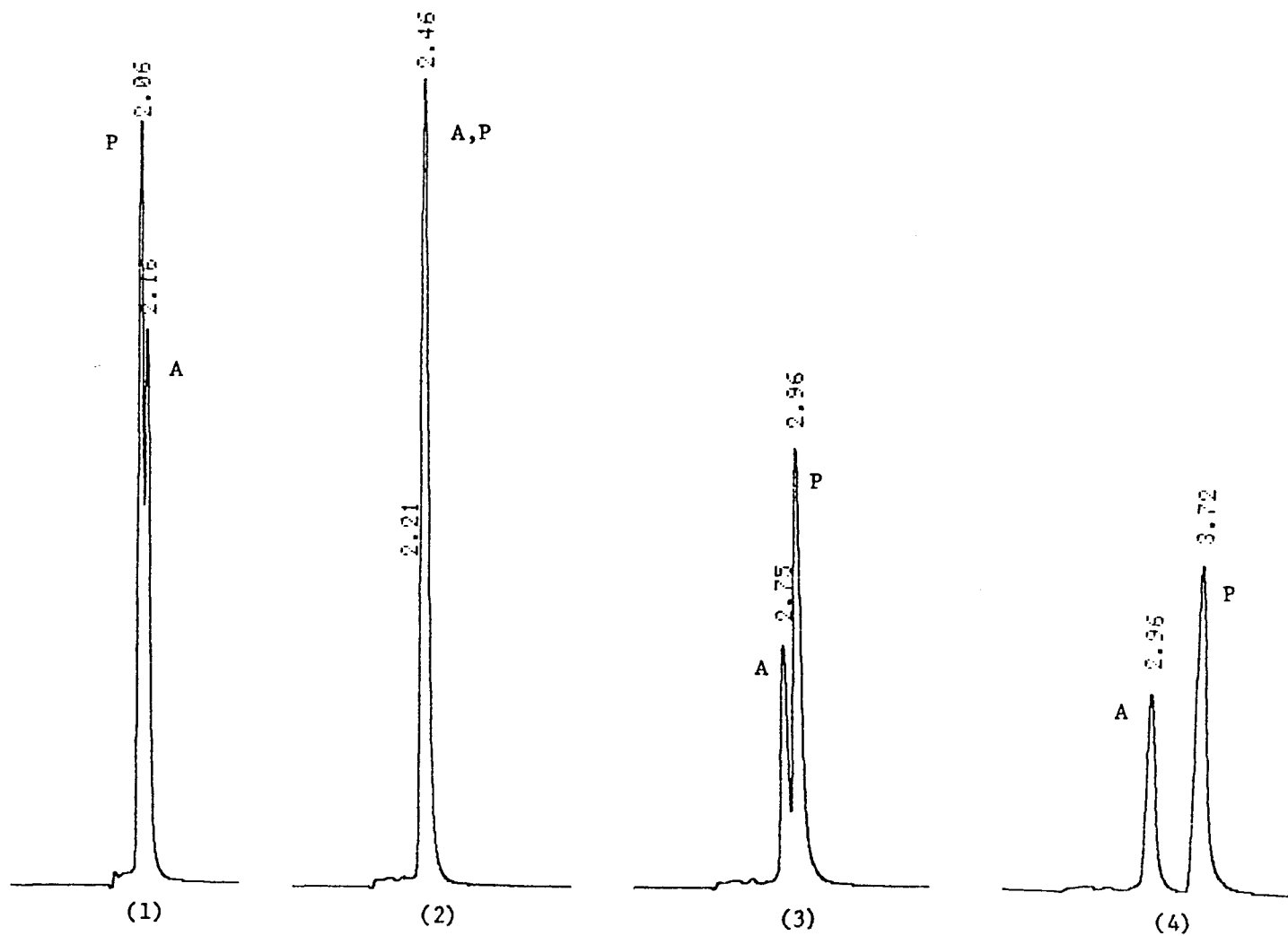


Figure II-12. Separations of PHenol (P) and Aniline (A) on Keytone Hypersil - CN Column With Hexane / Ether Eluent :
 (1) 60/40, (2) 78/22, (3) 88/12 and (4) 93/7.

II-3. Summary

The presence of residual silanol groups on non-capped cyano bonded-phase columns contributes to retentivity and selectivity for normal bonded-phase liquid chromatography. Moreover, the existence of polar residual silanols acting as strong adsorption sites confuses the interpretation of chromatographic data and prediction of chromatographic performance of normal bonded-phase packings.

The residual silanol groups, however, can be effectively eliminated by an on - column end-capping reaction with a silylation reagent --- BSA (N,O-bis(trimethylsilyl)acetamide). On the basis of our experimental observations by comparing the non-capped and end-capped columns, the results indicate that the residual silanols provide a positive contribution to retention time for all compounds and the apparent column efficiency for most of compound is genenally improved following end-capping of residual silanols. These are especially true for the compounds which have strong interaction with silanols. Furthermore, a retention reversal of phenol and aniline on non-capped and end-capped cyano columns was observed. Thus, the elimination

of residual silanol groups significantly alters the column selectivity and retention behavior.

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CHAPTER III

STUDY OF MECHANISM OF RETENTION AND SEPARATION ON CYANO AND AMINO NORMAL BONDED PHASE COLUMNS

III-1. Retention Variables On Normal Bonded - Phase Liquid Chromatography

In order to characterize the retention behavior and selectivity of chemically bonded phases in HPLC, it is necessary to understand the factors governing retention on normal bonded-phases. Based on our observations, the retention behavior is determined by five main factors: mobile phase composition, functional group on stationary phase, nature of solute, amount of residual silanols, and temperature.

Generally, the variation of retention in liquid chromatographic separations depends upon the free energy change of the solute in partitioning from the mobile phase to the stationary phase. Thermodynamically, this

is described by the van't Hoff equation:

$$\ln k' = - \Delta H^\circ / RT + \text{const} \quad (1)$$

The capacity factor, k' , is proportional to the distribution coefficient K , $k' = \beta K$, where β is the phase ratio, the ratio of volumes of stationary phase and mobile phase. The plot of $\ln k'$ versus $1/T$ should be linear if the enthalpy of the chromatographic process is constant. Changing the column temperature may also alter the separation selectivity. Since the change of enthalpy of is much smaller in HPLC than in GC, temperature generally has less effect on retention in LC than in GC. In practice, most LC separations are carried out at temperatures less than 60°C, usually ambient.

In normal bonded-phase liquid chromatography, as mentioned above, the mobile phase is usually a binary mixture which consists of a non-polar solvent and a polar solvent modifier. The mobile phase composition is the most important factor in sample retention and separation behavior, as discussed by Snyder [1-7] for liquid-solid chromatography. There are a number of chromatographic workers who investigated the effect of

mobile phase composition in normal bonded-phase chromatography on column performance [8-14], as reviewed in Chapter I. Further discussion will also be given in Section III-2.

III-2. Experimental Section

(1) Apparatus

The liquid chromatograph used was a Perkin-Elmer Series 400 HPLC equipped with a dual reciprocating pump, a Rheodyne model 7125 sample valve with a 6 ul fixed sample loop and a Perkin - Elmer LC 90 UV spectrophotometric detector. All data were obtained on a Perkin - Elmer LCI-100 computing integrator.

(2) Columns

The Hypersil-CN and Hypersil-NH₂ columns used in this part of study were purchased from Keystone Scientific Inc. (State College, PA). According to the manufacturer, the 25 cm x 4.6 mm i.d columns were packed with 5 um particles with a size distribution such that 90% were within ± 1.5 um. The pore size of particles is 120 ± 15 Å. The specific surface area of the Hypersil silica particles is $170 \text{ m}^2/\text{g} \pm 10 \text{ m}^2/\text{g}$. The percentage carbon loading of Hypersil - CN is 5% and of Hypersil-NH₂ is 10%. Hypersil - CN was end - capped by the manufacturer using trimethylchlorosilane (TMCS). The

Hypersil - NH₂ had 20 - 30 Å cross linked polymeric coating. Both had very low residual silanol activity based on chromatographic performance.

(3) Reagents and Standards

All standards were purchased from Aldrich Chemical Co. (Milwaukee, WI) and, being at least 97% pure, were used without further purification. Mobile phase solvents were HPLC grade n-hexane, n-heptane, chloroform, 2-propanol, methylene chloride, acetonitrile, methanol and ethyl acetate were purchased from J.T. Baker (Philipsburg, NJ). High purity distilled water used in reversed phase eluents was obtained from a Corning Mega-PureTM water purification system. Most of the standard solutions were prepared in 80% hexane / 20% 2-propanol. For insoluble compounds, other solvent compositions were used. The concentration of each standard was about 0.1 mg/ml - 1.0 mg/ml.

(4) Chromatographic Systems Studied

1. Normal - Phase Mode

The Keystone Hypersil-CN and Hypersil-NH₂ columns

with hexane/2-propanol mobile phase of various compositions and other normal phase solvent systems were all studied at a flow rate of 2.0 ml/min.

2. Reversed - Phase Mode

The Keystone Hypersil -CN and Hypersil -NH₂ with acetonitrile/water mobile phase of various compositions were studied at a flow rate of 2.0 ml/min.

(5) Chromatographic Procedures

Before the collection of experimental data, the columns as received from Keystone were washed with 1ml/min of 2-propanol for 6 hours. This washing process removes residues. All retention data was obtained using injections of 6.0 ul of the standard solutions into the chromatograph. The capacity factor k' was calculated from $k' = (t_R - t_0) / t_0$, where t_R is the peak maximum retention time and t_0 is the column dead time. The t_0 was assumed to equal the retention time of n-nonane as non-retained sample for the normal phase systems and of methanol as non-retained sample for the reverse phase systems. Both n-nonane and methanol are not UV-adsorbing compounds. t_0 measurements on based on the pseudopeaks of n-nonane and methanol due to the

refraction effects that affect the detection signal.

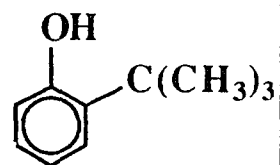
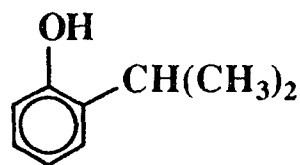
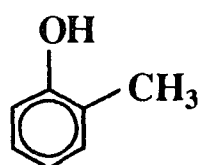
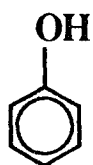
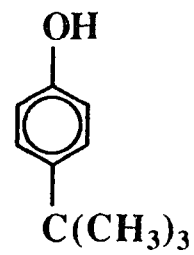
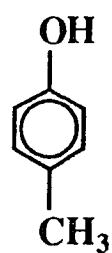
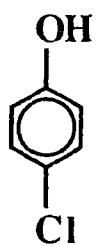
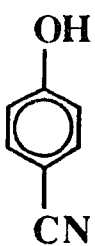
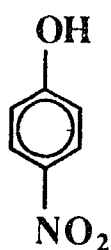
III-3. Dependence of retention on the Structure of Solute Molecules

To investigate the specific molecular interactions between solute molecules and the stationary phase functional groups, compounds with different functional groups were selected for study, as illustrated in Figures III-1 and III-2. A discussion of the different possible interactions associated with hydrogen bonding (acid-base interaction), dipole-interactions, and charge transfer between the solute molecules and the polar terminal groups is given in the following.

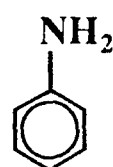
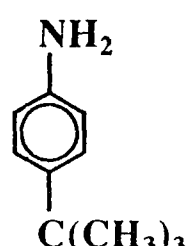
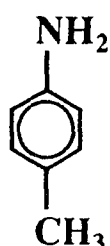
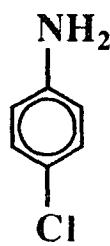
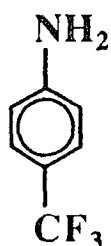
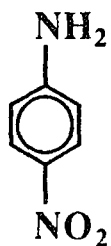
III-3-1. Hydrogen Bonding

Phenols and anilines are the two most important groups for study because phenols are weakly acidic and anilines are weakly basic. The specific molecular interactions between phenols and anilines and cyano- and amino- packings can be associated with hydrogen bonding between the polar packing surface and solute molecules, which might be considered an acid-base interaction. The possible hydrogen-bonding interactions of phenols and anilines on cyano and amino bonded-phase column are

[1] PHENOLS



[2] ANILINES



[3] SUBSTITUTED BENZENES

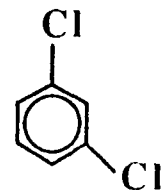
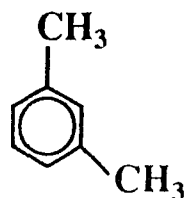
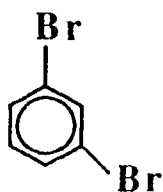
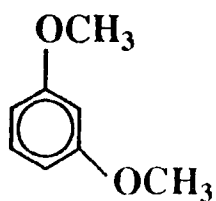
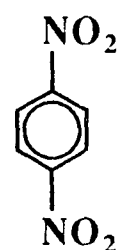
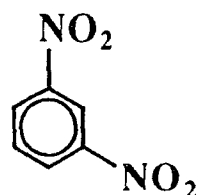
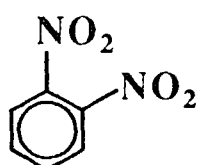
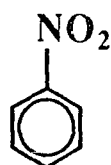
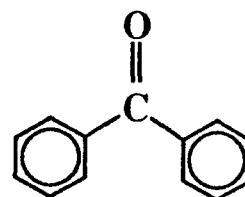
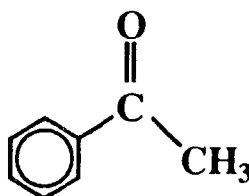
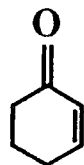
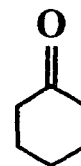
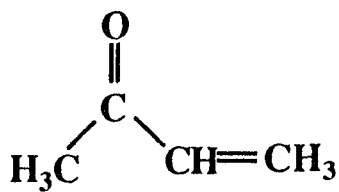
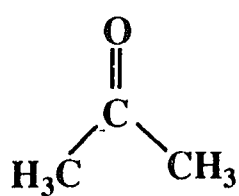
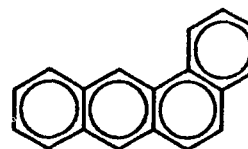
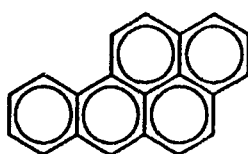
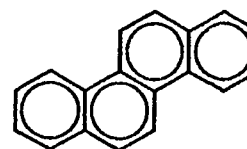
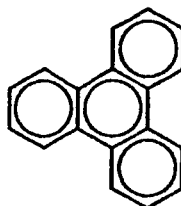
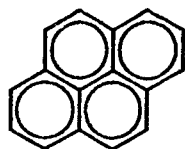
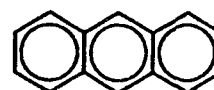


Figure III-1. Structures of Organic Compounds (1)

[4] KETONES



[5] PAHs



[6] AZAARENES

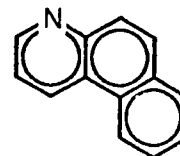
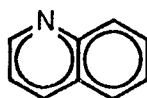


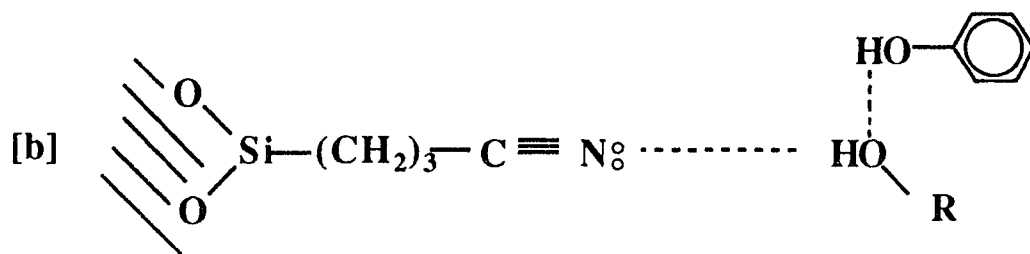
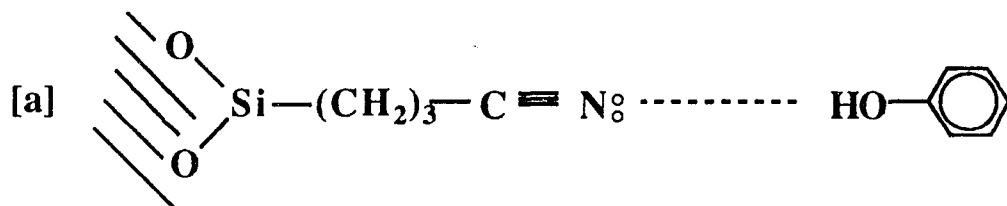
Figure III-2. Structures of Various Organic Compounds (2)

illustrated in Figures III-3 and III-4. It is clear that the hydrogen-bonding interaction on the amino phase (Fig. III-4) is more complicated than that on cyano phase (Fig. III-3) since more than one possible hydrogen-bonding site is available on amino phase.

The k' values of a series of substituted phenols on the cyano column are listed in Table III-1. On the cyano column, the acidic phenols can interact with the lone-pair electrons on the nitrile group. It is expected that the stronger the acidity of solutes, the stronger the interaction. This is seen in the data in Table III-1. Phenols substituted with electron-withdrawing groups ($-\text{NO}_2$, $-\text{CN}$, $-\text{Cl}$) or electron-donating groups ($-\text{CH}_3$, $-\text{C}(\text{CH}_3)_3$), which respectively increase and decrease the phenol acidity, have correspondingly higher and lower retention times in comparison to phenol itself. Thus, 4-nitrophenol ($\text{p}K_a = 7.14$) is more strongly retained than phenol ($\text{p}K_a = 10.85$) and 4-t-butylphenol ($\text{p}K_a = 11.59$) is less retained than phenol.

Hydrogen bonding or acid-base interaction also occurs with anilines, although the interaction is different from that of the phenols because the anilines are weak bases. The NH_2 group can hydrogen bond to the

PHENOL



ANILINE

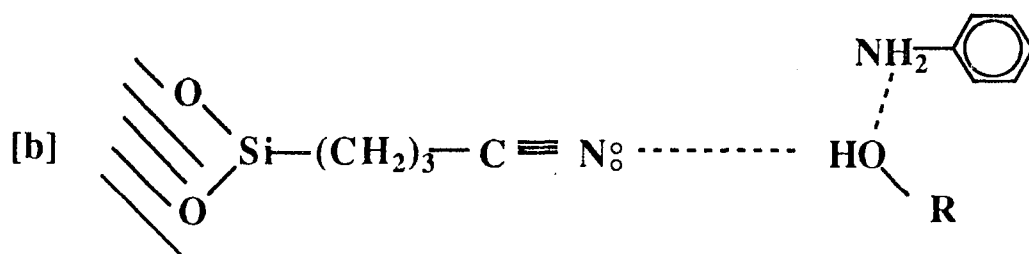
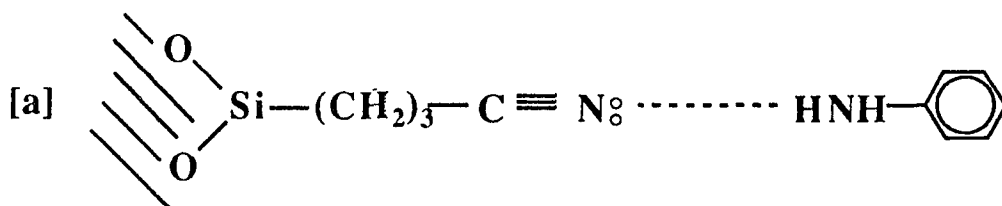


Figure III-3. Suggested major Hydrogen-Bonding Interactions of Phenol and Aniline with Cyano Stationary Phase

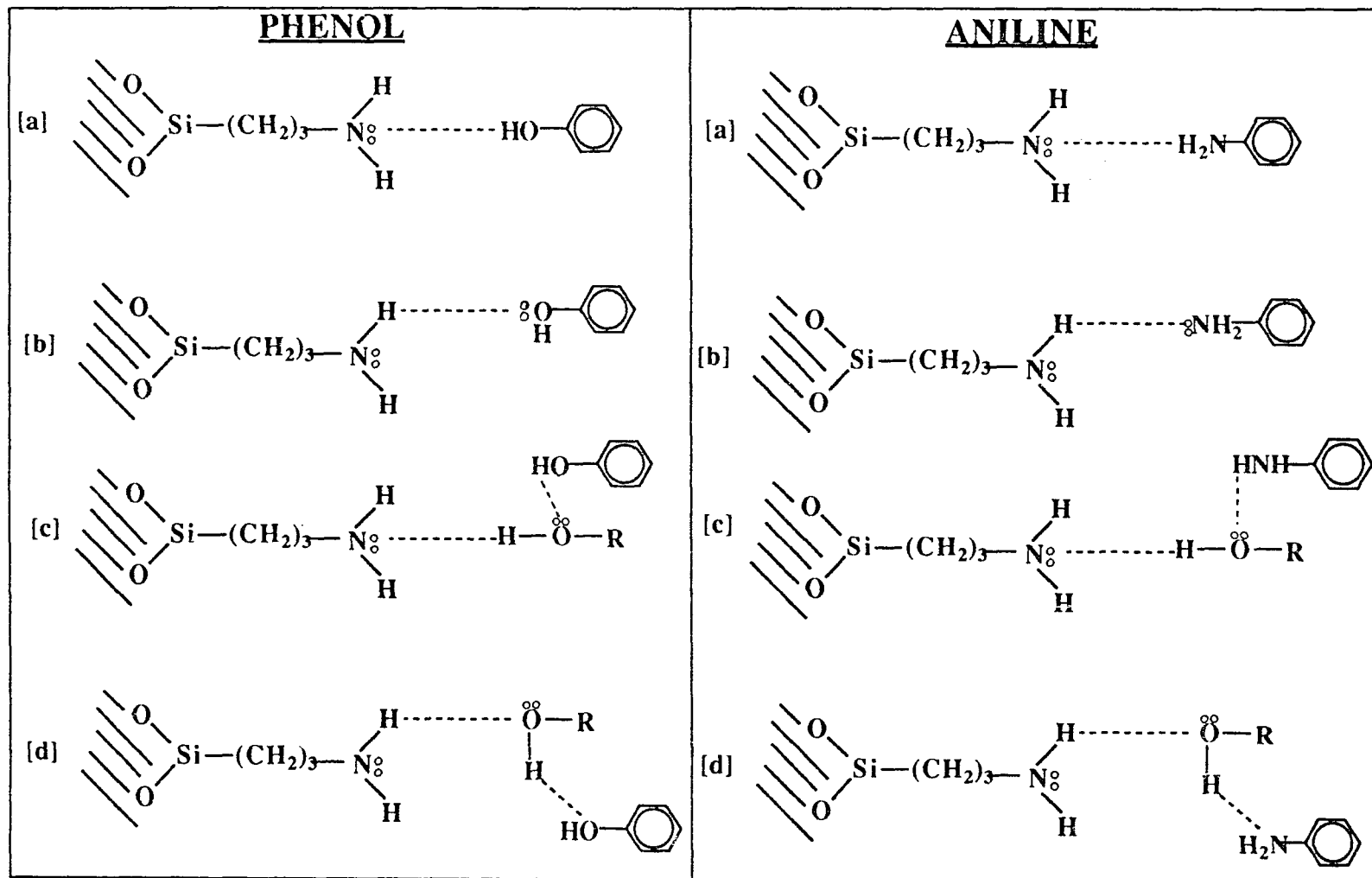


Figure III-4. The Suggested Major Hydrogen Bonding Interactions of Phenol and Aniline with Amino Stationary Phase

Table III-1. k' Values of Substituted Phenols on
Hypersil - CN Column With Hexane/2-
Propanol Mobile Phase at 2.0 ml/min.

<u>Compound</u>	<u>pK_a</u>	<u>k' (HEX/IPA)</u>			
		<u>80/20</u>	<u>90/10</u>	<u>95/5</u>	<u>99/1</u>
4-nitrophenol	7.14	1.43	3.33	4.83	11.90
4-cyanophenol	7.95	1.29	2.12	3.62	7.09
4-chlorophenol	9.38	0.52	1.09	1.44	4.36
phenol	10.85	0.51	0.77	1.12	3.23
4-t-butylphenol	11.59	0.39	0.64	0.94	2.79

lone-pair electrons on nitrile groups. As expected, the stronger the basicity of anilines, the weaker the interaction. As the aniline is substituted with electron - withdrawing groups or electron -donating groups, the basicity will decrease and increase, respectively. This is seen by comparing the pK_b and k' values in Table III-2. For example, 4-nitroaniline ($pK_b = 12.76$) is less basic and more strongly retained than 4-t-butylaniline.

The same two groups of compounds were also chromatographed on the Hypersil-NH₂ column. On the amino column, the acidic phenols interact more strongly with the electron-rich, basic -NH₂ groups than the basic anilines. Again, the more acidic phenols have the longer retention time, and the more basic the aniline the shorter the retention time, as demonstrated in Table III-3 and III-4.

In all these cases, the amount of polar solvent (2-propanol) greatly affects the sample retention. The larger the amount of polar solvent, the less the retention of the solute. As seen in Tables III-1 - III-4, the retention time for all compounds is largely decreased with increasing the concentration of 2-propanol from 1% to 20%. Further discussion of the role

Table III-2. k' Values of Substituted Anilines on
Hypersil - CN Column With Hexane/2-
Propanol Mobile Phase at 2.0 ml/min.

<u>Compound</u>	<u>pK_b</u>	<u>k' (HEX/IPA)</u>			
		<u>80/20</u>	<u>90/10</u>	<u>95/5</u>	<u>99/1</u>
4-nitroaniline	12.76	3.61	9.80	18.17	—
4-trifluoromethyl -aniline	11.43	0.86	1.44	1.93	2.67
4-chloroaniline	10.19	0.77	0.83	1.33	1.91
aniline	9.37	0.64	0.80	0.99	1.19
4-t-butylaniline	9.35	0.58	0.72	0.94	1.04

Table III-3. k' Values of Substituted Phenols on
Hypersil - NH_2 Column With Hexane/2-
Propanol Mobile Phase at 2.0 ml/min

<u>Compound</u>	<u>pK_a</u>	<u>k' (HEX/IPA)</u>					
		<u>70/30</u>	<u>80/20</u>	<u>90/10</u>	<u>95/5</u>	<u>97/3</u>	<u>99/1</u>
4-nitrophenol	7.14	25.31	-	-	-	-	-
4-cyanophenol	7.95	18.41	26.21	-	-	-	-
4-chlorophenol	9.38	0.89	1.43	3.29	6.79	12.60	18.47
phenol	10.85	0.88	1.37	3.02	5.86	10.89	11.36
4-methylphenol	11.14	0.59	0.90	1.96	3.90	6.87	10.21
4-t-butylphenol	11.59	0.47	0.74	1.72	3.39	5.91	9.92

Table III-4. k' Values of Substituted Anilines on
Hypersil - NH₂ Column With Hexane/2-
Propanol Mobile Phase at 2.0 ml/min.

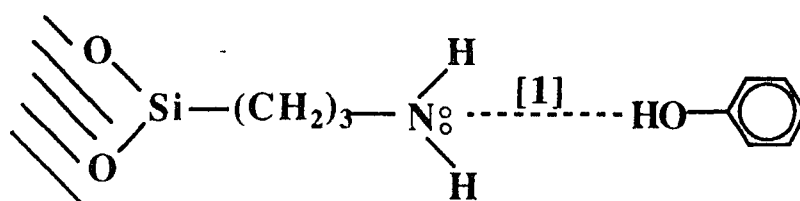
<u>Compound</u>	<u>pK_a</u>	<u>k' (HEX/IPA)</u>			
		<u>80/20</u>	<u>90/10</u>	<u>95/5</u>	<u>97/3</u>
4-nitroaniline	12.76	25.69	43.97	-	-
4-trifloroaniline	11.43	6.67	9.96	11.17	13.87
4-chloroaniline	10.19	3.31	7.00	9.98	11.05
aniline	9.37	2.18	3.52	4.75	5.97
4-t-butylaniline	9.35	1.40	2.30	3.86	4.47

of the mobile phase solvent on solute retention in normal phase system is given in Section III-4.

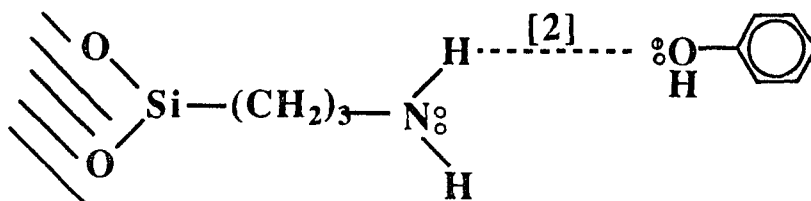
It should be noted that since hydrogen bonding between $\text{-OH} \cdots \text{:NH}_2\text{-}$ is generally stronger than that of $\text{-HNH} \cdots \text{:NH}_2\text{-}$, one might expect that phenols should elute later than the corresponding anilines. However, this is true only at small percentages of the polar solvent, 2-propanol. Comparing k' values in Tables III-3 and III-4, we find that at concentrations of 2-propanol less than about 5%, phenols elute later than anilines; at higher concentration of 2-propanol, elution order reverses.

