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The Influence of Sorbed Organic Modifier on Solute Retention on an Octadecylsilyl Bonded Phase in HPLC

By

Natalia Felitsyn 

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Doctor of Philosophy

Department of Chemistry

Edmonton, Alberta

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Natalia Felitsyn

vul. Proektna 4/6
Staryj Sambir
L'viv Region
UKRAINE 292700

Date: Oct. 4/99

University of Alberta

Faculty of Graduate Studies and Research

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled The Influence of Sorbed Organic Modifier on Solute Retention on an Octadecylsilyl Bonded Phase in HPLC by Natalia Felitsyn in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Dr. Frederick F. Cantwell

Dr. Liang Li

Dr. Charles A. Lucy

Dr. George Kotovych

Dr. Carl Mendoza

Dr. Susan V. Olesik, External Examiner, Ohio State University

Date: Sept. 24/99

Abstract

The influence of organic modifier (OM) on the retention of non-ionic solutes by an octadecylsilyl (C_{18}) bonded phase HPLC packing is quantitatively studied. Solute transfer activity coefficients are used to model the mobile phase contribution of OM to the solute retention. They are obtained from analyte solubility in the mobile phase, measured by the shake-flask and cloud point methods. Stationary phase contribution of sorbed OM to the solute retention is obtained from simultaneous sorption experiments performed by the column equilibration technique. Individual sorption isotherms of all compounds studied are also measured. For these, infinite dilution standard state activity is used in place of mobile phase concentration.

Two models are proposed for the stationary phase contribution of OM. In one model, the sample and OM are sorbed in different planes within the stationary phase, which is viewed as an ideal solution of C_{18} chains, sorbed OM and sorbed sample. Solute sorption decreases exponentially with the volume fraction of organic modifier in the stationary phase, similar to bulk partitioning. This model describes the system in which 1-butanol and eucalyptol serve alternatively as sample solute and as OM. Only the C_4 group of 1-butanol is dissolved within the stationary phase. It was observed that the interaction of sorbed solute with the C_{18} chains was much stronger than its interaction with the sorbed organic modifier. The model is consistent with the experimentally observed Langmuir isotherm of 1-butanol and with the "associative-bilayer isotherm with limited solubility in the aqueous phase" observed for eucalyptol.

The second model applies when both sample and OM are sorbed at the C_{18}/mobile phase interface. Competition for space occurs. This model describes the influence of 1-

propanol OM on 1-hexanol sample. In this case, competition for space in the stationary phase is partially offset by the rearrangement of C_{18} chains, caused by the sorbed OM (1-propanol), leading to a decrease in space occupied by OM in the stationary phase. A sorption isotherm equation incorporating these effects is derived for 1-propanol. It is in excellent agreement with the experimental data. Sorption of 1-hexanol is described by a Langmuir isotherm.

In the mobile phase concentration range of 0-15 % v/v 1-propanol, the changes in retention of 1-hexanol are controlled exclusively by changes in the stationary phase. For 15-30% v/v 1-propanol in the mobile phase, retention of 1-hexanol is controlled by both mobile and stationary phase contributions.

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Glossary of Symbols

a - activity in solution, mol/L

a^{ID} - infinite dilution standard state activity in solution, mol/L

A - area, m^2

A_i - preexponential term in equation 4.14, $A_i = \phi_{i,m}\alpha_i(\overline{V}_{solv} / \overline{V}_i)$

$\overline{A}_{ROH,s}$ - area in the stationary phase occupied per mol of sorbed alcohol ROH, m^2/mol

$\overline{A}^{o}_{ROH,s}$ - area in the stationary phase occupied per mole of sorbed alcohol ROH in the absence of perturbation of alkyl bonded phase by sorbed alcohol, m^2/mol

A_s - area in the stationary phase available for sample sorption, m^2/kg

A_{sp} - specific surface area of the stationary phase, m^2/kg

α_i - preexponential term in equation 4.11, $\alpha_i = \exp(-(\mu^{o}_{i,C_{18}} - \mu^{o}_{i,H_2O}) / RT)$

$\alpha_{j/i}$ - chromatographic selectivity between compounds *j* and *i*, $\alpha_{j/i} = k'_j / k'_i$

β_i - exponential multiplier term in equation 4.11, $\beta_i = (\mu^{o}_{i,C_{18}} - \mu^{o}_{i,solv}) / RT$

$C_{Eu,H_2O,sat}$ solubility of eucalyptol in water, mol/L

$C_{Eu,org}$ - the concentration of eucalyptol in the organic liquid layer for the liquid-liquid partitioning of eucalyptol between the eucalyptol-rich organic layer and aqueous layer, mol/L

$C_{i,added}$ - in the solubility experiments, the concentration of compound *i* in the mobile phase calculated under the assumption that the dissolution of compound *i* was complete, mol/L

$C_{i,dissolv}$ - in the solubility experiments, the concentration of compound *i* in the mobile phase measured experimentally after the dissolution equilibrium was achieved, mol/L

$C_{i,m}$ - concentration of compound *i* in a mobile phase, mol/L

$C_{i,s}$ - concentration of compound *i* in the stationary phase, mol/L

$C_{j,m,eqilib}$ - in the solubility experiments, the concentration of mobile phase component *j* after the dissolution equilibrium was achieved, mol/L

$C_{solv,m}$ - concentration of organic modifier solvent in the mobile phase, mol/L

$C_{solv,s}$ - concentration of organic modifier solvent in the stationary phase, mol/kg

$C_{i,s,max}$ - monolayer concentration of compound *i* in the stationary phase if the sorption of i can be described by Langmuir equation, mol/kg

$C_{i,s,1,max}$ - maximum concentration of compound *i* in the first monolayer in the stationary phase in "associative-bilayer isotherm with limited solubility in the aqueous phase" model (equations 4.23 and 4.24), mol/kg

d - density, mol/L

$d_{H_2O+PrOH}$ density of 1-propanol/water mixture, mol/L

ϕ - volume fraction, mL/mL

$\phi_{i,m}$ - volume fraction of compound *i* in the mobile phase, mL/mL

$\phi_{i,s}$ - volume fraction of compound *i* in the stationary phase, mL/mL

$\phi_{solv,m}$ - volume fraction of organic modifier solvent in the mobile phase, mL/mL

$\phi_{solv,m}$ - volume fraction of organic modifier solvent in the stationary phase, mL/mL

$\Phi_{i,m}$ - true volume percent of component *i* in solution calculated as the ratio, times 100, of the added volume of *i* to the final, mixed total solution volume

$\Phi'_{i,m}$ - apparent volume percent of component *i* in solution calculated as the ratio, times 100, of the added volume of *i* to the sum of added volumes of all solution components

$\gamma^{ID}_{i,m}$ - infinite dilution standard state activity coefficient of compound *i* in the mobile phase

$\gamma^{PS}_{i,m}$ - pure solute standard state activity coefficient of compound *i* in the mobile phase

$\gamma^{T}_{i,m}$ - transfer activity coefficient of compound *i* in the mobile phase

g_{H_2O} - weight/volume fraction of water in 1-propanol/water solutions, g H_2O / mL of solution

ΔG^{o}_{HexOH} the free energy of 1-hexanol sorption into the Partisil-10 ODS-3 stationary phase from pure water mobile phase, kJ/mol

$\Delta G^{Pr\,OH}_{HexOH}$ the free energy of 1-hexanol sorption into the Partisil-10 ODS-3 stationary phase from a pure water mobile phase when there is 1-propanol sorbed in the stationary phase, kJ/mol

h - height of gas chromatographic peak, mm

$K_{BuOH,1}$ - distribution coefficient of 1-butanol between the stationary and the mobile phases for the first layer in the "associative-bilayer" sorption model, L/kg

$K_{BuOH,2}$ - distribution coefficient of 1-butanol between the stationary and the mobile phases for the subsequent layers in the "associative-bilayer" sorption model, L/kg

K'_1 - the distribution coefficient of 1-butanol between the stationary and

the mobile phases in Langmuir isotherm, L/kg; $K_1' = C_{BuOH,s} / a^{ID}_{BuOH,m}$

K_1 - the first eucalyptol distribution coefficient between the stationary and the mobile phases in the "associative-bilayer isotherm with limited solubility in the aqueous phase" (equations 4.23 and 4.24), L/kg

K_2 - the second eucalyptol distribution coefficient between the stationary and the mobile phases in the "associative-bilayer isotherm with limited solubility in the aqueous phase(equations 4.23 and 4.25), L/kg

k_i' - chromatographic retention factor of compound *i*

K_i - volume-mass distribution coefficient of compound *i* between the stationary and the mobile phases, L/kg; $K_i = C_{i,s} / C_{i,m}$

$K_{D,i}$ - volume-area distribution coefficient of compound *i* between the stationary and the mobile phases, L/m^2 ; $K_{D,i} = K_i / A_s$

K_i^{ϕ} - volume fraction distribution coefficient of compound *i* between the stationary and the mobile phases; $K_i^{\phi} = \phi_{i,s} / \phi_{i,m}$

$K_{Eu,dist}$ - equilibrium constant for the liquid-liquid distribution of eucalyptol between organic and aqueous layers, $K_{Eu,dist} = C_{Eu,m}/C_{Eu,org}$

$k_{1,HexOH}^{Pr\ OH}$ the free energy difference for the sorption of 1-hexanol per unit concentration of sorbed 1-propanol, $kJ \cdot mol^{-2} \cdot kg^{-1}$ (equation 5.14 and 5.15)

$k_{1,ROH}$ - the free energy difference for the sorption of alcohol ROH per unit concentration of sorbed ROH, $kJ \cdot mol^{-2} \cdot kg^{-1}$ (equation 5.24)

$k_{2,ROH}$ - the fractional change in $\bar{A}^{o}_{ROH}$ per mol of ROH sorbed, equation 5.23

p - the fraction of eucalyptol molecules in the second sorbed layer that are not able to undergo further association with additional sorbed eucalyptol in the equation 4.23

R - the universal gas constant, 8.314 $J \cdot K^{-1} \cdot mol^{-1}$

$R_{s,j/i}$ - resolution between the peaks for sample compound *j* and the sample compound *i* in linear chromatography, with *j* being the later eluting sample

T - temperature, K

$\overline{V}_i$ - the molar volume of compound *i*, mL/mol

V_{HU} - holdup volume, L

V^o_{HU} - holdup volume in the 1-hexanol and 1-propanol sorption studies for the case when no 1-propanol was added to the mobile phase, L

$V_{HU,PrOH}$ holdup volume in the 1-hexanol and 1-propanol sorption studies in the presence of 1-propanol in the mobile phase, L

V_{void} - void volume of the packed bed, L

W_s - the weight of packing in precolumn, kg

$W_{s,t}$ - the effective weight of packing in precolumn at time t, kg

$W_{s,o}$ - the weight of packing in the freshly packed precolumn, kg

List of commonly encountered subscripts

C_{18}	-	bonded octadecylsilyl chains
BuOH	-	1-butanol
Eu	-	eucalyptol
HexOH	-	1-hexanol
H_2O	-	aqueous (mobile phase)
i	-	sample compound
j	-	sample compound; in definitions of chromatographic selectivity and resolution the sample with the longer retention time
m	-	mobile phase (comprised of water and an organic modifier solvent)
ROH	-	straight-chain alcohol, in Chapter 5 either 1-propanol or 1-hexanol
s	-	stationary phase (normally Partisil-10 ODS-3)
solv	-	organic modifier solvent (commonly, the additive to the mobile phase in HPLC)

1. Preface

Reversed phase chromatography is by far the most widely used branch of high performance liquid chromatography, with bonded phase packings being one of the most popular choices[1,2]. One of the first rigorous attempts to relate liquid chromatographic retention to physical chemistry principles was the solvophobic theory[3]. This theory attributs the retention process mostly to the mobile phase, ignoring the contributions from the bonded phase stationary phase such as bonding density, alkyl chain length and conformation, the changes in the stationary phase properties caused by the sorption of organic modifiers. Although solvophobic theory is still being used[4,5], there is extensive experimental evidence that the stationary phase plays an important role in the reversed-phase bonded phase retention, including one of the recent studies demonstrating that over the entire range of solvent composition, most of the free energy of retention in reversed-phase chromatography arises from attractive dispersive interactions between the solute and the stationary phase, and not from net repulsive interactions in the mobile phase[6].

The present work focuses on stationary phase processes, namely the influence of the sorption of organic modifiers from the mobile phase on the retention of analytes, and on the comparison of the relative magnitudes of the mobile and stationary phase contributions to changes in the retention of analytes as a function of the mobile phase composition. We have chosen to look at the influence of 1-propanol and 1-butanol on the sorption of two different analytes because of the common use of short-chain alcohols added to the mobile phase in order to improve separations in reversed-phase bonded phase chromatography. For example, 3% 1-propanol in an acetonitrile/water mobile

phase greatly improved selectivity in the separation of phenols and naphthols[7]; 4% 1-propanol greatly improved the efficiency of columns used with highly aqueous mobile phases[8,9]; 3% 1-propanol dramatically enhanced the efficiency of separations involving micellar mobile phases[10]; and 10% 1-butanol in a methanol/water mobile phase significantly improved the selectivity in separations of retinol isomers[11]. In a recent study an octadecylsilyl bonded phase (ODS) column was used with highly aqueous mobile phases for the separation of mixtures of 14 nucleosides or seven alkylsulfonates[12]. Pretreatment of the column with 5% v/v 1-propanol and 4% v/v 1-butanol was shown to reduce retention and improve separation. The authors suggest that sorption of the alcohol onto the stationary phase changes its hydrogen bonding and hydrophilic characteristics. In the gradient elution method addition of constant 3% v/v of 1-propanol to the aqueous mobile phases containing 0-100% methanol or acetonitrile greatly reduced reequilibration time by providing consistent solvation of the reversed-phase stationary phase with 1-propanol[13].

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2. Introduction

2.1. Reversed-Phase Bonded Phases

Reversed phase bonded phase liquid chromatography utilizes stationary phases in which alkyl chains are chemically attached to a silica backbone[1]. The surface-active sites on silica are Si-OH groups (silanols) which serve as attachment points for the covalent silyl ether bonds that anchor bonded phases to the silica support. Because of steric hindrance, the density of silanols on chromatographic-grade silica (≈8 μmol/m^2) is much greater than the maximum possible concentration of alkyl groups in a bonded phase (≈4.5 μmol/m^2 for C_{18} ligands). The properties of bonded phases are influenced by the properties of the original silica (i.e. particle size, pore diameter and unreacted silanol groups) as well as by the type of bound alkyl group(s) and their surface density[1,2]. They also depend upon the derivatization process, in particular on the choice of silanization reagent and synthetic procedure[2].

The two most common types of stationary phase morphologies are generally referred to as "monomeric" and "polymeric" phases[3]. Monofunctional silanes, containing one leaving group such as chloro- or methoxy- along with the alkyl group of interest and two side groups (most often methyl), produce monomeric phases characterized by a single bond linkage for each silane molecule with the silica surface (Figure 2.1). Monomeric phases can also be prepared by the reaction of silica with di- or trifunctional silanes (silanes containing two or three leaving groups), if care is taken to exclude water from the reaction.

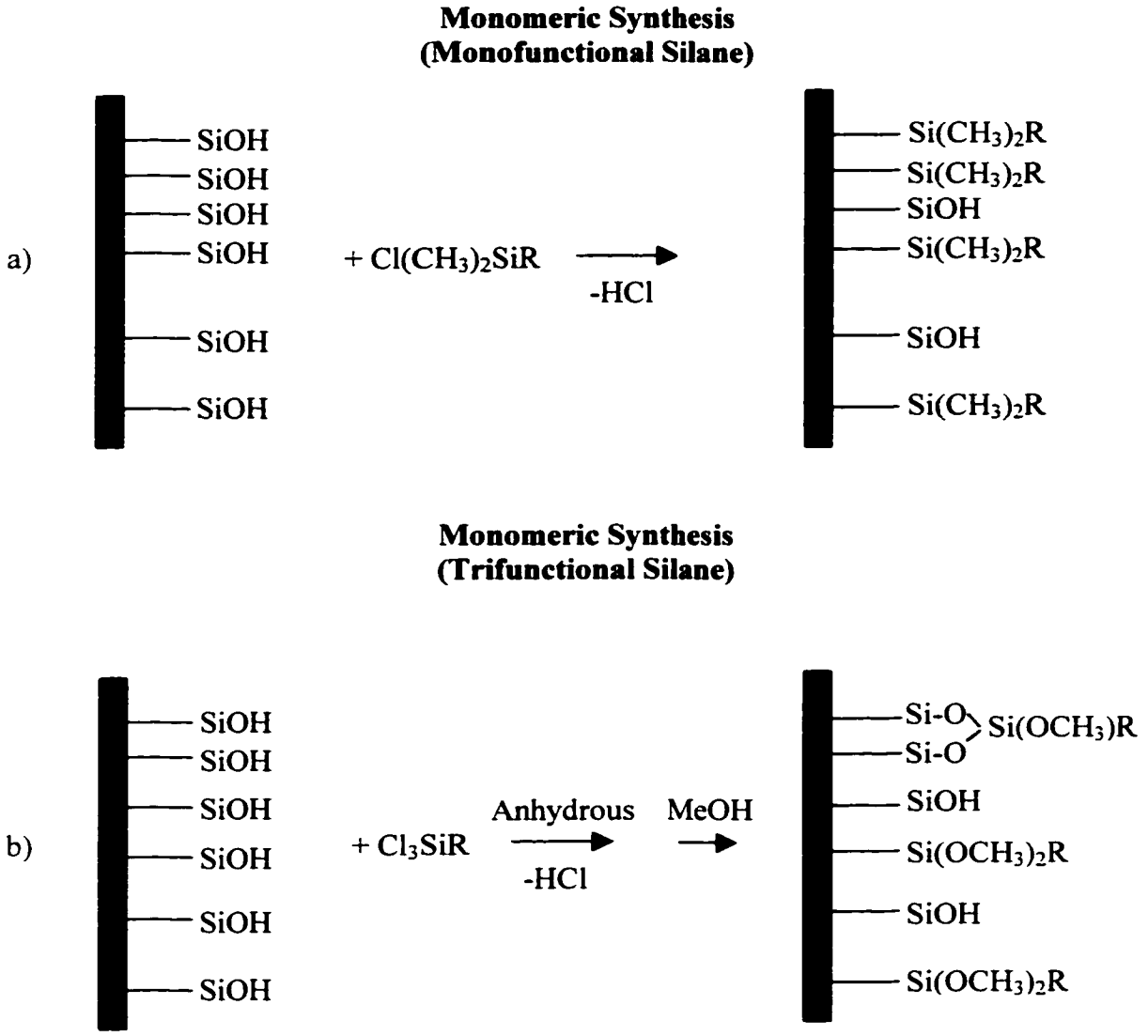

Figure 2.1. Schematic representation of a monomeric bonded phase synthesis from monofunctional silane (a) and trifunctional silane (b). **R** represents a bonded alkyl chain that can be of various length. (Adapted from reference 4).

Polymeric Synthesis
(Trifunctional Silane)

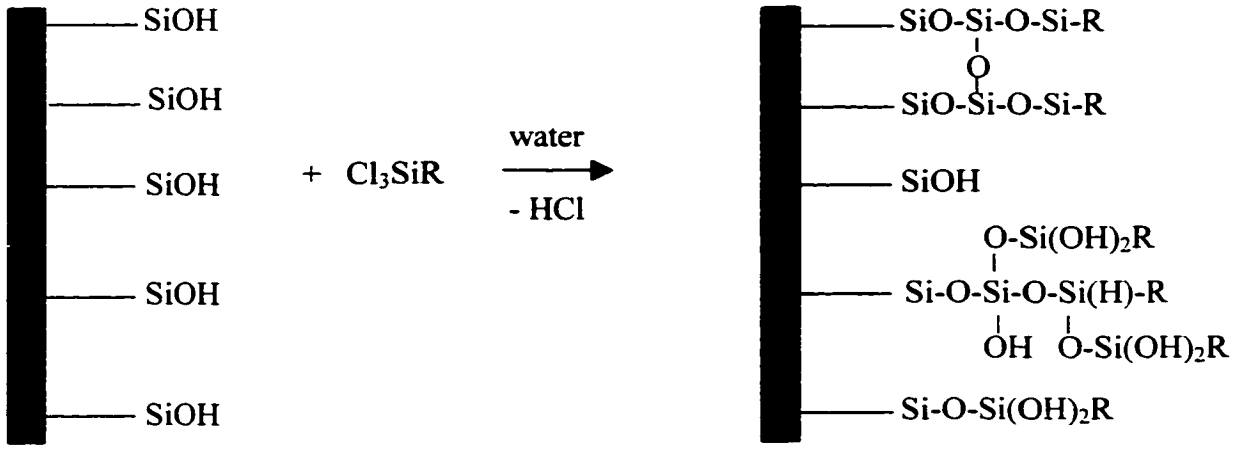

Figure 2.2. Schematic representation of a polymeric bonded phase synthesis from a trifunctional silane in the presence of water. **R** represents a bonded alkyl chain that can be of various length. (Adapted from reference 4).

A second approach involves a polymeric reaction in which di- or trifunctional silane reacts with the surface in the presence of water[3,4]. This provides for a more complicated surface chemistry and makes it possible to have two bonds with the silica for one bonded ligand, but more importantly, the leaving groups can hydrolyze before reaction with the surface to create -OH sites that will react with leaving groups from other silanes, resulting in a polymeric network extending away from the surface of silica (Figure 2.2). This yields more bonded mass but less well-defined surface coverage, because different silanes may anchor to the surface or to other silanes at some distance from the surface[1]. The implications of polymeric vs. monomeric phase morphology, surface coverage and length of bonded alkyl chain for retention, selectivity and retention mechanism will be discussed later.

Because manufacturers do not routinely provide details of preparation of chromatographic phases, and the phase morphology influences the properties of the bonded phase, chromatographers must somehow determine it[4]. A number of tests have been designed to aid in this task. Two of them, selectivity toward polyaromatic hydrocarbons and ^{13}C NMR spectroscopy, will be discussed here.