This observation can be rationalized as follows. When the concentration of 2-propanol is less than 5%, the predominant molecular interaction is hydrogen bonding between the stationary phase -NH_2 and solute molecules (see Figure III-5). Presumably interaction [1] is stronger than [1'] since the hydrogen on phenol is more acidic than that on the amino group. Thus, phenol is retained longer than aniline as discussed above. As the concentration of 2-propanol is increased, however, the surface of the stationary phase becomes covered with 2-propanol molecules because of the strong interaction of the polar solvent with the amino groups

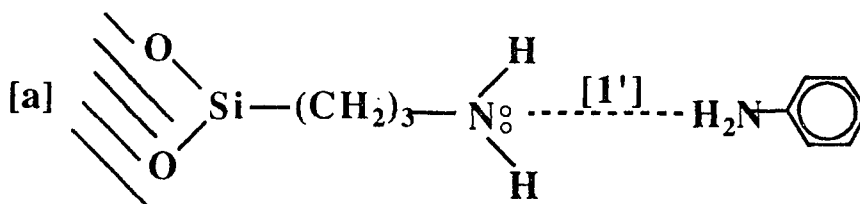
PHENOL



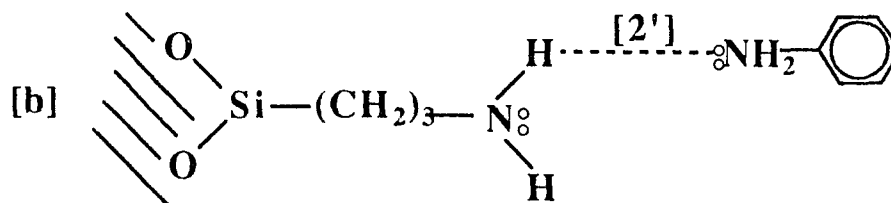
$$[1] > [2]$$



ANILINE



$$[1'] \approx [2']$$



$$[1] > [1']$$

Concentration of 2-propanol < 5 %

Figure III-5. Suggested Molecular Interactions of Phenol and Aniline with Amino Functional Group at Low Concentration of 2-propanol

($\text{H}_2\text{N}:$... $\text{HO}-\text{R}$, HNH ... $:\text{OH}-\text{R}$). Now the interaction of solute molecules with the stationary phase is through a surface layer of polar solvent. It is shown in Figure III-6 that at intermediate concentrations of polar solvent (2-propanol), hydrogen-bonding interactions take place between the surface layer of 2-propanol and lone-pair electrons of the amino group of aniline (interaction (4')) and the surface layer and the lone-pair electrons of the hydroxyl group of phenol (interaction (4)). It is assumed that the interaction (4') is stronger than (4) because the Lewis base $-\text{H}_2\text{N}:$ on aniline is stronger than that of $-\text{HO}:$ on phenol. This predicts that aniline is retained longer than phenol. However, if the polar solvent is present at high concentration, the stationary phase becomes covered with a layer of polar solvent, into which the solute molecules partition. It is our belief that this interaction mechanism can change with a change of mobile phase polarity; and the exact process should depend on several factors: (1) the solute structure, (2) the % polar solvent, (3) the solubility of solute in the layer of stationary phase, (4) the solubility of solvent in the layer of stationary phase and (5) the solubility of solute in bulk mobile phase.

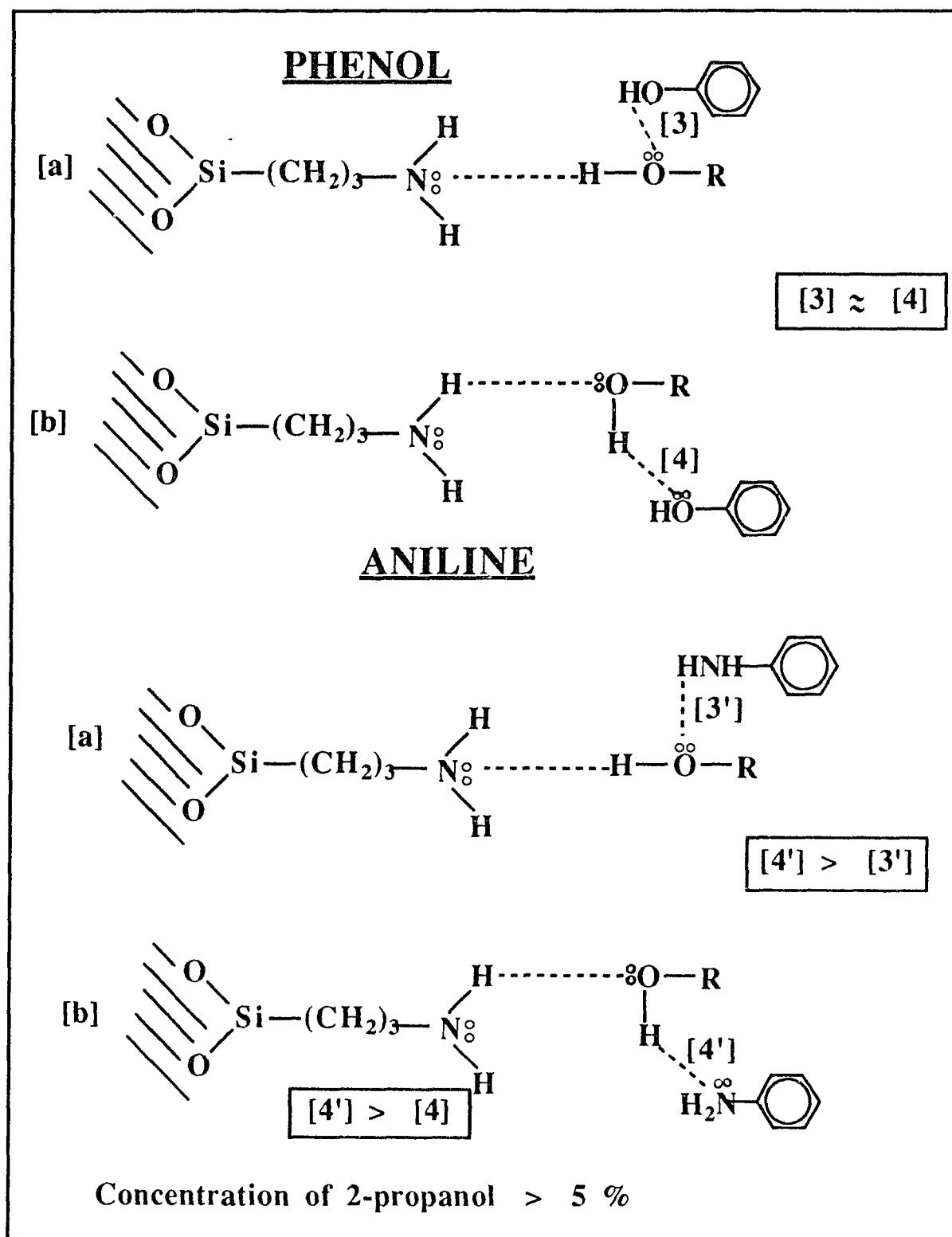


Figure III-6. Suggested Molecular Interactions of Phenol and Aniline with Amino Functional Group at High Concentration of 2-propanol

To further demonstrate the occurrence of specific hydrogen bonding molecular interactions on cyano and amino columns, a group of ortho - substituted phenols with different degrees of steric hindrance was chromatographed on these two columns. Table III-5 lists the k' value on the cyano column with various mobile phase compositions. The retention of 2-substituted phenols decreases relative to phenol itself as the phenolic group becomes progressively shielded. As expected, the order of increasing shielding, $C(CH_3)_3 > -CH(CH_3)_2 > -CH_2CH_3 > -CH_3$, is the reverse order of retention. In addition, the effect of electron-donation also decreases the retention of phenols since alkyl phenols are less acidic than phenol itself. Thus, the combination of shielding and electron-donating decreases the retention of substituted phenols.

The effect of steric hindrance of ortho-substituted phenols on the amino column is demonstrated in Table III-6. The elution order of these compounds on the amino column is the same as on the nitrile column, for the same reason.

Table III-5. k' Values of Alkylated Phenols on Hypersil-CN Column with Hexane / 2-propanol Mobile Phase at 2.0 ml/min

<u>Compound</u>	k' (Hex/IPA)			
	<u>80/20</u>	<u>90/10</u>	<u>95/5</u>	<u>99/1</u>
Phenol	0.51	0.77	1.21	3.23
O-cresol	0.43	0.67	0.97	2.09
2-ethyl-phenol	0.41	0.64	0.93	1.96
2-isopropyl phenol	0.38	0.59	0.86	1.72
2-t-butyl-phenol	0.38	0.47	0.75	1.12

Table III-6. k' Values of Alkylated Phenols on Hypersil-NH₂ Column with Hexane / 2-propanol Mobile Phase at 2.0 ml/min

<u>Compound</u>	<u>k' (Hex/IPA)</u>			
	<u>80/20</u>	<u>90/10</u>	<u>95/5</u>	<u>99/1</u>
Phenol	1.37	3.02	5.86	11.36
O-cresol	1.91	3.29	7.49	8.57
2-ethyl-phenol	1.57	2.96	6.40	7.20
2-isopropyl phenol	1.39	2.58	5.03	6.71
2-t-butyl-phenol	1.25	2.20	4.33	6.01

III-3-2. Dipole - Dipole Interactions

The retention of polar and moderately polar compounds on polar bonded-phase columns results from dipole - dipole interactions or dipole - induced dipole interactions, rather than hydrogen-bonding interactions experienced by phenols and anilines. These dipolar interactions occur between molecules that have a permanent or an induced dipole moment, and the polar group of the stationary phase. The strength of the dipole-dipole attraction depends on the magnitude of the dipole moment. In general, dipole-dipole attractions are weaker than hydrogen bonding interactions.

To study retention governed by dipole interactions on cyano and amino bonded-phase columns, groups of compounds with different dipole moments were selected for chromatography. Table III-7 illustrates the retention of a group of meta-di-substituted benzenes on the cyano column. The retention order of the compounds is the order of increasing dipole moment, which is m-dimethyl- < m-dibromo- < m-dimethoxy- < m-dinitro-benzene. It is most likely that the larger the value of dipole moment, the more polar the solute, and the longer the retention time.

Table III-7. k' Values of Meta - Disubstituted Benzenes on Hypersil-CN column With Hexane/2-Propanol Mobile Phase at 2.0 ml/min.

<u>Compound</u>	Dipole* moment (Debye)	k' (HEX/IPA)			
		<u>80/20</u>	<u>90/10</u>	<u>95/5</u>	<u>99/1</u>
m-dinitrobenzene	3.84	1.20	1.64	2.07	2.60
m-dimethoxybenzene	1.60	0.28	0.31	0.33	0.36
m-dibromobenzene	1.47	0.19	0.20	0.21	0.22
m-dimethylbenzene	0.37	0.17	0.16	0.17	0.18

* The values of dipole moment are from A.L. McClellan in "Tables of experimental Dipole Moments", W.H. Freeman, San Francisco, 1963.

To further investigate the dipole - dipole interaction between the solute molecules and the polar stationary phase, a group of nitrobenzenes was chromatographed on the cyano and amino columns. As can be seen from Tables III-8 and III-9, the order of increasing retention on both columns is para- < meta- < ortho-dinitrobenzene. This order is the same as the order of increasing dipole moment. Comparing the retention of nitrobenzene and p-dinitrobenzene, however, p-dinitrobenzene is retained longer than nitrobenzene. This is not what is expected on the basis of dipole moment -- p-dinitrobenzene has zero dipole moment whereas the dipole moment of nitrobenzene is 4.22. Thus, it should be pointed out that the retention order can not be interpreted simply by consideration only of the values the dipole moment. The dipole moment is a measure of the polarity of the whole molecule. Normal phase retention, however, is a function of specific functional group interaction between stationary phase and solute molecules. The dipole moment could be used as a reference parameter for solute polarity, but it is not always a useful parameter for predicting sample retention.

Table III-8. k' Values of Nitrobenzenes On Hypersil-CN
 Column with Hexane / 2-Propanol Mobile
 Phase at 2.0 ml/min

<u>Compound</u>	Dipole* moment (Debye)	k' (HEX/IPA)			
		<u>80/20</u>	<u>90/10</u>	<u>95/5</u>	<u>99/1</u>
o-dinitrobenzene	6.10	2.43	3.79	4.78	6.58
m-dinitrobenzene	3.84	1.20	1.64	2.07	2.60
p-dinitrobenzene	0.00	1.10	1.48	1.73	1.93
nitrobenzene	4.22	0.55	0.65	0.71	0.78

* The values of dipole moment are from A.L. McClellan in
 "Tables of Experimental Dipole Moments", W.H.Freeman,
 San Francisco, 1963.

Table III-9. k' Values of o-, m- and p-dinitrobenzenes
On Hypersil-NH₂ Column with Hexane / 2-
Propanol Mobile Phase at 2.0 ml/min

<u>Compound</u>	Dipole* moment (Debye)	k' (HEX/IPA)			
		<u>80/20</u>	<u>90/10</u>	<u>95/5</u>	<u>99/1</u>
O-dinitrobenzene	6.10	3.23	5.79	9.09	14.51
m-dinitrobenzene	3.84	1.04	1.59	2.27	3.03
p-dinitrobenzene	0.00	0.78	1.12	1.50	2.10
nitrobenzene	4.22	0.36	0.45	0.52	0.57

* The values of dipole moment are from A.L. McClellan in
"Tables of Experimental Dipole Moments", W.H. Freeman,
San Francisco, 1963.

III-3-3. Charge - Transfer Interactions

Retention characteristics of a series of polycyclic aromatic hydrocarbons (PAH) were studied on the cyano and amino stationary phases. According to a number of chromatographers [15-19], the predominant molecular interaction of solutes with these stationary phases is a charge transfer interaction between the functional group of stationary phase and the π -electron cloud of the PAHs. To study this interaction, a group of PAHs having different condensed ring systems was selected.

The values of the capacity factor of various PAHs chromatographed on the cyano and amino columns with the hexane/2-propanol mobile phase are listed in Tables III-10 and III-11. As can be seen, elution times increase with increasing number of aromatic rings. Benzo-[a]-pyrene (five benzene rings) eluted a little later on both columns than other PAH with fewer than five rings. The increase in retention is related to the increased density of π -electrons with increasing the ring size. The charge transfer interaction between the PAHs and cyano stationary phase can be attributed to two possible interactions: (1) the lone-pair electrons or π -electrons on nitrile group delocalize into the π^* antibonding

empty orbitals of PAHs; (2) the π -electrons on PAHs delocalize into the π^* antibonding empty orbital of nitrile group. It is assumed that the interaction (1) tends to predominate because the energy of π^* antibonding orbitals of conjugated aromatic rings is lower than that of nitrile group. For the amino stationary phase, however, the charge transfer interaction can only be due to that the lone-pair electrons on the amino group delocalizing into PAH π^* antibonding empty orbitals. With both columns, the retention should increase with increasing number of condensed rings because the number of π -electrons and π^* orbitals will increase as increasing the ring size and the energy of π^* antibonding orbitals will decrease as increasing the ring size.

It is evident from the retention data in Tables III-10 and III-11, that the retention of PAHs is weaker on amino than that on cyano column. This can be rationalized in terms of the fact that there are two possible charge transfer interactions between the PAHs and the nitrile group ($-\text{CN}:$), in contrast to the amino group (see above discussion). π -electrons on PAHs can transfer into the π^* orbital on nitrile group and π -electrons or lone-pair electrons on nitrile group can

also transfer into the π^* orbital on PAHs; this results in increased in retention.

Table III-10. k' Values of PAHs on Hypersil-NH₂ Column
with Hexane / 2-Propanol Mobile Phase at
2.0 ml/min

<u>PAH</u>	<u>RING SIZE</u>	<u>k' (HEX / IPA)</u>			
		<u>80/20</u>	<u>90/10</u>	<u>95/5</u>	<u>100/0</u>
Benzene	1	0.01	0.03	0.04	0.06
Naphthalene	2	0.08	0.09	0.11	0.16
Anthracene	3	0.16	0.19	0.23	0.29
Pyrene	4	0.24	0.28	0.33	0.44
Triphenylene	4	0.34	0.41	0.49	0.59
Benzo-[a]- Anthracene	4	0.29	0.36	0.43	0.51
Benzo-[a]- Pyrene	5	0.43	0.52	0.63	0.77

Table III-11. k' Values of PAHs on Hypersil-CN Column
with Hexane / 2-Propanol Mobile Phase at
2.0 ml/min

<u>PAH</u>	<u>RING SIZE</u>	<u>k' (HEX / IPA)</u>			
		<u>80/20</u>	<u>90/10</u>	<u>95/5</u>	<u>100/0</u>
Benzene	1	0.18	0.18	0.19	0.21
Naphthalene	2	0.22	0.23	0.25	0.30
Anthracene	3	0.28	0.32	0.33	0.35
Pyrene	4	0.32	0.36	0.39	0.43
Chrysene	4	0.38	0.44	0.49	0.70
Triphenylene	4	0.39	0.45	0.50	0.71
Benzo-[a]- Anthracene	4	0.39	0.45	0.50	0.71
Benzo-[a]- Pyrene	5	0.44	0.51	0.57	0.81

III-4. The Role of The Mobile Phase on Sample Retention in Normal-Phase Systems

III-4-1. Physicochemical Properties of Mobile Phase Solvents

Variations in sample retention on polar bonded-phase columns are achieved by changes in the mobile phase. In general, eluents used in normal bonded-phase chromatography are binary solvents consisting of a non-polar solvent and a polar modifier. As stated by Snyder [2,6], control of the mobile phase composition is a versatile tool for adjusting sample retention and enhancing the overall separation in normal phase chromatography. Thus, solvent properties are the most important factors governing sample retention. The most significant solvent property is probably its polarity, as measured by its dielectric constant and eluotropic value.

Dielectric constant can be used to represent the solvent polarity, with high values of the dielectric constant reflecting high polarity of the solvent. In polar normal bonded-phase chromatography, retention of polar samples decreases with increasing solvent

polarity, which correlates with increasing dielectric constant, as the experimental data show. For a binary solvent mixture, however, we assume that the solvent strength can be evaluated on the basis of the linear combination of the dielectric constant of pure solvent, that is:

$$D_{AB}^e = N_A D_A^e + N_B D_B^e \quad (1)$$

where N_A and N_B are molar fractions of solvent A and B, respectively. D_A^e and D_B^e are dielectric constants of pure solvent A and B, respectively, and D_{AB}^e is dielectric constant of binary solvent mixture.

Snyder [1] developed a scale of solvent strengths called an eluotropic series, based on retention values of naphthalene determined on alumina columns using n-pentane as a reference solvent ($E^\circ = 0$). For a binary solvent system consisting solvents A and B, the solvent strength of the binary mixture, E_{AB}° , is defined in terms of the pure solvent strengths E_A° and E_B° , according to Snyder:

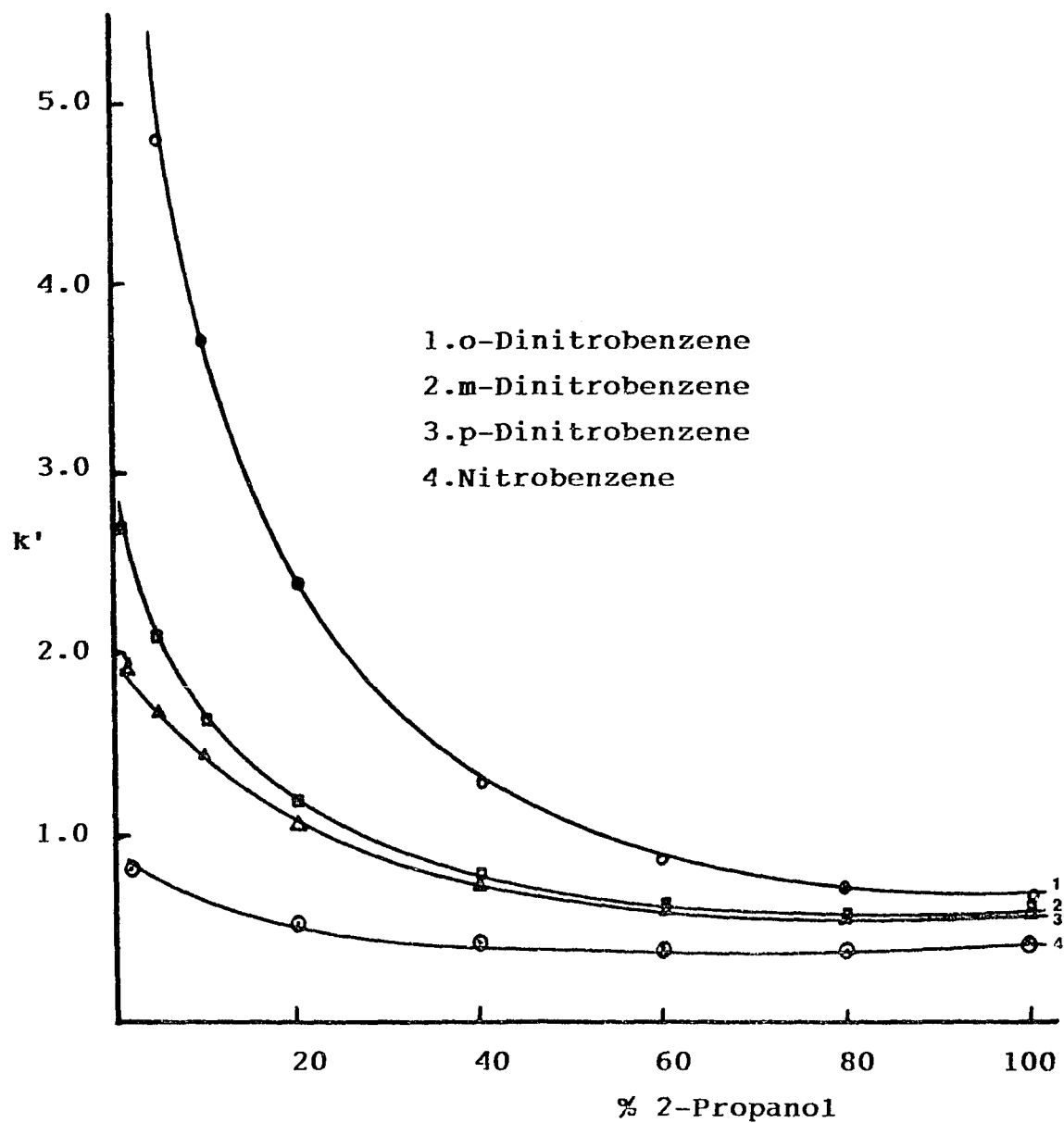
$$E_{AB}^\circ = E_A^\circ + \frac{\log (N_B 10^{\tilde{a}' n_b} (E_B^\circ - E_A^\circ) + 1 - N_B)}{\tilde{a}' n_b} \quad (2)$$

where $\tilde{a}'$ is the surface activity which is a measure of the interaction strength of the adsorbent surface; n_b is the molecular cross - sectional area of solvent B molecules; and N_B is mole fraction of the stronger solvent B.

To investigate the effect of mobile phase on sample retention in bonded-cyano and amino columns, a variety of mobile phase solvents was selected. The properties of these solvents are summarized in Table III-12.

III-4-2. Dependence of Sample Retention on Mobile Phase Composition using Hexane / 2-Propanol Eluents

The mobile phase composition in normal-phase chromatography is the most important factor in sample retention. To demonstrate this, various organic compounds were run on the cyano- and amino- bonded silica columns with various mobile phase compositions. Figure III-7 illustrates the retention of three different dinitrobenzenes on Hypersil-NH₂ column. As the percentage of polar solvent (2-propanol) is increased, the retention values decrease. It is assumed that the sample retention on polar bonded - phase



FigureIII-7. Retention Of Nitrobenzenes On Hypersil - CN Column With Hexane / 2-Propanol Eluent.

Table III-12. Mobile Phase Solvent Properties

<u>Solvent</u>	<u>MW</u>	<u>η(cp)</u>	<u>D^e</u>	<u>E°</u>
Water	18.0	1.00	78.5	-
Acetonitrile	41.0	0.36	37.5	0.65
Methanol	32.0	0.58	32.6	0.95
Ethanol	46.1	1.19	24.3	0.88
2-Propanol	60.0	2.39	18.3	0.82
Ethyl Acetate	88.1	0.46	6.02	0.38
Dichloromethane	84.9	0.45	9.08	0.42
Chloroform	119	0.58	4.81	0.40
Cyclohexane	84.2	0.66	2.02	0.04
n-Heptane	100	0.41	1.92	0.01
n-Hexane	86.0	0.33	1.89	0.01

η --- Viscosity at 20°C

D^e --- Dielectric constant

E° --- Eluotropic Value

column is largely due to the specific molecular interaction between the functional group on the solute molecules and polar stationary phase, as discussed in Section III-3. Therefore, increasing the polar solvent content will facilitate the displacement of solute molecules by solvent. Decreased retention results.

Figures III-8 to III-11 demonstrate the retention of four different groups of compounds on cyano column with various compositions of 2-propanol in hexane. Increasing polar modifier concentration decreases retention, especially for the more polar compounds. However, it should be noted that for less polar solutes, such as ketones and PAHs, the retention increases rather than decreases when 2-propanol composition exceeds 60%. We attribute this to a change in the fundamental molecular interaction between solutes and stationary phase at high concentration of polar solvent. A detailed discussion is given in Section III-5.

III-4-3. Dependence of Sample Retention on the Nature of Mobile Phase Solvent

To study further the effect of mobile phase on sample retention, ethanol and ethyl acetate were

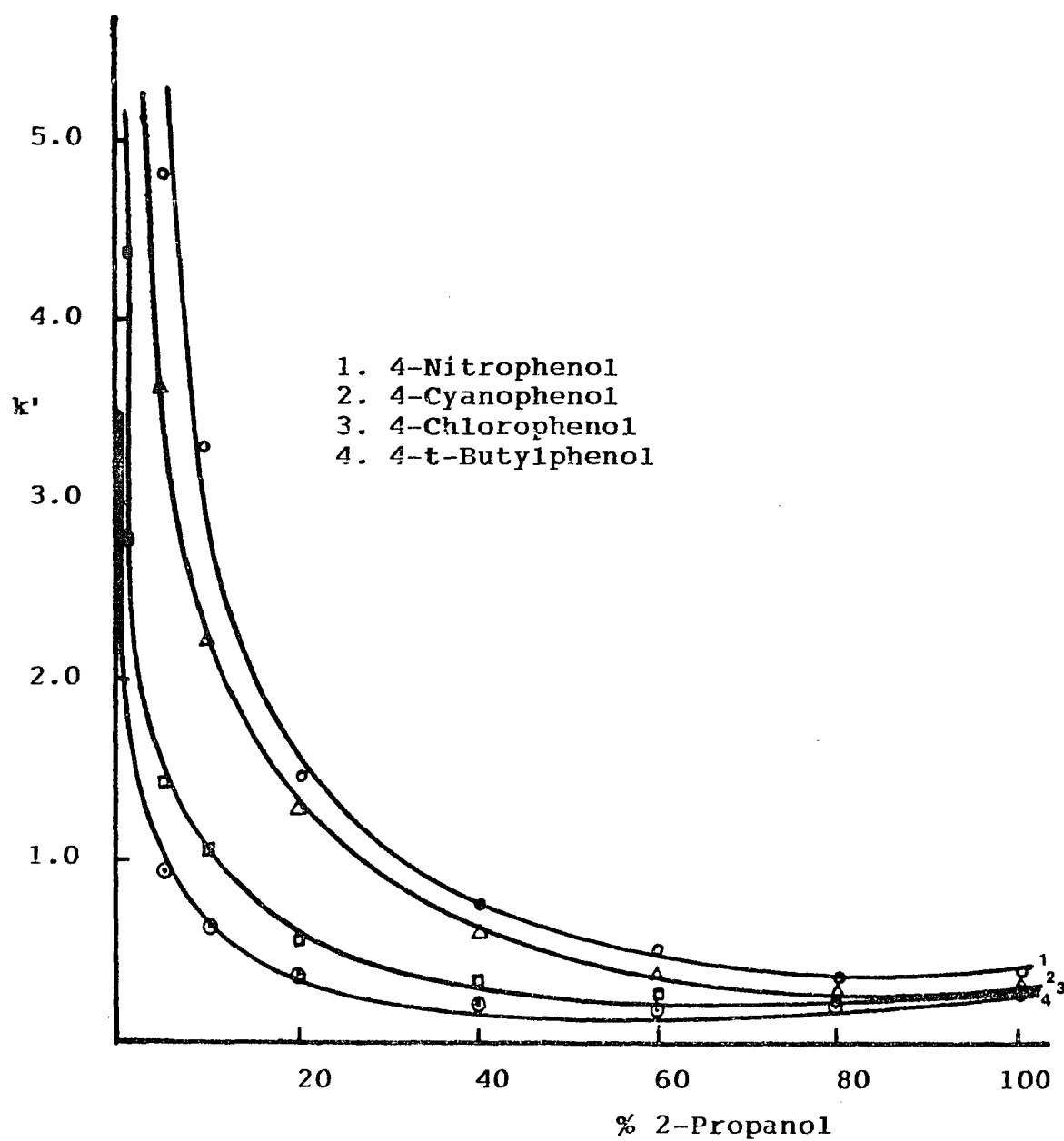


Figure III-8. Retention Of Substituted Phenols On Hypersil - CN Column With Hexane / 2-Propanol Eluent.