The retention of benzo[a]pyrene (BaP; planar conformation), relative to 1,2:3,4:5,6:7,8-tetrabenzonaphthalene (TBN, non-planar conformation) and phenantro[3,4-*c*]phenantrene (PhPh, non-planar conformation) provides a sensitive measure of the polymeric versus monomeric character of the phase[4]. Phases prepared using monomeric surface modification chemistry give the elution order BaP $\leq$ PhPh $<$ TBN; while phases prepared using polymeric surface modification chemistry give the order PhPh $<$ TBN $\leq$ BaP. The first category has been described as "monomeric-like selectivity", and the

second as "polymeric-like selectivity". Phases with intermediate properties, such as densely loaded monomeric C_{18} phases or lightly loaded polymeric C_{18} phases, are indicated by the elution order PhPh < BaP < TBN. A quantitative measure of shape selectivity can be calculated to enable relative comparisons between different C_{18} phases. The shape selectivity factor $\alpha_{TBN/BaP}$ (defined as k'_{TBN}/k'_{BaP} where k' is a chromatographic retention factor) has been shown to correlate with phase type. Values for $\alpha_{TBN/BaP} \leq 1$ reflect moderately loaded polymeric C_{18} phases, and values for $\alpha_{TBN/BaP} \geq 1.7$ reflect monomeric C_{18} phases. For values $1 < \alpha_{TBN/BaP} < 1.7$ the synthesis scheme is less certain, and may indicate light polymerization with di- or trifunctional reagents, or densely loaded monomeric phases.

Solid state ^{13}C NMR with cross-polarization (CP) and magic angle spinning (MAS) has been shown useful in identifying bonded phase morphology[5]. Carbon atoms at different positions relative to the attachment site are numbered, with the atom next to silicon atom having a number of 1, and the numbers for the following carbon atoms going up, to 18 for the atom at the free unattached end of C_{18} alkyl chain. The NMR signals of the trifunctional octadecylsilyl bonded phase (ODS) give very few signals compared to the number of signals found in the spectra of the difunctional and monofunctional ODS. The NMR spectra of the trifunctional polymeric phases do not have any signals for C1 and C2 carbon atoms, and only a very weak signal for C3. The monofunctional ODS have intensities of signals at C1, C2 and C3 higher than those of C16, C17 and C18. The difunctional polymeric ODS gives an intermediate pattern between tri- and monofunctional ODS phases, rather close to that of monomeric phase. NMR spectra will also show a signal for the endcapping trimethylchlorosilyl groups, if present. Generally,

the main C4-C15 peak is narrow for monomeric and difunctional phases, indicating weak interaction between bonded chains; and broad for trifunctional phases, indicating stronger interactions within the bonded phase. The coverage density may influence the interactions between chains, with polymeric phases of low coverage density having narrow C4-C15 peaks.

2.2. Influence of Mobile Phase on Retention and Selectivity

Since the study of mobile phase effects on retention in RPBP LC does not constitute a major part of this thesis, the current chapter will only introduce some of the treatments used to model the mobile phase contribution to the retention and selectivity in RPBP LC. This chapter is not intended as a review of such a broad subject.

First, Hildebrand solubility parameter theory will be introduced. Then Snyder-Rohrschneider polarity scheme and solvent selectivity triangle will be discussed, along with the concept of linear solvent strength. Solvophobic theory and its current state will be briefly examined next. The solvatochromic measurement of mobile phase polarity based on ET-30 scale and its correlation with retention will be introduced. Then, multiparameter linear solvation energy relationships and their use in RPLC will be discussed, followed by an introduction of a solvatochromically based solvent selectivity triangle approach. Finally, methods that directly measure mobile phase activity of solute compounds and relate it to chromatographic retention, will be mentioned.

2.2.1. Solubility Parameter Theory

The solubility parameter concept was established back in the thirties by the work of Hildebrand and Scatchard[6]. The original concept covers regular solutions, that is,

solutions that do not show an excess entropy effect upon mixing. Attraction forces between molecules that form regular solutions result primarily from dispersive interactions. In practice, regular solutions are rare.

The Hildebrand solubility parameter δ is defined as follows[6]:

$$\delta = \sqrt{\frac{-\mathbf{E}}{\overline{\mathbf{V}}}} \quad , \text{cal}^{1/2} \cdot \text{cm}^{-3/2} \tag{2.1}$$

where **E** is the cohesive energy required to transfer one mole of a substance from the ideal gas phase to its liquid state, a quantity that will always be negative; and $\overline{\mathbf{V}}$ is a molar volume of liquid.

Hildebrand solubility parameter can be used to give a quantitative meaning to the word polarity[7]. Compounds that are usually referred to as non-polar (e.g. *n*-alkanes) have a low (but a non-zero) cohesive energy, leading to a value for δ around 7 $\text{cal}^{1/2}\text{cm}^{-3/2}$. Polar compounds exhibit much stronger interactions in the liquid state, leading to an increase in δ up to a maximum value of 25 $\text{cal}^{1/2}\text{cm}^{-3/2}$ observed for water.

Retention factor **k′** is the basic retention parameter in liquid chromatography. It can be expressed in terms of Hildebrand solubility parameters of mobile phase, δ_m, and stationary phase, δ_s, as[7]:

$$\ln \mathbf{k}'_i = \frac{\overline{\mathbf{V}}_i}{\mathbf{RT}}(\delta_m + \delta_s - 2\delta_i)(\delta_m - \delta_s) + \ln \frac{\mathbf{n}_s}{\mathbf{n}_m} \tag{2.2}$$

where subscript *i* refers to solute, $\mathbf{n}_s$ is the number of moles of the stationary phase and $\mathbf{n}_m$ is the number of moles of the mobile phase.

In chromatographic practice solute often has an intermediate polarity between a mobile and a stationary phase. If $\delta_i \cong 0.5(\delta_s + \delta_m)$, differentiation of equation 2.2 with respect to δ_m at constant δ_s gives:

$$\frac{\Delta k_i'}{k_i'} = \frac{\overline{V}_i}{RT}(\delta_m - \delta_s)\Delta\delta_m \qquad (2.3)$$

Since δ_m - δ_s can easily be equal to 10 $cal^{1/2}cm^{-3/2}$, $\overline{V}_i$ is of the order of 100 cm^3mol^{-1} and **RT** for room temperature is $\cong$ 600 $cal \cdot mol^{-1}$, we find[7]:

$$\frac{\Delta k_i'}{k_i'} = 1.5\Delta\delta_m \qquad (2.4)$$

Consequently, even a minor variation in the mobile phase polarity can have a dramatic effect upon the retention factor. This is a known observation in chromatographic systems utilizing binary solvents[8].

Another conclusion to be drawn from equation 2.2 is the increase in retention with molar volume, or with solute size. It can also be noted, based on equation 2.3, that the relative variation of retention with mobile phase polarity increases with solute size. Indeed, if the water content of the mobile phase in RPLC is increased, diverging retention curves are observed, demonstrating that the more retained, larger solute is more susceptible to mobile phase influence than the smaller solute[8].

From equation 2.2 an expression for the relative retention (selectivity $\alpha_{j,i}$) of two solutes i and j with equal molar volumes is easily derived:

$$\ln \alpha_{j,i} = \ln\left(\frac{k_j'}{k_i'}\right) = \frac{2\overline{V}_i}{RT}(\delta_i - \delta_j)(\delta_m - \delta_s) \qquad (2.5)$$

It shows that the separation between two solutes can be influenced by two factors:

(a) the difference in polarity between the two solutes (δ_i - δ_j);

(b) the difference in polarity between the mobile and the stationary phase (δ_m-δ_s).

The polarity of the mobile phase can be continuously adjusted by mixing two or more solvents of different polarity[7]. The Hildebrand solubility parameter of a mixture may be approximated by[9]:

$$\delta_m = \Sigma\, \phi_p \delta_p \qquad (2.6)$$

where ϕ_p and δ_p are the volume fractions and Hildebrand solubility parameters of the mixture components **p**. On the basis of equations 2.2 and 2.6 an expression describing retention behavior as a function of composition for a binary mixture can be derived[8]:

$$\ln k'_i = A_i \phi^2 + B_i \phi + C \qquad (2.7)$$

where ϕ is the volume fraction of one of the mobile phase constituents. The constants A_i, B_i and **C** depend on the Hildebrand solubility parameters of the mobile phase components and of the solute. The complete expressions derived from the Hildebrand solubility parameter model show that B_i is strongly negative, whereas A_i is weakly, but significantly positive. As a result the retention factor decreases sharply and nonlinearly with increasing modifier content ϕ.

As seen from above, this simple model provides an insight into the influence of mobile and stationary phase polarity on retention and selectivity. However, the molecular interactions in the liquid state are much too complex to be described by a single parameter. It is one of the major limitations of the Hildebrand solubility parameter model. This simple treatment is not able to distinguish such different compounds as ethyl acetate (δ=9.53) and toluene (δ=9.57) or methylenechloride (δ= 10.68) and dioxane (δ= 10.65)[7].

Clearly, a refinement of the Hildebrand solubility parameter model is required that would offer the description of different types of interactions that exist in the mobile phase.

2.2.2. Snyder-Rohrschneider Polarity Scheme and Solvent Selectivity Triangle

One of the models that offer such refinement over Hildebrand solubility parameter theory is the empirical model of Snyder[10] based on Rohrschneider constants[11], which are the gas-liquid partition coefficients of several standard test compounds between the gas phase and common solvents, measured by head-space analysis. These test compounds were chosen to represent specific polar interactions: acidic (dioxane), basic (ethanol) and dipolar (nitromethane). By calculation, the dispersion contribution (effect of molecular weight of solute) was approximately removed from the measured partition coefficients of the test compounds between solvent and gas phase, and a corrected distribution coefficient K_g'' for each test compound was obtained[10]. Polarity index P' for a solvent was defined as

$$P' = \log (K_g'')_{ethanol} + \log (K_g'')_{dioxane} + \log (K_g'')_{nitromethane} \qquad (2.8)$$

The selectivity parameter χ for the solvent is defined as the fraction of P' contributed by the interaction associated with ethanol, dioxane or nitromethane:

$$\chi_e = \log (K_g'')_{ethanol}/P'; \quad \chi_d = \log (K_g'')_{dioxane}/P'; \quad \chi_n = \log (K_g'')_{nitromethane}/P' \qquad (2.9\text{-}11)$$

As a result, values of χ sum to one. It is assumed, that this "polarity normalization" results in a separation of solvent strength from solvent selectivity. The total selective interaction strength of the solvent with e.g. ethanol is given by $P' \cdot \chi_e$[10].

Polarity of the solvent mixture is a weighed (on volume fraction basis) average of that for the component solvents A and B:

$$P' = \phi_A P_A' + \phi_B P_B' \qquad (2.12)$$

According to the linear solvent strength approximation of this theory[12], a logarithm of solute retention factor linearly depends on volume fraction of a particular solvent in a binary solvent mixture. In RPLC the weakest eluent is water, and the common mobile phases are the mixtures of organic solvents (solv) with water. In such case:

$$\log k'_m = \log k'_{H_2O} - S\phi_{solv} \qquad (2.13)$$

where subscript 'm' is for a mixed solvent and subscript 'H_2O' is for water. **S** is a solvent-dependent quantity, and if the Snyder solvent strength model is obeyed, **S** would be equal to $(P'_{H_2O} - P'_{solv})/2$.

A plot using triangular coordinates to display values of χ_{solv} for different solvents results in a solvent-selectivity triangle (SST)[13]. Solvents falling near the corners of such triangle are assumed to exhibit primarily one kind of selectivity (acidic, basic or dipolar), while solvents within the triangle are capable of all three interactions. Classification of solvents by the SST is in principle useful for two reasons[13]:

(a) choosing a solvent of different selectivity, in order to separate two sample bands that overlap one another in an initial solvent;

(b) selecting some minimum number of solvents for a systematic approach to selectivity optimization; three such solvents (each close to one of the corners of the SST) should provide a broad range of solvent selectivity; blends of these three solvents in various proportions should then allow the continuous variation of solvent selectivity over the widest possible limits.

Even though the SST scheme provides a general background for solvent selection, it has been appreciated for some time that each of test solutes that form the basis of classification are capable of more than one type of interaction. For example, ethanol is clearly dipolar, protic and a good proton acceptor, in turn raising questions about the reliability of solvent classification by means of SST[13]. A comparison[14] of solvent acidity, basicity and dipolarity as measured by SST[15] and the solvatochromic approach[16] revealed that the SST procedure is, indeed, based on test compounds that have multiple interactions.

Another problem inherent in the SST approach is that the acidity, basicity and dipolarity of the organic modifiers used in RPBP LC are only partly responsible for mobile phase selectivity. Changes in the mobile-phase concentration of the organic modifier often lead to significant changes in separation selectivity[17]. This is contrary to the assumption that solvent strength can be varied by varying the percent of water in the mobile phase, without changing selectivity[13]. Water as solvent is far from inert. Direct spectroscopic studies of solvatochromism in mixtures of water with the four more common organic modifiers in RP HPLC show very considerable variations in their dipolarity, hydrogen bond acidity and hydrogen bond basicity as the volume fraction of organic modifier is varied[18,19]. These criticisms imply that the SST approach to adjusting solvent strength and selectivity in RPLC is overly simplified.

2.2.3. Solvophobic Theory

The fundamentals of this approach are based on the thermodynamics of a two step process: binding of the sample solute (eluite in this treatment) to the stationary phase in vacuum followed by transferring of the participating species into the mobile phase[20]. The

theory focuses in particular detail on solvation of sample solute by mobile phase[21,22]. Mobile phase contribution to retention of the sample compound is comprised of the cavity term (energy required for the formation of solute-sized cavity in the mobile phase), solute-solvent interaction term, mixing term (net free energy of mixing of solute species and eluent molecules of the different size), reduction term (responsible for the reduction in gas phase interaction of solute and stationary phase upon transferring into the mobile phase), and the free volume change for the whole process. The first two terms are modeled based on the molecular area of the sample and surface tension of the mobile phase. Surface tension is further corrected to its microthermodynamic, molecular scale equivalent. According to the solvophobic theory[22], free energy of retention depends linearly on the product of the nonpolar water accessible surface area of the solute and the difference in molecular surface tension of the hydrocarbonaceous portion of the solute molecule and the hydrocarbon/eluent interface.

Analysis of data presented in reference 22 shows that the retention of alkylbenzenes on C_{18} bonded phase from hydroorganic mobile phases having various concentrations of methanol, acetonitrile, tetrahydrofuran (THF) and 2-propanol correlates with the nonpolar water accessible surface area of solutes. It was proved experimentally[23] that for solutes without π electrons the plots of **log k'** versus volume percent of organic modifier are correlated with the plots of surface tension versus volume percent of organic modifier. However, a number of inconsistencies were also reported[24]. Compared to methanol and acetonitrile, tetrahydrofuran is a stronger eluent than expected from the surface tension curves alone. So is acetonitrile compared to methanol when solutes

contain π electrons. These observations are due to a rearrangement of stationary phase structure caused by THF, and to π-π interactions of acetonitrile with sample solutes[24].

2.2.4. Solvatochromic Models

This treatment is an extension of the Hildebrand solubility parameter model in that it assumes that retention is controlled by solvent polarity and that polarity can be approximated by a single parameter such as a spectroscopically measured value of absorbance of a solvatochromic dye[25]. One of the most widely used polarity scales is known as the $E_T(30)$ scale, and is based on the charge transfer absorption of 2,6-diphenyl-4-(2,4,6-triphenyl-N-piridino)phenolate[26]. This molecule exhibits one of the largest observed solvatochromic effects of any known molecule, as the charge transfer absorption maximum shifts from 453 nm in water, a very polar solvent, to 810 nm in dyphenyl ether, a very nonpolar solvent. It also has been shown to be sensitive to both solvent dipolarity/polarizability and as well as solvent hydrogen bond donor ability[26]. The $E_T(30)$ polarity values are commonly reported as the energy value of the charge transfer absorption, and are calculated as[26]:

$$\mathbf{E_T(30) = 28{,}592 / \lambda_{max}} \quad \text{, kcal/mol} \tag{2.14}$$

where λ_{max} is the measured maximum of absorbance in nm, and the constant is a product of Avogadro's number, the speed of light, and Planck's constant.

Correlations between **log k'** and both volume percent of organic modifier and $E_T(30)$ polarity were reported for 332 different retention data sets[27]. These involved many different solutes on eight different stationary phases with C_2, C_4, C_8 and C_{18} bonded chain lengths, with mobile phases of methanol-water and acetonitrile-water. For the 332 data

sets, plotting **log k′** versus $E_T(30)$ polarity gave significantly better linearity, with an average value of R^2 of 0.9910 as opposed to an average value of 0.9783 when plotted versus volume percent organic modifier.

2.2.5. Linear Solvation Energy Relationships (LSER)

This treatment views the retention process as the sum of differential interactions of a solute with the mobile phase and with the stationary phase[28]. These include the cavity formation, dipolarity/polarizability, hydrogen bond donor acidity, and hydrogen bond acceptor basicity processes.

The basic premise of LSER is that the free energy of a phase transfer process can be correlated with various fundamental solute descriptor properties[29]. When the LSER method is applied to RPLC, the logarithmic retention factors, **log k′**, are separated into several molecular interaction terms:

$$\log k' = \log k'_0 + M(V_s - V_m)V_2 + S(\pi^*_s - \pi^*_m) + A(\beta_s - \beta_m)\alpha_2 + B(\alpha_s - \alpha_m)\beta_2 \qquad (2.15)$$

The subscripts 's' and 'm' denote bulk stationary and mobile phase properties, respectively; the subscript '2' denotes a solute property. **V** is molar volume, π^* - dipolarity/polarizability, α - hydrogen-bond acidity, and β - hydrogen bond basicity. In this formalism each solute property is multiplied by a term that represents the difference in the complementary property between the stationary and the mobile phase. The coefficients **M**, **S**, **A** and **B** as well as $\log k'_0$ are fitting parameters that ought to be independent of the solute and nature of the chromatographic phases if the formalism is rigorously correct[30].

In a recent study[28] this model was put to a test with 73 solutes that included both aliphatic and aromatic alcohols, aldehydes, amides, esters, ethers, ketones, nitriles, nitro and halogenated compounds, and alkylbenzenes, phenols and polyaromatic hydrocarbons. These solutes were chosen to span as wide a range as possible in the various solute characteristics, and cover both aliphatic and aromatic subsets. Mobile phases were mixtures of methanol, acetonitrile and THF with water at four volume/volume ratios for each organic modifier (20, 30, 40 and 50%). The stationary phase used was a C_8. The solvatochromic parameters π^*, α and β were generally obtained from gas chromatographic measurements. It was argued that the use of these parameters is more justifiable, because they are not back-calculated from RPLC data or subjected to parameter estimation rules.

The regression results for equation 2.15 were excellent with the average residuals in the range of 0.05-0.09; the correlation coefficients were always better than 0.99 which supports the efficacy of LSERs in modeling the retention behavior of BP RPLC using different organic modifiers.

It was found that the solute size V_2 and hydrogen bond basicity were the most important solute descriptors governing retention; i.e. the large solutes with small hydrogen bond acceptor ability were significantly more retained. Solute acidity and dipolarity/polarizability had minor significance in establishing retention, and their contributions were virtually constant over the whole range of the explored mobile phase composition. It was also shown that coefficient **M** in the equation 2.15 is well correlated with the cohesive energy density of the mobile phase, which is a square of Hildebrand Hildebrand solubility parameter of the mixed solvent. Therefore, cohesive energy density

was considered a good complementary parameter to solute volume, and a good descriptor of the **M** coefficient. Hydrogen bond acidity of the mobile phase was the other important factor influencing solute retention.

2.2.6. Solvatochromically Based Solvent Selectivity Triangle

In this approach the original SST of Snyder (section 2.2.2) is reconstructed on the basis of a set of three solvent parameters devised to describe solvent hydrogen bond acidity (α), basicity (β) and dipolarity/polarizability (π^*), which were developed within the context of linear solvation energy relationships[13]. However, values for these parameters for different solvents are derived from spectroscopic (hence the name solvatochromic) and other measurements, that were specifically designed so as to measure only a single interaction. Furthermore, values of these parameters are averages over results obtained with several probe solutes for each parameter, in contrast to the original SST approach, where each parameter is based on a thermodynamic property of a single solute. The solvatochromic parameters have been used to correlate literally hundreds of chemically distinct processes[31]. It can therefore be argued that α, β and π^* are inherently better measures of solvent acidity, basicity and dipolarity than are χ_d, χ_e and χ_n (section 2.2.2)[13]. However, in a later paper[28] it was shown that solvent basicity is not important in describing reversed-phase bonded phase retention, an that a parameter describing dispersive interactions should be used instead.

The essential concept of the SST approach is to use mixtures of solvents with maximal differences in their properties to explore the full range of available mobile phase induced selectivity and to optimize a separation. Neither the original nor the

solvatochromically based SST method is capable of making quantitative predictions of relative retention (selectivity). Part of the reason is that the bonded phases in RPBP LC sorb considerable amounts of organic modifier, and this influences retention and selectivity[13].

2.2.7. Relationship of RPBP Retention and Solute Activity Coefficients in the Mobile Phase

The thermodynamic equilibrium constant for a partition model can be written as a ratio of solute activity coefficients in the stationary and mobile phases[32]. Since the chromatographic retention factors are a product of the thermodynamic equilibrium constant and of the phase ratio (a ratio of stationary and mobile phase volumes or masses), the relationship between mobile phase activity coefficients and chromatographic retention provides an insight into the retention process.

Among the practical methods of measuring mobile phase activity coefficients are head-space analysis[33] and solubility measurement[34], which will be discussed in chapter 5.

Comparison of retention factors of a number of aromatic solutes on an ODS bonded phase in aqueous mobile phases containing 40-100% v/v methanol, 60-95% v/v acetonitrile, 30-100% v/v isopropanol and 30-90% THF with the activity coefficients measured by head-space analysis in the same mobile phases revealed that retention closely followed mobile phase activity of sample solutes[32]. It was concluded that over the mobile phase composition range studied the changes in solute environment in the mobile phase greatly outweigh the changes in environment sensed by a solute immersed in the stationary phase. However, the variation in solute activity coefficients in a bonded stationary phase with the mobile phase composition was also noted.

In another study[34] that compared mobile phase activity coefficients, measured by the solubility method, of a number of nonpolar aromatic compounds in 30-90% v/v of methanol/water mixtures with the retention of these compounds on a C_{18} bonded phase, a linear correlation was observed. It was concluded that in these mobile phases retention of the above solutes is predominantly controlled by the solute activity coefficient in the mobile phase. For comparison to the discussions of stationary phase effects, which appear later in these thesis, it is useful to keep in mind that examples given so far in this section involved the concentration of organic modifier in the mobile phase 30% v/v and greater.

Solute activity coefficients in the mobile phase will be used to describe the influence of mobile phase on the changes in chromatographic retention in chapters 4 and 5 of this work.

2.3. Influence of Stationary Phase on Retention and Selectivity

The importance of the stationary phase in reversed phase bonded phase chromatography was recognized early in the development of this technique due to a number of experimental observations. Chromatographic retention factors, which are a product of distribution coefficient and phase ratio, increase with the length of the bonded chains[35,36] and surface coverage (bonding density) [35,37]. Both of these factors influence the phase ratio, but they also may contribute to changes in the distribution coefficient. Chromatographic selectivity, i.e. the ratio of retention factors for two solutes, also depends on surface coverage and the length of the bound alkyl ligand[35,37]. The effect of surface coverage and related phenomena will be discussed first.

2.3.1. Effect of Surface Coverage and Ordering of Bonded Chains on Retention and Selectivity

In this section, when the effect of bonding density is discussed, only the bonded phases with the same chain length are compared.

It was observed for smaller solutes such as toluene, benzene and *m*-xylene that the slope of the dependence of retention factors on the density of the bonded phase (m^2/g) decreases as the bonding density is increased[37]. The authors suggested that at low bonding density both the phase ratio and the distribution coefficients are increasing with the increase in bonding density, and at higher bonding density only the phase ratio keeps increasing.

To study the influence of surface coverage on the distribution coefficients alone, phase ratios must be carefully determined. An evaluation of the dependence of distribution coefficients of nonpolar solutes into monomeric octadecylsilyl bonded phases on bonding density in the range of 1.6-4.1 $\mu mol/m^2$ was carried out in methanol/water and acetonitrile/water mobile phases[38]. After compensating for the dependence of retention on the phase ratio, the authors found a maximum in distribution coefficients at approximately 3.1 $\mu mol/m^2$. Below this critical value the distribution coefficients increase with bonding density due to the increase in interactions between the solutes and the bonded chains. Above the critical bonding density conformational constraints of bonded chains affect partitioning. Larger free energy changes are required to create a solute cavity in the bonded phase so distribution coefficients decrease. This interpretation assumes penetration of solutes into the bonded alkyl chains.

An easy way of eliminating the effect of phase ratios on retention factors is by studying the selectivity among the members of a homologous series. In a study of the influence of alkyl chain bonding density of monomeric octadecyl bonded phases on methylene selectivity and phenyl (shape) selectivity for nonpolar solutes[39] it was found that phenyl selectivity increases with increasing bonding density, whereas methylene selectivity remains approximately constant. The increase in phenyl selectivity was attributed to the increased anisotropic chain ordering at the higher surface coverage values and therefore higher shape selectivity.

A similar stationary phase effect that is thought to be related to chain density is observed in the separation of polyaromatic hydrocarbons. It is well established that for the separation of polyaromatic hydrocarbons polymeric bonded phases yield better selectivity than monomeric ones[40,41]. A difference in selectivity for polymeric and monomeric phases is observed for another class of rigid molecules, the carotenes: the separation of *cis* -and *trans*-carotenes can be accomplished in nonaqueous mobile phases on polymeric bonded phases but not on monomeric ones[42]. This is thought to be due to the higher local bonded chain density of polymeric phases versus monomeric ones, contributing to the higher ordering of alkyl bonded chains for the polymeric packings and therefore higher shape discrimination[3]. This finding agrees with the observation that polyaromatic hydrocarbon selectivity on polymeric bonded phases increases with the phase bonding density and the polyaromatic selectivity of monomeric phases approaches that of polymeric ones as the bonding density increases[3]. Moreover, plots of methylene group selectivity for homologous series versus the carbon atom number are different on monomeric and polymeric phases[43], but identical for different monomeric ones[44].

A different variable influencing bonded chain ordering is temperature. Reduced temperature results in a more ordered stationary phase as well as more effective intermolecular interactions among chains. It was observed that selectivity of monomeric bonded phases toward polycyclic aromatic hydrocarbons and polyphenyl isomers improved at subambient temperatures and the improvement was more pronounced for the phase with a higher bonding density of 4.4 $\mu mol/m^2$ compared to the phase with a lower bonding density of 1.5 $\mu mol/m^2$ [45]. Similarly, the ability of several commercial columns, prepared by using both monomeric and polymeric surface chemistry, to separate closely related isomers of polycyclic aromatic hydrocarbons was observed to be highest at subambient temperature[46]. When a C_{30} bonded phase was used for the separation of the vitamin A acetate isomers, different retention behavior was observed at different temperatures. At low temperatures (2° C), the most angled 9-*cis* isomer was the most retained, whereas at high temperatures (>40° C) the linear all-*trans* isomer exhibits the strongest interaction with the stationary phase[47].

Studies of molar enthalpy of retention of ethylene and benzene homologues on octadecylsilyl bonded phases of low (2.7 $\mu mol/m^2$) and high (5.4 $\mu mol/m^2$) bonding density have shown that the differential change for the difference of the molar enthalpy in the stationary and mobile phases per both methylene and benzene group is significantly greater for the high bonding density phase than for the lower density one[48]. The average value of the differential change in molar enthalpy per ethylene group of even-numbered saturated fatty acids is ten times greater for the high density bonded phase than for the low density one.

In conclusion, chromatographic retention generally increases with the increase of bonding density [37,38]. A maximum on the plot of distribution coefficients versus bonding density can be reached due to entropic constraints in bonded phases with very high surface coverage[38]. The increase in chain ordering either through increase of bonding density or through decrease of temperature improves the selectivity of rigid molecules such as PAHs and carotenes[39,42,45,46,47].

2.3.2. Effect of Bonded Chain Length on Retention and Selectivity

Retention of analytes increases with an increase in chain carbon number at low values of chain lengths and plateaus at high chain lengths[36]. The critical chain length after which retention becomes constant depends on the size of analytes. Larger and more strongly retained analytes reach the plateau at higher bonded chain length[36]. Such an observation is consistent with at least partial embedding of analytes into the bonded chains.

An interesting variation of the above experiments is the one in which the retention of homologous series of analytes is studied on monomeric stationary phases with different bonded chain length. A break is observed in the plots of logarithm of retention factor or methylene selectivity vs. the carbon number of the member of homologous series. This break is observed at a sample carbon number corresponding to the length of the bonded alkyl chain of the stationary phase[43]. This number is independent of the homologous series studied and is characteristic only of the bonded phase chain length. The observed effect indicates vertical penetration of the solute molecules into the bonded layer.

Studies of the dependence of chromatographic selectivity on the bonded phase chain length at constant mobile phase composition show an increase in selectivity with the bonded phase chain length[35,36,37,49,50] and in some cases an approach to plateau values[35,37]. Conversely, if the chain length of the bonded phase is held constant and the number of carbon atoms of the member of homologous series is increased, selectivity between homologues decreases[51] to a constant value such that further increase in the carbon number of homologue causes no changes in the selectivity. The carbon number at which the onset of constant selectivity occurs is related to the bonded chain length and is independent of the homologous series investigated; it is larger for longer bonded chains. This indicates an intercalation of solute among the bonded phase chains, with the portion of solute that does not penetrate the chains not contributing to selectivity.

All of these experimental observations suggest a significant role of the stationary phase in the reversed-phase bonded phase retention process, and in particular a penetration of the solute into the chains of the bonded phase.

2.4. Sorption of Organic Modifiers into the Stationary Phase

2.4.1. Distribution Isotherms

Many workers have studied the sorption of components from the typical mobile phases used in reversed phase chromatography, e.g. methanol-water, acetonitrile-water and tetrahydrofuran-water[52,53,54,55,56.] Isotherms for the distribution of methanol (MeOH), acetonitrile(AN) and tetrahydrofuran (THF) from their respective aqueous mobile phases into an octyl bonded phase, measured by several procedures, show that significant

quantities of organic solvents are extracted into the stationary phase. The amount is dependent on the solvent strength of the modifier and its concentration in the mobile phase[55,56]. THF has the highest distribution coefficient into the stationary phase followed by acetonitrile and then methanol[55,56]. It was also observed that sorption of the organic modifiers is influenced by the alkyl chain length and surface coverage of the bonded phase, as well as the availability of the surface silanols[56,57]. In one of these studies[55] it was found that the quantity of water extracted into the stationary phase is small and reaches a plateau at concentrations greater than ≈ 3% v/v of H_2O in the mobile phase.

In a different study it was confirmed that the amount of acetonitrile sorbed by C_8 and C_{18} stationary phases is 2.5-3 times higher than the amount of methanol[54]. The authors also found that a small amount of water was sorbed in the stationary phase at high organic modifiers concentration (>70% acetonitrile and >90% methanol in the mobile phase). This was attributed to the preferential interaction of water with the residual silanols once the bonded chains become solvated[54]. An interesting observation was made that even though the amount of sorbed acetonitrile in mmoles per gram of packing was similar for C_2, C_8 and C_{18} phases, the compositions of the stationary phases with regard to volume fractions of alkyl chains, sorbed water and sorbed acetonitrile were strikingly different for the three phases. For the region 50-80% v/v acetonitrile in the mobile phase the following are the volume fraction compositions of the stationary phases: C_2 : AN : H_2O = 29:62:9; C_8 : AN : H_2O = 40:53:7; C_{18} : AN : H_2O = 65:32:3 (%, v/v). The authors concluded that the change in solvent composition in the sorbed phase may be partly responsible for the increase in retention of sample compounds on going from C_2 to C_{18} when using the same mobile phase composition [54].

Similar results were found in two other studies[58,59]. Solvent distribution isotherms from binary water-acetonitrile and water-methanol mixtures onto C_{18} bonded phase were defined in terms of surface excess of the mobile phase components in the stationary phase[58]. In the mobile phase composition range 0-70% v/v of acetonitrile and 0-90% v/v methanol there is a large surface excess of the organic modifier. At the high organic modifier concentration range (>70% acetonitrile and >90% methanol) there is also a significant surface excess of water, presumably by hydrogen bonding to the remaining non-alkylated surface silanol groups[58]. Again, the amount of sorbed acetonitrile is larger than the amount of sorbed methanol. It was also observed that the ethanol distribution isotherm into C_{18} bonded phase from ethanol-water solutions lies below acetonitrile distribution isotherm from aqueous solutions[59].

Distribution isotherms of four short chain *n*-alcohols on a monomeric C_{18} stationary phase were measured over the low mobile phase concentration region of 0-100 g/L[60]. It was confirmed that sorption is increasing with the size of the alkyl chain in the order methanol<ethanol<propanol<butanol. The data were successfully fitted to the Langmuir isotherm equation. Butanol and propanol appear to be approaching a plateau, whereas methanol and ethanol isotherms are still rising in the range of the concentrations studied.

A 1-butanol isotherm on a polymeric stationary phase, measured over the range of 0-0.654 mol/L (0-50 g/L) solute in the aqueous mobile phase, did not approach a plateau. Its earlier part in the concentration region of 0-0.1 mol/L (0-7.4 g/mL) was fitted to the Langmuir isotherm equation, but above this concentration the isotherm did not follow Langmuir behavior[61].

Authors frequently stress the importance of careful choice of the method for the measurement of void volume in isotherm studies[58,59,62] as well as of the method for the determination of the retention of organic modifiers. However, there seems to be no agreement as to what the suitable method for the measurement of void volume should be[62]. In this work, water will be used as an unretained component of the mobile phase for the measurements of holdup volume, and justification of this will be given in the following section.

2.4.2. Spectroscopic Measurement of the Sorption of Organic Modifiers into the Reversed-Phase Bonded Phases

While there are difficulties in using chromatographic methods for the determination of the exact amount of modifier in the stationary phase, due to the uncertainty of the void volume measurement, the sorption of modifier and its concentration-dependence can also be demonstrated independently by spectroscopic techniques[63,64] . Fluorescence studies of chemically attached[65,66] and physiosorbed[67,68] probes reveal that the nature of the mobile phase can affect the polarity of the stationary phase. Octadecylsilyl surfaces are significantly more polar than analogous liquid alkanes[66]. In the presence of water, the polarity of the C_{18} surface was unchanged from the dry state, indicating no sorption of water[66]. The surfaces were also examined with an overlay of a 50% mixture of acetonitrile, methanol or tetrahydrofuran with water. These C_{18} surfaces gave emission maxima that were similar to those obtained for the C_{18} surfaces exposed to the pure organic solvents, indicating extraction of the organic modifier by the stationary phase[66]. In the study of the polarity of monomeric and polymeric C_{18} phases in the presence of 50-80% of methanol, 20-70% of acetonitrile or

25-45% of tetrahydrofuran in water, the effective polarity experienced by physically sorbed pyrene decreased as the amount of water in the mobile phase increased, indicating preferential sorption of organic modifier but not water by the stationary phase. However, the polarity of the stationary phase was significantly greater than either a dry C_{18} or a bulk alkane[67]. The polarity was always lower than the surrounding bulk solution, indicating that the C_{18} chains protected the pyrene from exposure to bulk solvent[67]. This study was extended to probe the 2-80% range of methanol concentrations in the mobile phase[68]. The results were compared to the effectiveness of pyrene fluorescence quenching by the ionic quencher, potassium iodide, believed to be unretained. Polarity experienced by pyrene molecules sorbed in the polymeric C_{18} environment was lowest at 50% v/v methanol in the mobile phase. As the amount of methanol in the mobile phase increased above this concentration, the sorption of methanol led to the increase in polarity of the stationary phase; and at lower concentrations of methanol the increase of polarity of pyrene surroundings was explained by partial exposure to the mobile phase as the bonded phase collapsed. This was correlated with the increase in fluorescence quenching by potassium iodide at mobile phase concentrations of methanol below 50%[68].

Studies of flat silica surfaces derivatized with C_{18} at chromatographic densities allow one to measure the angle of bonded moieties with respect to the surface normal[69,70]. Frequency-domain fluorescence anisotropy measurements of the orientational distribution of a long hydrophobic probe physically sorbed at the derivatized surface were used to sense the orientational distribution of the alkyl chains. When pure water is in contact with the C_{18} surface, the orientational distribution of the probe is broad and centered close to the plane of the surface, with the angle to the surface normal 70±20

degrees, confirming the widely held notion of collapsed alkyl chains. There is little change in the orientational distribution of the probe when the surface is in contact with aqueous solutions containing 20% v/v methanol or 5% v/v 1-propanol. The angle with respect to the normal for the surface in contact with methanol solution is 70±30 degrees and for the surface in contact with 1-propanol solution is 60±20 degrees[69]. In contrast, long chain *n*-alcohols such as *n*-heptanol, *n*-octanol and *n*-decanol sorbed from their saturated aqueous solutions caused the C_{18} chains to tilt upward toward the surface normal and the orientational distribution of the probe to become narrower. The angle with respect to the normal for the surface in contact with saturated aqueous solutions of the above alcohols was 50±15 degrees[70]. This was explained by the ordering of the surface when alcohols interpenetrated with the C_{18} chains, and by partial reorientation of the bonded alkyl chains.

Acridine orange is a probe expected to reside at the interface between the polar solvent and the octadecylsilyl chains, rather than partition into the chains, as was the case with the probe discussed above. Spectroscopic study of acridine orange sorbed at the C_{18} surface in contact with water or 5% 1-propanol solution indicated the increase of surface roughness in the presence of alcohol[71], consistent with the sorption of 1-propanol at the C_{18}-solution interface .

NMR is another technique widely used to investigate alkyl bonded phases[64]. Studies of solvent uptake by bonded phases can be done by monitoring either the bonded chains or the solution components. NMR spectra of bonded chains in the presence of solvents are somewhat difficult to interpret unambiguously, but studies monitoring the mobile phase components allow a determination of the types and degrees of interactions

between mobile and stationary phases. NMR measurements made on mobile phase components can not generally be used to quantify the amount of sorbed organic modifier, but are rather used to determine the extent to which mobile phase components interact with the stationary phase[64].

Spin-lattice relaxation time T_1 of the nuclei of interest is a useful parameter for such measurements. It is expected that a distribution of relaxation times of any solute in contact with RPLC stationary phase would result from the varying degrees of solute association with that phase[72]. In going from a three-dimensional bulk liquid to a two-dimensional surface, the T_1 of the quadrupolar nucleus of interest in the mobile phase is expected to decrease[73]. By monitoring the deuterium nuclei of ^2H_2O in acetonitrile solutions containing 50-100% acetonitrile in contact with monomeric C_1 and monomeric C_{18} the degree of water association with the stationary phases was qualitatively determined [73]. The T_1 values for the solvent mixtures decreased with increasing percent ^2H_2O and it was deduced that the amount of water associated with the stationary phase was decreasing with the increased percentage of water in the mobile phase due to stronger self-association of alkyl stationary phase chains in the presence of mobile phases with higher water content. This self-association would cause some degree of expulsion of both organic co-solvent and water[73].

This study was expanded to measuring ^2H T_1 values for fully deuterated methanol, acetonitrile and water in binary aqueous mixtures and for those mixtures in contact with monomeric C_{18} bonded phases at pressures comparable to the ones used under normal chromatographic conditions over the entire range of binary compositions[74,75]. Measuring a change in ^2H T_1 (i.e. T_1 for the solution minus T_1 for the

solution in contact with the bonded phase) confirmed that the amount of methanol associated with the stationary phases of low (1.4 μmol/m^2) and high (4.4 μmol/m^2) bonding density was a function of the amount of methanol present in the mobile phase. In contrast, the amount of water was almost constant and was small, i.e. much smaller than the amount of sorbed methanol. For acetonitrile-water solutions, the degree of association with both low and high density stationary phases was much less for water than for acetonitrile. However, the degree of water association with the stationary phase was a function of bulk mobile phase concentration, whereas the degree of association of acetonitrile was relatively constant regardless of the mobile phase composition. This was opposite from the case with methanol-water mobile phases. The amount of organic modifier sorbed in the stationary phase differed between low and high density bonded phases, whereas the amount of sorbed water did not[74,74].

A similar study was conducted for the same stationary phases and deuterated tetrahydrofuran and water mixtures[76]. The observations are very similar to those of water-methanol solutions[74,74]. The amount of THF associated with the stationary phase increases with the increase of THF mobile phase concentration. The increase is similar for low density and high density bonded phases in spite of the higher phase ratio of the high density bonded phase. This was explained by the increase in the energy needed for the disruption of stationary phase chain interaction at the high density. The amount of water associated with the stationary phases was small and nearly constant over 0 to 80 % THF concentration and slightly increased in the region of 80-100% THF in the mobile phase[76], following the same trend as for methanol-water mobile phase[75].

2.5. Influence of Sorbed Organic Modifiers on Retention and Selectivity

As discussed above, the stationary phase is composed of a combination of bonded organic moiety, sorbed mobile phase components and residual silanols on the silica surface. Therefore, sorbed mobile phase components will influence retention and selectivity.

In a study of methylene selectivity, α_{CH_2}, for members of a homologous series of *n*-alcohols on C_{18} and C_8 stationary phases in methanol-water mobile phases it was found that C_{18} has the higher selectivity at low % MeOH, but a cross-over occurs at approximately 70% methanol v/v in the mobile phase, and from then on C_8 has the larger α_{CH_2} value[57]. The authors argue that the enrichment of the stationary phase with non-aqueous modifier does have a definite effect on the separation process. The higher the concentration of methanol in the stationary phase, the smaller the difference in free energy of transfer of one methylene group from the mobile to the stationary phase. C_8 phase has a greater concentration of methanol (mol MeOH per gram of dry packing) in the stationary phase up to 70% modifier concentration in the mobile phase, and therefore has a lower selectivity for CH_2 than the C_{18} phase has. At higher modifier concentrations, the uptake of methanol by C_8 phase levels off, but C_{18} keeps sorbing more methanol, and its methylene selectivity falls below the one for C_8[57].

In a study of the sorption of methanol, acetonitrile and THF into the C_{18} bonded phase it was noted that methylene selectivity for *n*-alcohol homologues is inversely proportional to the amount of sorbed organic modifier, i.e. THF mobile phases had the highest amount of organic modifier sorbed in the stationary phase and the lowest selectivity[56]. The authors argue, as in the above case, that as the amount of organic

modifier in the stationary phase increases, the difference in solvent strength between the mobile and stationary phase decreases, and so does the selectivity.

The influence of sorption of various mobile phase additives on the distribution equilibrium of the main organic modifier component of the mobile phase into a C_8 stationary phase was studied in order to gain an insight into polar group selectivity[77]. In this study the mobile phase containing the main organic modifier component was continuously pumped through the C_8 column. Then a pulse of second organic modifier, the additive, was injected on the column. This pulse would create either a displacement or a vacancy band of the main modifier, which would travel down the column separately from the peak containing additive compound, at a rate determined by the distribution coefficient of the main organic modifier.

It was observed that, depending on the nature of the mobile phase additive, the sorption of the main modifier either increased or decreased, thus producing either a vacancy or a displacement band. For example, injection of mobile phase containing 1% THF into the column equilibrated with methanol/water resulted in a displacement peak for methanol, as a direct consequence of competitive sorption of THF. The authors also measured the amounts of sorbed methanol and THF from the mobile phases containing a constant concentration of methanol and varying amounts of THF. As the THF concentration in the stationary phase increases, the sorption of methanol decreases. However, the amount of displaced methanol is less than the increase in the amount of sorbed THF. Analogous results were obtained for addition of THF and benzyl alcohol to acetonitrile-water mobile phase. On the other hand, addition of benzene increased the

amount of acetonitrile sorbed. As the concentration of benzene in the stationary phase increases, the amount of sorbed acetonitrile increases as well.