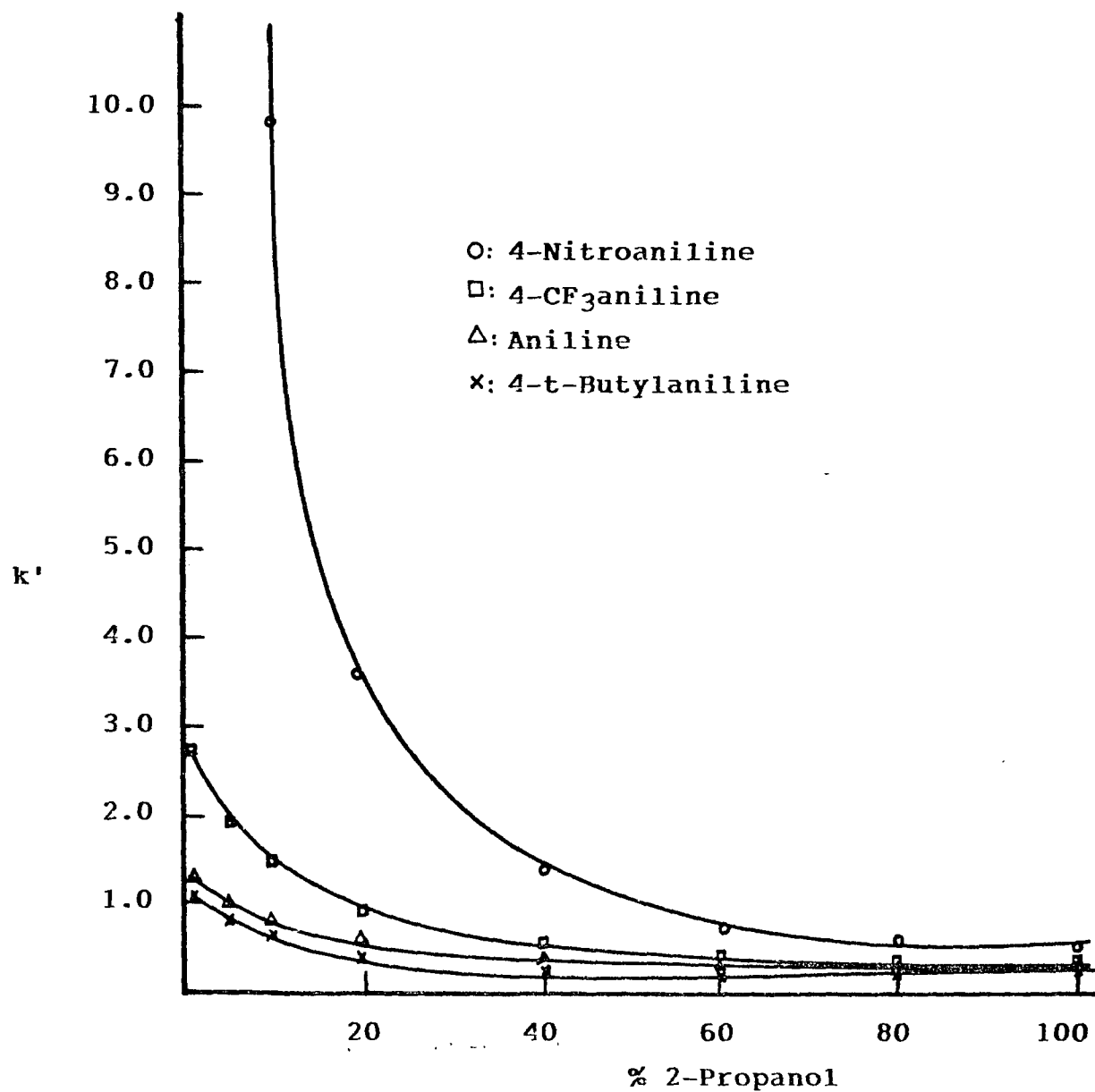


Figure III-9. Retention Of Substituted Anilines On Hypersil - CN Column With Hexane / 2-Propanol Eluent.

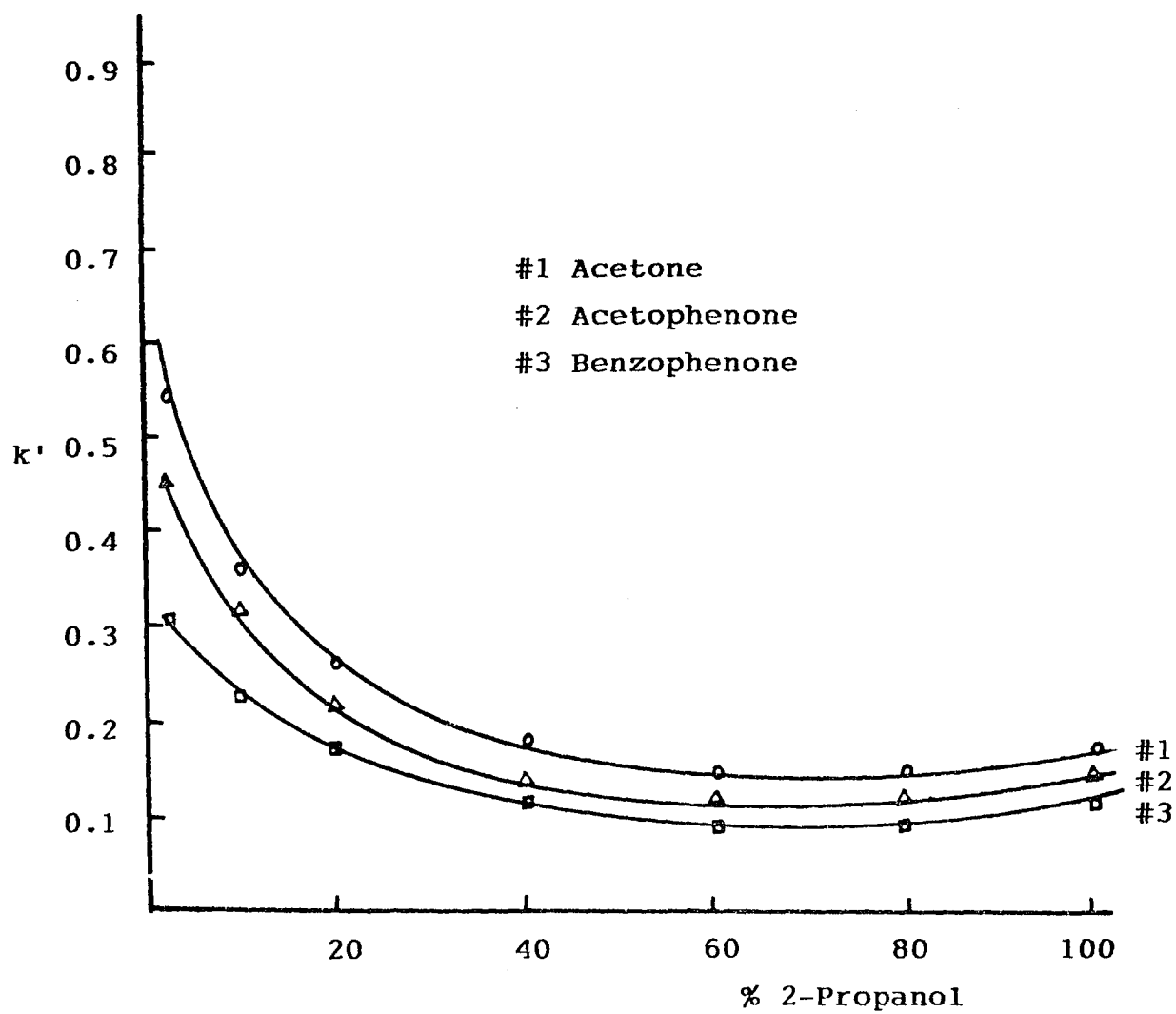


Figure III-10 Retention Of Various Ketones On Hypersil - CN Column With Hexane / 2-Propanol Eluent.

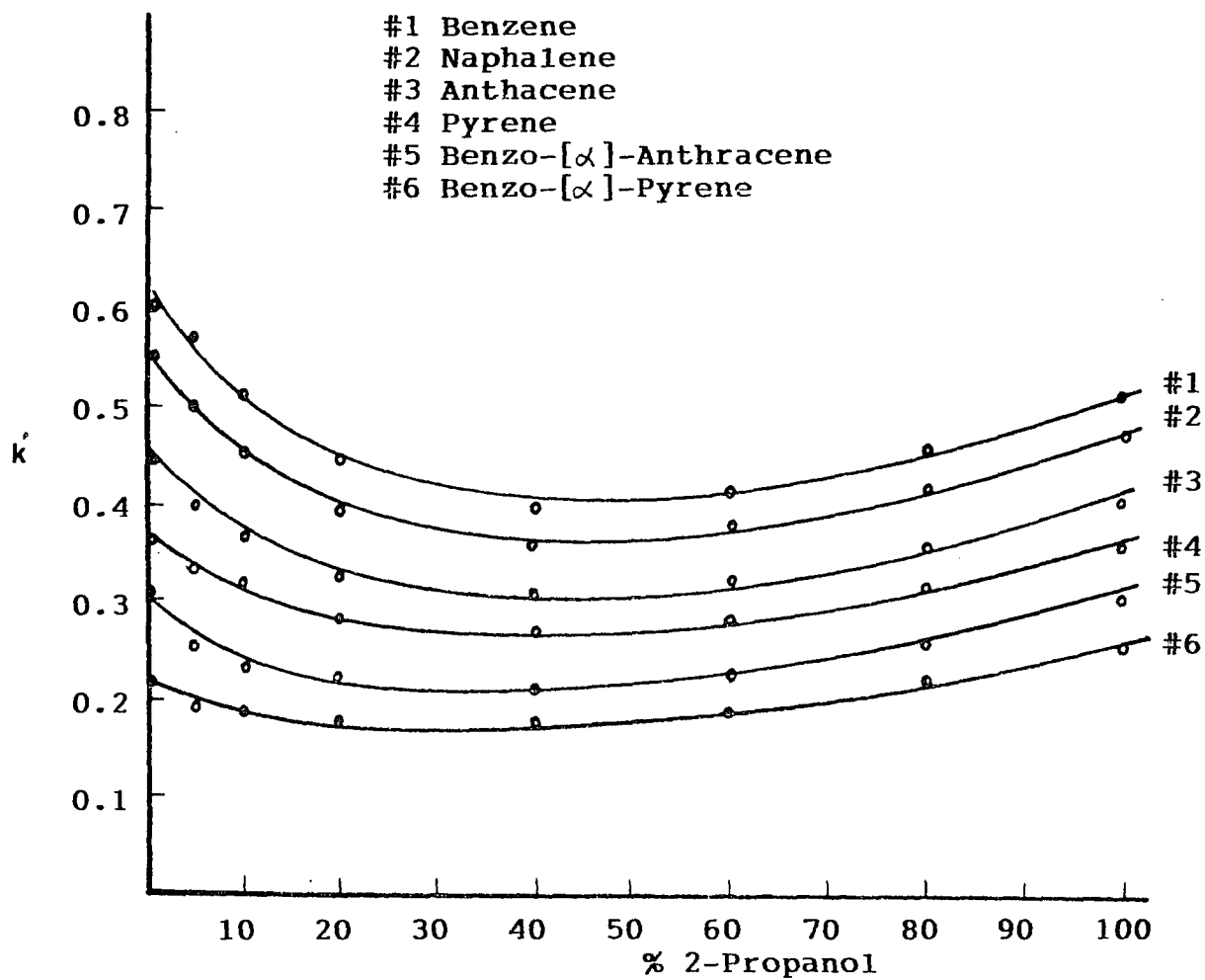


Figure III-11. Retention Of Various PAHs On Hypersil
- CN Column With Hexane/2-Propanol Eluent

compared with 2-propanol as the polar solvents. The polarity of these solvents is expected increase with the dielectric constant: ethanol (24.30) > 2-propanol (18.30) > ethyl acetate (6.02). Dichloromethane, chloroform, cyclohexane, n-heptane and n-hexane were employed as non-polar solvents. The non-polar solvent also affects retention in a manner related to their dielectric constants, that is dichloromethane (9.08) > chloroform (4.81) > cyclohexane (2.02) > heptane (1.92) > hexane (1.89).

In Tables III-13 and III-14, the k' values of several compounds, on cyano and amino columns, respectively, are compared in three different solvent systems: hexane / ethanol, hexane / 2-propanol and hexane / ethyl acetate. It is clear that the retention decreases as the polar organic modifier is changed from ethyl acetate to ethanol. Ethyl acetate has the lowest dielectric constant and is the weakest solvent because it cannot form hydrogen bonds in competing for the active sites. Ethanol and 2-propanol are both proton donating solvents, ethanol being the stronger. The retention is least with the hexane / ethanol mobile phase and greatest with the hexane / ethyl acetate, as seen in Table III-13 and III-14.

Table III-13: k' Values of Various Compounds on
Hypersil - CN Column

<u>Solute</u>	<u>Mobile Phase</u>	<u>k' (nonpolar/polar)</u>				
		<u>70/30</u>	<u>80/20</u>	<u>90/10</u>	<u>95/5</u>	<u>98/2</u>
Pyridine	I	0.33	0.47	0.66	0.80	0.92
	II	0.37	0.51	0.78	0.97	1.43
	III	0.60	0.75	1.22	1.58	2.34
Quinoline	I	0.30	0.38	0.50	0.58	0.62
	II	0.34	0.44	0.54	0.66	0.72
	III	0.33	0.44	0.56	0.73	0.85
7,8-benzo- Quinoline	I	0.26	0.29	0.37	0.42	0.44
	II	0.28	0.33	0.44	0.48	0.54
	III	0.33	0.38	0.46	0.52	0.57
Phenol	I	0.32	0.41	0.61	0.95	1.27
	II	0.39	0.51	0.77	1.21	3.23
	III	0.41	0.55	0.91	1.35	3.87
Aniline	I	0.37	0.51	0.71	0.84	1.05
	II	0.52	0.64	0.80	0.99	1.19
	III	0.59	0.66	0.90	1.10	1.26
Acetone	I	0.32	0.39	0.48	0.52	0.59
	II	0.42	0.49	0.59	0.66	0.75
	III	0.45	0.53	0.60	0.71	0.80

Mobile phase: I: Hexane / Ethanol

II: Hexane / 2-Propanol

III: Hexane / Ethyl Acetate

Table III-14. k' Values of Various Compounds on
Hypersil - NH_2 Column

Solute	Mobile Phase	k' (Hex/Polar Solvent)				
		70/30	80/20	90/10	95/5	99/1
Quinoline	I	0.34	0.42	0.59	0.90	1.17
	II	0.35	0.47	0.73	0.98	2.01
	III	0.74	1.04	1.86	6.17	--
7,8-benzo- Quinoline	I	0.22	0.26	0.34	0.42	0.51
	II	0.27	0.31	0.40	0.48	0.66
	III	0.41	0.56	0.89	1.29	1.62
Phenol	I	0.66	1.08	2.11	4.16	6.90
	II	0.88	1.37	3.02	5.86	11.36
	III	3.54	5.96	14.66	24.66	--
Aniline	I	1.22	1.69	2.41	3.38	6.20
	II	1.58	2.18	3.58	4.75	7.27
	III	2.01	2.75	5.35	9.37	--
4-chlorophenol	I	0.86	1.27	2.54	4.59	17.24
	II	0.89	1.43	3.29	6.79	18.47
	III	5.71	9.73	21.61	35.30	--
4-chloroaniline	I	1.30	1.83	3.17	4.73	7.21
	II	2.16	3.31	7.00	9.98	11.84
	III	2.39	4.41	8.34	18.83	--
o-dinitro- benzene	I	1.38	2.00	3.37	5.04	6.93
	II	2.23	3.23	5.79	9.09	14.51
	III	2.78	3.63	6.81	13.15	--
Acetone	I	0.13	0.15	0.25	0.32	0.41
	II	0.19	0.22	0.33	0.40	0.61
	III	0.29	0.39	0.59	0.76	1.32

Mobile phase: I: Hexane / Ethanol
 II: Hexane / 2-Propanol
 III: Hexane / Ethyl Acetate

The effect of polar solvent on retention of phenols on amino and cyano phases with the three binary solvent systems is shown in Figures III-12 and III-13. The curves for phenol on both columns exhibit the same profile: the retention decreases with increasing polar solvent concentration; at the same polar solvent concentration, the retention decreases with increasing the polar solvent polarity.

In order to evaluate the non-polar solvent effect on retention, several mobile phase systems containing 2-propanol in various non-polar solvents were studied. In Table III-15, k' data are given for five different phenols in three binary solvent systems: dichloromethane / 2-propanol, chlorform/2-propanol and hexane/2-propanol, on the amino bonded-phase column. Again, the retention decreases with increasing polarity of the non-polar solvent, as expected. Figure III-14 shows the retention curve of phenol with these three solvent systems on the amino column.

As a further demonstration of the effect of the non-polar solvent on sample retention, three non-polar solvents with similar dielectric constants, hexane, heptane, and cyclohexane, were used with 2-propanol.

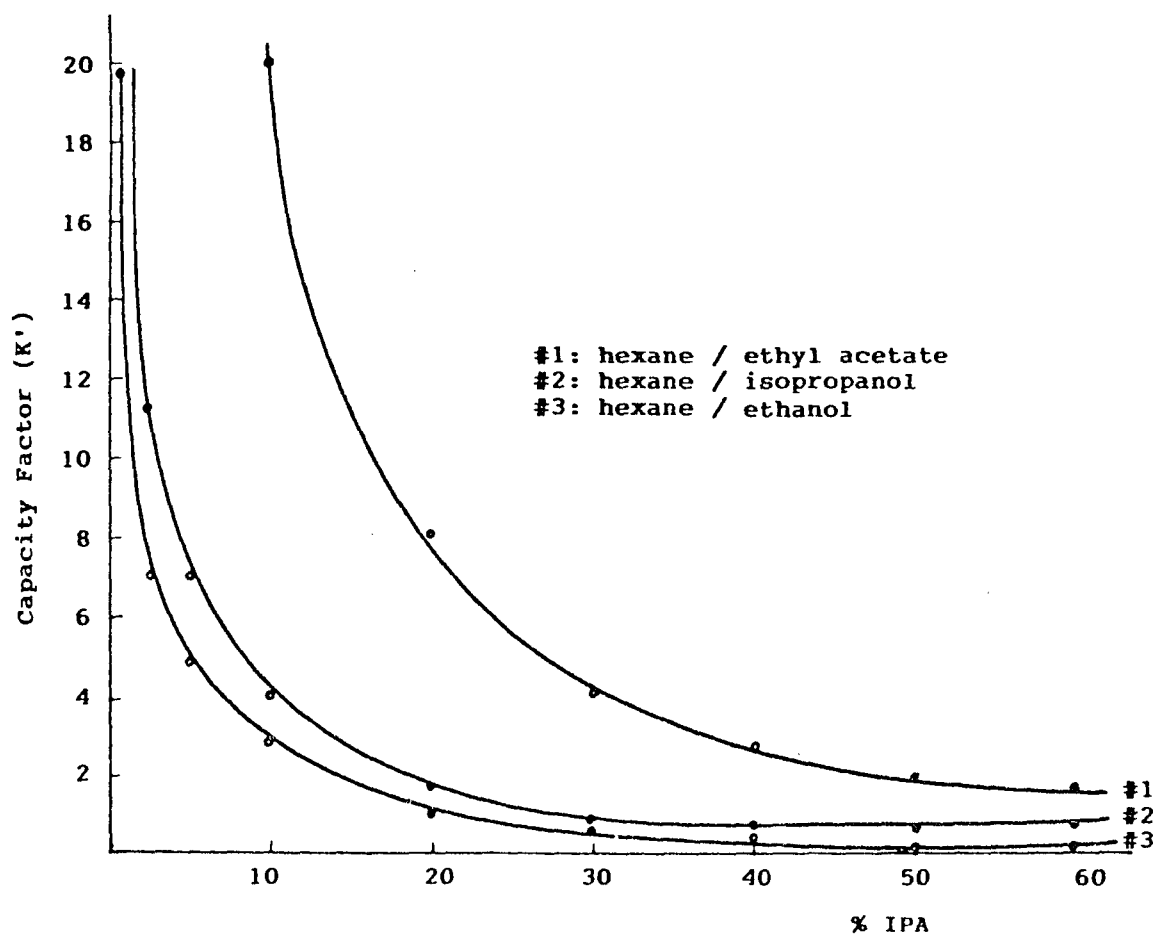


Figure III-12. Retention Of Phenol On Hypersil - NH_2 Column With Three Different Polar Solvents In Binary Mobile Phase System.

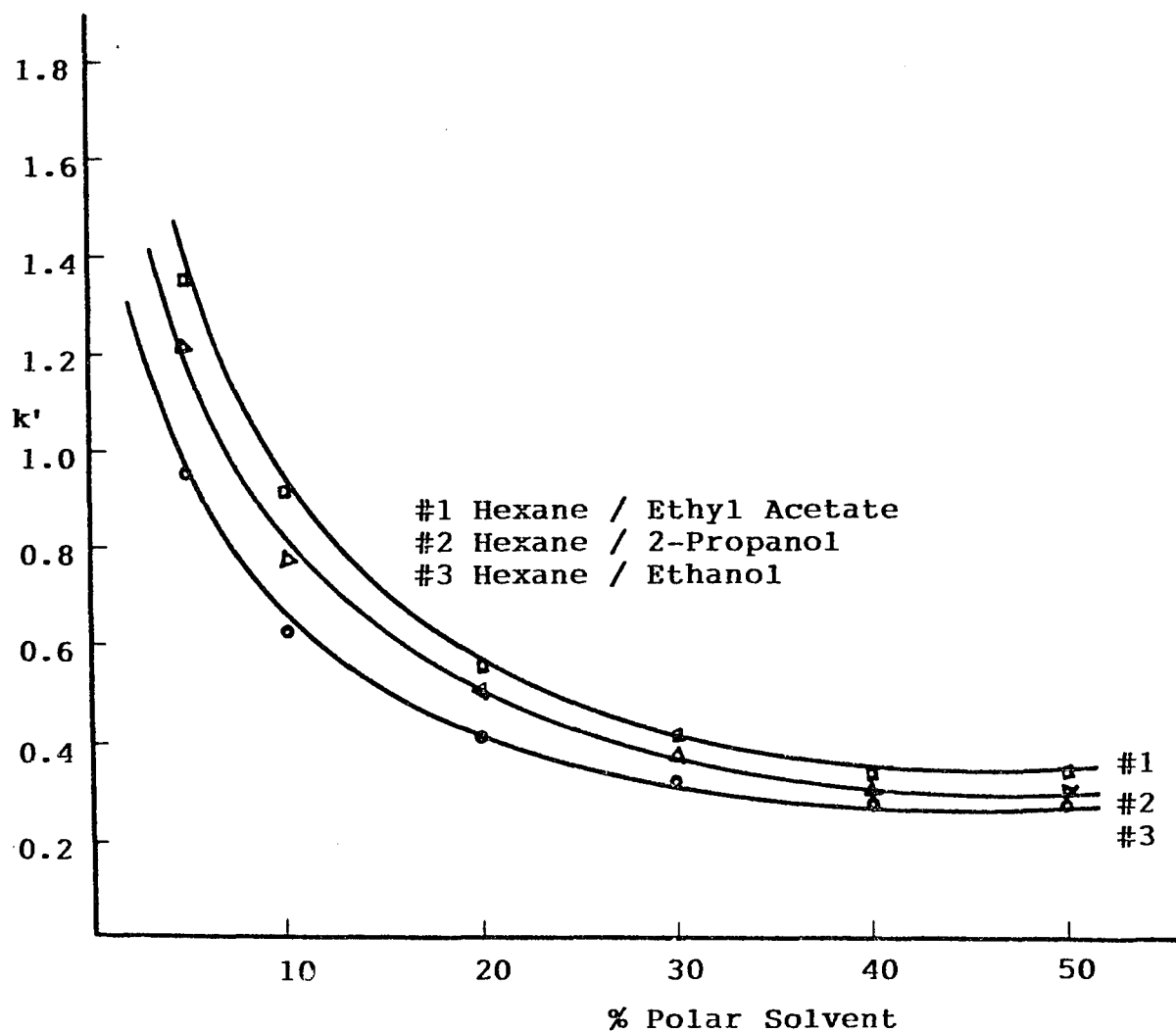


Figure III-13. Retention Of Phenols On Hypersil - CN Column With Three Different Polar Solvents In Binary Mobile Phase System.

Table III-15. k' Values of Various Phenols on
Hypersil - NH_2 Column

<u>Solute</u>	<u>Mobile Phase</u>	k' (Non-Polar Solvent / 2-Propanol)				
		70/30	80/20	90/10	95/5	99/1
phenol	I	0.28	0.39	0.71	1.43	2.17
	II	0.37	0.92	1.82	2.56	3.92
	III	0.91	1.37	3.02	5.86	11.36
o-cresol	I	0.21	0.28	0.41	0.72	0.31
	II	0.24	0.32	0.53	0.91	1.64
	III	0.61	0.94	1.91	3.29	8.57
2-ethyl-phenol	I	0.16	0.22	0.38	0.56	1.13
	II	0.19	0.26	0.41	0.66	1.29
	III	0.69	0.77	1.57	2.96	7.20
2-isopropyl-phenol	I	0.13	0.18	0.31	0.47	0.91
	II	0.16	0.21	0.33	0.52	1.04
	III	0.42	0.64	1.39	2.58	6.71
2-t-butyl-phenol	I	0.10	0.16	0.28	0.41	0.72
	II	0.14	0.19	0.29	0.46	0.88
	III	0.44	0.57	1.25	2.20	6.01

Mobile phase: I: Dichloromethane/2-Propanol

II: Chloroform/2-Propanol

III: Hexane/2-Propanol

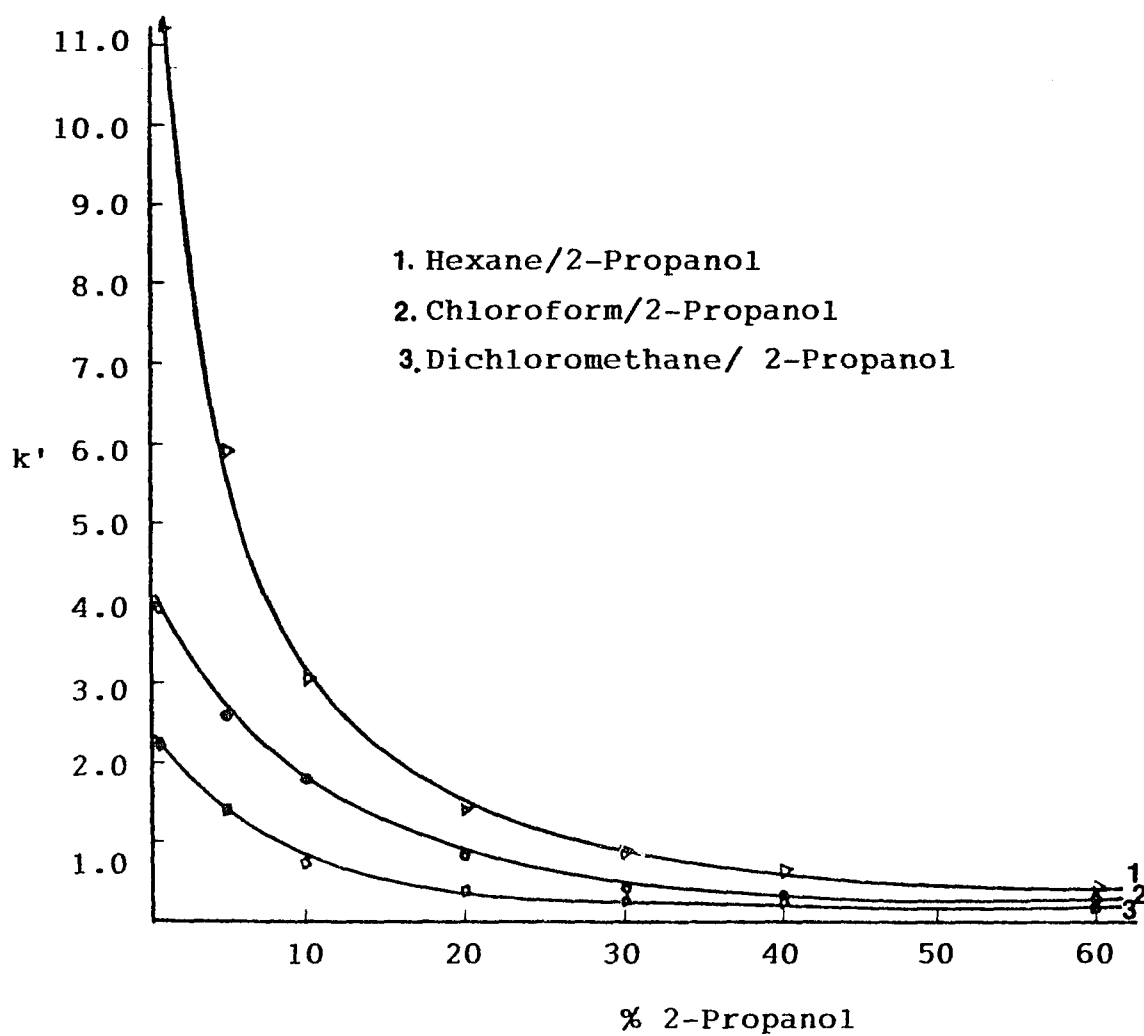


Figure III-14. Retention Of Phenol On Hypersil - NH_2 Column With Three Different Non-Polar Solvents In Binary Mobile Phase System.