The influence of n-alkyl diols on the sorption of THF was also investigated[77]. It was observed that additives, which elute immediately after THF in elution chromatography, produce a net displacement of THF from the stationary phase upon injection on the column equilibrated with THF/water mobile phase. As retention of successive homologue additives in the elution chromatography increases, the magnitude of the THF displacement peak that they produce diminishes, leading to THF vacancy peak for strongly retained solutes. This indicates that upon injection of additives significantly more hydrophobic than the organic modifier, there is a net increase in the amount of the organic modifier in the stationary phase over that in the absence of these. Two effects are observed. Injection of additives slightly more hydrophobic than the organic modifier causes a displacement of that modifier from the solute band due to competition. As the retention of the additive increases, more of it is sorbed in the stationary phase and this additive distribution will modify the hydrophobic character of the stationary phase, favoring a net flux of the organic modifier solvent into the bonded phase. Organic modifier displacement from the n-alkyl will be overshadowed by the enrichment of organic modifier in the bonded phase due to the change by the sorbed additive in the properties of the latter.

In the same work polar group selectivities were investigated based on the properties of mobile phase additives having a high distribution coefficient into the stationary phase[77]. Addition of THF to methanol-water mobile phase retarded acidic p-chlorophenol relative to benzene and accelerated basic methylbenzoate, though all eluted

earlier than in the absence of THF. This was explained by hydrogen bond interactions of the solutes with THF in the stationary phase. When other ternary mobile phases were studied, significant changes in retention and elution order were observed, many of which can be rationalized on the basis of solute interactions with the extracted mobile phase additive in the stationary phase. Additives possessing greater proton donating ability than methanol accelerate the migration of proton donating solutes through the column while retarding the migration of proton accepting solutes, relative to the solutes that are neither acidic nor basic. For the same mobile phase concentrations, larger effects are observed for the more hydrophobic and thus more strongly sorbed mobile phase additives, confirming that the effect occurs in the stationary phase.

In another study the influence of sorption of 1-pentanol onto a C_8 stationary phase on retention of carboxylic acids was investigated[78]. The authors first determined the distribution isotherm of 1-pentanol. At the relative saturation of 1-pentanol in the mobile phase of 0-60% a Langmuir isotherm is observed with a monolayer coverage giving the mean molecular area of about 26 $Å^2$/molecule of sorbed 1-pentanol. At concentrations above 60% of relative saturation the slope of the isotherm starts to increase and above 85% it increases dramatically. The authors attribute this latter behavior to the formation of a multilayer of 1-pentanol and a filling of the pores of the support with 1-pentanol. The total amount of 1-pentanol sorbed at the saturation point corresponds to a mean layer thickness of about four layers if it were evenly distributed over the surface. The retention of carboxylic acids in the region corresponding to the formation of the monolayer of 1-pentanol (0-60% relative saturation in the mobile phase) decreases due to competition from 1-pentanol for the support surface. When the multilayer forms at the higher

concentrations of 1-pentanol, the retention increases since the multilayer acts as a bulk phase into which the solutes can be partitioned. The distribution coefficients at 100% relative saturation were compared to distribution ratios between water and 1-pentanol determined by a batch experiment and corrected for water solubility in 1-pentanol. The values closely agree.

The influence of sorption of methanol from aqueous mobile phase on the separation of polyaromatic hydrocarbons on a monomeric C_{18} stationary phase was recently investigated[79]. The authors found that the slope of the mole fraction of methanol in the stationary phase versus its mobile phase concentration is higher in the mobile phase concentration range 0-0.5 mole fraction of methanol, and then decreases as the mole fraction of methanol in the mobile phase is increased. When the logarithms of retention factors of polyaromatic hydrocarbons, **log k′**, were plotted vs. mole fraction of methanol in the mobile phase, a similar change was observed. There are two concentration intervals in which the values of **log k′** change linearly with a change in mobile phase composition. In the first interval, 0-0.5 mole fraction of methanol, values of **log k′** decrease faster than in the interval of 0.5-1 mole fraction of methanol in the mobile phase. The interval where **log k′** decreases more steeply occurs where the concentration of methanol in the stationary phase increases rapidly. The authors believe that in this interval changes in retention are dominated by the changes in the stationary phase composition.

An interesting study of relative retention of normal, alicyclic, polyaromatic and aromatic-aromatic (e.g. biphenyl) hydrocarbons on monomeric C_1, C_8 and C_{18} phases and its dependence on the mobile phase conditions led the authors to conclude that the

retention of aromatic hydrocarbons is influenced by solvent molecules in the stationary phase[80]. They observed that, in general, a longer alkyl chain in the stationary phase provided a longer retention time. The retention of rigid planar aromatic hydrocarbons relative to retention of both non-planar aromatic compounds and alkanes was much larger on the C_{18} phase than on the C_1 or C_8 phases in a methanolic mobile phase. When the mobile phase was changed from 60% to 80% methanol with the C_1 phase, the retention of alicyclic compounds decreased just as much as that of n-alkanes, and all aromatic compounds showed a much larger decrease. The effect on the C_{18} phase was completely different. As the methanol content was increased, polyaromatics showed a smaller decrease in relative retention compared with alkanes. The authors explained these differences based on the changes in the stationary phase structure caused by sorption of organic modifiers[81]. The C_{18} stationary phase is known to be relatively tangled, especially for mobile phases with low organic modifier content. As organic solvent content increases, the alkyl chains sorb organic solvents, swell and become more ordered in their conformation. As a result, PAHs can more readily penetrate the stationary phase and their retention relative to the more bulky and/or flexible solutes increases.

As mentioned before, the methylene selectivity is a useful parameter for comparisons of different stationary phases, as it does not require knowledge of the phase ratio - a parameter that is difficult to determine experimentally. This can be taken one step further, by comparing the selectivity obtained on bonded phases with the selectivity of the liquid-liquid partitioning process in order to gain an insight into the retention process[82,83]. From methylene selectivity the ratio (**F**) of the free energy for the transfer of a methylene group the from mobile phase into hexadecane, to the free energy for the

transfer of a methylene group from mobile phase into the stationary phase of interest can be derived. When **F** is close to one, the retention process is similar, i.e. partitioning. A deviation of this ratio from one can give an insight into the differences between liquid-liquid partitioning and reversed phase bonded phase retention[82,83]. It was observed for a series of *n*-alkylbenzenes on a number of monomeric octyl bonded phases that **F** was very close to unity over 0-70% v/v methanol in the aqueous mobile phase. When the concentration of methanol exceeded 70% v/v, **F** steeply increased from an average value of about 1.3 to values between 2.9 and 4.5 for the different octyl phases studied[82]. A similar observation was made for octadecyl bonded phases, with **F** values of about 1.2 in 0-70% v/v methanol in the mobile phases and approaching 2 in 100% methanol. Such trend was absent with either the horizontally-polymerized stationary phase or polybutadiene-coated zirconia stationary phase[83]. The authors suggest that the C_{18} chains are solvated by methanol, and that the increase in **F** is caused by a change of retention process from predominantly bulk-liquid partition-like at lower methanol mobile phase concentrations to surface-partition at high methanol concentrations. In the surface partition process the solute is still extensively embedded within the bonded phase layer, but it senses an environment that is chemically and physically different from a bulk fluid analog such as hexadecane. In surface partitioning the chemical potential of the solute is influenced by the water and organic solvent associated with the surface bonded chains or with surface silanol groups, whereas the solubility of water and methanol in bulk liquid hexadecane is very minor[83] compared to their sorption on the bonded phases.

It was also observed that for 50% v/v methanol/water mobile phase the **F** ratio is close to unity for both monomeric and polymeric stationary phases with bonded chain

lengths of 8 or more carbon atoms, but as the number of carbon atoms in the bonded chain decreases, **F** increases for the same mobile phase composition[82]. The authors concluded that the retention mechanism of a methylene group becomes somewhat less partition-like as the bonded phase chain length decreases.

The dependence of **F** ratios on the surface coverage was also studied[82]. It was noted that for the monomeric octadecyl packings with lower bonding density (0.6 $\mu mol/m^2$) the **F** ratios varied with methanol volume fraction in the mobile phase from the value of 1.8 in pure water to the value of 3.4 in pure methanol. These values were significantly larger over the entire range of methanol-water mobile phase compositions compared to the values for the two packings with higher bonding densities (1.4 and 2.3 $\mu mol/m^2$) that had essentially the same **F** ratios varying from the value of 1.3 in pure water to the value of 2.1 in pure methanol. The authors suggest that the retention mechanism becomes more "partition-like" as the coverage density increases. However, once a critical surface coverage for the bonded phase is achieved, the retention mechanism for a methylene group becomes constant.

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3. Experimental

3.1. Introduction

In this Chapter, the details of all experiments that were done are presented. Three basic types of experiments were performed. First, in Section 3.4 are described the column equilibration experiments that were used to study competitive sorption of 1-butanol and eucalyptol, and to study the influence of 1-propanol on the sorption of 1-hexanol. Second, in Section 3.5.1 is described the measurement of the solubility of eucalyptol in water by the shake-flask method. Third, in Section 3.5.2 are described the measurement of the influence of 1-butanol on the solubility of eucalyptol in water and the determination of the solubility of 1-hexanol in water/1-propanol mixtures, performed by the cloud point method. The details of the gas chromatographic determination of water (void volume measurement), 1-butanol, eucalyptol, 1-propanol and 1-hexanol are given in Section 3.6.

3.2. The Octadecylsilyl Packing

The octadecylsilyl packing used as the stationary phase in all experiments was Whatman Partisil-10 ODS-3 (Batch No. 101409, Whatman Inc., Clifton, NJ) which has a 10 μm particle diameter and about 8.5 nm (85 Å) pore diameter. This packing is prepared from a trifunctional silane and is reported to be a polymeric bonded phase[1]. It is described by the manufacturer to be "highly end-capped" with 95% surface coverage[2]. The average surface density of octadecylsilyl groups is 1.45 μmol/m^2, as calculated from a surface area of 350 m^2/g and a carbon loading of 10.5%[3].

As was discussed in Section 2.1, above, the shape selectivity toward polyaromatic hydrocarbons is different for polymeric and monomeric stationary phases[4]. The shape selectivity of an RPBP column can be assessed by performing a separation of tetrabenzonaphthalene and benzo[a]pyrene (see reference 5 and Section 2.1). The shape selectivity factor $\alpha_{TBN/BaP}$ on a column packed with Partisil-5 ODS-3 was 1.93, and this packing was identified as "monomeric-like"[5]. Partisil-5 ODS-3 differs from Partisil-10 ODS-3 only in the packing particle size (5 μm rather than 10 μm). This inconsistency with the synthetic scheme can be understood by realizing that shape discrimination is dependent on the ordering of bonded phase chains, which in turn depends on the bonding density (Section 2.3.1). Typically, polymeric phases have a higher bonding density than monomeric phases, and thus an improved shape selectivity compared to monomeric phases. Partisil-10 ODS-3 has a low bonding density of 1.45 μmol/m^2 and poor shape selectivity, just like most monomeric phases.

A solid state CP/MAS ^{13}C NMR determination of Partisil-10 ODS-3 bonded phase morphology was carried out to give an additional information about the phase morphology. It was performed with a Bruker AM 300 instrument at room temperature. Sample spinning rate was 5000 Hz, the proton 90° pulse length was 6.6 μs, contact times and delay times were 2 ms and 2 s respectively and the broadening factor was 3.00 Hz. Peaks observed and their assignment according to Jinno[6] were: 50.9 ppm - OCH_3, 33.1 ppm - C3, 32.3 ppm - C16, 30.1 ppm - C4-15 (main peak), 22.8 ppm - C17, 13.1 and 12.6 ppm - C18 and 1.7 ppm - endcapping CH_3 groups. Since no signals can be observed for C1 or C2, the signal for C3 is weak and broad and there is a strong signal for C18, this spectrum belongs to a polymeric phase. The spectrum of Partisil-10 ODS-3 also bears a

close resemblance to the spectrum of a THE phase[6], which is an endcapped trifunctional polymeric phase with low bonding density. Comparison to the spectra of monomeric and polymeric octadecylsilyl phases reported by Pursch et. al.[7] confirms that Partisil-10 ODS-3 is a polymeric phase.

The extent of inter-chain interaction among the bonded chains (i.e. whether the phase is "liquid" or "solid") can not be unambiguously determined using the guidelines provided by Jinno[6], as no numerical values for peak widths for the main C4-15 peaks were given by Jinno, only a general description of peaks as "broad" for polymeric phases and "sharp" for monomeric phases. However, the spectrum of a THE phase which has a morphology similar to Partisil-10 ODS-3, shows fairly narrow C4-15 peak compared to other spectra in the work[6], and therefore qualitatively can be considered more "liquid" than "solid". Pursch et. al.[7] give a quantitative guideline for the determination of the extent of the interaction among bonded chains. They specify that the position of C4-15 peak close to 30.0 ppm corresponds to a high proportion of gauche conformations (more disordered), whereas a shift toward 32.0 ppm indicates a high proportion of ordered *trans* conformations. The position of C4-15 in Partisil-10 ODS3 is at 30.1 ppm, indicating a high degree of gauche conformations characteristic of a disordered, or liquid-like, state. Therefore, according to solid state NMR analysis Partisil-10 ODS-3 is a polymeric "liquid-like" bonded phase.

3.3. Chemical Reagents and Solvents

Eucalyptol (Fluka), 1-pentanol (Aldrich) and 2-pentanol (Aldrich) were reagent grade and were used as received. 1-Propanol (Sigma-Aldrich) and 1-butanol (Aldrich) were HPLC grade, 1-hexanol (Fluka) was puriss. grade (>99% by GC), and all were used

as received. Water was distilled and then deionized by a Series 550 Barnstead Nanopure water system (Dubuque, IA) to a resistance of 18.0 kΩ or higher. Methanol (Fisher Scientific) and ethanol (Commercial Alcohol Ltd.) were reagent grade and were distilled before use. Sodium hydroxide (Fisher Scientific) and glacial acetic acid (BDH Inc. Toronto, ON) were reagent grade and used as received.

3.4. Column Equilibration Technique

3.4.1. Apparatus and Procedure

The column equilibration apparatus[8,9] is shown in Figure 3.1. It consists of a pump P1 (Model 590, Waters Chromatography Division of Millipore) for the loading solution, a pump P2 (constant pressure pump operated at 30-50 psig of nitrogen), an injection valve V (Part N0. 7010, Rheodyne Inc.) and a precolumn C inserted in place of the injection loop of valve V. Precolumn C was modified from a standard type B refillable preconcentration column (Chrompack International) and was dry-packed with Partisil-10 ODS-3 packing. For the studies involving eucalyptol and 1-butanol the precolumn contained 29.29 mg of packing (precolumn#1), and for the studies involving 1-propanol and 1-hexanol it contained 30.73 mg of packing (precolumn#2). The precolumn and injection valve were placed in a water bath whose temperature was maintained at 25.00±0.04 °C.

The basic procedure for the column equilibration experiment is as follows. With valve V in the "load" position (indicated by the dashed lines in Figure 3.1), a loading

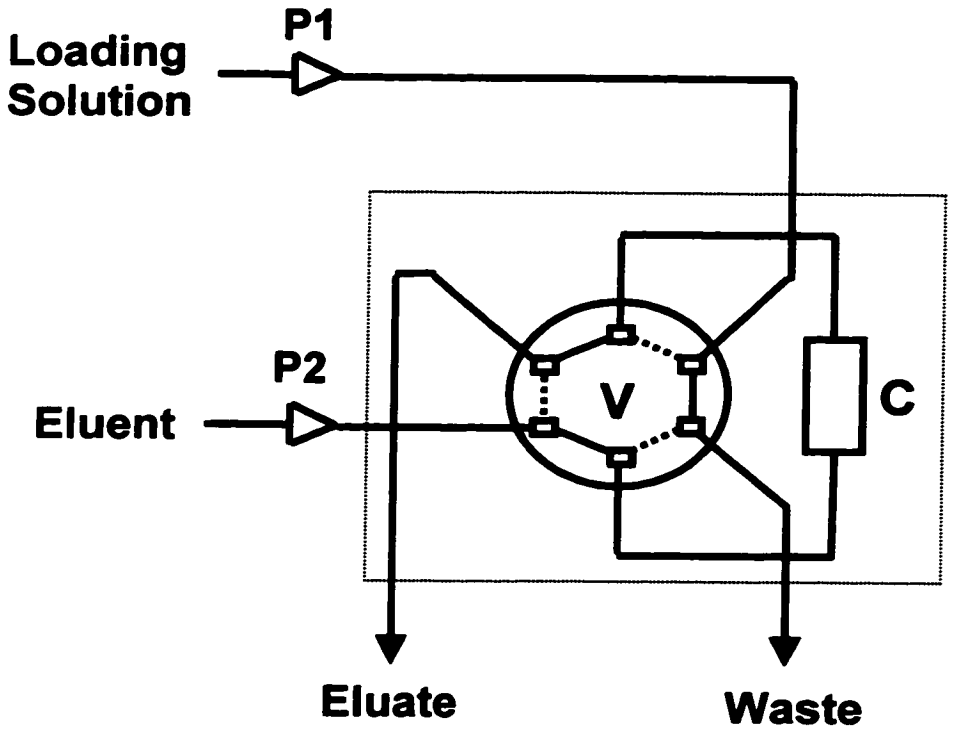

Figure 3.1. Column equilibration apparatus. P1 is an HPLC pump, P2 is a constant pressure pump, V is a six port sample injection valve and C is a small column packed with Partisil-10 ODS-3 packing. Dotted line surrounds the parts of the apparatus that were immersed in a constant temperature bath.

solution is pumped through the precolumn to waste until the equilibrium is achieved between the ODS packing and solution. This is the *loading step.* The flow rate during the loading step was always 12 mL/min. Valve V is then switched to the "inject" position (indicated by the solid lines in Figure 3.1) and eluent is pumped through the precolumn to elute whatever has sorbed on the packing. The eluate is collected up to the calibration mark in a volumetric flask, to which a measured amount of internal standard has been added. This is the *elution step.* The total amount of each species eluted from the precolumn, including that in the holdup volume, is then measured by determining the concentration of each of the species in the solution in the volumetric flask.

3.4.2. Eluent and Loading Solutions

The eluent for the studies involving eucalyptol and 1-butanol was always a mixture of 60% methanol and 40% water v/v, and for the study of the influence of 1-propanol on the sorption of 1-hexanol the eluent was always a mixture of 70% methanol and 30% water v/v. These mixtures were prepared by combining measured volumes of methanol and water. Loading solutions contained varying amounts of eucalyptol and/or 1-butanol or 1-propanol and/or 1-hexanol in aqueous pH=5 sodium acetate/acetic acid buffer of concentration $1 \cdot 10^{-4}$ mol/L. Even though there is no evidence in our studies to suggest that residual silanol groups play any role in the sorption of solutes, we have continued the practice of including a low concentration of buffer as a means of preventing variations in the degree of ionization of any residual silanol groups that may be present. At 10^{-4} mol/L concentration neither acetate nor acetic acid will significantly affect the properties of either the aqueous mobile phase or the ODS bonded phase. The

exact composition of loading solutions for loading and elution experiments is specified in the following sections; the composition of loading solutions for the studies of competitive sorption of 1-butanol and eucalyptol is presented in Sections 4.3.4 and 4.3.5, and the composition of loading solutions for the study of influence of 1-propanol on sorption of 1-hexanol is presented in Section 5.3.

All solutions, for both loading and elution, were filtered through a 0.45 μm pore size Nylon 66 filter (Alltech Associates Inc., Guelph, ON) before passing through the precolumn. Loading solutions and eluents were also degassed by sparging with helium (prepurified, Linde), after which a blanket of helium was maintained over the solution.

3.4.3. Loading Experiments

For the measurement of eucalyptol and 1-butanol loading curves (loading experiment #1), a solution containing $1.00 \cdot 10^{-5}$ mol/L eucalyptol and $2.00 \cdot 10^{-4}$ mol/L 1-butanol in the aqueous acetate buffer (see above) was pumped through the precolumn for various times. Valve **V** was then switched to "elute" position, and the eluent was collected up to mark into 2 mL volumetric flasks to which 0.200 mL of $2.5 \cdot 10^{-3}$ mol/L aqueous 2-pentanol solution was added as an internal standard. The eluent then was analyzed by GC. The results are plotted in Figure 3.2. Precision of the measurements can be estimated from the scatter among the points on the horizontal plateau. The scatter is relatively large because the precolumn temperature wasn't constant, even though the precolumn was immersed in the constant temperature bath. This was caused by the high flow rate during the loading step (12 mL/min). It was determined experimentally that placing a 120 cm long stainless steel coil (i.d. 0.5 mm) inside the constant temperature

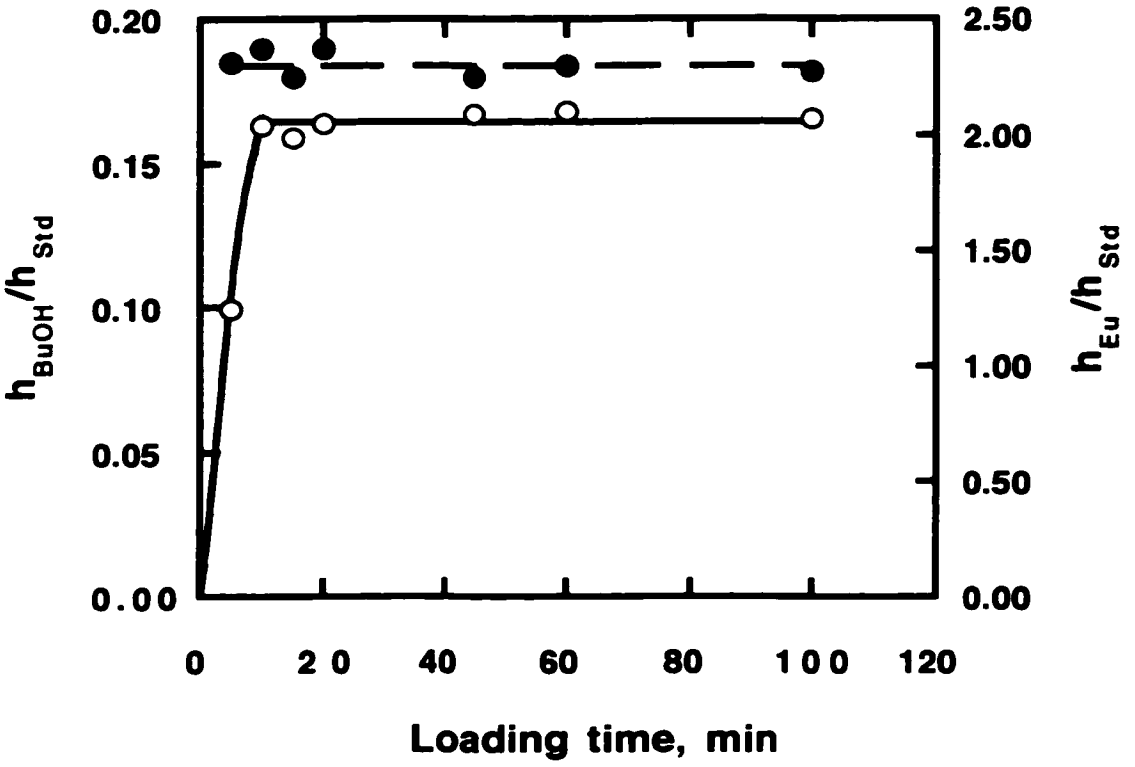

Figure 3.2. Results of the loading experiment on precolumn #1 for the solution containing $1.00 \cdot 10^{-5}$ mol/L eucalyptol and $2.00 \cdot 10^{-4}$ mol/L 1-butanol in the aqueous acetate buffer (loading experiment #1). The ratios of peak heights of eucalyptol sample and 2-pentanol standard in the eluate, determined from GC, are plotted on the left-hand vertical axis. The same ratios for 1-butanol sample and 2-pentanol standard are plotted on the right hand vertical axis. Open circles and solid line correspond to the eucalyptol, closed circles and dashed line to 1-butanol.

bath between the loading pump and the precolumn provided the necessary temperature equilibration and improved precision. This was done in all the subsequent experiments. The improvement of precision can be observed from the decrease of scatter in Figures 3.3 and 3.4.

In Figure 3.2 ratios of peak heights for the sample compounds (eucalyptol or 1-butanol) to the peak heights of standard (2-pentanol) are plotted on the vertical axis, and loading times are plotted on the horizontal axis. From this experiment it can be seen that the loading equilibrium is achieved for eucalyptol in less than 10 minutes and for butanol in under 5 minutes. To ensure the completeness of the loading step, the loading times were 25 minutes both for the study of the influence of 1-butanol on the sorption of eucalyptol and for the study of the influence of lower concentrations of eucalyptol on the sorption of 1-butanol (mobile phase concentration of eucalyptol $< 1 \cdot 10^{-3}$ mol/L).

The eucalyptol isotherm shows that the eucalyptol distribution coefficient decreases significantly at higher concentrations. Since the length of the time required to achieve loading equilibrium is expected to be approximately proportional to the sample distribution coefficient, it was expected that the loading times could be reduced for higher eucalyptol concentrations. Therefore, another loading experiment was run for a loading solution containing $1.00 \cdot 10^{-3}$ mol/L eucalyptol and $1.20 \cdot 10^{-3}$ mol/L of 1-butanol in the same buffer as above. After various loading times the eluate was collected into 2 mL volumetric flasks to which 0.200 mL of solution containing 2.00 mL/L of aqueous 2-pentanol was added as an internal standard for the GC analysis. The results are presented in Figure 3.3 in the same format as above. The loading times for eucalyptol and butanol at this higher concentration in the mobile phase are under 5 minutes. Therefore, in all the

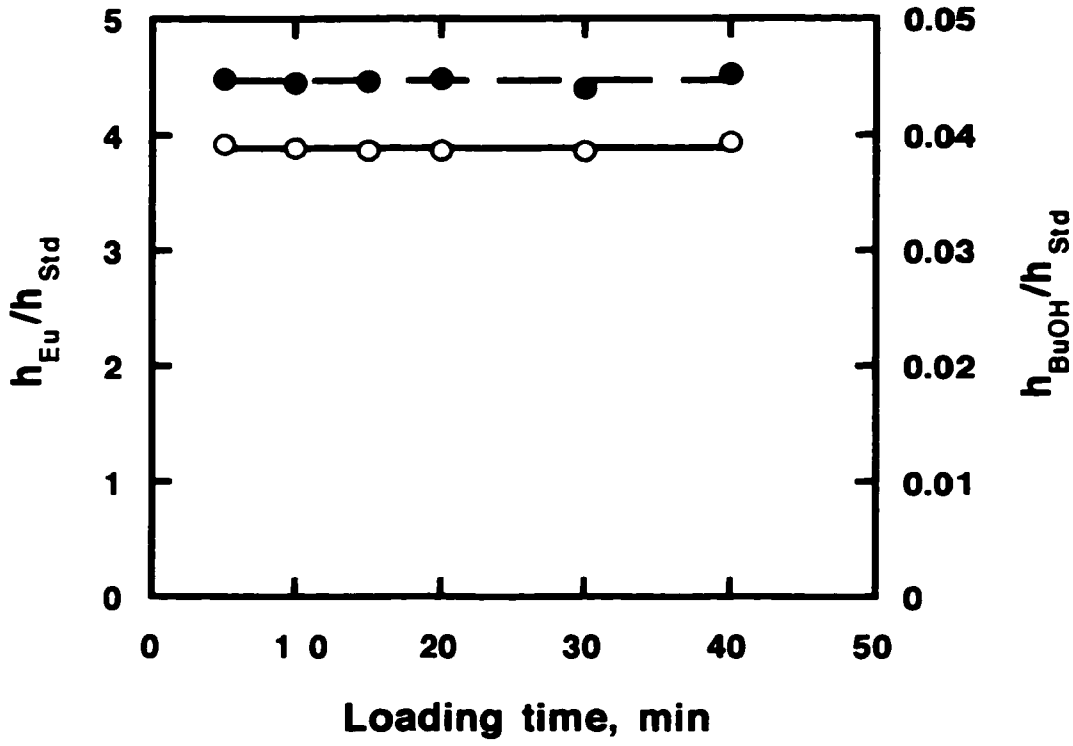

Figure 3.3. Results of the loading experiment on precolumn#1 for the solution containing $1.00 \cdot 10^{-3}$ mol/L eucalyptol and $1.20 \cdot 10^{-3}$ mol/L 1-butanol in the aqueous acetate buffer (loading experiment #2). The ratio of peak heights of eucalyptol sample and 2-pentanol standard in the eluate, determined from GC, is plotted on the left-hand vertical axis. The same ratio for 1-butanol sample and 2-pentanol standard is plotted on the right hand vertical axis. Open circles and solid line correspond to the eucalyptol, closed circles and dashed line to butanol.

experiments that contained > $1.00 \cdot 10^{-3}$ mol/L of eucalyptol in the mobile phase a loading time of 8 minutes was used.

Since the loading times are approximately proportional to the distribution coefficients and the distribution coefficient of 1-propanol is expected to be much smaller than the distribution coefficient of 1-hexanol, for the study of the influence of 1-propanol on the sorption of 1-hexanol only the 1-hexanol loading curve was measured. The loading solution contained $1.00 \cdot 10^{-4}$ mol/L of 1-hexanol and 0.10% v/v of 1-propanol in aqueous sodium acetate/acetic acid buffer, but only eluted 1-hexanol was measured by gas chromatography. The loading step was performed for various times and was followed by the elution step. The eluate was collected into 2 mL volumetric flasks to which 0.200 mL of $3.00 \cdot 10^{-3}$ mol/L 2-hexanol solution was added as an internal standard for the GC analysis. The results of the GC analysis of the eluates are plotted in the Figure 3.4 as the ratios of 1-hexanol to 2-hexanol peak heights vs. the loading times. It is seen that 1-hexanol loads in less than 3 minutes. For the experiments involving 1-hexanol and/or 1-propanol a loading time of 8 minutes was used.

3.4.4. Elution Experiments

To determine the volume of eluent that is required to completely elute the sorbed eucalyptol and butanol from the precolumn, a solution containing $1.00 \cdot 10^{-5}$ mol/L eucalyptol and $1.00 \cdot 10^{-3}$ mol/L 1-butanol in the sodium acetate/acetic acid buffer was first loaded by pumping it through the precolumn for 25 min. Then valve V was switched into "elute" position and the eluent was pumped through the precolumn with the constant pressure pump set at 30 psig, which gave a flow rate of ≈ 0.5 mL/min. The eluate was

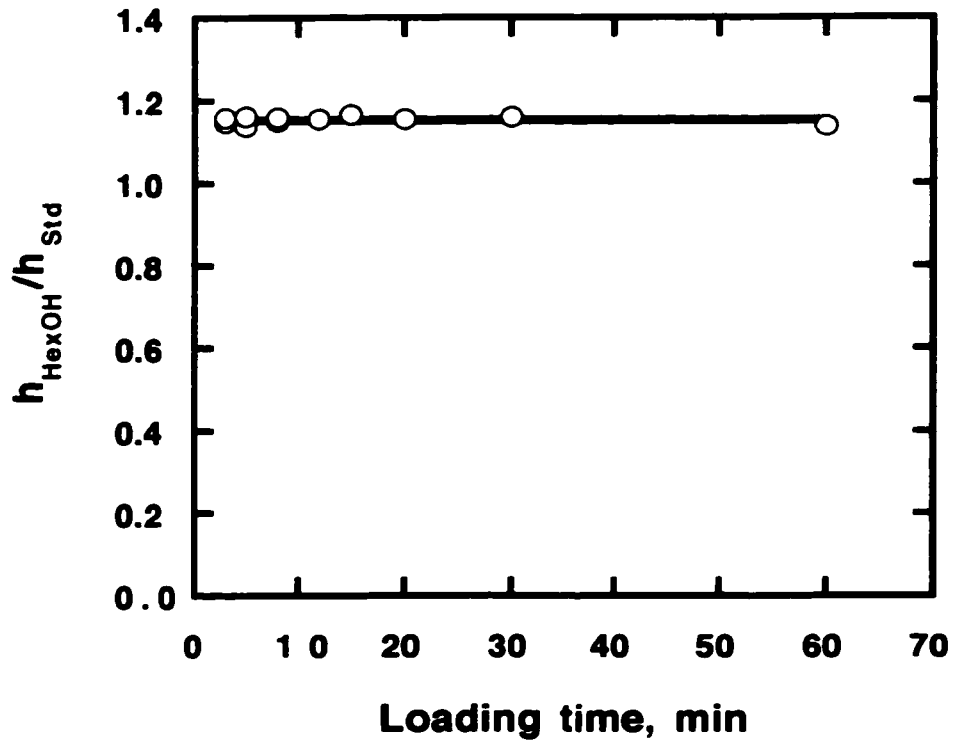

Figure 3.4. Results of the loading experiment on precolumn#2 for the solution containing $1.00 \cdot 10^{-4}$ mol/L 1-hexanol and 0.10% v/v 1-propanol in the aqueous acetate buffer. Open circles represent the ratio of peak heights of 1-hexanol sample and 2-pentanol standard in the eluate, as determined by GC. The solid line is the average of h_{HexOH}/h_{PrOH} for all data points.

collected in 20 drops fractions into 7 volumetric flasks of 2 mL volume, so that the total volume of collected eluate would give 140 drops or ≈ 1.7 mL. Each of the flasks contained 0.200 mL of $5 \cdot 10^{-3}$ mol/L aqueous 2-pentanol as an internal standard for gas chromatography. After collecting the eluate, the volumetric flasks were brought to volume with eluent and analyzed by GC. The results are presented in Figure 3.5. It is seen that eucalyptol is completely eluted within 4 fractions of 20 drops, or approximately 1 mL, of eluent and 1-butanol is completely eluted within 2 fractions of 20 drops, or approximately 0.5 mL, of eluent. In all the subsequent experiments involving eucalyptol and/or 1-butanol the eluate was collected into 2 mL volumetric flasks up to the calibration mark at a flow rate of 0.5 mL/min.

For the 1-hexanol plus 1-propanol case, a solution containing $1.00 \cdot 10^{-3}$ mol/L 1-hexanol and 0.500 % v/v 1-propanol in the sodium acetate/acetic acid buffer was pumped through the precolumn for 8 min. Then the sorbed compounds were eluted in consecutive fractions into seven 1 mL volumetric flasks, to each of which 0.100 mL of 1.60 mg/mL aqueous 2-propanol and 0.100 mL of 1.60 mg/mL aqueous 1-pentanol were added as internal standards for gas chromatography. The first three volumetric flasks contained eluate fractions of 15 drops and the rest contained 20 drops fractions. The flasks were brought to volume with eluent and analyzed by GC. The results are presented in Figure 3.6. 1-Propanol was completely eluted with 30 drops, or approximately 0.35 mL, of eluent and 99.7% of 1-hexanol were eluted with 50 drops, or approximately 0.6 mL, of eluent. In all the subsequent experiments either 1 mL vials with PTFE lined septum closure (to reduce the evaporation of 1-propanol), or 2 mL glass volumetric flasks with PTFE stoppers were used to collect the eluates.

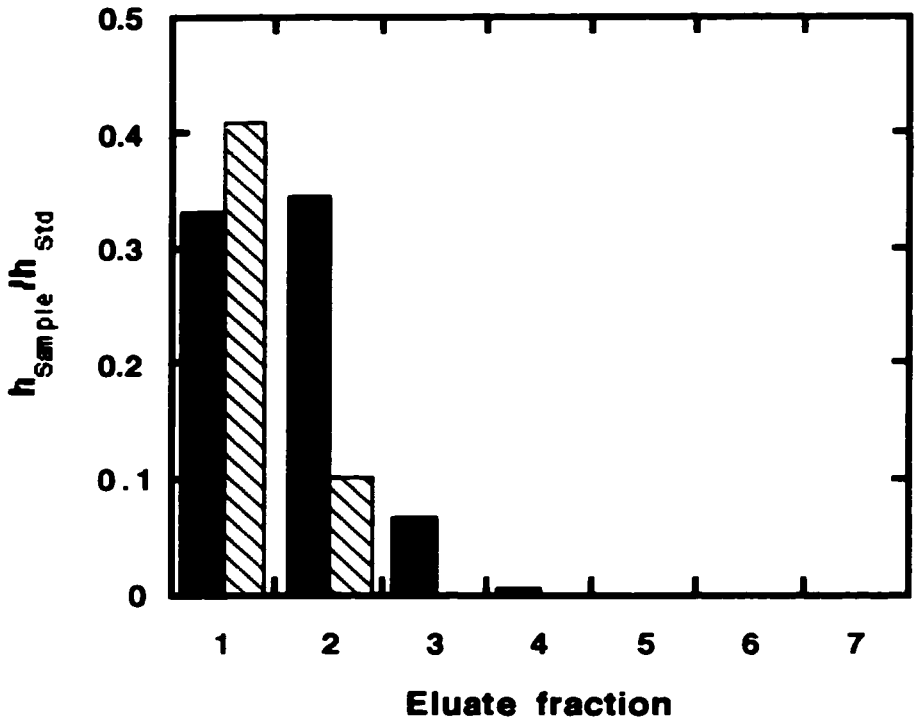

Figure 3.5. Results of the elution experiment for the solution containing $1.00 \cdot 10^{-5}$ mol/L eucalyptol and $1.00 \cdot 10^{-3}$ mol/L 1-butanol in the aqueous acetate buffer that was loaded on precolumn#1 for 25 min. The ratio of GC peak heights of either eucalyptol or butanol sample and 2-pentanol standard in the respective eluate fraction is plotted on the vertical axis, and the corresponding eluate fraction on the horizontal axis. Solid bars represent data for eucalyptol and dashed bars – for 1-butanol.

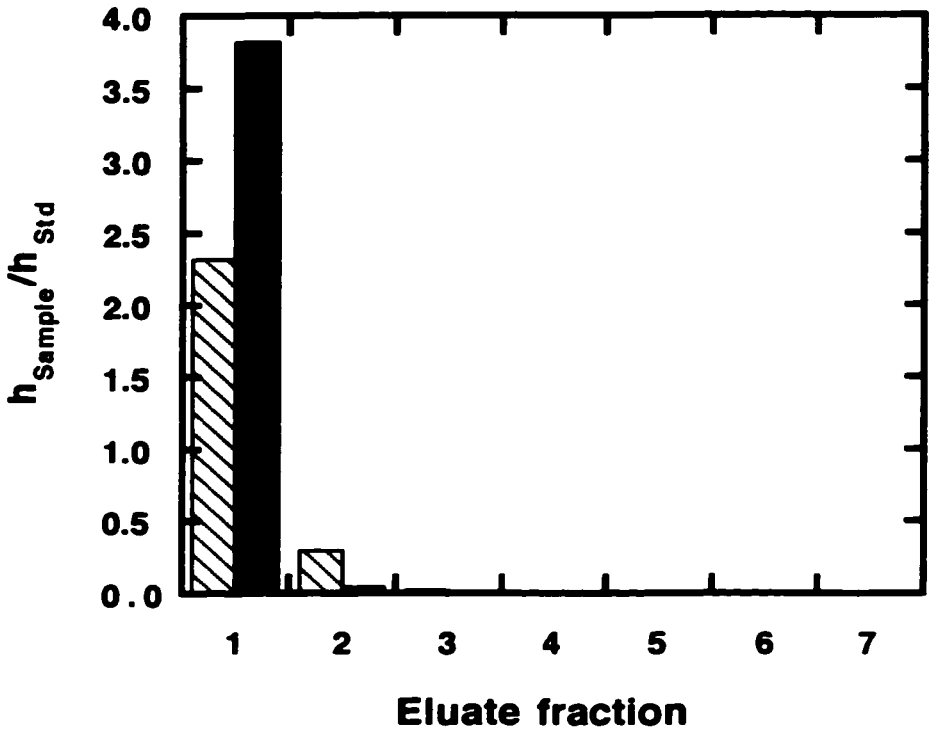

Figure 3.6. Results of the elution experiment for the solution containing $1.00 \cdot 10^{-3}$ mol/L 1-hexanol and 0.10% v/v 1-propanol in the aqueous acetate acid buffer that was loaded on precolumn#2 for 8 min. The ratios of GC peak heights of either 1-hexanol sample and 1-pentanol standard or 1-propanol sample and 2-pentanol standard in the respective eluate fractions are is plotted on the vertical axis, and the corresponding eluate fraction on the horizontal axis. Solid bars represent data for 1-hexanol and dashed bars – for 1-propanol.

3.4.5. Determination of Stationary Phase Concentration

The sorbed concentration of either 1-propanol or 1-hexanol was determined using the equation:

$$C_{X,s} = \frac{n_{T,X} - C_{X,m} V_{HU}}{W_s} \qquad (3.1)$$

where $n_{T,X}$ is the total number of moles of compound X sorbed (X = either 1-propanol or 1-hexanol), including that in the holdup volume, $C_{X,m}$ is the mobile phase concentration of X in mol/L, V_{HU} is the holdup volume in L and W_s is the weight of packing in the precolumn in kg.

As before[10] passage of mobile phase through the precolumn for long periods of time was found to slowly deteriorate the ODS stationary phase so that the effective number of sorption sites was reduced, although no evidence was found for dissolution of the silica substrate itself and the volume of packing in the precolumn was not visibly reduced. This decrease in the number of sorption sites can formally be taken into account as a decrease in the weight of stationary phase (W_s) in equations 3.1 and in 3.3 below. To do this, a solution which contained 1×10^{-3} *M* 1-butanol in acetate buffer, but no eucalyptol (for the experiments described in Chapter 4) or 3×10^{-4} mol/L of 1-hexanol in acetate buffer, but no 1-propanol, was run between experiments. Using the amount of 1-butanol or 1-hexanol sorbed from this standard by the precolumn at age **t**, the effective weight of packing $W_{s,t}$ to be used in place of W_s in equations 3.1 and 3.3 was calculated as:

$$W_{s,t} = \frac{n_{X,t}}{n_{X,o}} W_{s,o} \qquad (3.2)$$

where $n_{X,t}$ is the amount of 1-butanol or 1-hexanol sorbed at age **t**, $n_{X,0}$ is the amount of 1-butanol or 1-hexanol sorbed by the freshly packed precolumn, and $W_{s,0}$ is the weight of packing in the precolumn.

3.4.6. Holdup Volume Measurements

The holdup volume V_{HU} includes the void volume of the packed bed and frits V_{void} and the volume of the connecting tubing. In the holdup volume measurements, water is used as an unretained component and is determined by gas chromatography in ethanol solvent using methanol as an internal standard.

The apparatus used to determine the holdup volume of the precolumn is the same as that shown in Figure 3.1 and described in Section 3.4.1. For the precolumn used in the eucalyptol/butanol experiments the solution pumped through the precolumn was water and the eluent was ethanol. With valve V in the "load" position (dashed lines) water is pumped through the precolumn at a flow rate of 12 mL/min for 7 minutes. Valve V is then switched to the "elute" position (solid lines), and ethanol is pumped through the precolumn with the constant pressure pump operated at 30 psig of nitrogen (flow rate ≈0.5 mL/min) into a 2 mL volumetric flask containing 0.250 mL of methanol as an internal standard. Eluate was collected up to the calibration mark of the volumetric flask. The amount of water in the volumetric flask was determined by gas chromatography as described in Section 3.6, below. The holdup volume was determined to be 65.6±0.5 μL as the average of four replicate experiments.