The retention data of six compounds on the Hypersil-CN column with the three mobile phases are listed in Table III-16. Hexane, heptane and cyclohexane have slightly different dielectric constants: 1.89, 1.92 and 2.02, respectively. The data in Table III-16 are interesting evidence that the retention decreases slightly as the dielectric constants increases, even though the differences are small.

III-4-4. Dependence of Separation Mode on the Mobile Phase Solvent

The previous discussion of mobile phase effects have addressed the effect of the mobile phase solvent and its composition are the most important factors of sample retention. However, the chromatographic systems studied were only the cyano and amino polar bonded phase columns used in normal phase mode; namely, the mobile phases consisted of binary mixtures of polar solvent modifiers and non-polar solvents.

The potential of multi-mode separations on polar bonded-phase columns with appropriate mobile phases was also explored by further study. The polar bonded-phase columns can behave not only as normal phase columns but

Table III-16: k' Values of Various Compounds on
Hypersil - CN Column

<u>Solute</u>	<u>Mobile Phase</u>	<u>70/30</u>	<u>80/20</u>	<u>90/10</u>	<u>95/5</u>	<u>97/3</u>
phenol	I	0.42	0.51	0.77	1.21	2.16
	II	0.38	0.48	0.69	1.14	1.52
	III	0.35	0.44	0.68	1.02	1.15
aniline	I	0.52	0.64	0.80	0.99	1.08
	II	0.52	0.64	0.79	0.94	1.12
	III	0.44	0.51	0.67	0.81	0.89
pyridine	I	0.37	0.51	0.78	0.97	1.24
	II	0.37	0.45	0.73	0.87	0.95
	III	0.35	0.41	0.54	0.66	0.71
o-dinitro benzene	I	1.75	2.43	3.79	4.78	5.54
	II	1.75	2.40	3.64	4.60	5.42
	III	1.36	1.87	2.85	3.59	3.96
acetone	I	0.42	0.49	0.59	0.66	0.71
	II	0.41	0.49	0.58	0.62	0.67
	III	0.36	0.43	0.52	0.58	0.62
cyclohexanone	I	0.39	0.44	0.54	0.62	0.66
	II	0.38	0.44	0.54	0.59	0.66
	III	0.31	0.36	0.44	0.50	0.53

Mobile phase: I: Hexane / 2-Propanol

II: Heptane / 2-Propanol

III: Cyclohexane / 2-Propanol

columns but also effectively as reversed phase columns, depending on the selection of the mobile phase.

To achieve reversed phase mode conditions on polar columns, the columns were first flushed with 60 ml dichloromethane, 60 ml 2-propanol and 60 ml acetonitrile, in that order. The retention times of various compounds were then measured using various compositions of acetonitrile in water. The acetonitrile/water mobile phase is more polar than stationary phase and is, therefore, referred to as a reversed phase eluent.

Comparison of retention behaviors of various compounds in both normal and reversed phase modes is made in Tables III-17 and III-18. A reversal of retention order of phenols and anilines on the cyano phase is observed. As can be seen in Table III-17, the retention order of a series of phenols in the normal phase mode is that of increasing phenol acidity. The retention order of anilines is that of decreasing basicity. The retention behavior of phenols and anilines in the normal phase mode has been explained earlier in terms of the specific molecular interaction between the solute molecules and the stationary phase. In general, more polar solutes have stronger retention

in normal phase mode. The retention order of phenols and anilines in the reversed phase mode is the reverse of that in the normal phase mode.

The reversal of retention order for these compounds provides insight into the retention mechanism. The specific molecular interactions operative in the normal phase mode are different in highly polar mobile phase. In the acetonitrile/water systems, a nonspecific liquid-liquid partition effect seems to be more important than a specific interaction with the bonded group. Thus, the relatively polar solutes (more acidic compounds having stronger interactions with stationary phase in normal phase mode) are more likely to partition into the polar aqueous mobile phase, resulting in less retention. The elution order in an aqueous polar solvent system is determined by the solubility of solutes in the mobile phase.

The elution order of PAHs on cyano and amino columns (Table III-17 and III-18) is the same in both modes; the retention times increase with increasing number of condensed rings. This order is exactly the same as that in typical reversed phase columns in which retention is attributed to a partition type mechanism. Retention data of PAHs on cyano and amino columns in acetonitrile

Table III-17. Comparison of k' Values of Various Compounds in Normal and Reversed Phase on Hypersil-CN Column.

<u>Compound</u>	<u>normal phase Hex/IPA(99/1)</u>	<u>reverse phase H₂O/ACN(80/20)</u>
4-nitrophenol	11.90	1.67
4-cyanophenol	7.09	2.17
4-chlorophenol	4.36	2.67
phenol	3.23	3.28
4-t-butylphenol	2.79	3.79
o-cresol	2.09	3.50
2-ethylphenol	1.96	5.52
2-isopropylphenol	1.72	6.43
2-t-butylphenol	1.12	9.13
4-trifloromethylaniline	2.67	2.89
4-chloroaniline	1.91	3.86
aniline	1.19	5.01
4-t-butylaniline	1.04	5.86
pyridine	1.49	2.34
quinoline	0.74	2.69
7,8-benzoquinoline	0.55	3.41
o-dinitrobenzene	6.58	5.72
m-dinitrobenzene	2.60	3.65
p-dinitrobenzene	1.93	3.35
benzene	0.19	1.22*
naphthalene	0.25	2.24
anthracene	0.34	4.21
pyrene	0.41	4.70
benzo-[a]-pyrene	0.61	10.01

*: The data for benzene and PAHs in the reversed phase mode refers to 60/40 H₂O/ACN.

Table III-18. Comparison of k' Values of Various Compounds in Normal and Reversed Phase on Hypersil-NH₂ Column.

<u>Compound</u>	<u>normal phase</u> <u>Hex/IPA(99/1)</u>	<u>reverse phase</u> <u>H₂O/ACN(80/20)</u>
phenol	11.36	0.72
o-cresol	8.57	0.85
2-ethylphenol	7.20	1.03
2-isopropylphenol	6.71	1.38
2-t-butylphenol	6.01	2.72
acetone	0.61	0.15
cyclohexanone	0.55	0.23
2-cyclohexen-1-one	0.43	0.28
acetophenone	0.38	0.46
benzophenone	0.32	0.62
o-dinitrobenzene	3.03	0.86
m-dinitrobenzene	2.10	0.64
p-dinitrobenzene	0.57	0.58
benzene	0.06	0.10
naphthalene	0.16	0.25
anthracene	0.29	1.05
pyrene	0.44	1.28
benzo-[a]-pyrene	0.77	2.86

/ water solvent systems is presented graphically in Figure III-15 and III-16. On the cyano column (Fig. III-15), the retention of each PAHs increases as solvent polarity increases. Increasing solvent polarity in the reversed phase mode is actually increasing its water content. This is consistent with reversed phase chromatographic behavior, that is, a partition mechanism based on a hydrophobic or solvophobic effect. By increasing the solvent polarity, the solutes preferably partition into the less polar stationary phase (or adsorbed-surface layer) due to the hydrophobic effect.

On the amino phase (Figure III-16), however, the results are slightly different. In pure acetonitrile, the retention time of each PAH is greater than that at 80% acetonitrile. This is presumably a solubility effect, i.e., the nonpolar PAH is more "soluble" in the propyl amino stationary phase, which is less polar than acetonitrile. As the mobile phase composition changes from 100% to 80% acetonitrile, the stationary phase surface is partially covered with a water layer hydrogen-bonded to the NH_2 groups, which could inhibit PAH solutes contacting the stationary phase. Thus, solute retention decreases. As water content is increased further, the mobile phase becomes a poorer

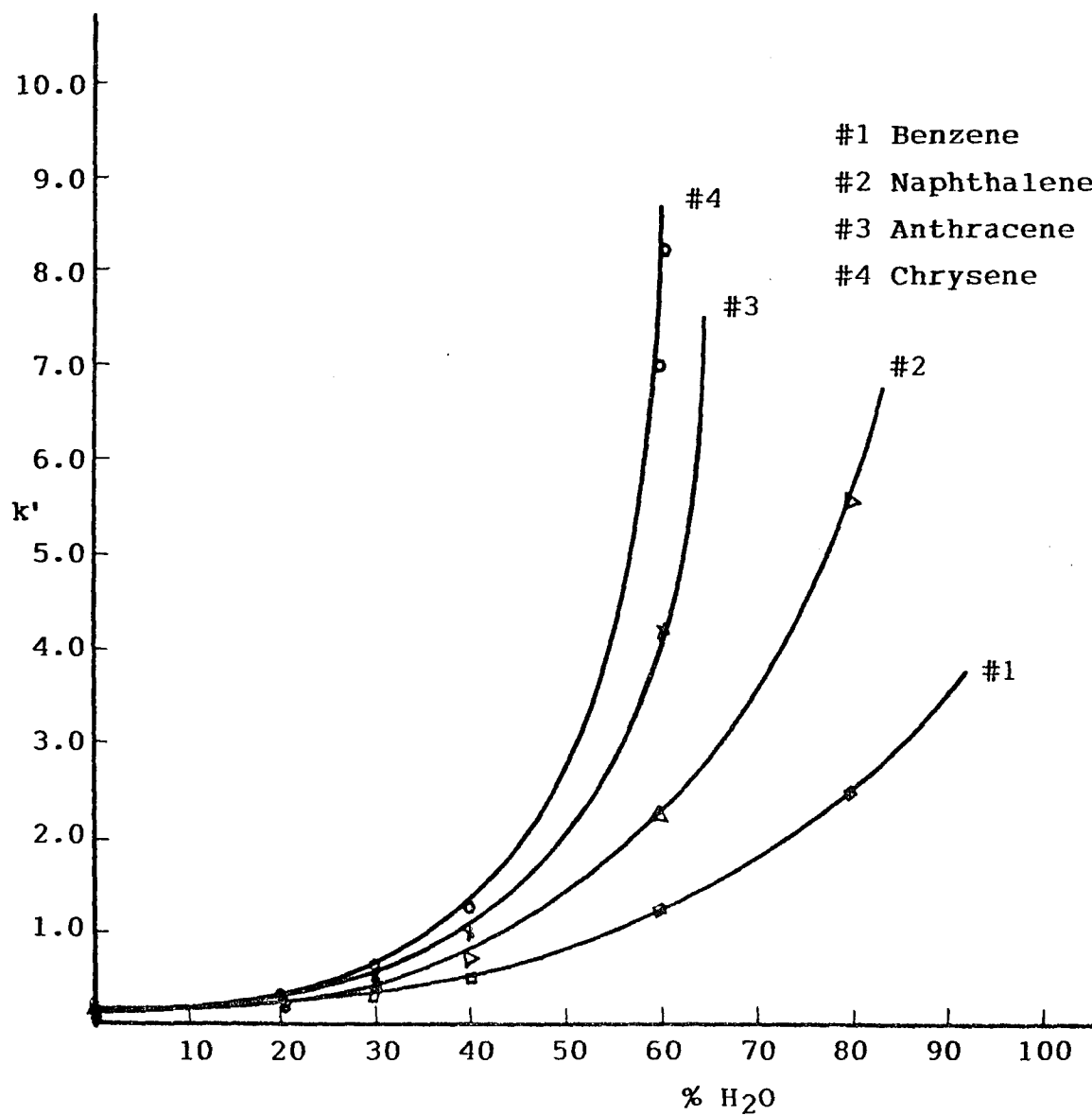


Figure III-15. Retention Of PAHs On Hypersil - CN Column In Reverse Phase Mode (Acetonitrile/Water).

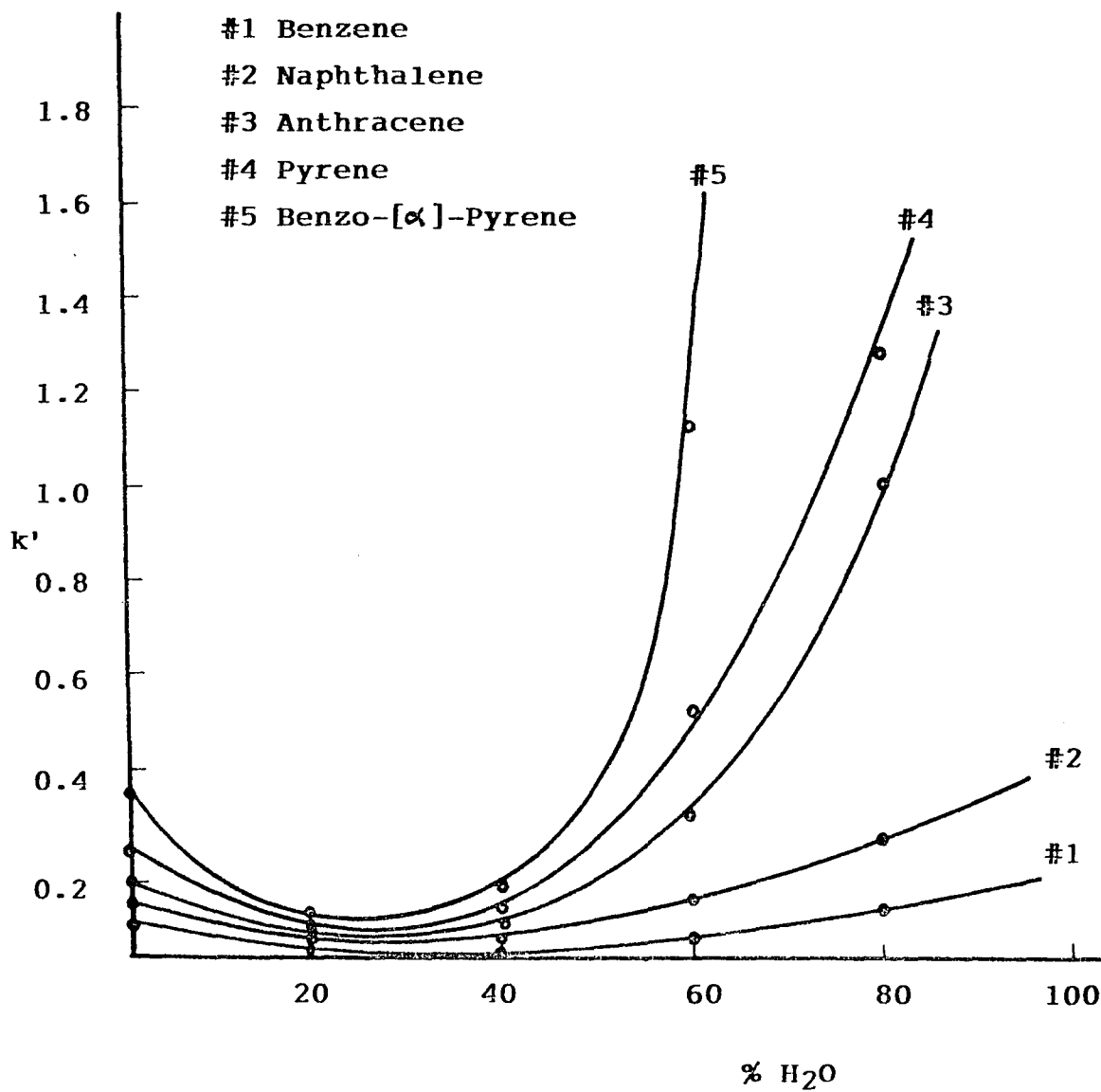


Figure III-16. Retention Of PAHs On Hypersil - NH₂ Column In Reverse Phase Mode (Acetonitrile/Water).

solvent, and solutes preferentially partition into the stationary phase. Solute retention increases.

Figures III-17 and III-18 demonstrate the separation of phenols and of anilines on the cyano column with the normal phase and reversed phase systems. As mentioned earlier, the reverse phase operation often yields an elution sequence inverse to the normal phase order, as in the case of phenols (Fig. III-17) and anilines (Fig. III-18). The separation sequence obtained in the normal phase mode is the order of increasing solute polarity. However, the separation sequence achieved in the reversed phase mode shown on the top of Figures III-17 and III-18 is most likely a "distorted mirror image" of the normal phase mode.

Thus, polar normal bonded-phase columns can be used in a reversed phase mode with a highly polar mobile phase, such as acetonitrile/water or methanol/water. The retention mechanism in the reversed phase mode seems to be changed, from one involving specific molecular interactions with the bonded group to a liquid-liquid-like partitioning effect. However, the precise eluent composition at which partitioning begins to occur cannot be distinguished. Rather, partitioning and adsorption are in competition and the transition between the two is

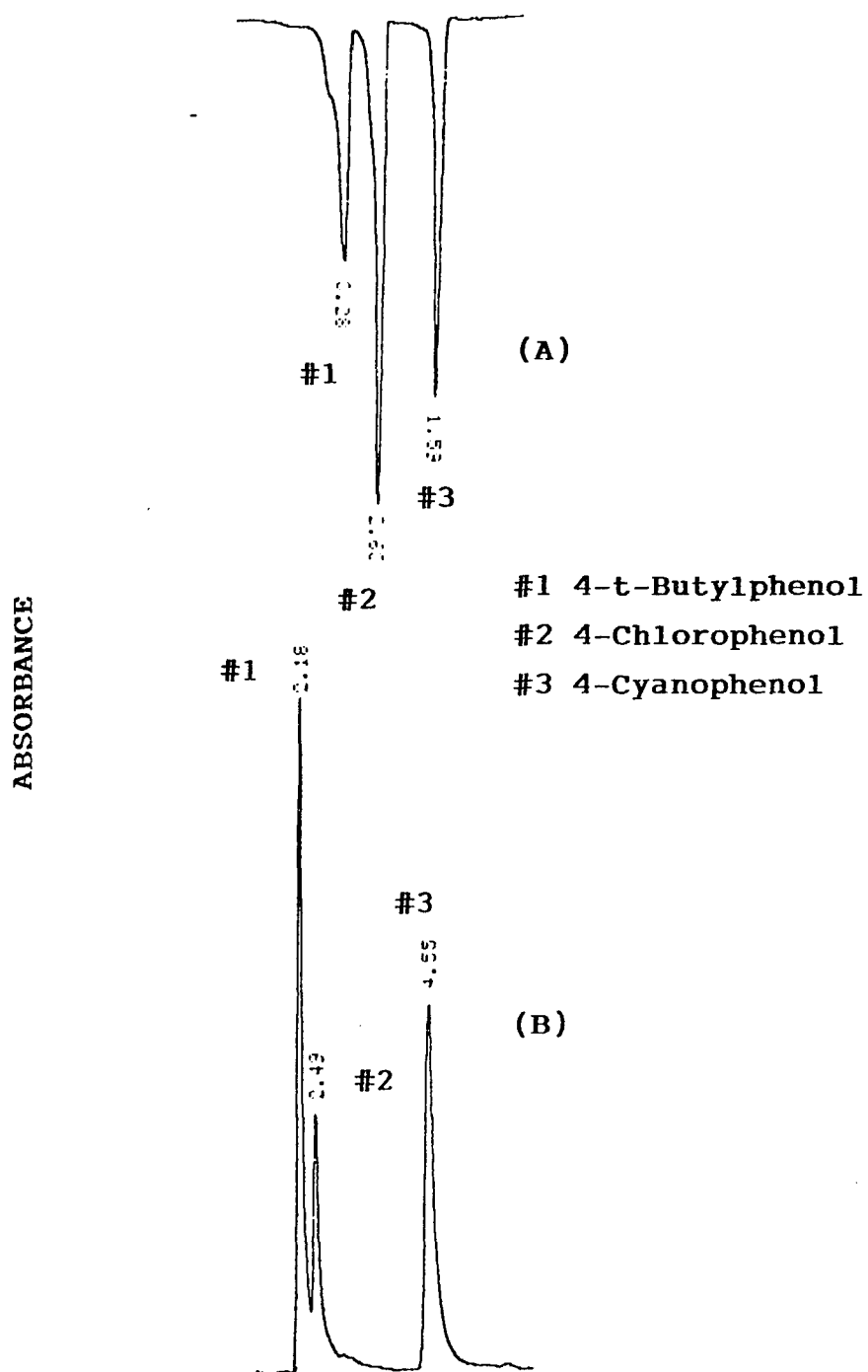


Figure III-17. Separations of Substituted Phenols on Hypersil - CN Column in Reverse Phase (A): Acetonitrile/Water (40/60) and Normal Phase (B): Hexane/2-Propanol (90/10).

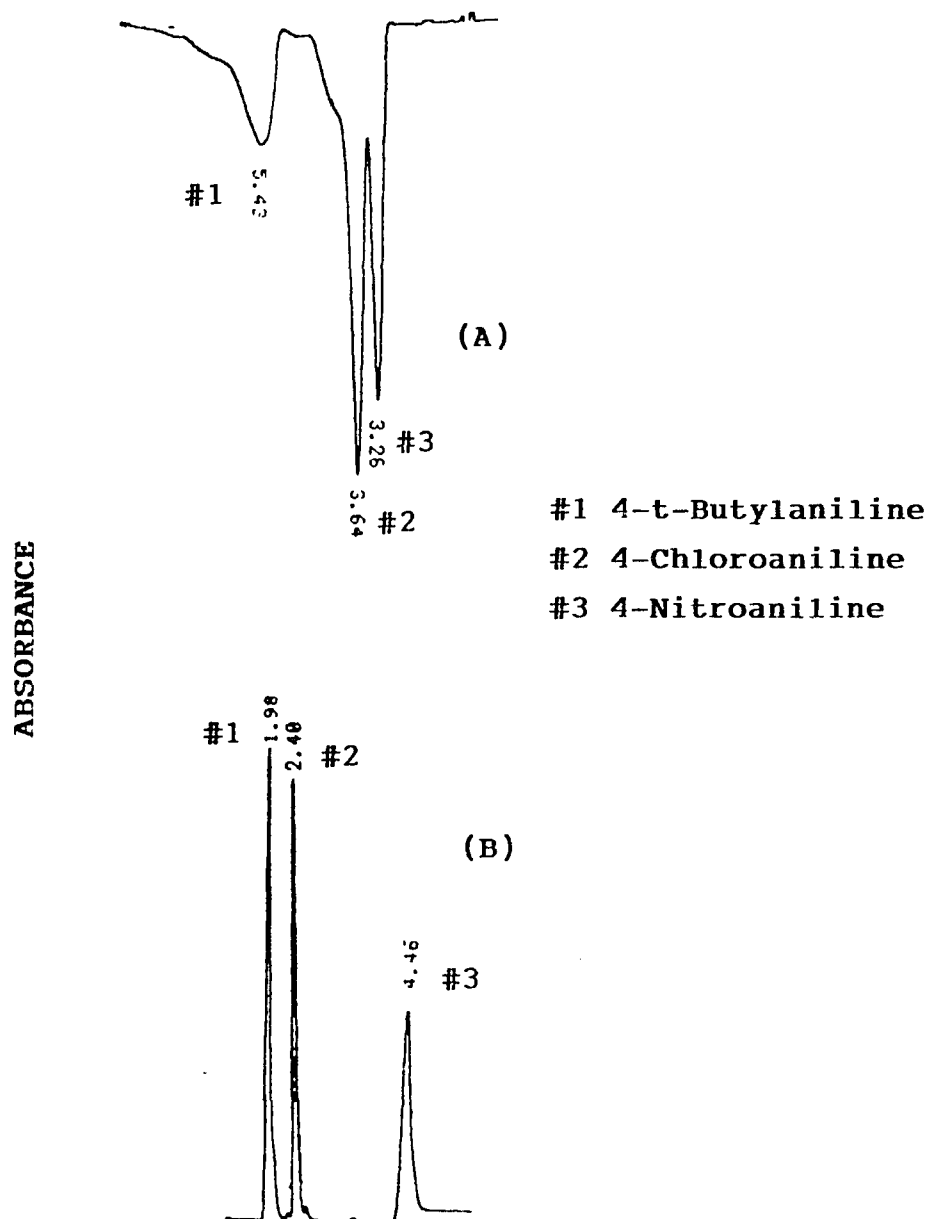


Figure III-18. Separations of Substituted Anilines on Hypersil-CN Column in Reverse Phase (A): Acetonitrile/Water (30/70) and Normal Phase (B): Hexane/2-Propanol (75/25).

gradual, and depends on the nature of the solute. The reversed phase separation on polar bonded-phase columns often offers an elution sequence inverse to that of the normal phase. This can not be generalized for all compounds. In fact, the elution sequence is not easily predicted in the reverse-phase mode. Although the above discussion is supported experimentally, further study of multi-mode separations on a polar bonded-phase column is needed for more generalized conclusions.

Finally, the potential of multi-mode separations using various phases is yet to be developed. It is interesting that the cyano and amino polar bonded-phase columns can play different separation roles because their retention characteristic can be dramatically altered by varying the mobile phase.

III-5. A New Model of Normal Bonded-Phase Liquid Chromatography

III-5-1. Introduction

The basic theory of liquid-solid chromatography (LSC) retention was originally developed by Snyder [1] in 1968. The Snyder displacement model describes LSC systems in which solvent and solute molecules form an adsorbed monolayer; sample retention is controlled by the competition between solute and solvent molecules.

The original Snyder model, allows the accurate prediction of solvent effects using alumina as an adsorbent. A modification of this model of retention in LSC was presented a few years later by Soczewinski [25-26]. The Soczewinski model represents a special case of the original Snyder model. Soczewinski proposed that the surface silanol groups on the silica packing interact with proton-acceptor solutes and solvents to form discrete, one-to-one complexes. The solvent binaries consist of a strong solvent B and a weak solvent A. However, it has been shown [27] that the Snyder and Soczewinski models are essentially equivalent. While the Snyder-Soczewinski (S-S) model is

usually referred to as the displacement model, in this dissertation, it is referred to as the Snyder displacement model. The Snyder displacement model will be described in detail in the following.

The third model of retention in LSC was offered by Scott and Kucera [28] in 1973. The Scott and Kucera (S-K) model differs fundamentally from the S-S model. It was further developed on the basis of a considerable amount of experimental data [29-35]. Most of LSC systems discussed by Scott and Kucera are binary solvent systems. Their considerable study was focused on the coverage of the adsorbent surface by the stronger solvent component B which varies from 0 to 100%. A monolayer of B is formed when there is only a few percent of B in the binary mixture. Instead of interacting directly with the sorbed adsorption surface, the solute molecules interact with the monolayer of B after the completion of this monolayer.

The S-K model assumes that the solute retention is largely determined by solution interactions between molecules of mobile phase and solute. As the concentration of strong solvent B increases, the strength of solvent-solute interaction increases and k' values decrease. The retention mechanism was referred

to as "sorption".

According to Snyder and Poppe [3], the scope of the S-S model is much broader than that of the S-K model. They stated that with regard to the kinds of LSC systems that can be described by the S-S and S-K models, the latter applies only to a single case: silica as adsorbent and hydrogen-bonding solvents as mobile phase. The S-S model, however, has been shown to be useful for a wide range of situations: a variety of adsorbents such as silica, alumina, magnesia, and even bonded-phase materials with polar groups with any non-polar and polar mobile phases.

Normal-phase separation in liquid chromatography, involves two different modes, liquid-solid (LSC) and various polar normal bonded-phase chromatography (NBPC). Retention behavior and separation mechanism on normal bonded-phase columns, such as cyanopropyl and aminopropyl stationary phase, have been studied by a number of chromatographers [8-10, 20-24, 37-38]. Hammers, et al. [37] conclude that the retention of most samples on the amino phase packing is weaker than on silica, but the separation sequence or selectivity is similar. Hurtubise and his co-workers [8] studied the effects of mobile phase composition (n-heptane/2-

propanol and n-heptane/ethyl acetate) on the NH_2 phase of alkylphenols and naphthols. They stated that Snyder displacement model could describe interactions of solutes with NH_2 column. Linear relationships were obtained from graphs of $\log k'$ vs. $\log N_B$ (N_B is mole fraction of the polar solvent) for both n-heptane/2-propanol and n-heptane/ethyl acetate mobile phases up to the maximum 10% of polar solvent content. The retention of various non-polar or moderately polar solutes (such as benzyl alcohol, naphthyl acetate, 1-cyanonaphthalene, etc.) using a less polar mobile phase, hexane/dichloromethane, with the maximum CH_2Cl_2 content of 20%, has been studied by Snyder, et al. [21]. They suggested that cyano-silica behaves as a weak silica and retention for cyano phase could proceed in the same way as for silica. Scolla and Hurtubise [38] investigated retention characteristics of several aromatic nitrogen compounds on an aminopropyl bonded-phase column with several n-heptane/ethyl acetate mobile phases. They concluded that the Snyder displacement model was effective in predicting the retention of nitrogen compounds in amino bonded-phase chromatography.