For the precolumn used in the experiments involving 1-butanol and eucalyptol the holdup volume was determined only in the presence of mobile phase buffer. However,

the sorption of large amounts of either eucalyptol or 1-butanol solvent would increase the volume of the stationary phase and reduce the void volume and the holdup volume[11,12,13]. Since the change of void volume due to the sorption of eucalyptol and/or 1-butanol was not directly measured, the concentration of eucalyptol or of 1-butanol sorbed onto the ODS packing, either as solute or as solvent, was calculated iteratively by the following equation:

$$C_{X,s} = \frac{n_{T,X} - C_{X,m}(V_{HU}^{H_2O} - n_{solv,s}\overline{V}_{solv})}{W_s} \qquad (3.3)$$

where $V_{HU}^{H_2O}$ is the holdup volume measured in aqueous buffer, as described above, $n_{solv,s}$ is moles of solvent sorbed and $\overline{V}_{solv}$ is the molar volume of solvent in mL/mol. The second term in the numerator of equation 3.3 represents a correction for the presence of sorbed solvent (*solv*) in the hold-up volume.

The holdup volume of the precolumn used in the 1-hexanol/1-propanol experiments was determined at more than one mobile phase composition in order to test the validity of equation 3.3. The loading solutions for the holdup volume determination contained $3.00 \cdot 10^{-4}$ mol/L of 1-hexanol and various volume percentages, $\Phi_{PrOH,m}$, of 1-propanol in the aqueous sodium acetate/ acetic acid buffer. The solutions were prepared by pipetting the necessary amount of $1.00 \cdot 10^{-3}$ mol/L solution of 1-hexanol into the volumetric flask, adding buffer and 1-propanol by volume, and diluting to mark with water. Care was taken to mix the solution thoroughly before adding the last portion of water so that the mixture would not change its volume once brought up to the calibration mark. Since the volume of the 1-propanol/water mixture is not equal to the sum of the volumes of its constituents, the amount of water in each solution had to be determined

separately. This was done by subtracting the measured weight of 1-propanol that was used to prepare the solution from the total solution weight. Fraction of water g_{H_2O} (grams of water per mL of solution) was calculated as a ratio of water weight to the total volume of solution. The g_{H_2O} for the loading solutions containing various volume percentages of 1-propanol are presented in Table 3.1.

The amount of water in the holdup volume of the precolumn, upon equilibration with an aqueous/1-propanol loading solution, was determined by gas chromatography of the ethanol eluate from the precolumn. The holdup volume was calculated from the expression:

$$V_{HU} = \frac{w_{H_2O}}{g_{H_2O}} \qquad (3.4)$$

where w_{H_2O} is the weight of water in the eluate-containing 2 mL volumetric flask as determined by gas chromatography and g_{H_2O} is for the loading solution with which the precolumn had been equilibrated. At least two replicate experiments were run for every loading solution concentration of 1-propanol.

3.5. Solubility Experiments

The solubility of eucalyptol in water was determined by the shake-flask method. The solubility of eucalyptol in 1-butanol/water was determined by both the shake-flask method and by the cloud point method. The solubility of 1-hexanol in water and in aqueous 1-propanol solutions was determined by the cloud point method only.

Table 3.1. Volume percent of 1-propanol in 1-propanol/water mixtures with a corresponding weight/volume fraction of water.

$\Phi_{PrOH,m}$, % v/v	g_{H_2O}, g/mL
1.00	0.9883±0.0004
5.00	0.9513±0.0003
10.00	0.9057±0.0002
20.00	0.8136±0.0001
30.00	0.7173±0.0001

3.5.1. Shake-Flask Method

The determination of the solubility of eucalyptol in water and in aqueous 1-butanol solutions was carried out in conical 250 mL flasks and in sealed 100 mL ampoules. The procedure for the shake-flask method using conical flasks is as follows. Increasing measured volumes of eucalyptol were delivered into flasks followed by a measured volume of either water or aqueous 1-butanol solution. The conical flasks were stoppered and then sealed with parafilm. They were placed in a constant temperature bath with mechanical agitation, and kept at 25.0±0.4 °C for four days. After that the liquid in the flasks was allowed to settle in the constant temperature bath without mechanical agitation for several hours. Then 40 mL of the liquid from the flask were centrifuged to separate the aqueous and the organic layers, and either both layers or only the aqueous layer was analyzed by gas chromatography. Care was taken not to contaminate the aqueous solution with the organic layer.

The procedure for the determination of 1-hexanol solubility in water by shake-flask method was different only in that the dissolution of 1-hexanol was carried out in an ice bath with magnetic stirring for three hours. The flasks were then transferred into the constant temperature bath with mechanical agitation, and kept at 25.0±0.4 °C overnight. Then the agitation was switched off for 2 hours to allow for the separation of the aqueous and the organic layers. The aqueous layer was analyzed by gas chromatography.

The procedure for the sealed ampoules was as follows. First, a saturated solution of eucalyptol in water was prepared by adding an excess of eucalyptol to the measured volume of water in a large flask. The flask was stoppered and sealed with parafilm, and agitated in a constant temperature bath at 25.0±0.4 °C for four days. Then most of the

organic layer was removed by a pipette and discarded. Using a volumetric pipette, 50.00 mL of the eucalyptol-saturated aqueous solution were added to an empty ampoule followed by a 0.100 mL excess of liquid eucalyptol. Then the ampoules were stoppered with corks and kept in an ice bath for 30 min after which the ampoule was sealed with a flame. Sealed ampoules were agitated in the constant temperature bath at 25.0±0.4 °C for ten days. After allowing the contents of the ampoules to settle at constant temperature without agitation, the organic layers were removed and the aqueous layers were mixed with methanol and analyzed by GC.

3.5.2. Cloud Point Method

In this method the temperature-dependence of the solubility of eucalyptol and 1-hexanol[14] was exploited. The solubility of eucalyptol in water decreases when the temperature is increased, i.e. the process is exothermic. The solubility of 1-hexanol in water and in the aqueous 1-propanol solutions containing 10% v/v or less of 1-propanol also decreases with the increase of temperature. In the range 10-15% v/v of 1-propanol in water an inversion in the temperature dependence of 1-hexanol solubility occurs. At concentrations of 1-propanol of 15% v/v and above, the solubility of 1-hexanol increases when the temperature rises, i.e. the dissolution of 1-hexanol becomes endothermic.

The procedure for the determination of eucalyptol solubility in water and in 0.300 mol/L of aqueous 1-butanol is as follows. A desired amount of eucalyptol is weighed into a dry 125 mL conical flask. A magnetic stirring bar is placed into the flask, and water or 0.300 mol/L 1-butanol solution is delivered by a volumetric pipette. The flasks are stoppered with a plastic stopper and then sealed with parafilm. The sealed flasks are

placed in an ice bath with continuous magnetic stirring for 36 hours. A periodic inspection of the flask contents and walls indicated that eucalyptol was completely dissolved in less than 12 hours. After the dissolution step, the plastic stopper was replaced with a teflon tape wrapped cork stopper holding a thermometer with the temperature range from -2.0 to 51.0°C. The flask was then placed in a water bath whose temperature was maintained at approximately 10 °C above the expected eucalyptol cloud point. With continuous magnetic stirring, the temperature in the flask was allowed to rise until the solubility of eucalyptol was reached, as evidenced by the formation of a fine cloud-like emulsion of eucalyptol in the solution. The temperature at which the cloud first appeared was recorded as the solubility temperature for the mixture of a given composition. Then the flask was placed into an ice bath for 20-25 minutes to allow the cloud to re-dissolve, and the measurement of a cloud point was repeated again.

According to this procedure, cloud point measurements were performed for several mixtures with varied concentrations of eucalyptol in water. The eucalyptol concentrations were chosen in such a way as to give cloud point temperatures that encompass the region above and below 25°C with a span of about 15-20 °C. Then a plot of the natural logarithm of the mole fraction solubility of eucalyptol in water, $\ln X_{Euc,sat}$, versus reciprocal Kelvin temperature was constructed. This plot was linear, as expected, since only a narrow temperature range was investigated. The eucalyptol solubility at 25.0 °C was obtained by interpolation from this plot.

Then two solutions in 0.300 mol/L aqueous 1-butanol containing the same concentration of eucalyptol as its aqueous solubility at 25.0 °C were prepared. The cloud

point temperatures for these solutions were measured and compared to the plot of cloud point temperature versus aqueous solubility of eucalyptol.

A similar procedure was carried out for the determination of 1-hexanol solubility in water and in 1-propanol/water solutions. The main difference was that for solutions containing 0-10% 1-propanol the excess of 1-hexanol was dissolved by placing the flask in an ice bath for ≈ 3 hours and then the flask was heated at a slow rate to obtain a cloud point; whereas solutions containing 15% v/v or more of 1-propanol were first heated at 45 °C for 10-15 minutes to dissolve the excess 1-hexanol, and then cooled to obtain a cloud point. All mixtures were continuously stirred through the dissolution and cloud point measurement steps to maintain a uniform temperature inside the flasks. For an aqueous mixture containing a given 1-propanol concentration a number of solutions, containing varying amounts of 1-hexanol, were tested such that the cloud points covered a temperature range of 15-20 °C with 25 °C falling inside the range. Then the plot of cloud point temperature versus 1-hexanol solubility was constructed, and the solubility of 1-hexanol at 25 °C was determined from the plot by interpolation.

While mole fraction composition is an unambiguous concept, volume/volume composition can be defined in more than one way and, so, requires clarification. In the cloud point experiments, a measured volume of water was added to a measured volume of 1-propanol. The ratio, times 100, of the added volume of 1-propanol to the sum of individually added volumes of 1-propanol and water, before mixing, can be defined as an apparent volume percent, $\Phi'_{PrOH,m}$. Because the solution changes volume upon mixing, the final, mixed total solution volume may be different than the sum of added volumes. The apparent volume percent was converted to the true volume percent of 1-propanol,

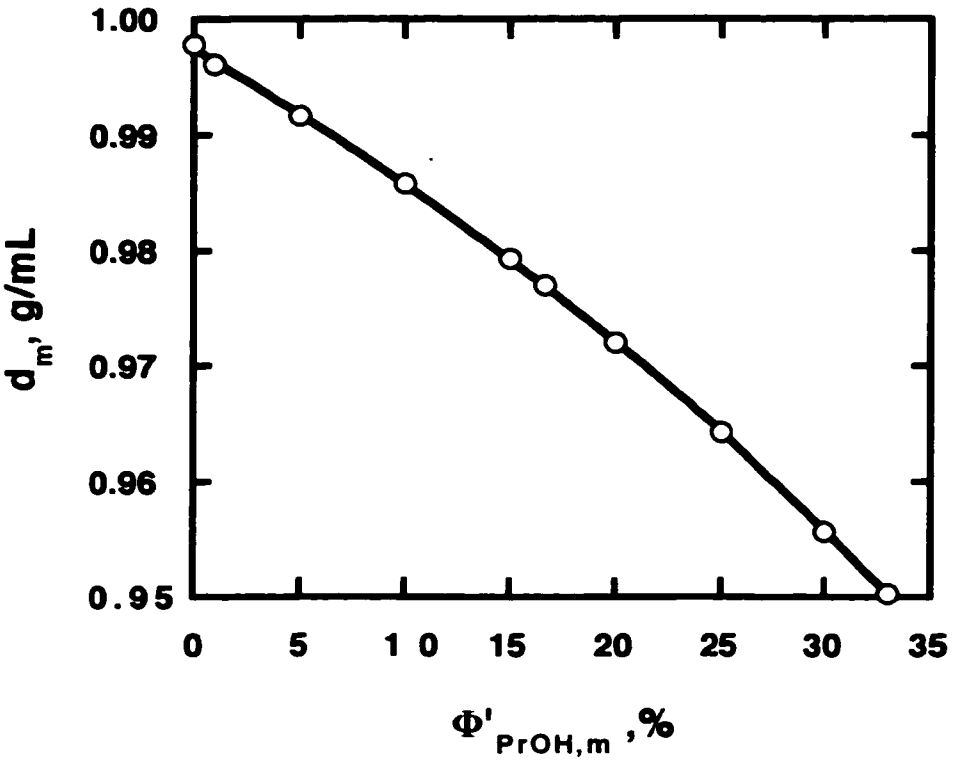

Figure 3.7. Plot of density of 1-propanol and water mixtures at 25 °C *versus* apparent volume percent of 1-propanol in solution. The density measurement is described on previous page.

Table 3.2. Apparent volume percent of 1-propanol for the mixtures used in the cloud point experiments with corresponding solution densities and true volume percent of 1-propanol calculated as described in section 3.5.2.

$\Phi'_{PrOH,m}$, %	d_{PrOH+H_2O}, g/mL	$\Phi_{PrOH,m}$, %
0.0000	0.9978	0.0000
0.9997	0.9961	0.9999
5.000	0.9917	5.020
10.00	0.9858	10.08
15.00	0.9793	15.17
16.67	0.9770	16.87
20.00	0.9721	20.28
25.00	0.9643	25.40
30.00	0.9557	30.53
33.00	0.9503	33.62

$\Phi_{PrOH,m}$, defined as 100 times the ratio of the volume of 1-propanol added to the final, mixed total solution volume, by employing the measured densities, $\mathbf{d_{PrOH+H_2O}}$, of 1-propanol/water mixtures. Density was measured by weighing 100 mL of a 1-propanol/water mixture of known $\Phi'_{PrOH,m}$ that was equilibrated at 25.0 °C. The resulting weight of 1-propanol/water mixture was divided by a true volume of 100 mL flask obtained by calibration with distilled water[15] in a separate measurement.

The plot of solution density $\mathbf{d_{PrOH+H_2O}}$ versus $\Phi'_{PrOH,m}$ is presented in Figure 3.7. The final, mixed total solution volume was calculated by multiplying the sum of added known weights of 1-propanol ($V_{PrOH} \times d_{PrOH}$) and water ($V_{H_2O} \times d_{H_2O}$) and dividing by the density of solution obtained by interpolation from the plot in Figure 3.7. Table 3.2 contains apparent volume fractions of 1-propanol for the mixtures used in the cloud point experiments with corresponding solution densities and calculated true volume fractions of 1-propanol.

3.6. Gas Chromatographic Analysis

Gas chromatographic analysis of the eluates of the column equilibration experiments were performed on a Perkin Elmer 8500 gas chromatograph equipped with flame ionization and thermal conductivity detectors or on a Shimadzu GC 14-A equipped with a thermal conductivity detector.

3.6.1. Columns

Several different gas chromatographic columns were used. For the determination of eucalyptol, 1-butanol and 1-hexanol column #1 was used. It was a 10 foot long, 1/8"

o.d., 0.082" i.d. stainless steel column packed with 5% Carbowax 20M (Chromatographic Specialties Inc., Brockville, Ontario) on Chromosorb WAW DMCS mesh 100/120 support (Manville, Denver, Colorado). It was prepared according to a standard technique[16]. Column #2 was a 10 foot long 1/8" o.d., 0.079" i.d. stainless steel column packed with Porapak QS mesh 50-80 (Waters Associates, Milford, MA). Column #3 was the same as column #2, but only 5 feet long.

3.6.2. Procedures

Injector and detector temperatures for all GC determinations were 230 °C. Helium was always used as a carrier gas.

For the determination of eucalyptol and 1-butanol column #1 was used in a Perkin Elmer 8500 gas chromatograph with flame ionization detector (FID). Helium inlet pressure was 37 psig, air and hydrogen were used as FID support gases at pressures of 22 and 18 psig respectively. The injection volume was 0.5 μL. After an injection the column oven was held at 70 °C for three minutes, then the temperature was raised at 2 °C/min untill the final temperature of 86 °C was reached. Sometimes small changes in this program were made to achieve a better baseline.

The determination of 1-propanol was carried out either simultaneously with the determination of 1-hexanol, using the Perkin Elmer 8500 gas chromatograph with flame ionization detector (FID) and column #1, or separately, using the same instrument and detector with column #2. For 1-hexanol and 1-propanol determination using column #1 the inlet pressures were: helium - 50 psig, air - 26 psig and hydrogen - 20 psig. The injection volume was 0.4 μL. The following temperature progam was used. Initially, the

column oven was kept at 65 °C for 5 minutes. Then the temperature was raised at 2 °C/min until the final temperature of 78 °C was reached. The 1-propanol determination using column #2 had the same inlet pressures for helium, air and hydrogen, and the same injection volume as for the analysis of 1-hexanol and 1-propanol on column #1, but the chromatographic runs were performed isothermally at 165 °C for the duration of 7 minutes.

The determination of water and methanol in ethanol solutions in the void volume experiment for the studies involving eucalyptol and butanol, was carried out on the Perkin Elmer 8500 gas chromatograph using column #2 and a thermal conductivity detector. Helium inlet pressure was 35 psig which gave a flow of 40 mL/min through the column. The injection volume was 1.5 μL. The column oven was held isothermally at 160 °C for 1.9 minutes, after which a heating ramp of 30 °C/min was applied until the final temperature of 200°C was reached.

Water and methanol determination in the void volume experiment for the studies involving 1-hexanol and 1-propanol was done on the Shimadzu GC-14 A with column #3 and a thermal conductivity detector. The carrier gas inlet pressure was 18 psig, injection volume was 1.5 μL, and the analysis was performed isothermally at 160 °C. Water, methanol and ethanol peaks eluted from the column in less than 2 minutes. Generally, the peak for 1-propanol was too small to be quantitated by the thermal conductivity detector.

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4. Simultaneous Sorption of 1-Butanol and Eucalyptol*

4.1. Introduction

The results of the studies of individual and simultaneous sorption of 1-butanol and eucalyptol on Partisil-10 ODS3, and the influence of small additions of 1-butanol on the aqueous solubility of eucalyptol are discussed in this Chapter. In the simultaneous sorption experiments the mobile phase concentration of one compound (sample) was kept low and constant, and the concentration of the other compound (organic modifier, or solvent) was varied. Then sample and solvent components were reversed. Therefore, information was obtained about the influence of 1-butanol organic modifier (solvent) on the sorption of eucalyptol sample, and the influence of eucalyptol organic modifier (solvent) on the sorption of 1-butanol sample. Of interest is the quantitative dependence of the amount of *sorbed solute* on the amount of *sorbed solvent.*

4.2. Theory

4.2.1. Introduction

A number physicochemical models have been proposed to describe the retention process of solutes on RPBPs. Lattice models derived from statistical thermodynamics involve detailed molecular-scale properties of bonded phases such as surface coverage, chain conformations and location of sorbed solvent modifier[1,2,3,4]. Models based on

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classical thermodynamics do not deal with molecular-scale properties, but they still require assumptions about what constitutes a component of the bonded phase and whether competitive displacement is involved in the sorption process[5,6,7].

The sorption model employed in the present work is based on the classical thermodynamic treatment of partitioning of a solute between two bulk liquid phases[8]. The stationary phase is assumed to be composed of three components: bound alkyl chains, organic modifier and solute. This assumption is consistent with the approach of Martire and Boehm[2] but differs from that of some other workers who have either excluded sorbed solvent[3] or bound chains[6] or who have included sorbed water[6]. Justification for neglecting the role of sorbed water when a water-rich mobile phase was used was given in the chapter 2.

4.2.2. Theoretical Model

Concentrations of all components are given as volume fractions, ϕ. In the stationary phase (s) the volume fraction of component X (where X is either solute, i; organic modifier, solv; or bound chains, C_{18}) is given by:

$$\phi_{i,s} \approx \frac{C_{X,s}\overline{V}_{X,s}}{C_{solv,s}\overline{V}_{solv,s} + C_{C_{18},s}\overline{V}_{C_{18},s}} \qquad (4.1)$$

Here, $C_{X,s}$ is concentration in moles/kg of dry packing, and $\overline{V}_X$ is molar volume of X in mL/mol. The term $C_{i,s}\overline{V}_i$ is omitted from the denominator in equation 1 because sorbed solute is present at trace concentrations in these studies. Therefore, in the stationary phase:

$$\phi_{C_{18},s} \approx 1 - \phi_{solv,s} \tag{4.2}$$

In the mobile phase (m) *i* is also present at trace concentrations so that the volume fraction of solute *i* is given by:

$$\phi_{i,m} \approx \frac{C_{i,m} \cdot \bar{V}_i}{C_{solv,m} \cdot \bar{V}_{solv} + C_{H_2O,m} \cdot \bar{V}_{H_2O}} \tag{4.3}$$

where concentration $C_{X,m}$ is in moles/L of solution. The equilibrium distribution coefficient for solute *i* between the stationary and mobile phase is:

$$K_i^{\phi} = \frac{\phi_{i,s}}{\phi_{i,m}} \tag{4.4}$$

When the volume fractions of *i* in both phases are kept very small, then the value of K_i^{ϕ} will be independent of the concentration of *i*. However, K_i^{ϕ} does depend on the concentrations of solvent in both phases.

In the *stationary phase,* the chemical potential of solute, $\mu_{i,s}^{o}$, is taken with respect to infinite dilution in the mixed phase which contains $\phi_{C_{18},s}$ of chains and $\phi_{solv,s}$ of solvent:

$$\mu_{i,s}^{o} = \mu_{i,C_{18}}^{o}\phi_{C_{18},s} + \mu_{i,solv}^{o}\phi_{solv,s} + Z \tag{4.5}$$

The standard chemical potentials $\mu_{i,C_{18}}^{o}$ and $\mu_{i,solv}^{o}$ are with respect to infinite dilution in pure ODS-chains and in pure solvent, respectively. The collective term **Z**, which gives the excess free energy, includes within it terms that are functions of $\phi_{C_{18},s}$ and $\phi_{solv,s}$ as well as terms that are constants. The phenomena contributing to **Z** include both the excess entropy, arising from the mixing of components having different molar volumes,

and the interaction energy between solvent and chains[7,8]. The magnitude of **Z** can be estimated using extra-thermodynamic models such as those of Scatchard and Hildebrand or Flory and Huggins[8]. However, when nonspecific interactions (i.e., dispersion forces) among solute, solvent and chains are the dominant interactions in the stationary phase, which may be true in the case of 1-butanol and eucalyptol, then Z may be small enough to neglect[6,7,8]. In that case, the stationary phase is an *ideal solution of solvent, solute and chains*, for which:

$$\mu^{o}_{i,s} = \mu^{o}_{i,C_{18}}\phi_{C_{18},s} + \mu^{o}_{i,solv}\phi_{solv,s} \qquad (4.6)$$

Because of its simplicity, equation 4.6 rather than equation 4.5 was tested first to see how well it describes the experimental behavior.

Combining equations 4.2 and 4.6 gives for the case of 1-butanol and eucalyptol:

$$\mu^{o}_{i,s} \approx \mu^{o}_{i,C_{18}} - (\mu^{o}_{i,C_{18}} - \mu^{o}_{i,solv})\phi_{solv,s} \qquad (4.7)$$

In the *mobile phase,* the standard chemical potential of solute, $\mu^{o}_{i,m}$, taken with respect to infinite dilution in the water/solvent mixture, is:

$$\mu^{o}_{i,m} = \mu^{o}_{i,H_2O} + \mathbf{RT}\ln\gamma^{T}_{i,m} \qquad (4.8)$$

where μ^{o}_{i,H_2O} is the standard chemical potential with respect to infinite dilution in pure water and $\gamma^{T}_{i,m}$ is the *transfer activity coefficient* for the transfer of *i* from infinite dilution in water to infinite dilution in the water/solvent mixture.

The equilibrium distribution coefficient for *i* is related to the chemical potentials by the expression:

$$\mu^{o}_{i,s} - \mu^{o}_{i,m} = -\mathbf{RT}\ln K^{\phi}_{i} \qquad (4.9)$$

Combining equations 4.4, 4.7, 4.8 and 4.9 and rearranging gives:

$$\frac{K_i^{\phi}}{\gamma_{i,m}^{T}} = \frac{\phi_{i,s}}{\phi_{i,m}\gamma_{i,m}^{T}} = \exp\left(-\frac{(\mu_{i,C_{18}}^{o} - \mu_{i,H_2O}^{o})}{RT}\right)\exp\left(+\frac{(\mu_{i,C_{18}}^{o} - \mu_{i,solv}^{o})}{RT}\phi_{solv,s}\right) \quad (4.10)$$

When an experiment is performed in which $\phi_{i,m}$ is held constant while the solvent concentration $\phi_{solv,m}$ and, consequently, $\phi_{solv,s}$ are changing, equation 4.10 can be written in the form:

$$\frac{K_i^{\phi}}{\gamma_{i,m}^{T}} = \frac{\phi_{i,s}}{\phi_{i,m}\gamma_{i,m}^{T}} = \alpha_i \exp(\beta_i \phi_{solv,s}) \quad (4.11)$$

where α_i and β_i are constants given by the expressions:

$$\alpha_i = \exp(-(\mu_{i,C_{18}}^{0} - \mu_{i,H_2O}^{o}) / RT) \quad (4.12)$$

and

$$\beta_i = (\mu_{i,C_{18}}^{o} - \mu_{i,solv}^{o}) / RT \quad (4.13)$$

Depending on whether $\mu_{i,C_{18}}^{o}$ is smaller or larger than $\mu_{i,solv}^{o}$, equation 4.11 predicts that $K_i^{\phi} / \gamma_{i,m}^{T}$ will either decrease or increase with $\phi_{solv,s}$.

Calculation of $\phi_{i,s}$ and $\phi_{solv,s}$ from equation 4.1 in which X = i and X = solv, respectively, requires a knowledge of concentrations and molar volumes. $C_{i,s}$ and $C_{solv,s}$ are measured experimentally and $\overline{V}_i$ and $\overline{V}_{solv}$ can be approximated from molecular weights and liquid densities. However, neither $C_{C_{18},s}$ nor $\overline{V}_{C_{18}}$ can be accurately estimated, *a priori*, in this way because the chains are covalently bound to the silica surface and experience conformational constraints that vary with location along the

chain[2,3,4]. Therefore, it is best to leave $C_{C_{18},s}\overline{V}_{C_{18}}$ as part of a fitting parameter. To do this, equation 4.11 can be combined with equation 4.1 for both $\phi_{i,s}$ and $\phi_{solv,s}$ to give the following expression:

$$\frac{C_{i,s}}{\gamma^{T}_{i,m}} = A_i (C_{solv,s} + S_i) \exp\left(\beta_i \frac{C_{solv,s}}{C_{solv,s} + S_i}\right) \tag{4.14}$$

where

$$A_i = \phi_{i,m}\, \alpha_i\, (\overline{V}_{solv} / \overline{V}_i) \tag{4.15}$$

and

$$S_i = C_{C_{18},s}(\overline{V}_{C_{18}} / \overline{V}_{solv}) \tag{4.16}$$

Equation 4.14, which predicts the dependence of $C_{i,s}/\gamma^{T}_{i,m}$ on $C_{solv,m}$ has three fitting parameters: A_i, β_i and S_i.

4.3. Results and Discussion

Three types of experiments have been performed: (i) Measurement of eucalyptol solubility in water and dilute aqueous solutions of 1-butanol by shake-flask and cloud point methods; (ii) Individual *sorption isotherms* for 1-butanol and eucalyptol between ODS-3 packing and aqueous solution; and (iii) Measurements of the *simultaneous sorption* of 1-butanol and eucalyptol from aqueous solutions on ODS-3 packing by a column equilibration technique. The solubility experiments provide information about the mobile phase contribution to the retention of eucalyptol. Sorption isotherms and the

simultaneous sorption curves provide information about the nature of the stationary phase and its contribution to the solute retention.

4.3.1. Solubility of Eucalyptol in Water and in Dilute Aqueous Solutions of 1-Butanol

4.3.1.1. Shake-Flask Experiments

4.3.1.1.1. Stoppered Flasks

First, the solubility of eucalyptol was determined in water. In this experiment, increasing amounts of eucalyptol were added to consecutive flasks containing 200 mL of water. After dissolution in a constant temperature bath, as described in Section 3.5.1, the aqueous layer was separated from the organic layer and analyzed for eucalyptol. It is important to note that there was a residue of undissolved eucalyptol in the flasks that contained the three highest amounts of eucalyptol. The plot of measured dissolved concentration of eucalyptol, $C_{Eu,dissolv}$, (mol/L) *versus* $C_{Eu,added}$ (mol/L), the concentration calculated from the amount of eucalyptol added to the flask under the assumption that all eucalyptol dissolved, is presented in Figure 4.1. It is observed that $C_{Eu,dissolv}$ is increasing at first with $C_{Eu,added}$, and then reaches saturation at $(1.87\pm0.02)\cdot10^{-2}$ mol/L (average $C_{Eu,dissolv}$ for the three highest points in plot 4.1). This value is the solubility of eucalyptol in water under the conditions of this experiment. However, $C_{Eu,dissolv}$ was consistently lower than $C_{Eu,added}$. This is true even for the solutions of lower concentration in which eucalyptol was completely dissolved. Eucalyptol evaporation is the suspected culprit.

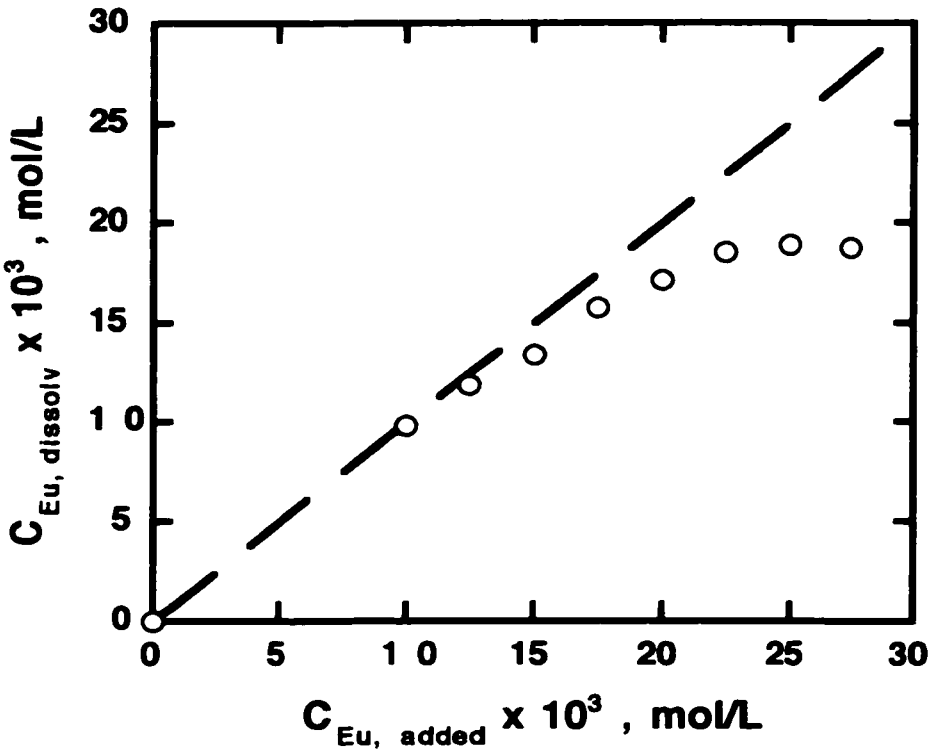

Figure 4.1. Shake-flask determination of eucalyptol solubility in water at 25 °C. Points are experimental, line is drawn according to equation $C_{Eu,dissolv} = C_{Eu,added}$.

A similar experiment was done to determine the solubility of eucalyptol in 0.300 and 0.600 mol/L aqueous 1-butanol. Excess of eucalyptol, above the solubility in water, was added to 1-butanol solutions and equilibrated as described in Section 3.5.1. Subsequent analysis of the aqueous layer revealed that the concentration of eucalyptol in it was lower than the concentration eucalyptol in saturated aqueous solution, as determined by the experiment above. Plots of equilibrium eucalyptol concentration, $\mathbf{C_{Eu,m}}$, in 0.300 and 0.600 mol/L aqueous 1-butanol solutions *versus* the percent excess eucalyptol added with regard to saturated aqueous solution are presented in Figure 4.2. The precision of the measurement was estimated to be ±0.0006 mol/L, as shown by error bars in the plot. The horizontal line is the concentration of eucalyptol in the saturated aqueous solution. The average value of $\mathbf{C_{Eu,m}}$ in 0.300 mol/L 1-butanol solution is 0.0175±0.0006 mol/L, and the average value of $\mathbf{C_{Eu,m}}$ in 0.600 mol/L 1-butanol solution is 0.0128±0.0006 mol/L. The average concentration of eucalyptol in 0.600 mol/L 1-butanol solutions was only about 70% of the average eucalyptol concentration in 0.300 mol/L 1-butanol solutions.

The concentration $\mathbf{C_{BuOH,m,equilib}}$ of 1-butanol in the aqueous layer at equilibrium was also determined. For the 0.300 mol/L 1-butanol solutions $\mathbf{C_{BuOH,m,equilib}}$ was close to the original concentration. However, $\mathbf{C_{BuOH,m,equilib}}$ for 0.600 mol/L 1-butanol solutions had decreased to 0.57±0.01 mol/L, with a tendency to lower 1-butanol concentration in the solutions containing more eucalyptol. The volume of eucalyptol added, $\mathbf{V_{Eu}}$, (μL), the percent excess added eucalyptol compared to the amount needed for saturated aqueous solution, **% Excess**, the original concentration of 1-butanol in the

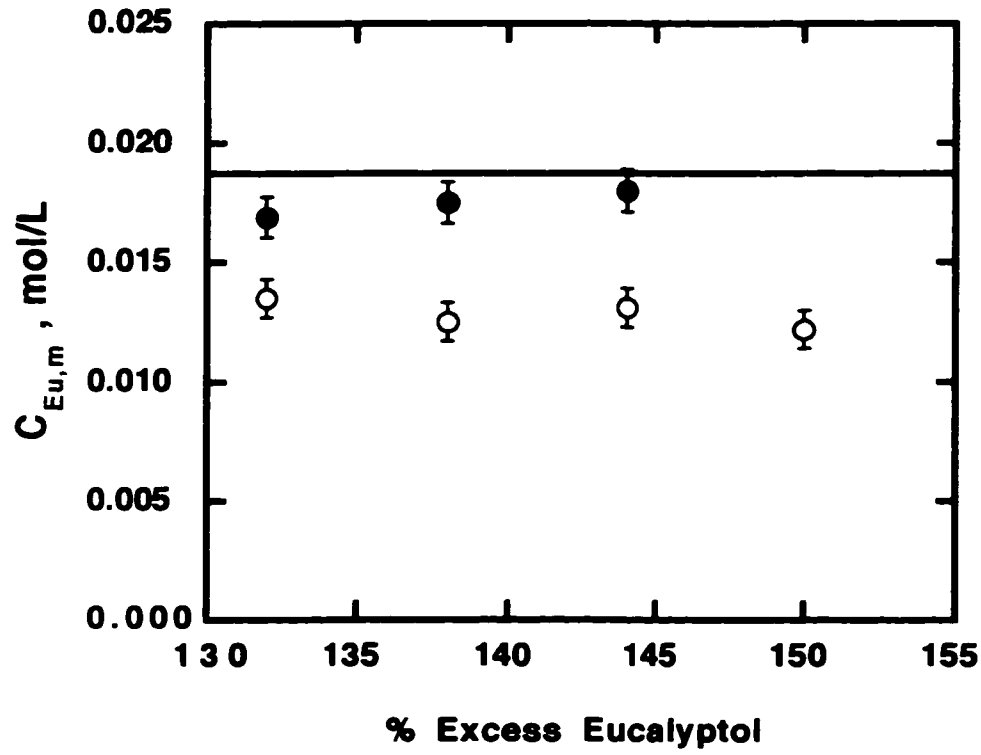

Figure 4.2. Shake - flask determination of eucalyptol solubility in 0.300 mol/L (●) and 0.600 mol/L (○) 1-butanol solutions in water at 25 °C. The horizontal line is the concentration of eucalyptol in the saturated aqueous solution.

Table 4.1. Data for the shake-flask determination of eucalyptol solubility in 0.300 and 0.600 mol/L solutions of 1-butanol in water at 25.0 °C.

V_{Euc} μL	% Excess Eucal.	$C_{BuOH,m}$ mol/L	$C_{BuOH,m,equil}$ mol/L	$C_{Eu,m}$ mol/L	$C_{Eu,H_2O,sat}$ mol/L
0.826	132	0.300	0.29_7	0.016_9	0.0187
0.864	138	0.300	0.29_7	0.017_5	0.0187
0.901	144	0.300	0.29_5	0.018_0	0.0187
0.826	132	0.600	0.57_7	0.013_5	0.0187
0.864	138	0.600	0.56_9	0.012_5	0.0187
0.901	144	0.600	0.56_6	0.013_1	0.0187
0.939	150	0.600	0.55_4	0.012_2	0.0187

aqueous layer, $C_{BuOH,m}$, the equilibrium concentration of 1-butanol in the aqueous layer, $C_{BuOH,m,equilib}$, the equilibrium concentration of eucalyptol in the aqueous layer, $C_{Eu,m}$, and the concentration of saturated aqueous eucalyptol solution, $C_{Eu,H_2O,sat}$, are presented in Table 4.2. Since the apparent solubility of eucalyptol in the 0.600 mol/L 1-butanol solutions was lower than in 0.300 mol/L 1-butanol solutions, and the equilibrium concentration of 1-butanol in 0.600 mol/L solutions was lower than the original, it was suspected that 1-butanol was extracted from the aqueous phase into the excess eucalyptol organic phase. To confirm this the organic layer was analyzed. Indeed, 1-butanol was extracted into the organic layer. The ratio of moles of eucalyptol to moles of 1-butanol in the organic layer in contact with 0.300 mol/L 1-butanol solution is 3.7±0.2. When 1-butanol mobile phase concentration is increased to 0.600 mol/L this ratio drops to 0.69±0.02. The decrease in apparent eucalyptol solubility can be understood if one considers the equilibrium described by:

$$K_{Eu,dist} = \frac{C_{Eu,m}}{C_{Eu,org}} \tag{4.17}$$

where $K_{Eu,dist}$ is the equilibrium constant for distribution of eucalyptol between the organic and aqueous layers and $C_{Eu,org}$ is the concentration of eucalyptol in the eucalyptol/1-butanol organic phase. When the organic layer in contact with the aqueous solution is pure eucalyptol, the aqueous concentration becomes the true solubility. Extraction of 1-butanol into the layer of eucalyptol causes $C_{Eu,org}$ to decrease, and $C_{Eu,m}$ decreases along with it, though not necessarily directly proportional to it.

4.3.1.1.2. Sealed Ampoules

Two problems were revealed in the above studies of eucalyptol solubility in water and aqueous 1-butanol solutions. First, there is evaporation of eucalyptol, and second, 1-butanol gets extracted by the excess liquid eucalyptol. The first problem can be circumvented by the use of sealed ampoules. Therefore, a determination of true eucalyptol solubility in water was carried out by shake-flask method in sealed ampoules as described in section 3.5.1.

The value of eucalyptol solubility in water at 25.0 °C is 0.0211±0.0004 mol/L as the average of three replicate determinations. This value is 13% above the value of solubility obtained by shake-flask experiment using stoppered flasks. It confirms that there was evaporation of eucalyptol in the experiments described above. Eucalyptol solubility in sealed ampoules is in good agreement with the literature eucalyptol solubility value of 0.022 mol/L also obtained by shake-flask method[9].

4.3.1.2. Cloud Point Experiment

In order to overcome the problem of 1-butanol extraction into the organic eucalyptol layer, a cloud point experiment was used. In this experiment eucalyptol solutions in water or aqueous 1-butanol are heated untill the eucalyptol solubility is reached, and a very fine cloud of insoluble eucalyptol is formed. Since the excess of eucalyptol is very small, and the process occurs rapidly compared to the several day equilibration in the shake flask method, the extraction of 1-butanol into organic eucalyptol layer is not expected to pose a problem, and true solubility of eucalyptol can be determined.

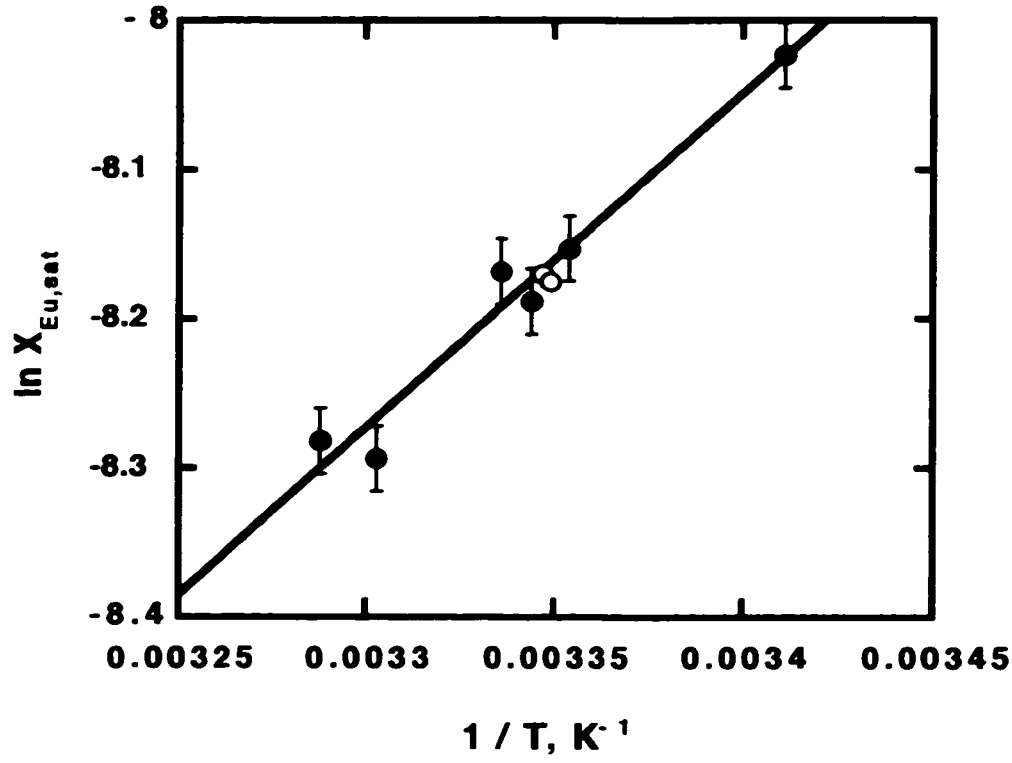

Figure 4.3. A plot of natural logarithm of mole fraction eucalyptol solubility in water $\ln X_{Eu,H_2O,sat}$ (●) and in 0.300 mol/L 1-butanol solution $\ln X_{Eu,m,sat}$ (○) *versus* reciprocal absolute temperature. The line is a linear least-squares fit to $\ln X_{Eu,H_2O,sat}$ *versus* 1/T. Error bars represent the standard error in the fit, equal to 0.021.

Over a small temperature interval the enthalpy of the eucalyptol dissolution process will be constant. Therefore, the plot of natural logarithm of the equilibrium constant *versus* the reciprocal temperature is expected to be linear[10]. When the organic phase in contact with the aqueous solution is pure eucalyptol, the activity of eucalyptol in that phase remains constant, and the variation of aqueous phase solubility reflects the variation in the equilibrium constant. In this case we expect a linear plot of the natural logarithm of the mole fraction eucalyptol solubility in water $\ln X_{Eu,sat}$ versus reciprocal absolute temperature (K). This plot is presented in Figure 4.3. It is linear, as expected. The standard error in the plot of $\ln X_{Eu,sat}$ *vs* $1/T$ for water was 0.021, meaning that the relative standard deviation of $X_{Eu,sat}$ is 2.1%. The solubility of eucalyptol in 0.300 mol/L 1-butanol solution relative to that in water, at 25 °C, was estimated from the deviation of the points obtained in the mixed solvent (open circles in Figure 4.3) from the linear regression line through the points obtained with water solvent (closed circles in the same Figure). At 25 °C, duplicate values of $X_{Eu,sat}$ for the 1-butanol/H_2O mixture fell, respectively, within 0.2 and 0.6 relative standard deviations of the value of $X_{Eu,sat}$ in water, demonstrating that the solubility of eucalyptol is the same in these two solvents and, consequently, that the value of $\gamma^{T}_{Eu,m}$ is the same in these two solvents[8,11]. That is,

$\gamma^{T}_{Eu,m} = 1.0_0$ for all aqueous solutions containing ≤ 0.300 mol/L of 1-butanol.