In recent years, Cooper and his co-workers [22-24] have proposed a selectivity matrix derived from the

Snyder displacement model for amino, cyano and diol normal bonded phase HPLC columns. Three solutes (phenol, aniline and nitrobenzene) and three polar solvents (chloroform, methy t-butyl ether and dichloromethane) chosen to represent the apices of the Snyder selectivity triangle were used to generate each selectivity matrix. Their selectivity matrix is a compilation of slopes, intercepts and solvent strengths which are obtained from the Snyder displacement equation. According to Cooper, the selectivity matrices provide a quantitative measure of localization and secondary solvent effects responsible for the selectivities.

In general, published studies of the retention mechanism of normal bonded-phase liquid chromatography correlate with the Snyder displacement model. On the basis of our observations and studies, however, consistency with the Snyder model was obtained for most solutes but only over a limited range of polar solvent compositions. Exceeding a critical percent of polar solvent for some solutes, significant retention differences were observed. In polar normal bonded-phase chromatography with a binary mobile phase mixture, we propose that two comparable processes govern the

retention mechanism. In addition to the Snyder mechanism, a partition mechanism in which the solute molecules distribute between a surface- adsorbed liquid layer and a bulk mobile phase liquid layer is suggested. The dominant mechanism is determined by the type of solute molecules and the concentration of the polar modifier in the mobile phase. A new model for retention on polar bonded phases is derived on the basis of our suggested retention mechanism. Detailed discussion is given in Section III-5-3.

III-5-2. Snyder Displacement Model

The displacement model of LSC retention is reviewed here in order to examine the retention mechanism of normal bonded-phase liquid chromatography and to develop a new model for normal bonded-phase chromatographic systems.

The Snyder model assumes that in LSC systems the adsorbent surface is covered by an adsorbate monolayer which consists of mobile phase solvent. Figure III-19 is a pictorial representation of the adsorption process in LSC. Retention of a solute molecule is controlled by the competition between the solute and solvent molecules

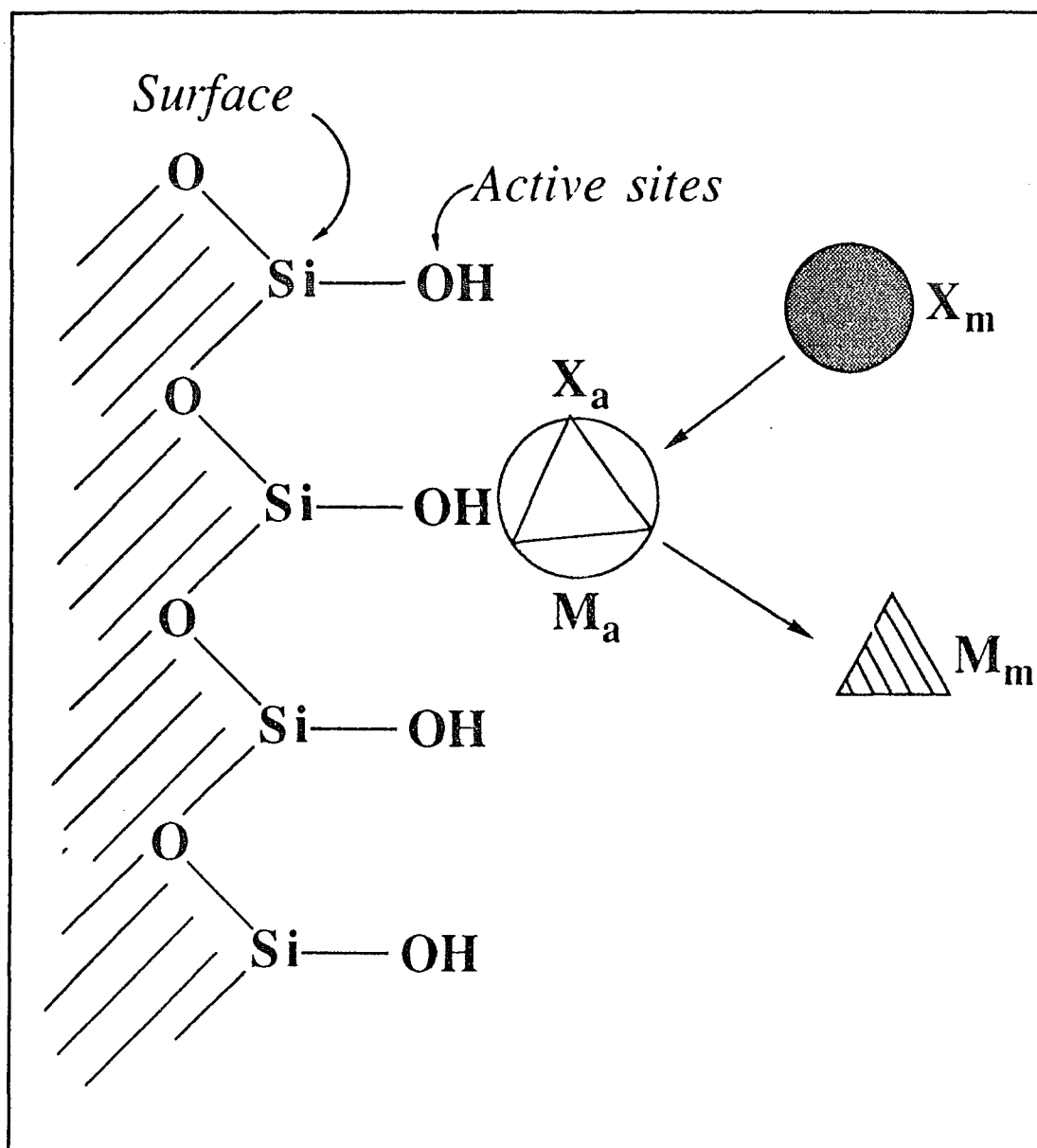


Figure III-19. Pictorial Representation of Snyder Displacement Process

for the active sites on the adsorbent surface. When a solute molecule is adsorbed, it displaces a roughly equal volume of solvent molecules from the monolayer. This displacement is described by

$$X_m + nM_a = X_a + nM_m \quad (3)$$

where X and M refer to solute and solvent molecules respectively, and subscripts m and a refer to mobile phase and adsorbed phase, respectively.

The net dimensionless free energy of adsorption corresponding to Eqn. 3 is

$$\Delta E = E_{Xa} + nE_{Mm} - E_{Xm} + nE_{Ma} \quad (4)$$

A further assumption of the Snyder model is that in most LSC systems the solution terms E_{Mm} and E_{Xm} are approximately equal resulting in cancellation of solvent-solute interaction terms in the two phases. Eqn. 4 then becomes

$$\Delta E = E_{Xa} - nE_{Ma} \quad (5)$$

where E_{Xa} and E_{Ma} refer the adsorption free energy of X

and M on the adsorbent surface. Corresponding adsorption energies on some other surface with surface activity $\tilde{a}'$ (relative to the standard adsorbent surface) are given as

$$\begin{aligned}\Delta E &= \tilde{a}' E_{Xa}^* - n \tilde{a}' E_{Ma}^* \\ &= \tilde{a}' (E_{Xa}^* - n E_{Ma}^*)\end{aligned}\quad (6)$$

Here, the superscript "*" refers to free energies for an adsorbant of standard activity. The quantity E can be related to the equilibrium constant K (chromatographic distribution coefficient) for the displacement equilibrium in Eqn. 3.

$$\begin{aligned}\Delta E &= -\Delta G^\circ / 2.3 RT = \log K \\ &= \tilde{a}' (E_{Xa}^* - n E_{Ma}^*)\end{aligned}\quad (7)$$

The capacity factor k' is proportional to the distribution coefficient K:

$$k' = (n_s/n_m) K \quad (8)$$

where n_s and n_m refer to the total number of moles of stationary phase and mobile phase within the column,

respectively. Therefore, the capacity factors for a given solute in two different mobile phases 1 (k_1') and 2 (k_2') are

$$\log (k_1' / k_2') = \log K_1 / K_2 \quad (9)$$

$$\log K_1 = \bar{a}' [E_{X1}^* - nE_{M1}^*] \quad (10)$$

$$\log K_2 = \bar{a}' [E_{X2}^* - nE_{M2}^*] \quad (11)$$

Combining Eqns.9-11,

$$\log(k_1' / k_2') = \bar{a}' n [E_{M2}^* - E_{M1}^*] \quad (12)$$

where E_{M2}^* and E_{M1}^* refer to E_{Ma}^* for mobile phase 2 and for mobile phase 1, respectively. If the mobile phase adsorption energy per unit molecular cross-sectional area is E° ,

$$E^\circ = E_{ma}^* / A_S \quad (13)$$

and

$$\log (k_1' / k_2') = \bar{a}' A_S (E_2^\circ - E_1^\circ) \quad (14)$$

Where A_S is the molecular area of solute X, and the

solvent strength E° of mobile phase 1 is E°_1 and of mobile phase 2 is E°_2 . Eqn 13 is the fundamental equation of the Snyder model. If the mobile phase is a binary mixture of a weak (A) and strong (B) solvent, the solvent strength of the mobile phase can be calculated [5] as already discussed in Section III-4-1.

$$E^\circ_{AB} = E^\circ_A + \frac{\log (N_B 10^{\bar{a}'n_b (E^\circ_B - E^\circ_A)} + 1 - N_B)}{\bar{a}'n_b} \quad (15)$$

It is important to note that Snyder's equation describes solute retention as a function of mobile phase solvent strength.

Following Soczewinski [25], for solvents are composed of binary mixtures of a strong solvent B in a weak solvent A, $E^\circ_B \gg E^\circ_A$ and $N_B \gg 0$. Thus Eqn. 15 reduces to

$$E^\circ_{AB} = E^\circ_B + (\log N_B) / \bar{a}'n_b \quad (16)$$

Now, if solvent 1 in Eqn.13 is a pure solvent B, and solvent 2 is a binary solvent mixture, substitution of Eqn.16 into Eqn.14, gives [2],

$$\log k'_2 = \log k'_1 - A_s/n_b \log N_B \quad (17)$$

where k'_2 is the capacity factor for a solute eluted with binary solvent phase, k'_1 is the capacity factor for a solute eluted with just the polar solvent, and A_s and n_b are the molecular area of the solute and the polar solvent respectively. Eqn.17 is the basic mathematical relation used to describe solute retention in chemically normal bonded phase chromatography at low concentration of the polar solvent. As we mentioned above, most chromatographers, who studied retention mechanisms on normal bonded-phase columns, have applied this model to interpret their experimental results.

III-5-3. Results and Discussion

To study the retention characteristics of amino- and cyano- propyl bonded phases, we examined systematically the retention of various solutes using the full range (0% - 100%) of compositions of polar solvent in non-polar solvent. The solute structure can greatly affect the sample retention because it is presumed that sample retention is based on the specific molecular interaction between the solute and the stationary phase. Thus, we classified the solutes studied into four groups:

hydrogen bond-donating compounds -- phenols; electron-rich compounds -- anilines, azaarenes and ketones; polar compounds -- nitrobenzenes; and - electron-rich compounds -- PAHs.

The retention curves (plots of k' vs. % of polar solvent) of each group of compounds on the NH_2 column with hexane / 2-propanol eluents are illustrated in Figures III-20 - III-24. By comparing the five sets of retention curves for these five different groups of compounds, the retention of phenols (Fig.III-20), anilines (Fig.III-21), nitrobenzenes (Fig.III-22) can be generalized as expected: solute retention decreases with increasing % polar solvent. The rate of decrease diminishes with increasing content of polar solvent. However, this does not apply to the less polar compounds such as PAHs. It is demonstrated in Figure III-23 (ketones) and Figure III-24 (PAHs) that the retention decreases for concentrations of polar solvent up to about 60% for ketones and about 40% for PAHs. Above these concentrations, the retention of these compounds increases instead of decreases. This is not expected from Snyder model.

To look at the data in a different form, plots of $\log k'$ for various solutes versus $\log N_B$ are shown in

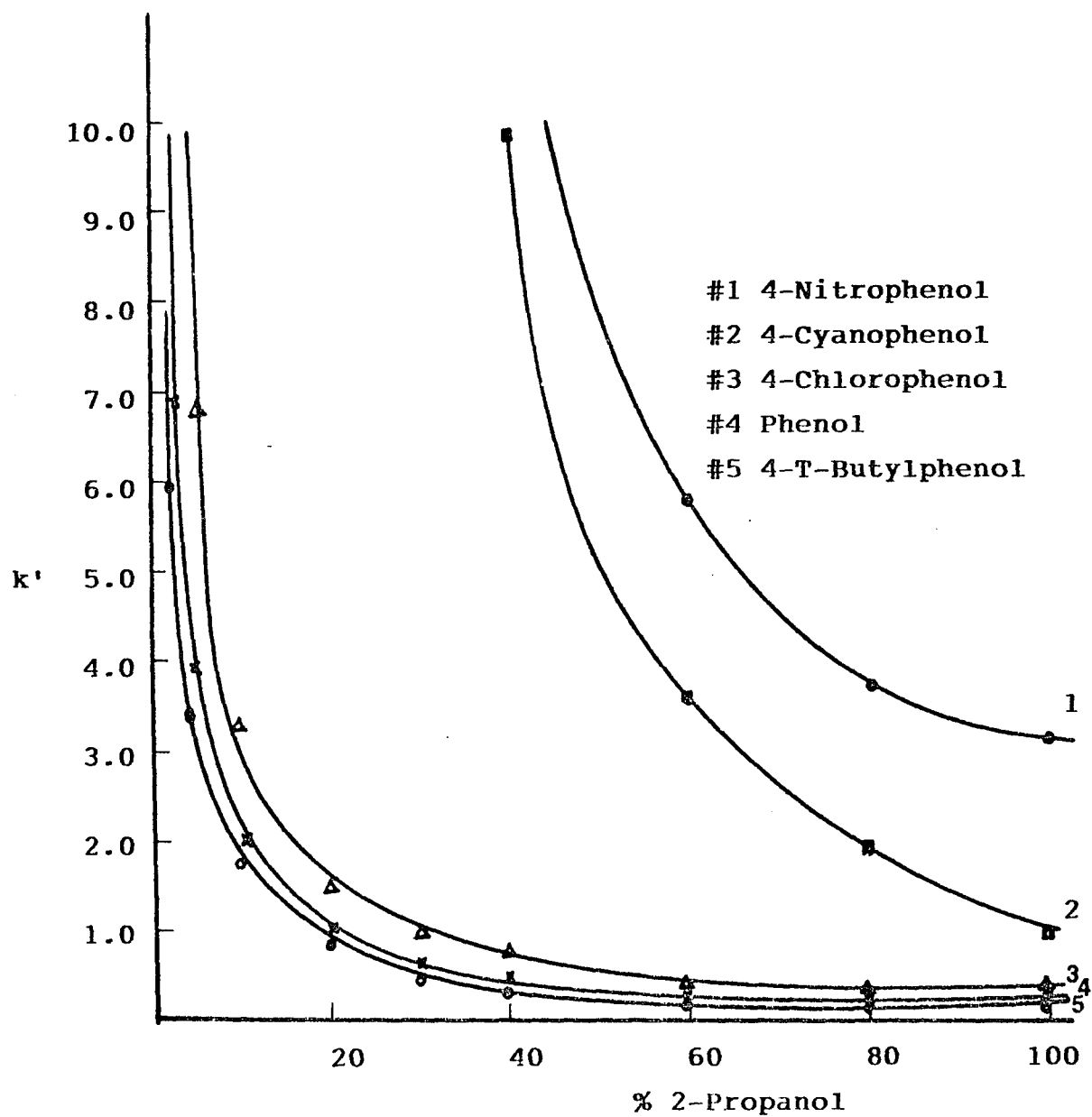


Figure III-20. Retention Of Substituted-Phenols On Hypersil - NH₂ Column With Hexane / 2-Propanol Eluent.

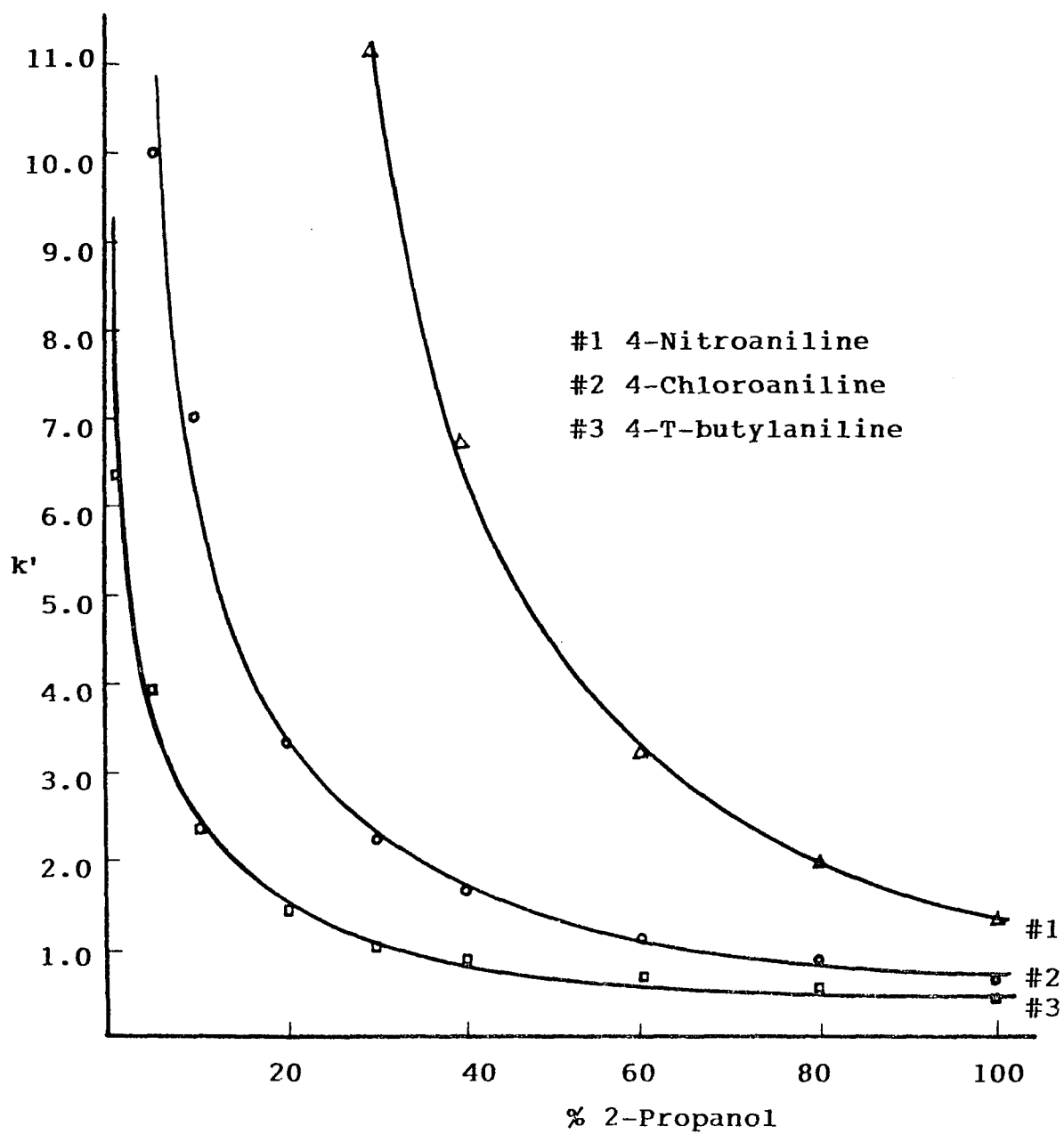


Figure III-21. Retention Of Substituted - Anilines
On Hypersil - NH₂ Column With
Hexane / 2-Propanol Eluent.

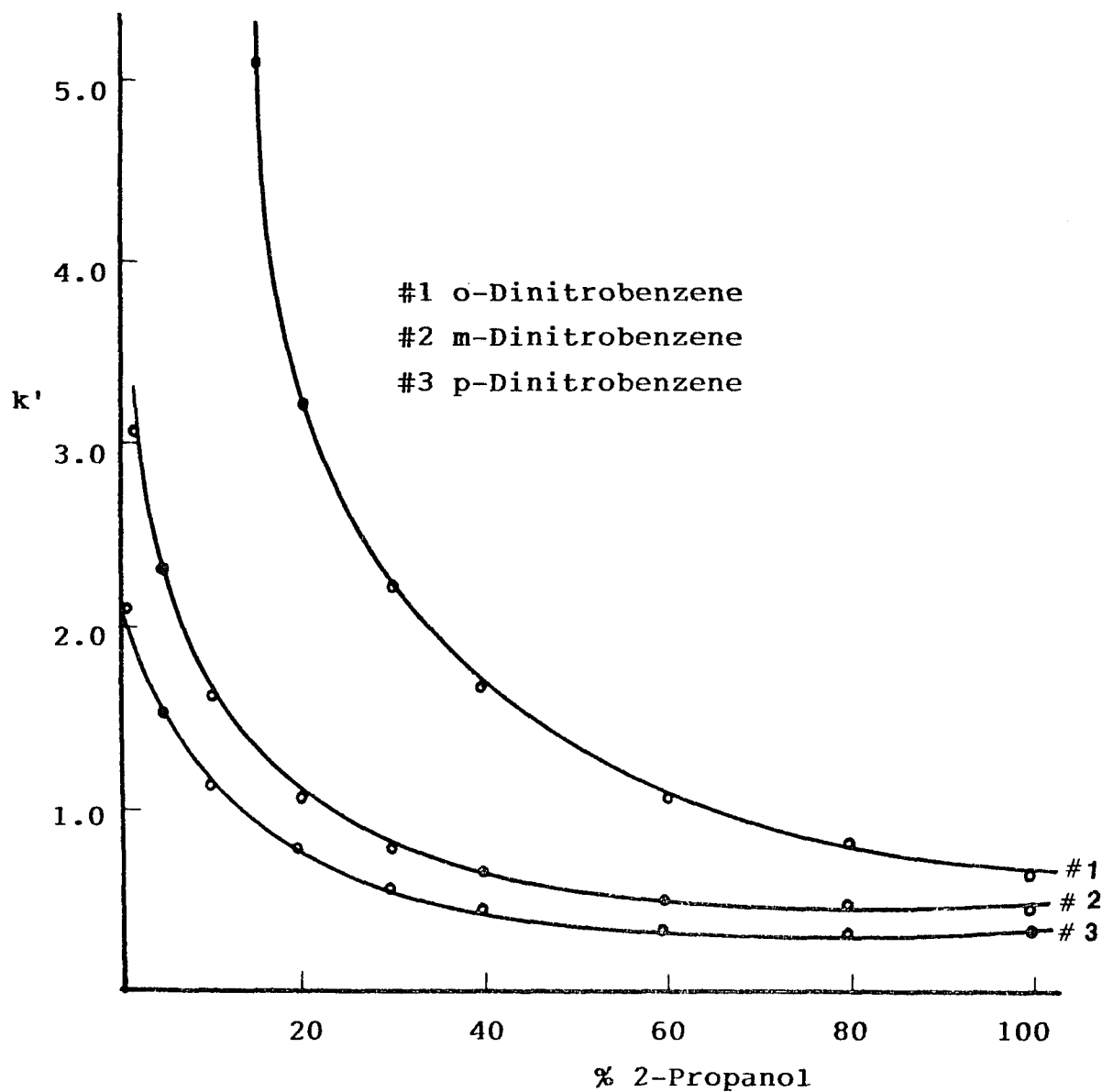


Figure III-22. Retention Of o-, m-, And p- Dinitrobenzene Isomers On Hypersil - NH₂ Column With Hexane / 2-Propanol Eluent.

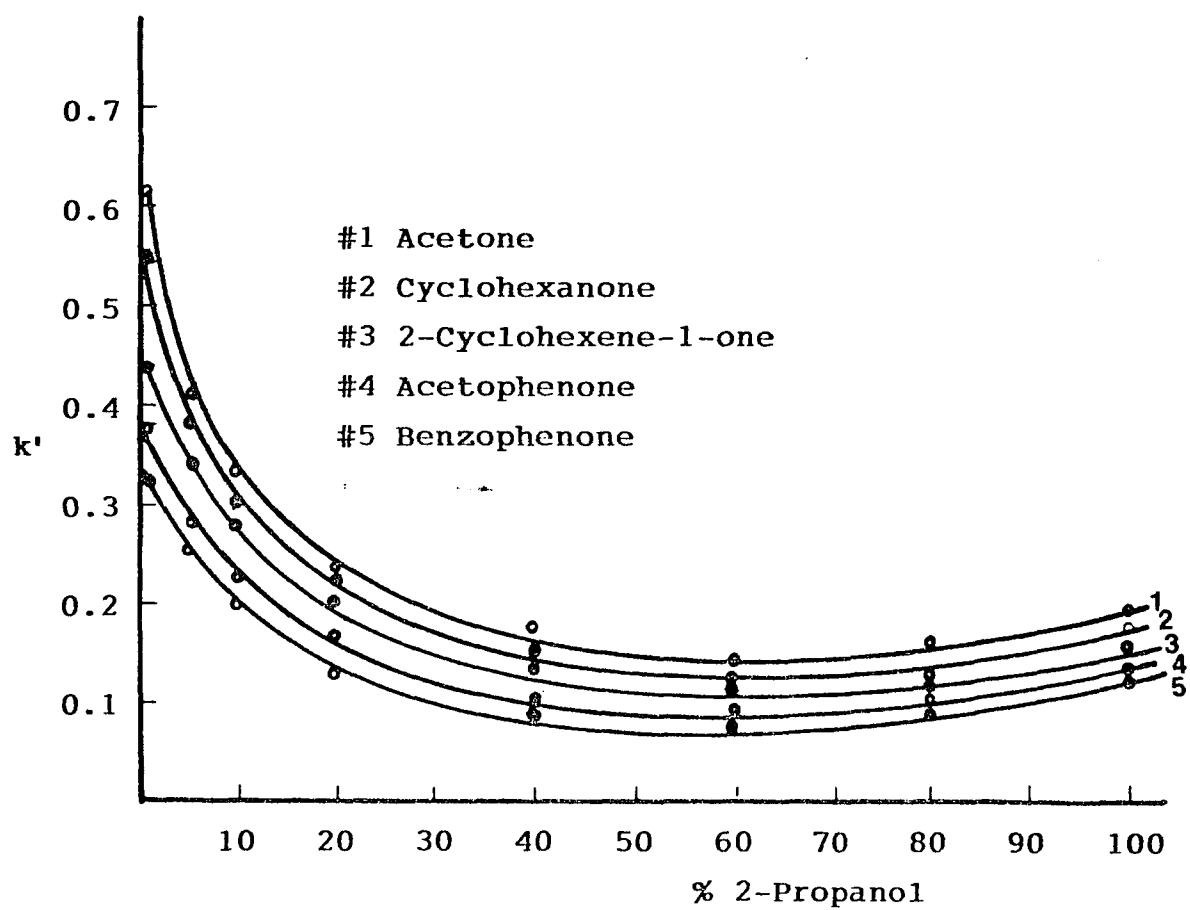


Figure III-23. Retention Of Ketones On Hypersil - NH_2 Column With Hexane / 2-Propanol Eluent.

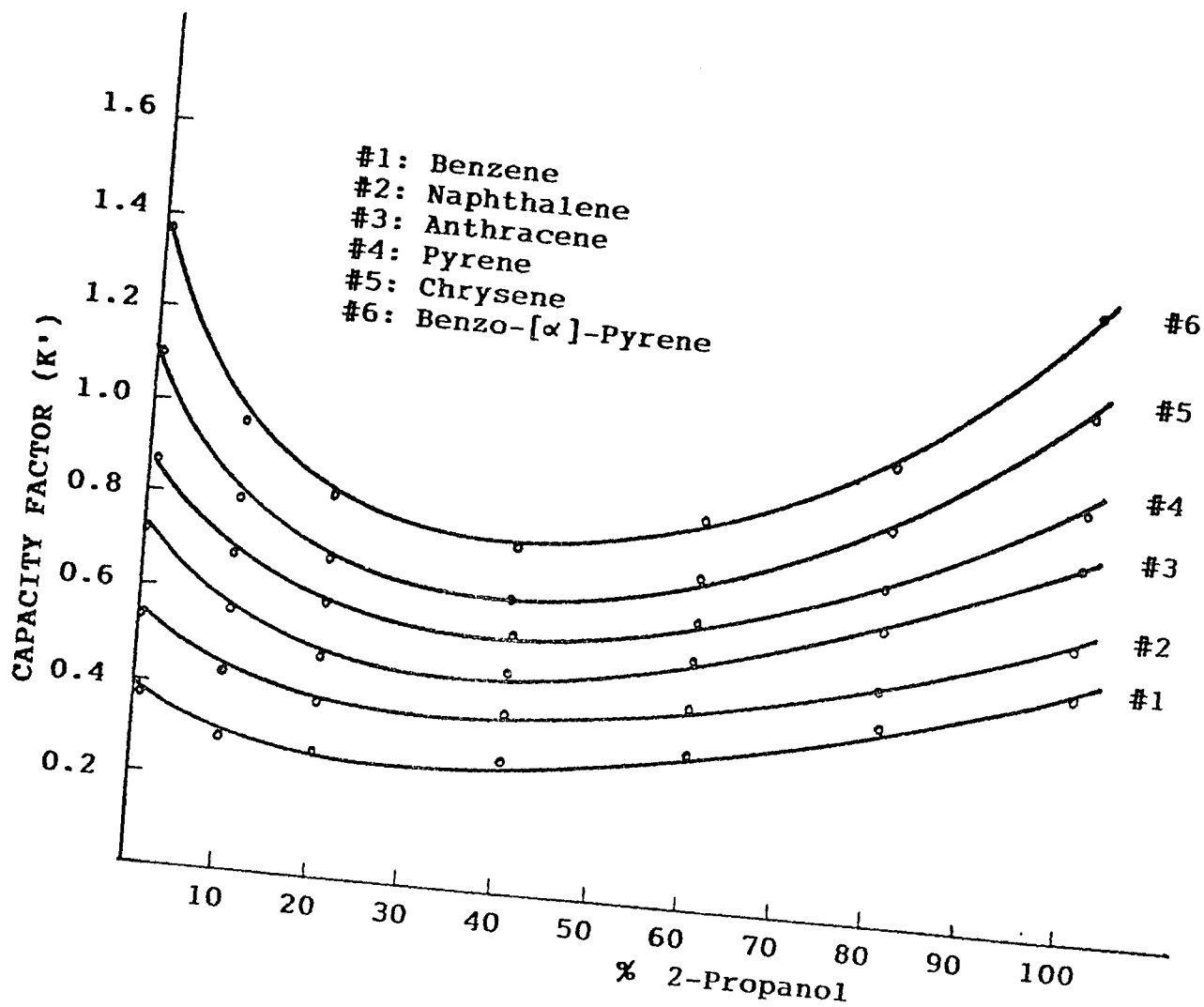


Figure III-24. Retention Of PAHs On Hypersil - NH₂ Column With Hexane / 2-Propanol Eluent.

Figures III-25 - III-29. Linear relationships, indicating correspondence with the Snyder model (Eqn. 17), are obtained for only a few compounds. It should be noted that the compounds which do show linearity are very polar compounds, compounds which undergo strong molecular interactions with the amino groups. The Snyder model represents these compounds well since this model is based on these molecular interactions.

In the case of 4-chloroaniline, 4-methylaniline and 4-t-butylaniline (Figure III-26), the linear relationships were obtained up to a concentration of 2-propanol of about 80%. For higher concentration the retention increases. The same is seen in Figures III-28 and III-29 for ketones and PAHs, in which linearity extends to about 60% 2-propanol for ketones and to 40% for PAHs. It is again interesting to note that the slope reverses for higher concentrations in each case. This means the Snyder model applies only over limited ranges of concentration of polar solvent. This observation of different retentivities for various compounds on propyl amino column with the hexane/2-propanol binary solvent systems applies also to the propylcyano column.

We repeated these measurements with the more polar

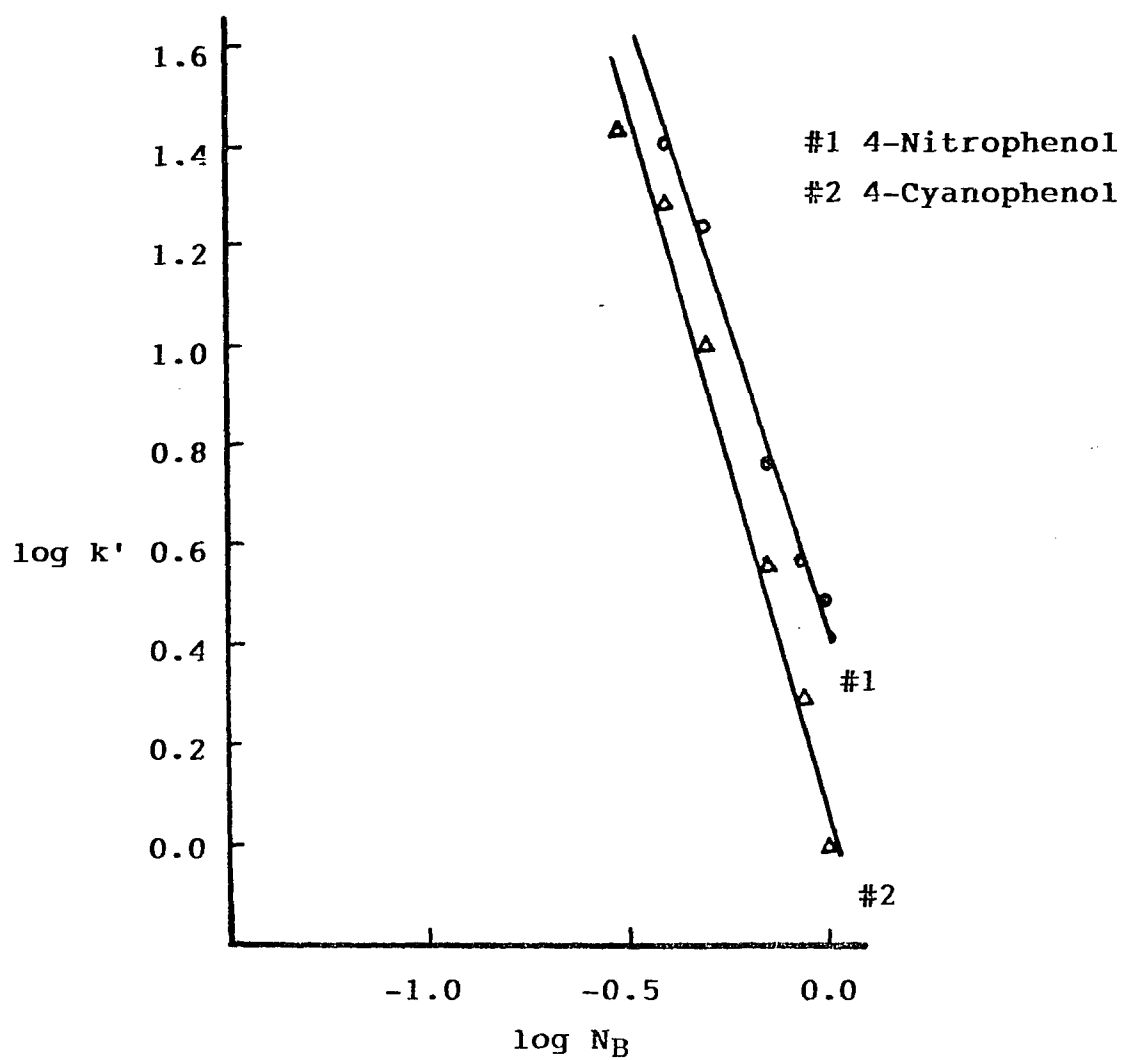


Figure III-25. Plots Of log k' Vs. log N_B Of 4-Nitrophenol And 4-Cyanophenol On Hypersil - NH_2 Column With Hexane / 2-Propanol Eluent.

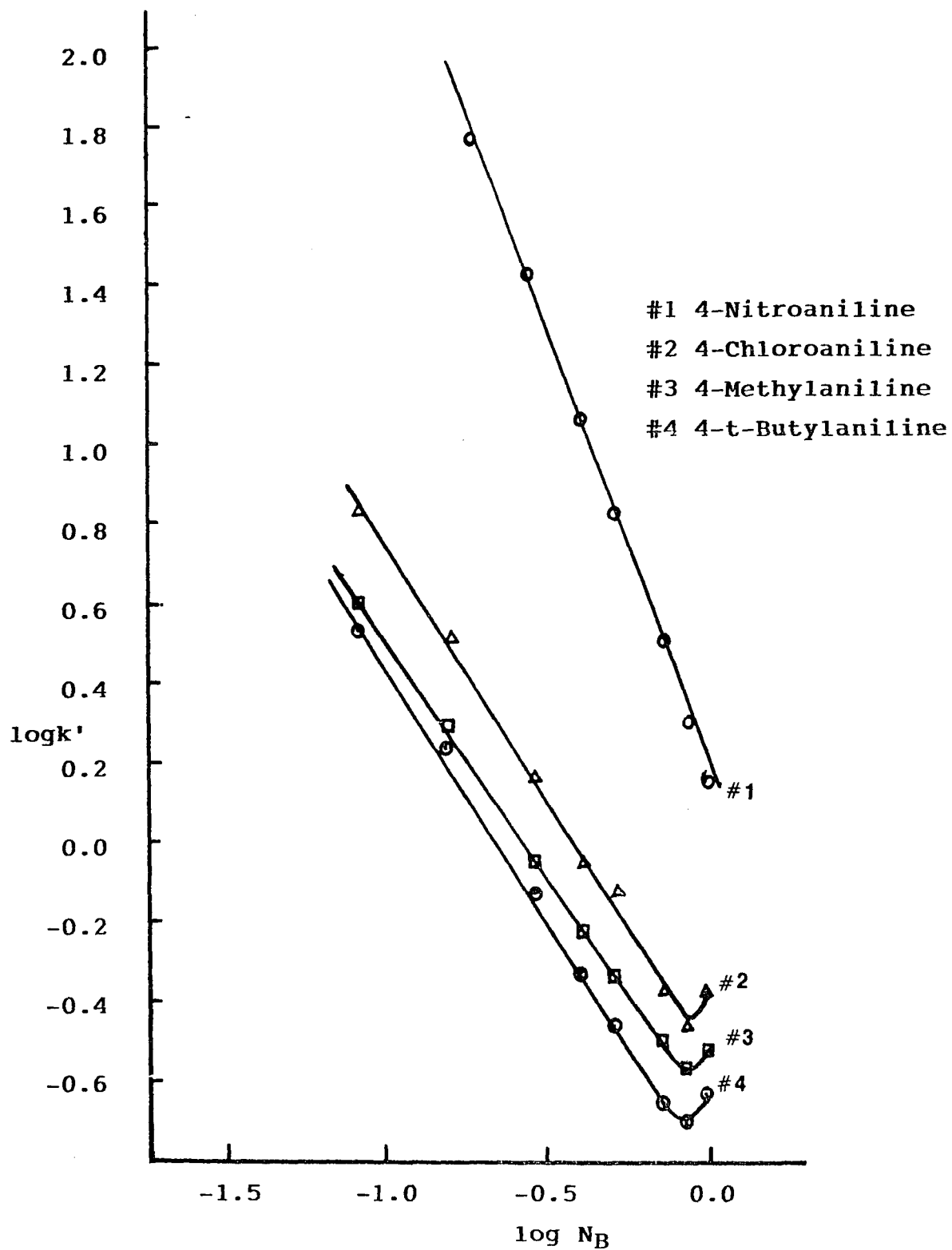


Figure III-26. Plots Of $\log k'$ Vs. $\log N_B$ Of Substituted Phenols On Hypersil - NH_2 With Hexane / 2-Propanol Eluent.

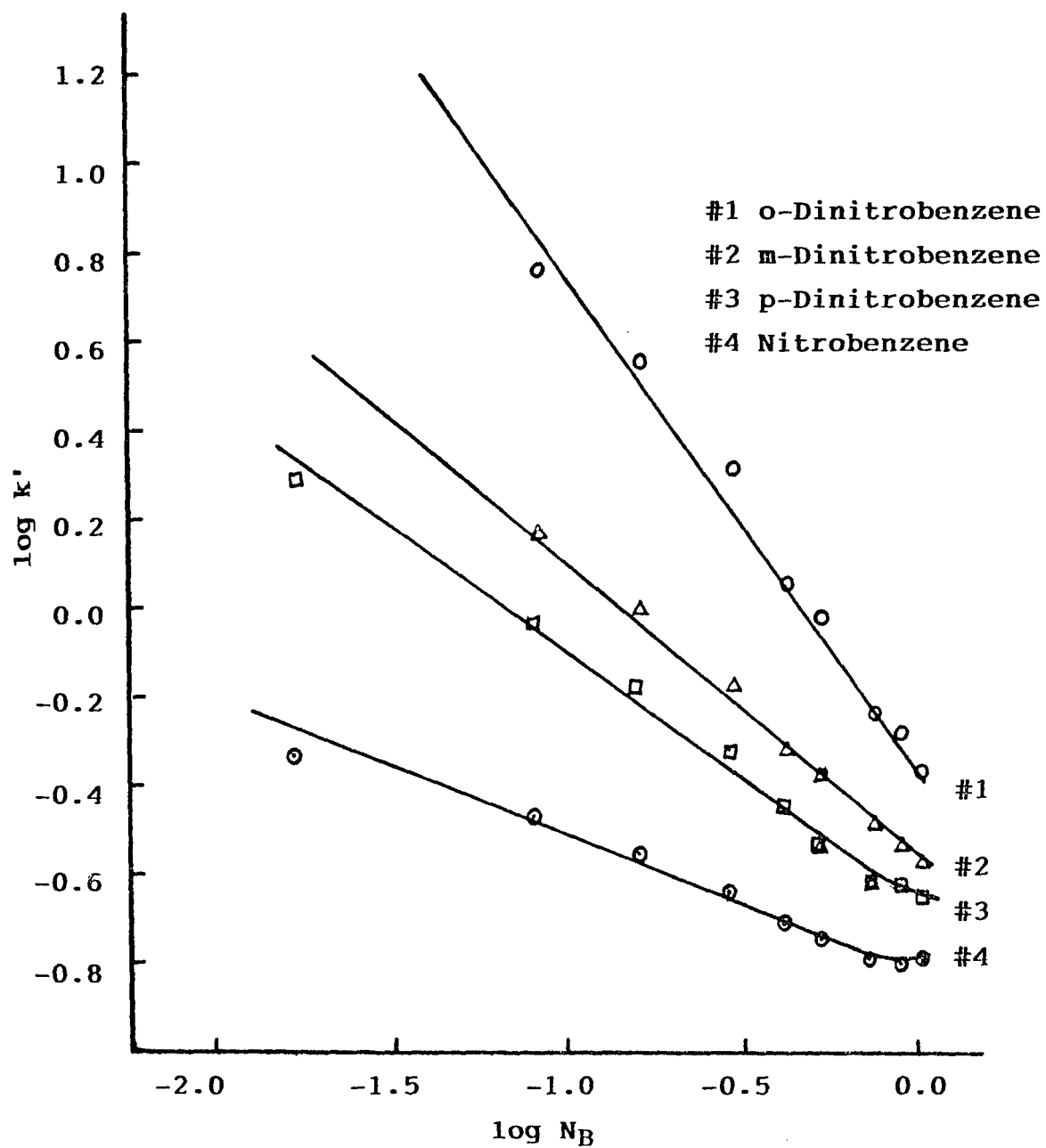


Figure III-27. Plots Of $\log k'$ Vs. $\log N_B$ Of Nitrobenzenes On Hypersil - NH_2 Column With Hexane / 2-Propanol Eluent.

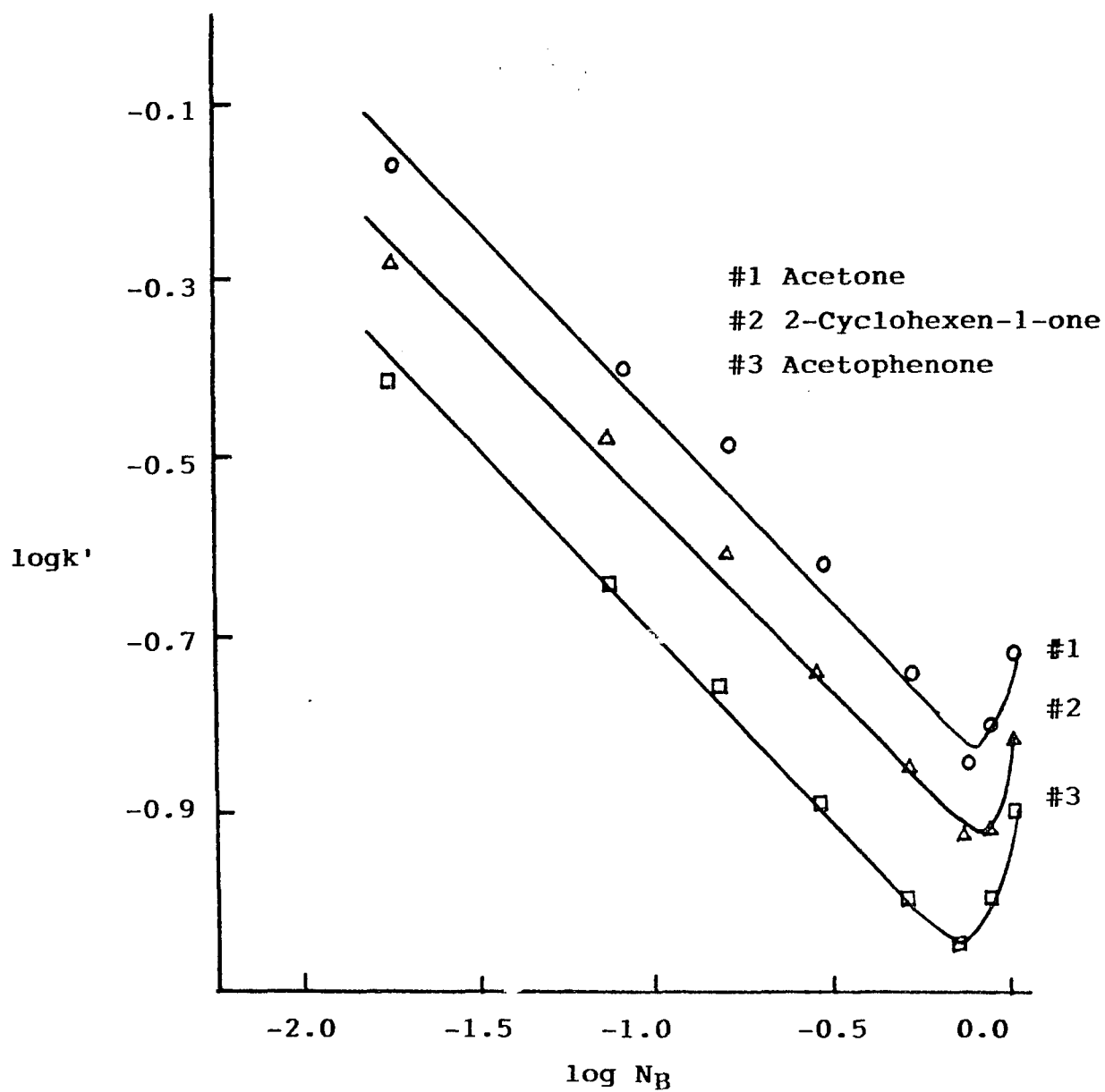
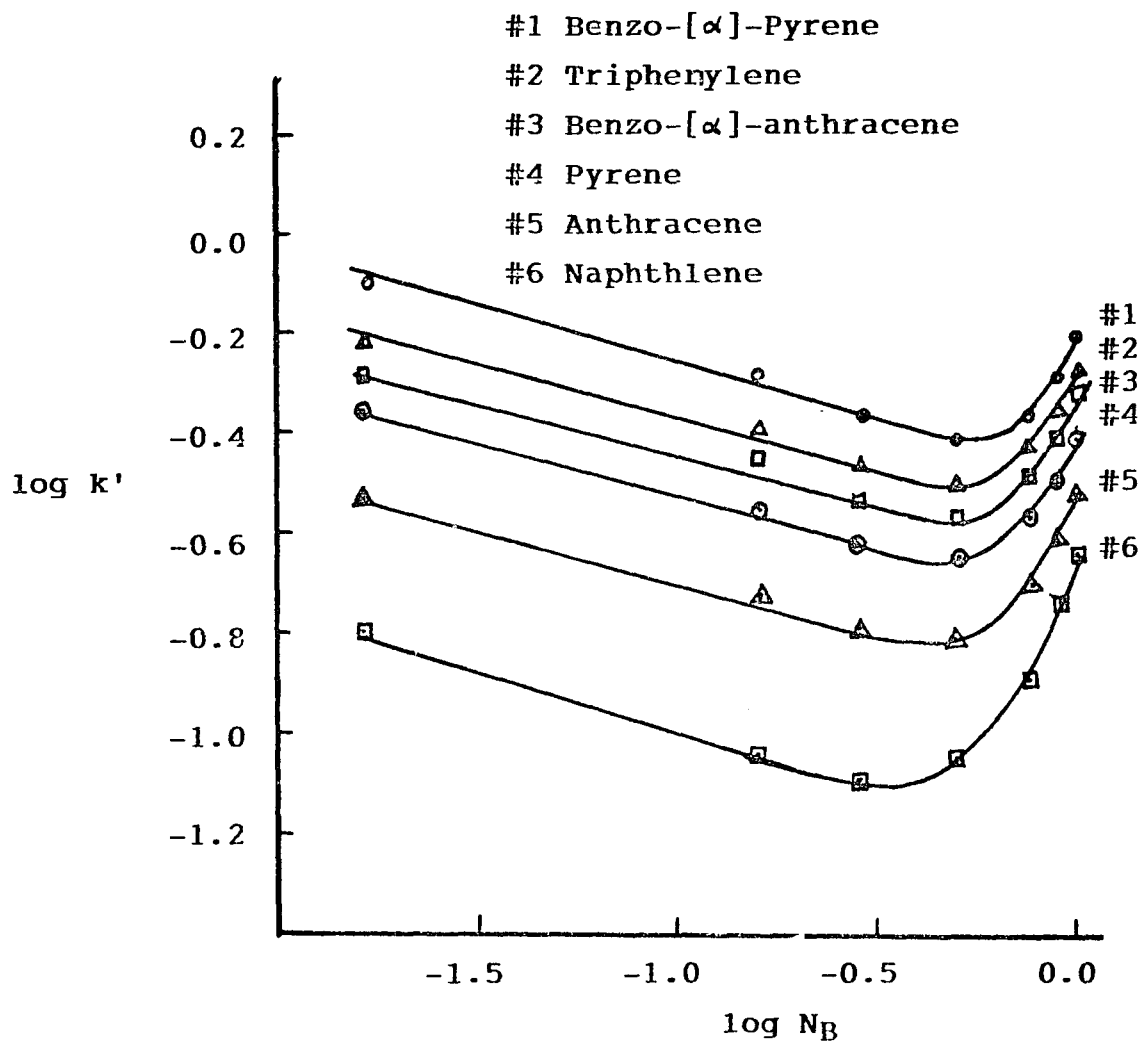


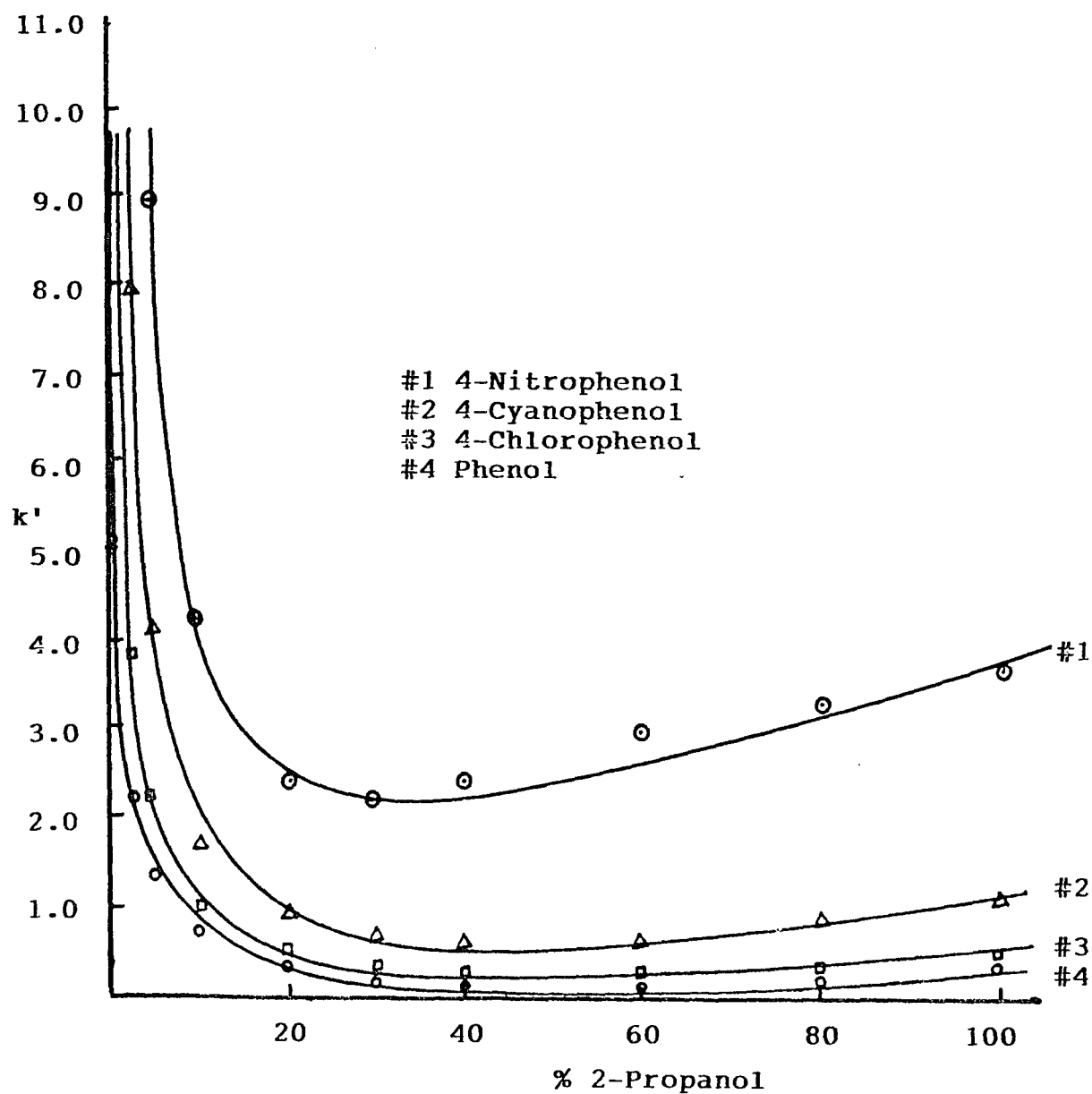
Figure III-28. Plots Of $\log k'$ Vs. $\log N_B$ Of Ketones On Hypersil - NH_2 Column With Hexane / 2-Propanol Eluent.



FigureIII-29. Plots Of log k' Vs. log N_B Of PAHs On Hypersil - NH_2 Column With Hexane / 2-Propanol Eluent.

dichloromethane / 2-propanol eluent. Figures III-30-III-34 give the retention curves for various compounds on the NH_2 column. However, as compared with the hexane/2-propanol eluent, the retention of all compounds studied gives a minimum with increasing % polar solvent. The amount of 2-propanol in the mobile phase at which the retention reverses is determined by the physicochemical properties of the solute. Different solutes, having different retentions, show different retention reversal compositions with different solvent systems. Strongly retained (very polar) compounds show a retention reversal at higher concentration than do weakly polar or non polar compounds.

The plots of $\log k'$ versus $\log N_B$ are shown in Figure III-35 - III-39. For phenols, comparing hexane/2-propanol eluent (Figures III-25) with dichloromethane/2-propanol (Figure III-35), it is seen that the retention reversal occurs only with the more polar solvent system. In Figure III-34, for 4-nitrophenol the critical % 2-propanol is about 25% ($\log N_B = -0.68$); for 4-cyanophenol, about 37% (-0.50), and for 4-chlorophenol and phenol, about 53% (-0.32). For the relatively weak polar solutes such as nitrobenzenes (Figure III-37) and ketones (Figure III-38), the



FigureIII-30. Retention Of Substituted Phenols On Hypersil - NH₂ Column With Dichloromethane / 2-Propanol Eluent.

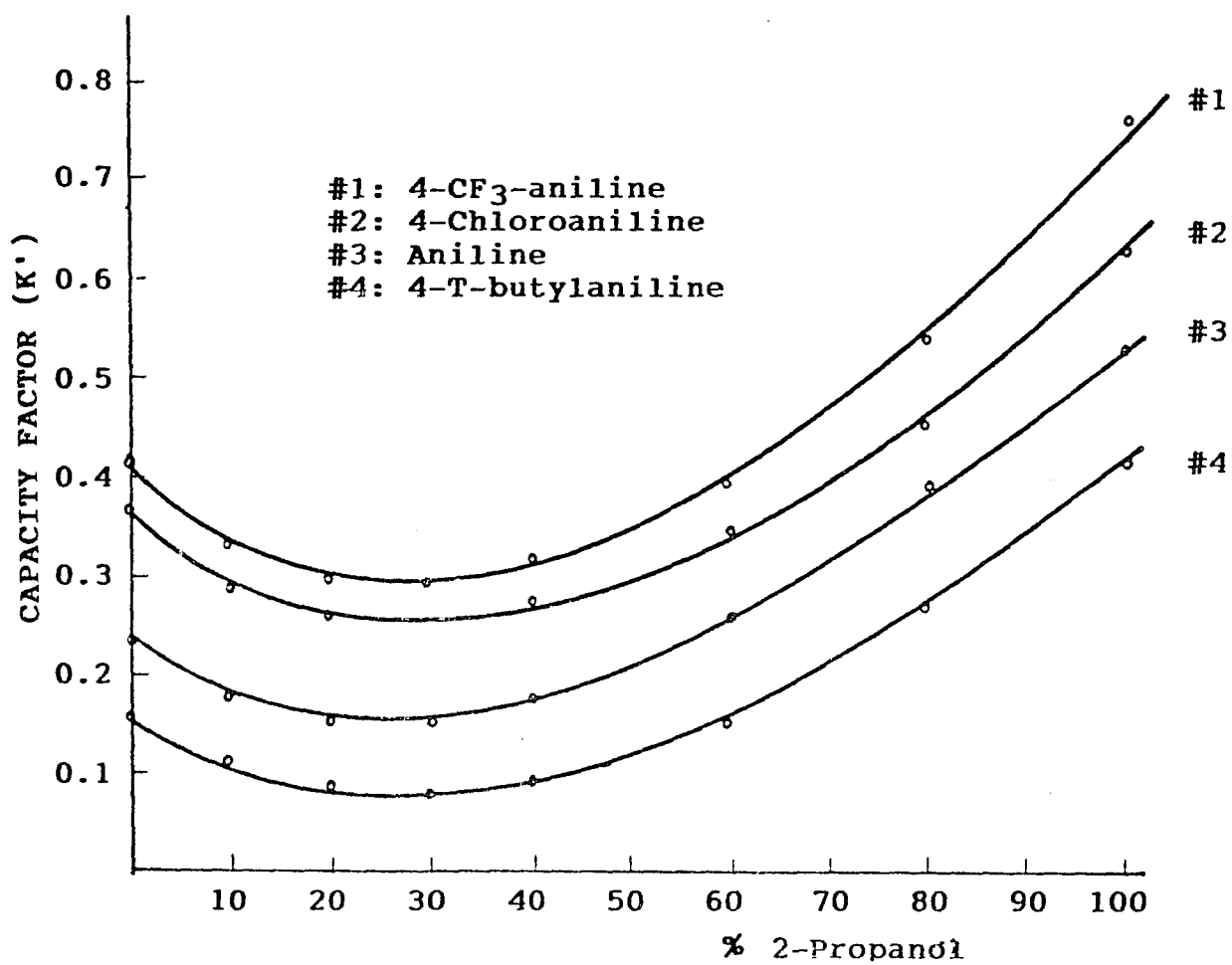
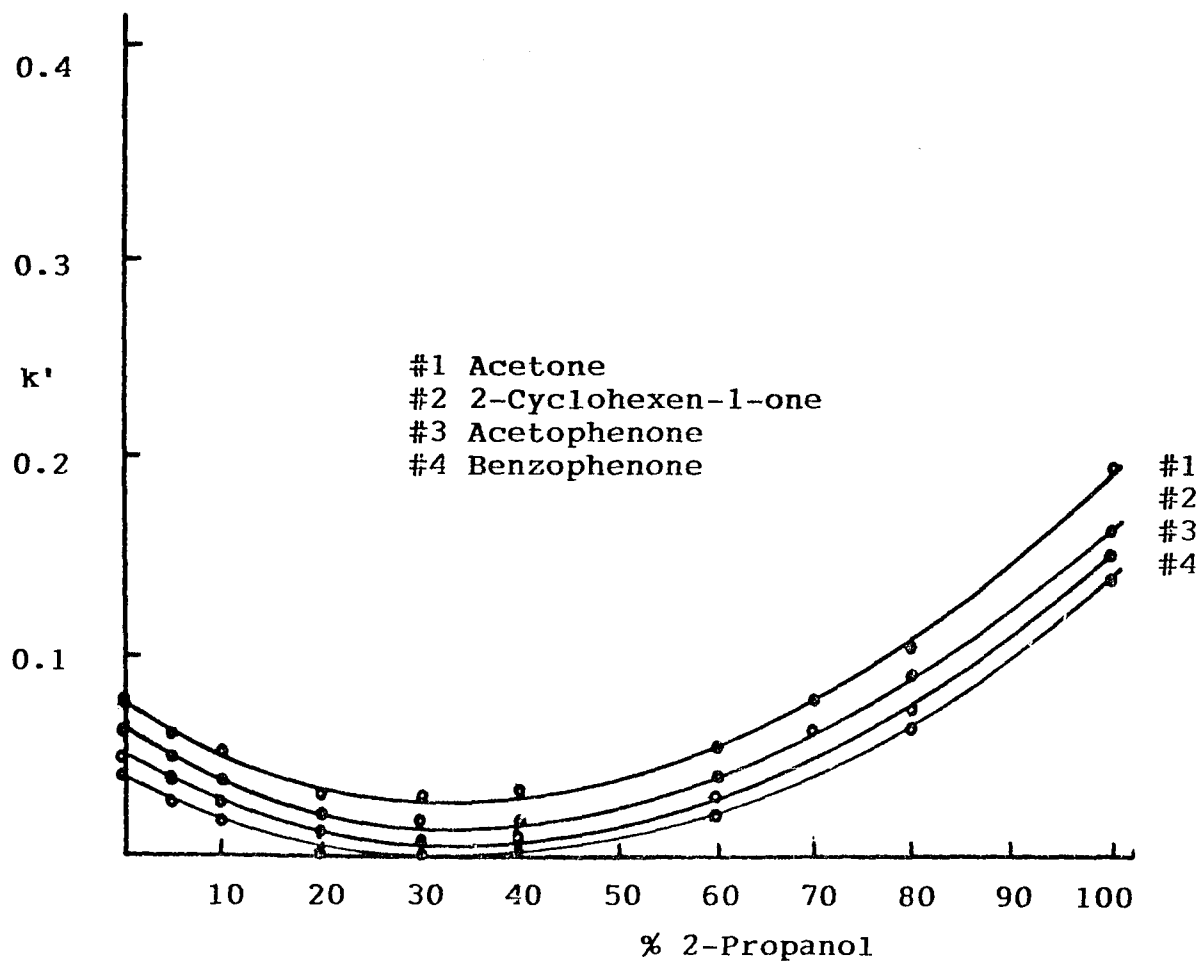


Figure III-31. Retention Of Substituted Anilines On Hypersil-NH₂ Column With Dichloromethane / 2-Propanol Eluent.



FigureIII-32. Retention Of Various Ketones On Hypersil - NH_2 Column With Dichloromethane / 2-Propanol Eluent.

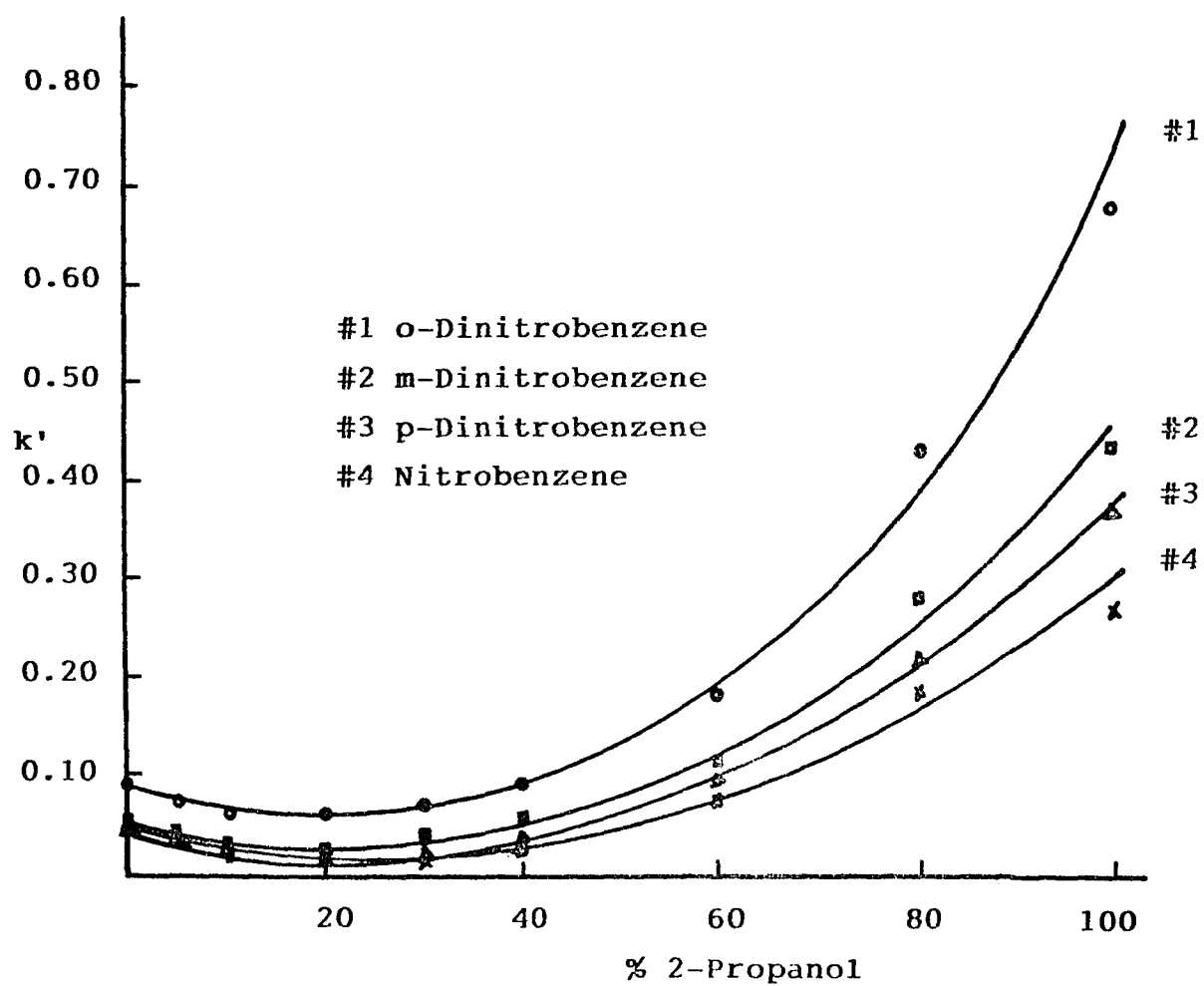
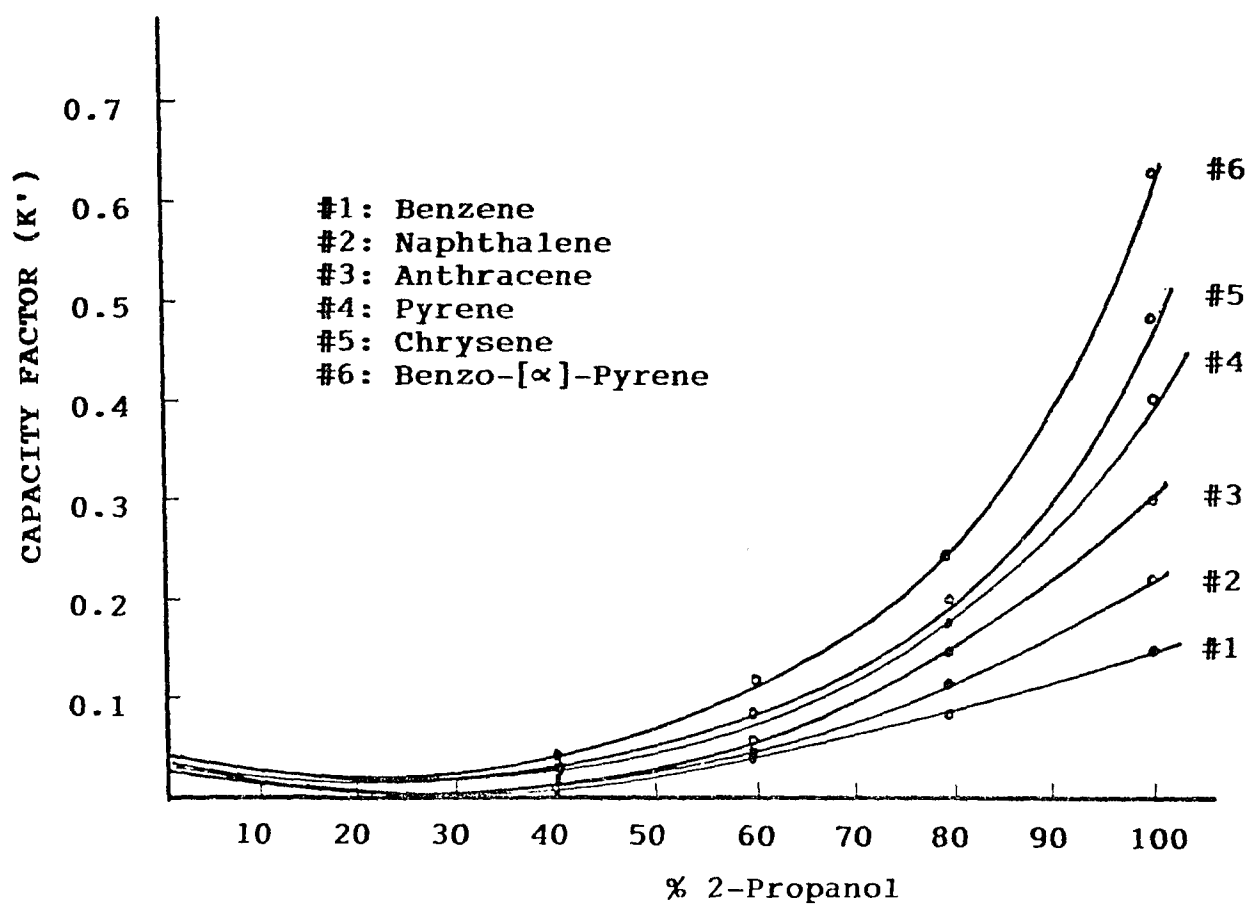


Figure III-33. Retention Of Nitrobenzenes On Hypersil - NH₂ Column With Dichloromethane 2-Propanol/Eluent.



FigureIII-34. Retention Of PAHs On Hypersil - NH_2 Column With Dichloromethane / 2-Propanol Eluent.

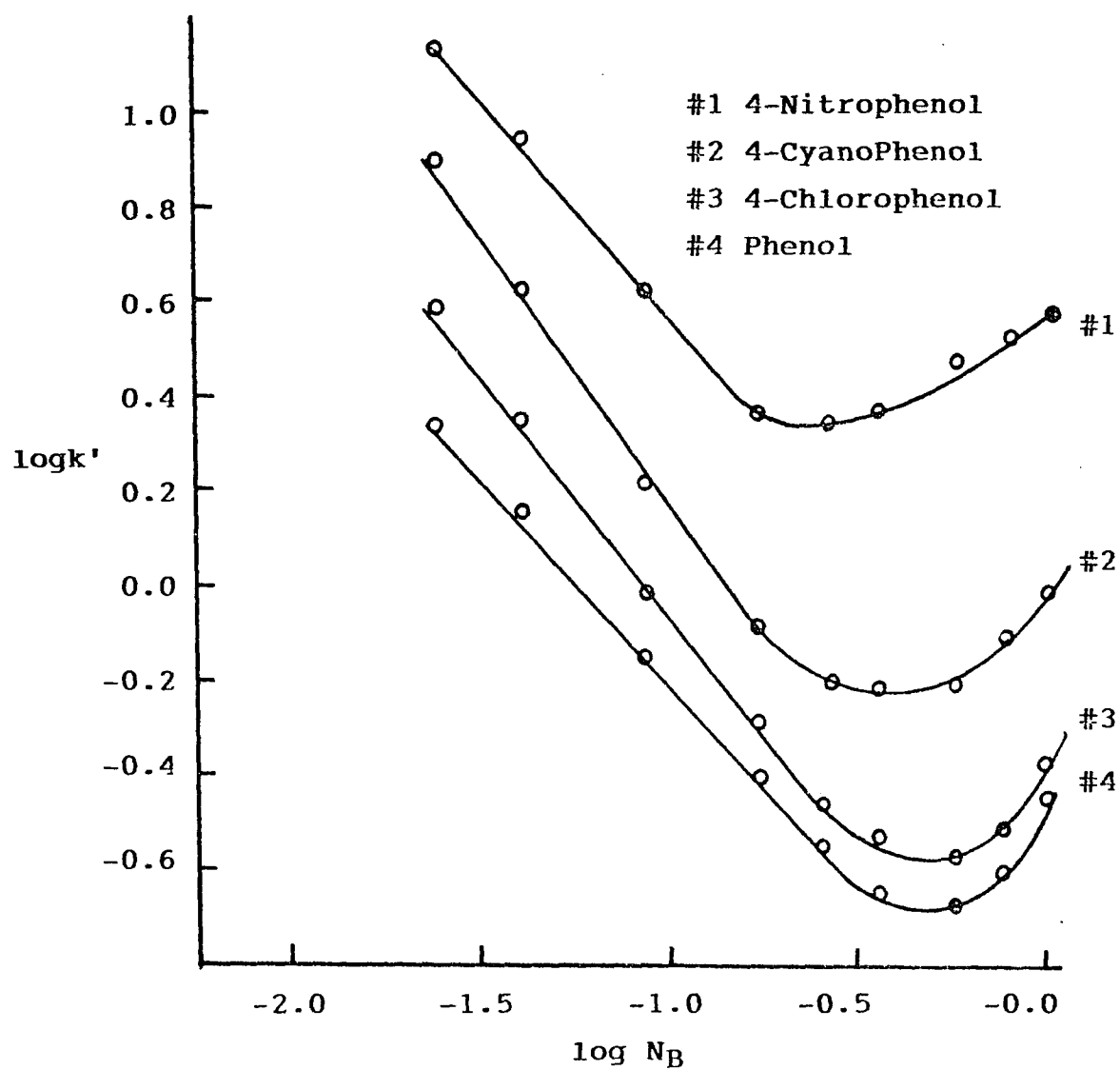


Figure III-35. Plots Of Several Phenols On Hypersil - NH₂ Column With Dichloromethane / 2-Propanol Eluent.

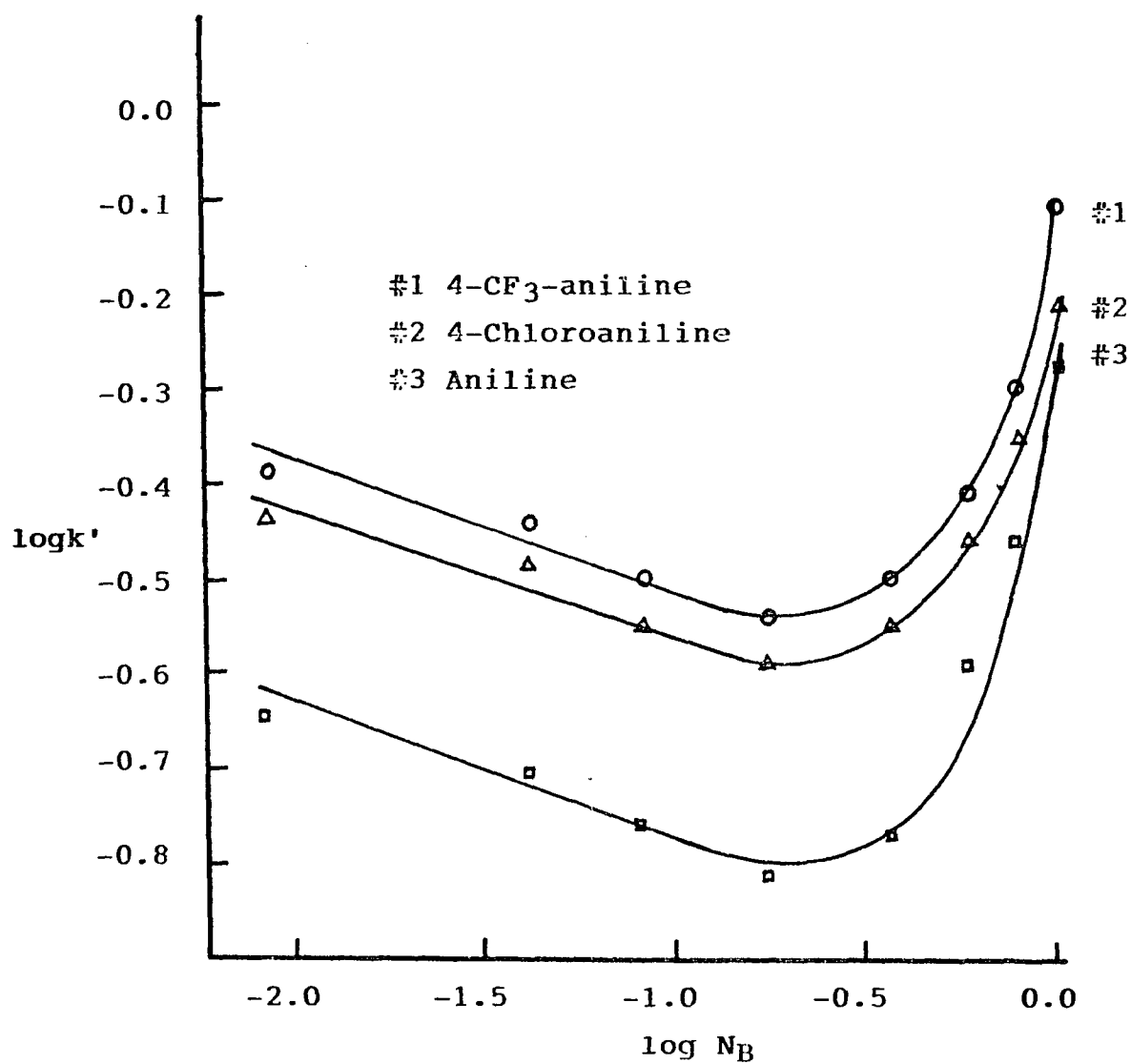


Figure III-36. Plots Of $\log k'$ Vs. $\log N_B$ Of Anilines On Hypersil - NH₂ Column With Dichloromethane / 2-Propanol Eluent.

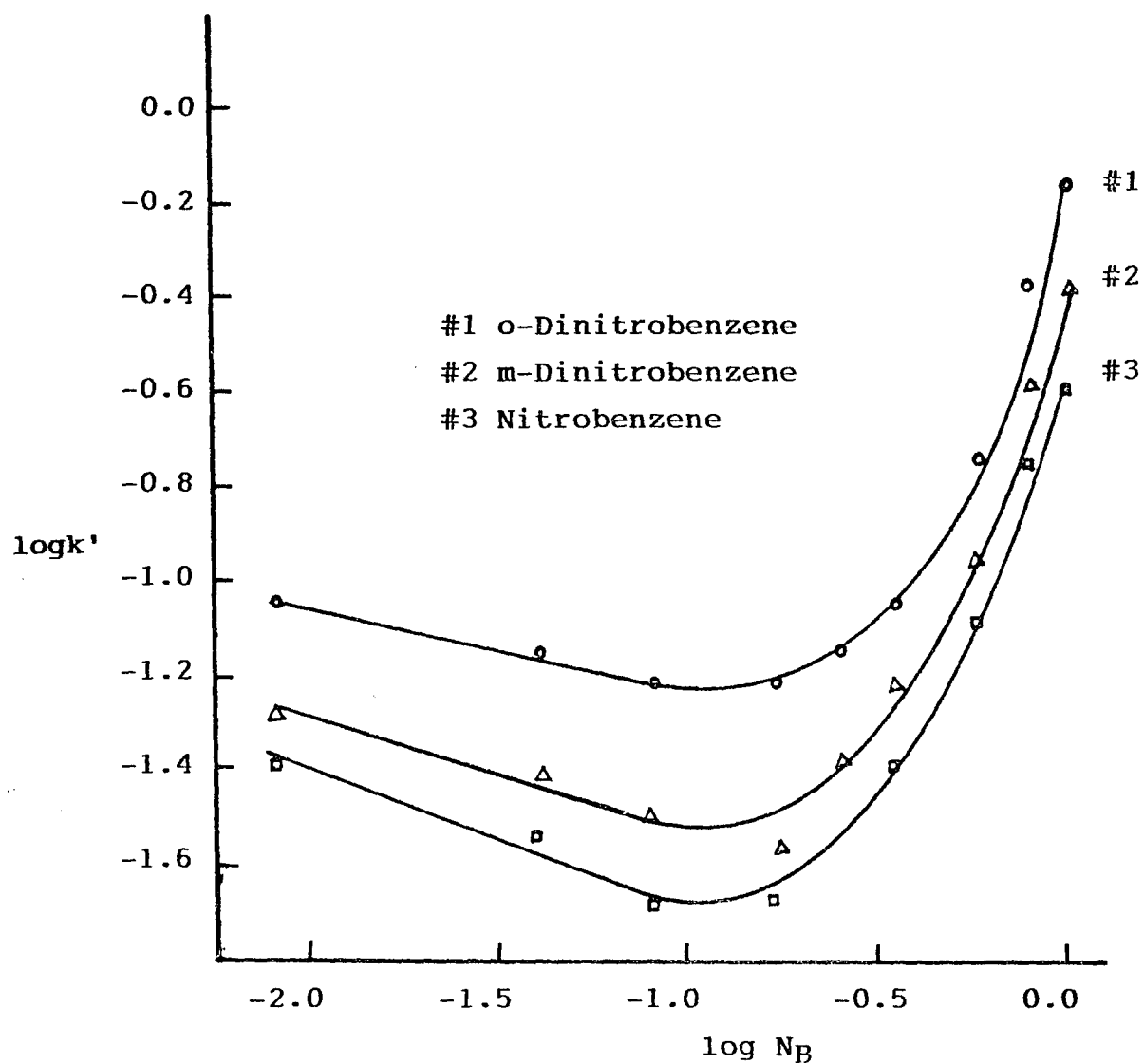
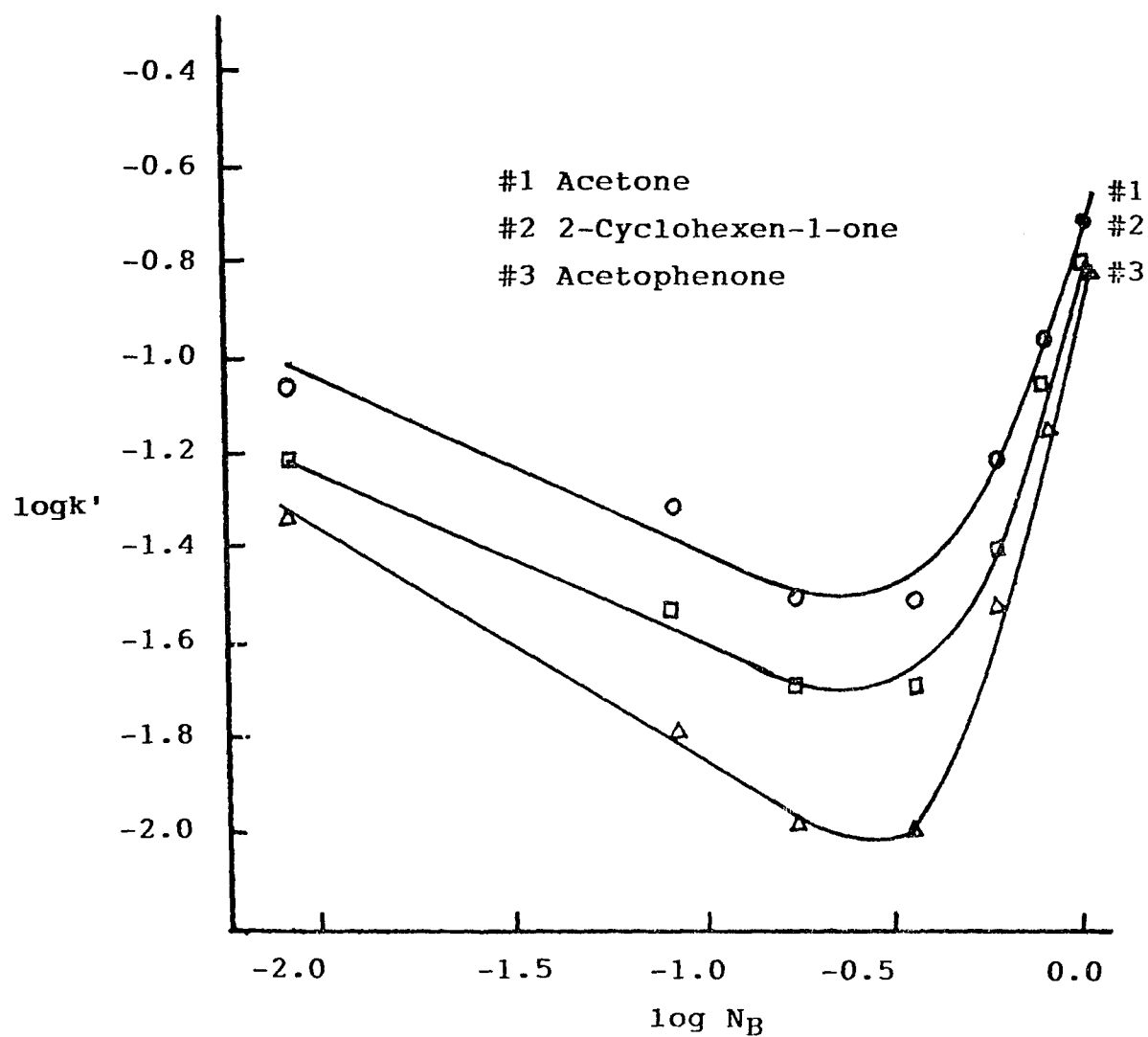


Figure III-37. Plots Of $\log k'$ Vs. $\log N_B$ Of Nitrobenzenes
 On Hypersil - NH_2 With Dichloromethane /
 2-Propanol Eluent.



1. Figure III-38. Plots Of $\log k'$ Vs. $\log N_B$ Of Ketones On Hypersil - NH_2 Column With Dichloromethane / 2-Propanol Eluent.

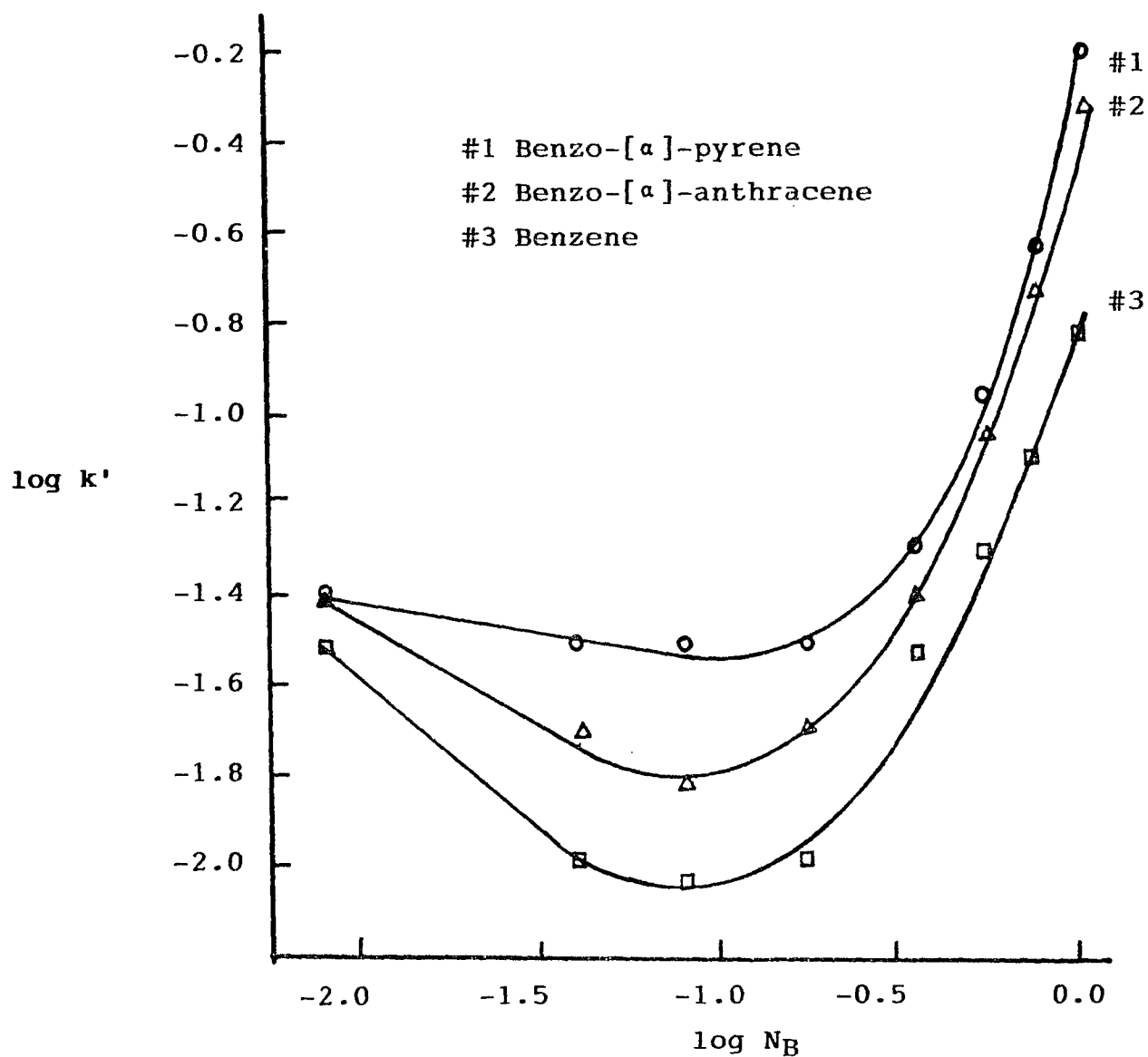


Figure III-39. Plots Of $\log k'$ Vs. $\log N_B$ Of PAHs On Hypersil - NH_2 With Dichloromethane / 2-Propanol Eluent.

critical values are even lower, about 20% 2-propanol. For the non-polar solutes such as PAHs (Figure 39), the upper limit is only 10-20%.

III-5-4. A New Model for Retention on Polar Bonded Phases

The retention behavior of various polar and non-polar compounds on aminopropyl- and cyanopropyl- bonded phase columns, with various mobile phases has been studied extensively. It is clear that over limited ranges of polar mobile phase compositions, the Snyder displacement model describes retention well. However, at high % polar modifier, significant retention differences are observed. The simple Snyder model no longer represents retention on polar bonded phases.

The Snyder model assumes that retention is governed by a specific molecular interaction process in which the solute molecules displace solvent molecules from active sites at the surface of the stationary phase. Our experimental results show that this model is accurate only up to certain polar solvent compositions. A model which can describe retention over the entire range of mobile phase composition, must go beyond the simple

displacement picture.

In polar bonded-phase chromatography with a binary mobile phase, we suggest that the retention mechanism consists of two comparable processes (see Fig. III-40). The first is the Snyder mechanism. The second is a partition mechanism in which the solute molecules distribute between a surface - adsorbed liquid layer and a bulk mobile phase liquid layer. The dominant mechanism is determined by the concentration and type of polar modifier in mobile phase and the type of solute molecules. At low concentration of polar solvent used in a binary mobile phase mixture, the molecules of the polar solvent (M) are adsorbed onto the surface of polar stationary phase (e.g. via hydrogen-bonding interaction for proton-donor polar solvents), resulting in the formation of a monolayer of adsorbed solvent molecules. The Snyder displacement mechanism then can occur. At the concentration of the polar solvent used in binary solvent mixture increases, however, the adsorbed-surface layer becomes more extensive and its thickness is determined by the % polar solvent. The partition mechanism then begins to predominate. As can be seen in Figure III-40, the solute both displaces solvent from active sites and distributes between the surface-

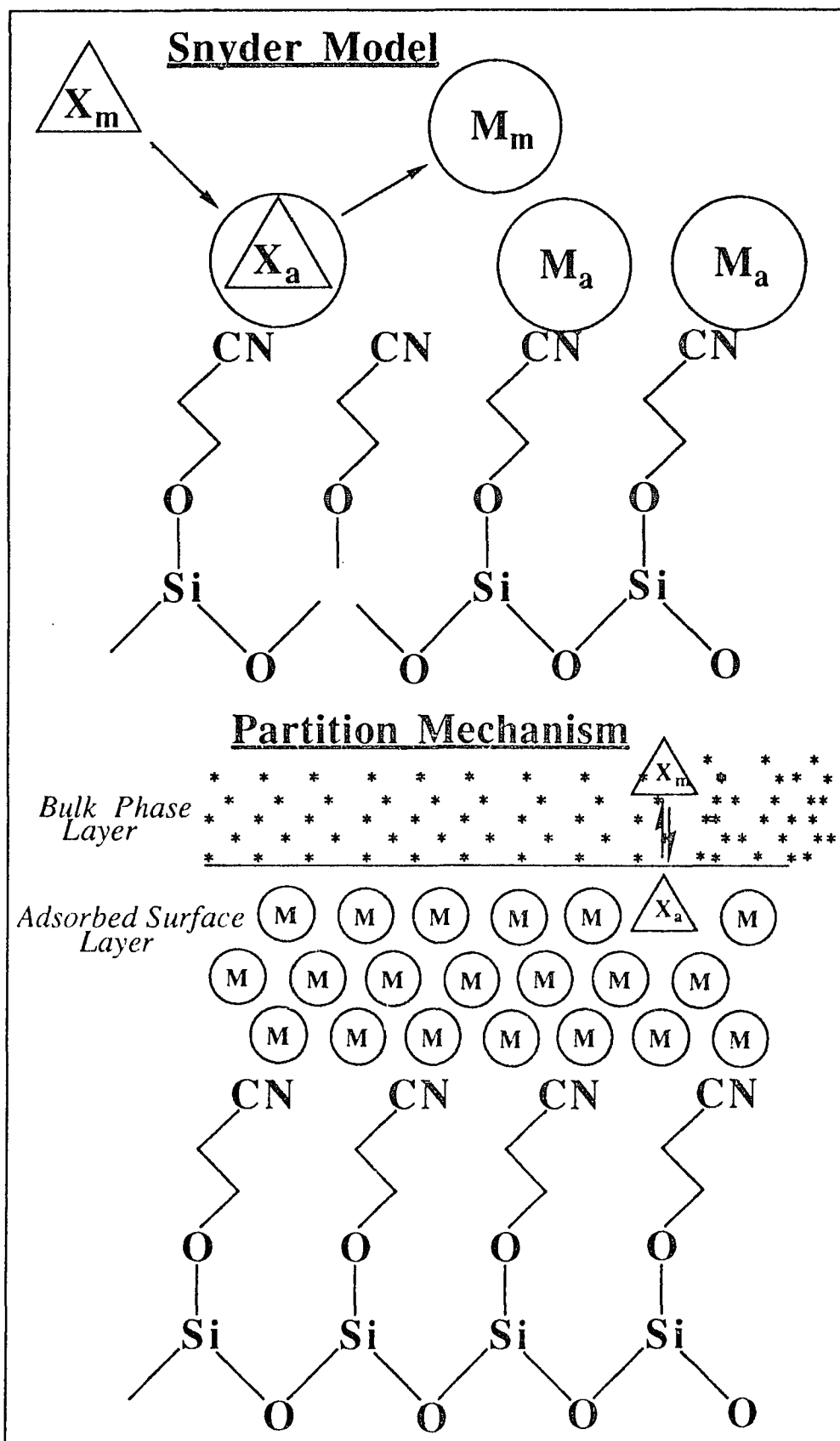


Figure III-40. Visualization of Two Comparable Processes
In Polar Bonded-Phase Liquid Chromatography

adsorbed liquid layer and the bulk mobile phase liquid layer. Both adsorption and partition mechanisms are in competition here, and both can occur simultaneously. At still higher concentrations of the polar modifier, a liquid-liquid partitioning effect seems to be most important.

Let us consider a chromatographic system consisting of molecules (X), a mobile phase mixture with polar solvent molecules (M) and the stationary phase with the surface-adsorbed liquid layer which thickness is determined by the polar mobile phase composition. We assume that solute retention occurs as a consequence of two processes, which are represented by the following equilibria:

$$X_m = X_{sm} \quad (18)$$

$$X_{sm} + nM_s = X_s + nM_{sm} \quad (19)$$

where m refers to mobile phase, sm refers to surface adsorbed liquid phase, and s refers to the stationary phase. Eqn. 18 represents solute partitioning between a surface-adsorbed liquid layer and a bulk liquid mobile phase; and Eqn.19 represents the Snyder displacement process. At small % polar solvent, the solute molecules

in the mobile phase adjacent to the surface monolayer could be expressed as X_m . As the % polar solvent is increased to certain percent at which an extensive adsorbed-surface layer can form, the solute molecules in the liquid layer adjacent to the surface of stationary phase can be then described as X_{sm} . Therefore, the Eqn. 18 and Eqn. 3 are essentially equivalent. The net retention process, however, is obtained from the combination of these two processes (Snyder displacement and partitioning):

$$X_m + nM_s = X_s + nM_{sm} \quad (20)$$

The distribution coefficient constants for the equilibria discribed in Eqn. 18-20 are

$$K_{pt} = \frac{[X_{sm}]}{[X_m]} \quad (21)$$

$$K_{sny} = \frac{[X_s] [M_{sm}]^n}{[X_{sm}] [M_s]^n} \quad (22)$$

$$K_{net} = \frac{[X_s] [M_{sm}]^n}{[X_m] [M_s]^n} = K_{sny} \cdot K_{pt} \quad (23)$$

where the subscript net refers to the net equation, pt

refers to the partition equilibrium and sny refer to Snyder displacement equilibrium. Expressed in log form,

$$\log K_{\text{net}} = \log K_{\text{Sny}} + \log K_{\text{pt}} \quad (24)$$

The first term, $\log K_{\text{Sny}}$, has been discussed in Section III-5-2. The second term, $\log K_{\text{pt}}$, is the partition term which describes a solute partitioning between the surface-adsorbed liquid phase and the bulk mobile phase. For a solute liquid-liquid partition equilibrium (Eqn. 18), we have

$$X_{\text{sm}} \cdot r^{\text{sm}} = X_{\text{m}} \cdot r^{\text{m}} \quad (25)$$

where r^{sm} and r^{m} denote the activity coefficients of a solute in surface-adsorbed liquid phase and in bulk mobile phase, respectively. The activity coefficient is a measure of deviation between a real solution (non-ideal solution) and an ideal solution. For an ideal solution, $r = 1$. Substituting Eqn.25 into Eqn.21, the distribution equilibrium constant becomes

$$K_{\text{pt}} = \frac{X_{\text{sm}}}{X_{\text{m}}} = \frac{r^{\text{m}}}{r^{\text{sm}}} \quad (26)$$

Two assumptions must be made up to this point. First, we assume that the solvent character of the surface-adsorbed liquid layer is uniform and once formed, is independent of further changes in mobile phase composition; this is because the surface-adsorbed layer (see Fig. III-40) consists essentially of the pure polar components. Therefore, r^{sm} is assumed to be a constant. The second assumption is that the solvent character of bulk mobile phase changes as the the binary solvent composition changes. This assumption has been expressed by an empirical equation proposed by Slaats [36]

$$r^m = \frac{1}{v_m (A - BN_B)} \quad (27)$$

Where v_m is the molar volume of the mobile phase, N_B is the mole fraction of the more polar solvent B, A and B are both constants in which $A = (r^{\circ,m} v_m)^{-1}$, here $r^{\circ,m}$ is the activity coefficient of pure non-polar solvent.

The distribution coefficient can be then obtained by combining Eqns. 25 and 26:

$$K_{pt} = \frac{r^m}{r^{sm}} = \frac{1}{v_m r^{sm}} \frac{1}{(A - BN_B)} \quad (28)$$

Combining this with Eqns.8, 17 and 24, the relationship between the capacity factor k' and the mole fraction N_B of polar solvent B for our new model is given as:

$$\begin{aligned} \log k' &= C' - A_s/n_b \log N_B \\ &\quad - \log v_{sm} r^{sm} - \log (A - BN_B) \end{aligned} \quad (29)$$

in which C' and $\log v_{sm} r^{sm}$ are assumed to be constants. Eqn. 29 is then rearranged to:

$$\log k' = C - A_s/n_b \log N_B - \log (A - BN_B) \quad (30)$$

This is the fundamental equation derived for our proposed retention mechanism. The form of the equation is consistent with our experimental data, i.e. the plot of $\log k'$ vs. $\log N_B$ has a point where the retention reversal occurs. The polar mobile phase composition at this point can be obtained by differentiating Eqn. 30 and setting equal to zero.

$$\frac{d \log k'}{d \log N_B} = - \frac{A_S}{n_b} + \frac{BN_B}{A - BN_B} = 0$$

$$N_B^\circ = \frac{AA_S}{B(n_b + A_S)} \quad (31)$$

where N_B° is the mole fraction of polar solvent in the binary mixture at the critical point. From Eqn.31, it is clear that the critical point at which the retention reversal occurs is the function of binary mobile phase system and solute molecules.

In order to obtain more information about constant A and B, we calculated the ratio of A/B from the following equation:

$$A/B = N_B^\circ (1 / A_S/n_b + 1) \quad (32)$$

The values of N_B° and A_S/n_b which is the slope representing the Snyder mechanism were evaluated from the experimental data. In Table III-19, are given the values of A/B for various compounds. As expected, the ratio of A/B is always greater than 1, i.e. solubility decreases as N_B increases. This implies that for thses systems, A is always greater than B, which is consistent with the fundamental equation (Eqn.30) describing the

Table III-19. Values of A/B for Various Compounds
on Hypersil-NH₂ Column

<u>Compound</u>	<u>A/B</u>
Phenol	1.55
O-cresol	1.55
2-Ethylphenol	1.56
2-isopropylphenol	1.45
2-t-Butylphenol	1.46
4-Chlorophenol	1.40
4-Methylphenol	1.40
4-t-Butylphenol	1.40
Acetone	1.60
2-Cyclohexan-1-one	1.58
Acetophenone	1.48
Benzo-[a]-pyrene	2.30
Triphenylene	2.30
Benzo-[a]-anthracene	1.98
Pyrene	1.88
Anthracene	1.88
Naphthalene	1.67

new model.

To illustrate Eqn.31, which indicates the N°_B value is dependent on the type of solute molecules on the basis of experimental data, the N°_B values of various compounds on the Hypersil-CN column are given in Table III-20. The data presented indicate that N°_B values for different compound is generally different. By comparing phenol and aniline, the N°_B value of phenol is 0.87 (80% IPA) and of aniline is 0.70 (59% IPA). This implies that Snyder displacement model applies for phenol up to 80% IPA, but aniline only up to 59% IPA. This can be rationalized by the fact that phenol has stronger interaction with cyano groups than aniline, as we discussed earlier, the adsorption effect for phenol based on Snyder model seems to be predominant over a wider range of % polar solvent. An interesting observation in Table III-20 is that the group of similar compounds have similar N°_B values, i.e. the five phenols have the N°_B values in the range of 0.79 - 0.87, corresponding to 70 - 80% IPA; anilines have the N°_B values in the range of 0.70 - 0.72, corresponding to 59-60% IPA; and PAHs have the N°_B values in the range of 0.53-0.66, corresponding to 40-54% IPA. This is

Table III-20. Values of Mole Fraction (N_B) at Critical Point for Various Solutes on Hypersil-CN Column.

<u>Solute</u>	<u>N_B</u>	<u>% IPA*</u>
phenol	0.87	80
o-cresol	0.87	80
2-ethylphenol	0.79	70
4-t-butylphenol	0.81	72
4-chlorophenol	0.87	80
aniline	0.70	59
4-t-butylaniline	0.70	59
4-chloroaniline	0.72	60
nitrobenzene	0.84	75
m-dinitrobenzene	0.87	80
pyrene	0.53	40
triphenylene	0.63	50
benzene	0.64	51
naphthalene	0.53	40
benzo-[a]-pyrene	0.66	54

* Hexane (HEX)/2-propanol (IPA) was used as binary mobile phase

expected on the basis of the new model.

To further verify that the nature of mobile phase system affects the N°_B values, these values for 4-chlorophenol in five different mobile phase systems on the amino propyl column are listed in Table III-21. It is obvious that different mobile phase systems demonstrate different N°_B value for a given solute. Comparing the three binary mixtures hexane/ethyl acetate, hexane/2-propanol and hexane/ethanol, ethanol is the strongest polar solvent and ethyl acetate is the weakest. In the hexane/ethyl acetate systems, there is no critical point for the plot of $\log k'$ vs. $\log N_B$. This means the Snyder adsorption model applies for the hexane/ethyl acetate mobile phase system for 4-chlorophenol over the full range of polar solvent (0%-100%). In the hexane/ethanol and hexane/2-propanol systems, however, the Snyder model holds only up to 30% ethanol ($N^{\circ}_B = 0.49$) and up to 80% 2-propanol ($N^{\circ}_B = 0.87$). This is exactly what would be expected since ethanol can undergo strong hydrogen-bonding interactions (acid-base type) with the NH_2 stationary phase to form a surface-adsorbed liquid layer. Thus the solute partitions between the surface adsorbed liquid phase and the bulk mobile phase. The liquid-liquid partitioning

Table III-21. N°_B Values of 4-Chlorophenol with Five
Different Mobile Phase Systems on
Hypersil - NH_2 Column

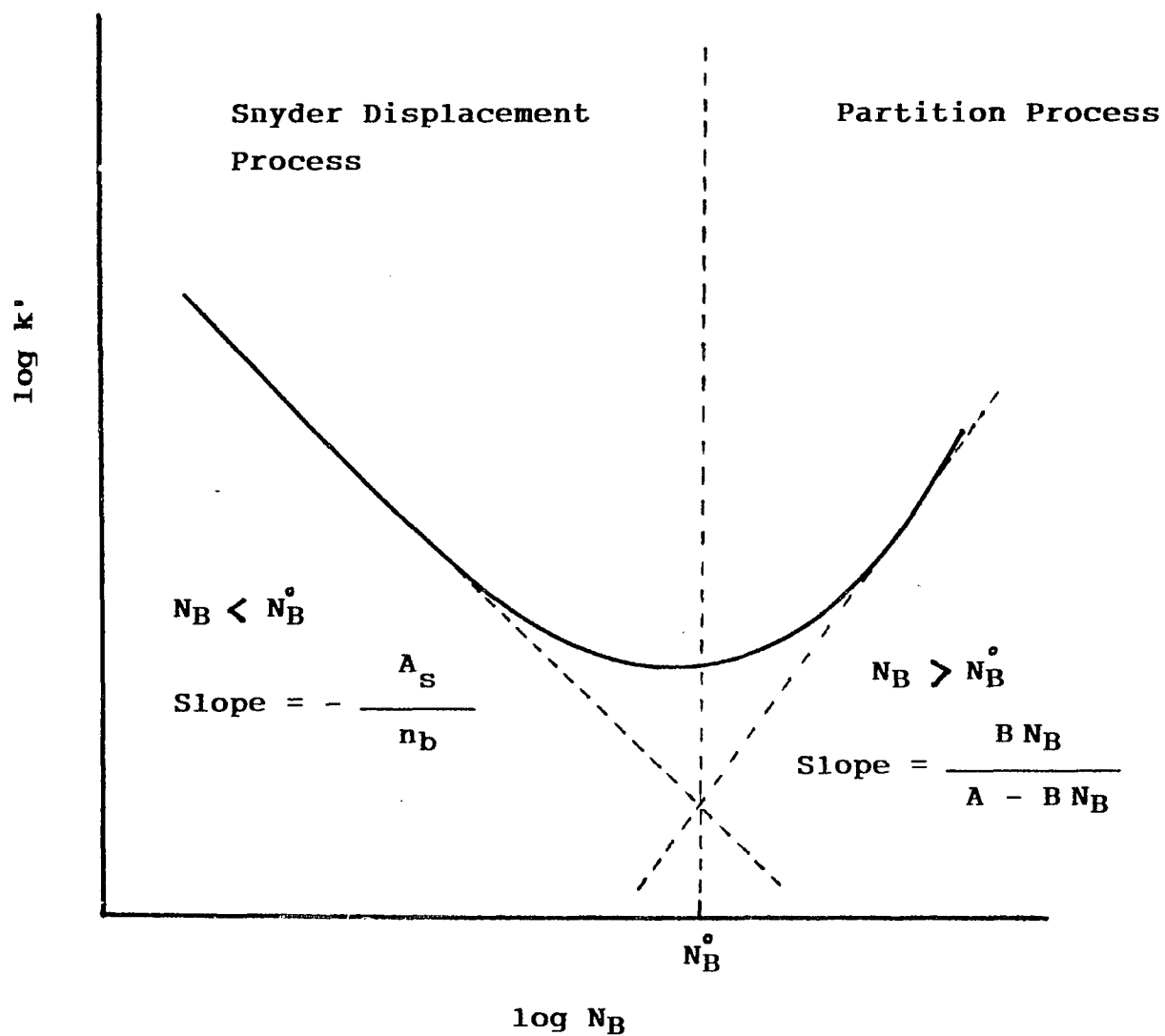
Binary Mobile Phase Mixture (A/B)	N°_B	$\%B$
Hexane/Ethyl Acetate*	none	none
Hexane/2-Propanol	0.87	80
Chloroform/2-Propanol	0.50	50
Dichloromethane/2-Propanol	0.42	47
Hexane/Ethanol	0.49	30

* There is no critical point (N°_B) for 4-chlorophenol
in hexane/ethylacetate mobile phase system.

effect seems to be the predominant above 30% ethanol.

To illustrate Eqn.30, a schematic plot of $\log k'$ vs. $\log N_B$ is given in Figure III-41. The experimental plots of $\log k'$ vs. $\log N_B$ for about 60 compounds are generally of the same form as that in Figure III-41. The plot consists of two parts, one the Snyder adsorption process in which retention decreases with increasing polar content of mobile phase; the other is the partition process which indicates retention increases with increasing the % polar solvent. For $N_B < N_B^0$, the amount of surface layer of polar solvent is insufficient to support the partitioning process and the Snyder displacement model will largely govern the retention. From Eqn. 30, the slope of plot $\log k'$ vs. $\log N_B$ when $N_B < N_B^0$ is equal to the ratio of the effective molecular area of the solute (A_S) and the polar solvent (n_b) at the adsorbent surface. This linearity is observed for plots of $\log k'$ vs. $\log N_B$. As $N_B > N_B^0$, however, partitioning effect becomes more important and retention mechanism depends on the solute liquid-liquid distribution. From the partition term in Eqn. 30, $-\log(A-BN_B)$, the slope of plot of $\log k'$ vs. $\log N_B$ corresponding to partition is given by

Eluent = Nonpolar + Polar



FigureIII-41. General Representation Of $\log k'$ Vs. $\log N_B$.

Eqn.31:

$$\text{Slope} = \text{BN}_B / \text{A-BN}_B \quad (31)$$

Thus, the plot of $\log k'$ vs. $\log \text{N}_B$ for partitioning is not linear; the slope increases as the mole fraction of polar solvent increases.

Our experimental data is generally consistent with the new model. The quantitative determination of the slopes on both sides has not yet made because of the lack of accurate solute molecular area data for aminopropyl and cyanopropyl stationary phases as compared to alumina and silica adsorbent phase.

III-5-5. Summary

Normal bonded-phase liquid chromatography plays a versatile and powerful role in chemical separations. The great potential of this technique is only now being explored. Polar normal bonded - phase HPLC has advantages which the reversed-phase technique may not have, as discussed in Chapter I.

In this chapter, we presented a systematic study of the effect of the nature and composition of the mobile phase on sample retention in normal-phase systems. According to our experimental results, it is evident

that the polar mobile phase solvent and its composition are the most important factors governing sample retention.

The potential of multi-mode separation on normal bonded-phase columns using appropriate solvents has also been noted. The polar bonded-phase columns can be operated not only in the normal phase mode but also in the reversed phase mode, depending on the selection of mobile phase. By using a highly polar mobile phase such as acetonitrile/water or methanol/water, a normal phase column can be converted to an effectively reversed phase mode. The retention mechanism in the reversed phase mode for a polar bonded-phase is presumably changed from one involving specific molecular interactions with the functional group on stationary phase in the normal phase mode to a liquid-liquid partitioning effect in reverse phase mode.

The retention behavior of various compounds on cyanopropyl and aminopropyl polar bonded-phase columns in the normal-phase mode with binary solvent mixtures has been examined in order to determine the mechanism of retention and the dependence of retention on the mobile phase composition. On the basis of the experimental measurements, a retention model was proposed. This new

model expands upon the Snyder displacement model to allow description of retention data over the entire range of mobile phase compositions.

The new model for retention mechanism of normal bonded-phase liquid chromatography is based on the possibility of two comparable processes, a Snyder displacement molecular interaction and a partitioning effect. The dominant process is determined by the mobile phase solvent polarity and the molecular structure of solutes. For binary mobile phase systems consisting of a small concentration of polar solvent, there appears to be a good agreement with the Snyder displacement model. The linear correlations were obtained between $\log k'$ and $\log N_B$ with the slope $-A_S/n_b$ for most of compounds.

For the high % polar solvent, retention is significantly changed to liquid-liquid partition arising from the distribution of a solute between a surface-adsorbed liquid phase and a bulk-liquid mobile phase. The experimental data is consistent with the equation describing this proposed model. For instance, the plot of $\log k'$ vs. $\log N_B$ has a point where a retention reversal occurs; a negative slope ($-A_S/n_b$) is obtained from the experimental plot of $\log k'$ vs. $\log N_B$ for the

process based on Snyder displacement model; and a positive, non-linear slope of $\log k'$ vs. $\log N_B$ is observed for the partition process.

In general, our data support the proposed retention mechanism on polar bonded-phase columns. However, quantitative verification of the theoretical equation representing the retention mechanism on the polar bonded phase column cannot be made because of the lack of accurate data on solute effective molecular areas for the cyanopropyl and aminopropyl stationary phase systems and the lack of information on the constants A and B in the partition term of Eqn.30. Nevertheless, use of our new model to explain the retention mechanism of solutes on polar stationary phases is expected to lead to the application of this technique more effectively and powerfully.

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