4.3.2. 1-Butanol Isotherm

Conventionally, sorption isotherms are plotted as *concentration* in the stationary phase *versus concentration* in the mobile phase. A plot of $C_{BuOH,s}$ *vs* $C_{BuOH,m}$ in Figure 4.4 can be fit very well ($R^2 = 0.9999$) by the "associative-bilayer isotherm" equation[12]:

$$C_{BuOH,s} = \frac{K_{BuOH,1} C_{BuOH,s,max} C_{BuOH,m}}{1 + K_{BuOH,1} C_{BuOH,m}} + K_{BuOH,2} C_{BuOH,m} \qquad (4.18)$$

where $K_{BuOH,1}$ and $K_{BuOH,2}$ are constants and $C_{BuOH,s,max}$ is the maximum monolayer coverage of 1-butanol. The values of constants obtained from the fit are: $K_{BuOH,1}$= 9.1±0.6 L/kg; $K_{BuOH,2}$=1.6±0.2 L/kg; and $C_{BuOH,s,max}$ =1.7±0.2 mol/kg. From this equation it might be inferred that 1-butanol is sorbed by the ODS stationary phase in more than one layer. Such an inference is based on the assumption that the entire non-linear shape of the isotherm arises only from processes occurring in the stationary phase. This would be true only if $C_{BuOH,m}$ is directly proportional to the activity of 1-butanol in the mobile phase; that is, if the activity coefficient of 1-butanol in the mobile phase is constant over the whole range of $C_{BuOH,m}$ involved. However, this assumption, and its dependent inference of associative-bilayer sorption for 1-butanol, are both false.

Activity coefficients of 1-butanol, with respect to pure 1-butanol standard state, $\gamma^{PS}_{BuOH,m}$, were calculated from vapor-liquid equilibrium data[13] at 25.0 °C using the Van Laar equation[14]. These activity coefficients were converted to activity coefficients with respect to infinite dilution of 1-butanol in water, $\gamma^{ID}_{BuOH,m}$, by the following expression:

$$\gamma^{ID}_{BuOH,m} = \frac{\gamma^{PS}_{BuOH,m}}{\gamma^{o,PS}_{BuOH,m}} \tag{4.19}$$

in which $\gamma^{o,PS}_{BuOH,m} = 61.09$ is the value of $\gamma^{PS}_{BuOH,m}$ at infinite dilution. The activity of 1-butanol with respect to infinite dilution is the product:

$$a^{ID}_{BuOH,m} = C_{BuOH,m} \cdot \gamma^{ID}_{BuOH,m} \tag{4.20}$$

Presented in Table 4.2 are the values of $\gamma^{PS}_{BuOH,m}$, $\gamma^{ID}_{BuOH,m}$, $a^{ID}_{BuOH,m}$ and $C_{BuOH,s}$ for the various $C_{BuOH,m}$ at which sorption was measured. Since $\gamma^{ID}_{BuOH,m}$ is seen not to be a constant over the range of $C_{BuOH,m}$ employed, it is necessary to plot the isotherm as $C_{BuOH,m}$ *vs* $a^{ID}_{BuOH,m}$, as in Figure 4.5, in order to understand what is happening in the stationary phase. The line in Figure 4.5 represents a non-linear least-squares fit of the data points by the Langmuir equation:

$$C_{BuOH,s} = \frac{K'_1 C_{BuOH,s,max}\, a^{ID}_{BuOH,m}}{1 + K'_1 a^{ID}_{BuOH,m}} \tag{4.21}$$

where

$$K'_1 = \frac{C_{BuOH,s}}{a^{ID}_{BuOH,m}} \tag{4.22}$$

The fit here is also very good, with $R^2 = 0.9996$, $K'_1 = 4.9 \pm 0.2$ L/kg and $C_{BuOH,s,max} = 3.2 \pm 0.2$ mol/kg. Using the specific surface area of $3.0 \cdot 10^5$ m^2/kg[16] the calculated area per mol of sorbed 1-butanol is $(9.4 \pm 0.6) \cdot 10^5$ m^2/mol. Dividing $\overline{A}_{BuOH,s}$

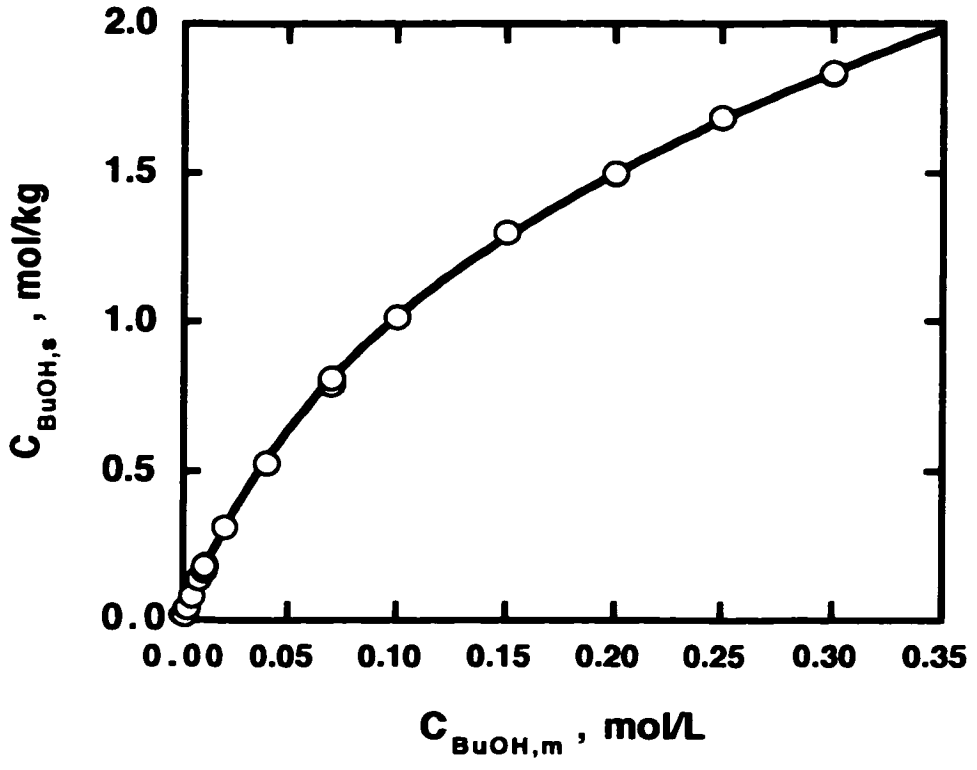

Figure 4.4. 1-Butanol isotherm plotted as stationary phase concentration versus mobile phase concentration (mol/L). The points are experimental, the line is a non-linear least squares fit of equation 4.18 to the data points.

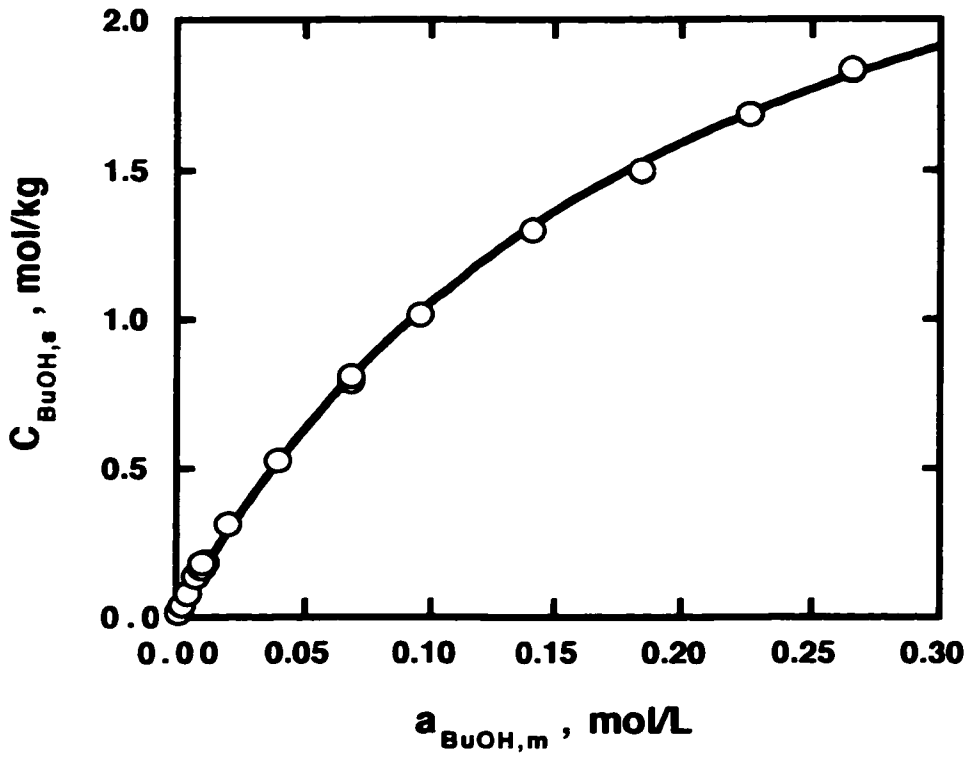

Figure 4.5. 1-Butanol isotherm plotted as concentration in the stationary phase versus activity of 1-butanol in the mobile phase. Points are experimental, the line is a non-linear least-squares fit of equation 4.21 to the data points.

Table 4.2. Data for the 1-butanol isotherm on Partisil-10 ODS-3.

$C_{BuOH,m}$, mol/L	$\gamma^{PS}_{BuOH,m}$	$\gamma^{ID}_{BuOH,m}$	$a^{ID}_{BuOH,m}$, mol/L	$C_{BuOH,s}$, mol/kg
0.000765	61.07	0.9997	0.000765	0.0179
0.00219	61.03	0.9991	0.00218	0.0419
0.00437	60.98	0.9982	0.00436	0.0801
0.00745	60.90	0.9969	0.00743	0.138
0.0109	60.83	0.9959	0.00996	0.185
0.0109	60.83	0.9959	0.00996	0.182
0.0100	60.84	0.9960	0.0996	0.181
0.0200	60.58	0.9917	0.0198	0.313
0.0400	60.08	0.9835	0.0393	0.526
0.0700	59.34	0.9714	0.0680	0.788
0.0700	59.34	0.9714	0.0680	0.806
0.150	57.43	0.9401	0.141	1.28
0.200	56.27	0.9211	0.184	1.47
0.300	54.04	0.0846	0.265	1.78
0.300	54.04	0.0846	0.265	1.79

by Avogadro number gives 16 ± 1 $Å^2$ per molecule of sorbed 1-butanol. This value is in good agreement with the experimentally measured molecular area of 20 $Å^2$ for lower normal alcohols sorbed at liquid-liquid interfaces[15]. In addition, the good fit of the data by the simple Langmuir equation, employing activity, implies that self-association of BuOH to form an additional sorbed layer does not occur at stationary phase concentrations corresponding to about 60% of a monolayer.

At high activities, the ODS sorbs quite a lot of BuOH. For example, a sorbed concentration of 1.8 mol/kg is equal to 133 grams of BuOH sorbed per kg of dry ODS-3 packing. In comparison, ODS-3 packing contains approximately 10.5% w/w carbon content[16], which corresponds to about 126 grams of C_{18} chains per kg of dry ODS-3 packing. Therefore, at this point the stationary phase on ODS-3 packing is composed of about 51% w/w BuOH and 49% w/w C_{18} chains.

4.3.3. Eucalyptol Isotherm.

A plot of sorbed concentration of eucalyptol *versus* its concentration in aqueous solution is shown by the points in Figure 4.6. The line represents the non-linear least squares fit of the "associative bilayer isotherm with limited solubility in the aqueous phase"[12] to the data points. The isotherm equation is:

$$C_{Eu,s} = \frac{K_1 C_{Eu,s,1,max} \frac{C_{Eu,m}}{C_{Eu,H_2O,sat}} + K_1 C_{Eu,s,1,max} (K_2(1+p)-1) \frac{C_{Eu,m}^2}{C_{Eu,H_2O,sat}^2}}{1 + (K_1 + K_2 p - 2) \frac{C_{Eu,m}}{C_{Eu,H_2O,sat}} + (K_1 - 1)(K_2 p - 1) \frac{C_{Eu,m}^2}{C_{Eu,H_2O,sat}^2}} \quad (4.23)$$

$$K_1 = \frac{C_{Eu,s,1}}{C_{Eu,m}} \quad (4.24)$$

$$K_2 = \frac{C_{Eu,s,2}}{C_{Eu,m}} \tag{4.25}$$

In these equations $C_{Eu,s,1}$ and $C_{Eu,s,2}$ are concentrations sorbed in the first layer and in the (associative) second layer, respectively, $C_{Eu,s,1,max}$ is the monolayer concentration of eucalyptol in the first layer, $C_{Eu,H_2O,sat}$ is the solubility of eucalyptol in water (i.e., 0.021 mol/L) and **p** is the fraction of the eucalyptol molecules in the second sorbed layer which are not able to undergo further association with additional sorbed eucalyptol. Since the highest value of eucalyptol concentration employed in measuring the isotherm (i.e., 0.012 mol/L) corresponds to a mole fraction of $2.2 \cdot 10^{-4}$ in water, eucalyptol is at "infinite dilution" in the mobile phase and $\gamma^{ID}_{Eu,m} = 1$ at all concentrations employed[8]. Thus, any deviation from linearity in curve A can be ascribed to processes occurring in the stationary phase.

The fit of equation 4.23 to the data is good, with $R^2 = 0.9998$, $K_1 = 60 \pm 5$ L/kg, $K_2 = 4.3 \pm 0.3$ L/kg, $C_{Eu,s,1,max} = 0.51 \pm 0.02$ mol/kg and $p = 0.098 \pm 0.003$. The value of 0.51 mol/kg for $C_{Eu,s,1,max}$ is comparable in magnitude to the value of 0.9 mol/kg which is predicted for a close-packed monolayer of eucalyptol molecules, based on an area of $4.5 \cdot 10^{-19}$ m^2 occupied by each approximately spherical eucalyptol molecule, as measured from a space-filling molecular model, and a specific surface area of $3 \cdot 10^5$ m^2/kg for ODS-3[16].

Theoretically, the parameter **p** in equation 4.23 can have values from 0 to 1. The measured value of 0.10 is much closer to 0 than to 1, indicating a high degree of

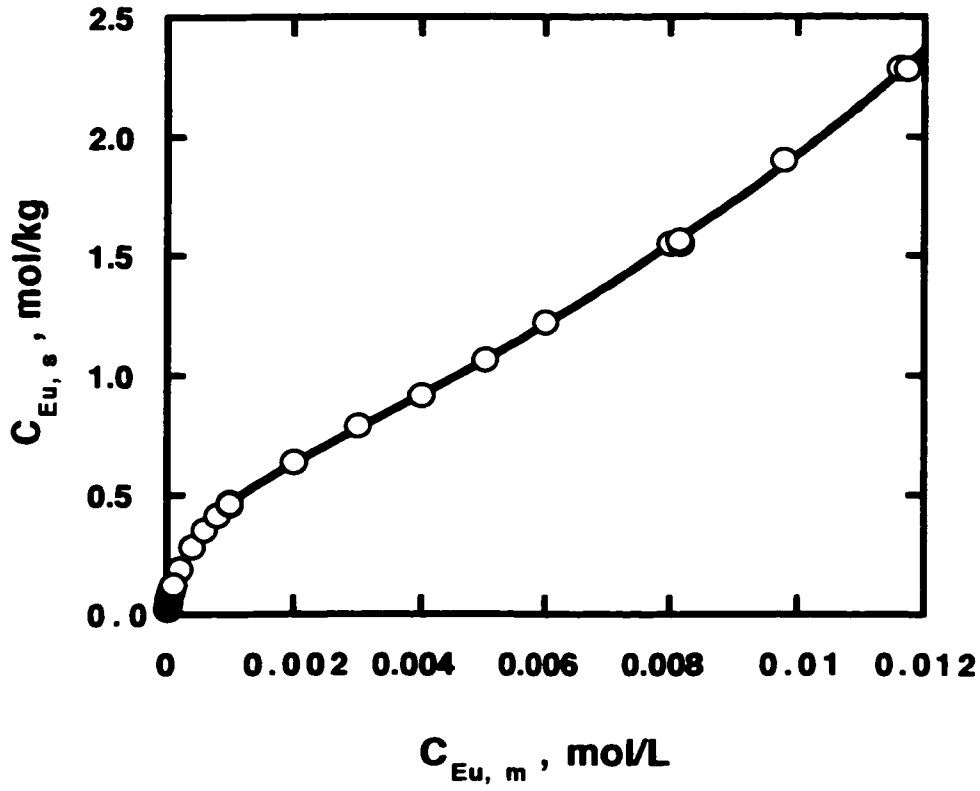

Figure 4.6. Eucalyptol isotherm. Points are experimental, the line is a non-linear least-squares fit of equation 4.23 to the data points.

association among sorbed eucalyptol molecules. This suggests that the relatively nonpolar eucalyptol molecule interacts in the stationary phase mainly by dispersion forces[12]. Because of this extensive association, the sorbed concentration of eucalyptol becomes considerably greater than $C_{Eu,s,1,max}$. For example, at $C_{Eu,m}$ = 0.0115 mol/L the stationary phase is composed of about 74% w/w of eucalyptol and 26% w/w of C_{18} chains.

4.3.4. Effect of Eucalyptol on 1-Butanol Sorption.

Shown in Figure 4.7 is a plot of $C_{BuOH,s}$ *versus* $C_{Eu,s}$ for the case in which $C_{BuOH,m}$ was held constant at the trace concentration of $1.0_0 \cdot 10^{-3}$ mol/L (*i.e.* $\phi_{Eu,m}$ = $9.2 \cdot 10^{-5}$) and $C_{Eu,m}$ was varied. In making this plot $\gamma^{T}_{BuOH,m}$ was assumed to be equal to 1.0_0 for all solutions because of the very low concentration of eucalyptol in the mobile phase (*i.e.*, $\phi_{Eu,m}$ = <0.003). The line in Figure 4.7 represents a non-linear least squares fit of equation 4.14 to the data points. The fit is good, with R^2 = 0.9987, A_{BuOH} = 0.0135 ± 0.0005, α_{BuOH} = 80.6 ± 0.3 (from equation 4.15), β_{BuOH} = -3.61 ± 0.03 and S_{BuOH} = 1.40 ± 0.05. Substituting this value of S_{BuOH} into equation 4.16, along with the value $\bar{V}_{Eu}$ = 167.5 mL/mol[17] gives $C_{C_{18},s}\bar{V}_{C_{18}}$ = (2.3 ± 0.1)·10^2 mL of C_{18} per kg of dry ODS-3 packing.

The values of $\phi_{Eu,s}$ and $\phi_{BuOH,s}$ corresponding to those of $C_{Eu,s}$ and $C_{BuOH,s}$, respectively, can be calculated *via* equation 4.1 using $\bar{V}_{BuOH}$ = 91.5 mL/mol[17] and

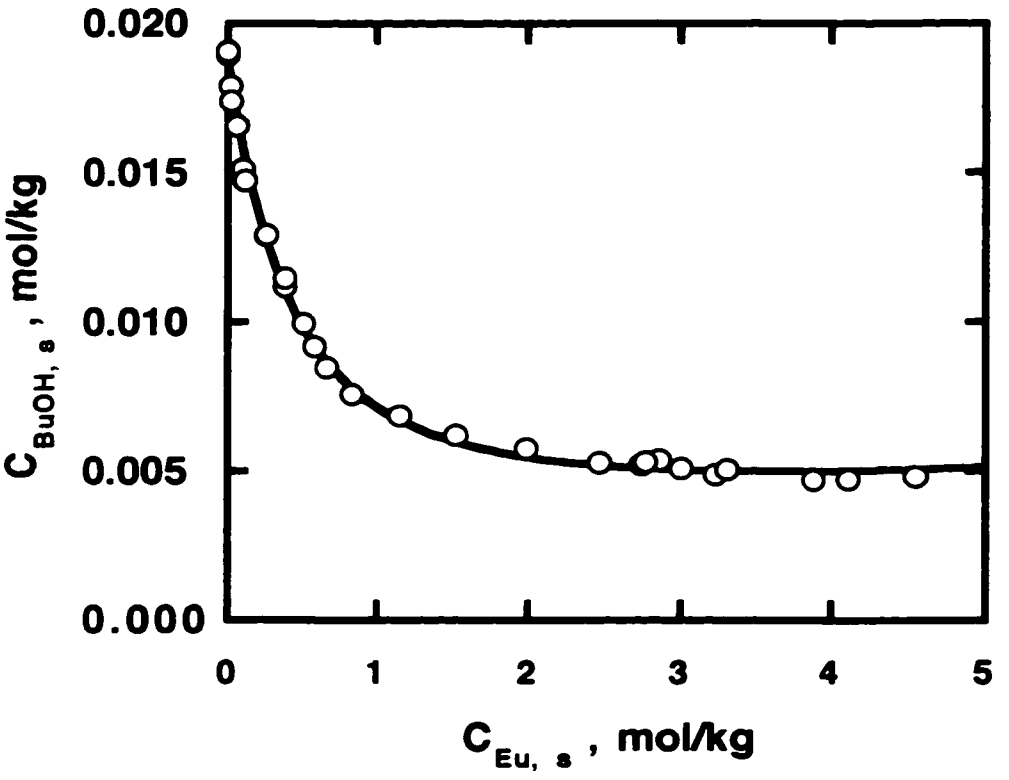

Figure 4.7. Sorbed concentration of 1-butanol versus sorbed concentration of eucalyptol from solutions in which 1-butanol concentration was kept constant at $1.00 \cdot 10^{-3}$ mol/L while eucalyptol concentrations were varied from 0 to 0.018 mol/L. The line is the fit to the data points by equation 4.14 with *i* = 1-butanol, *solv* = eucalyptol and $\gamma^{T}_{BuOH,m} = 1.0_0$.

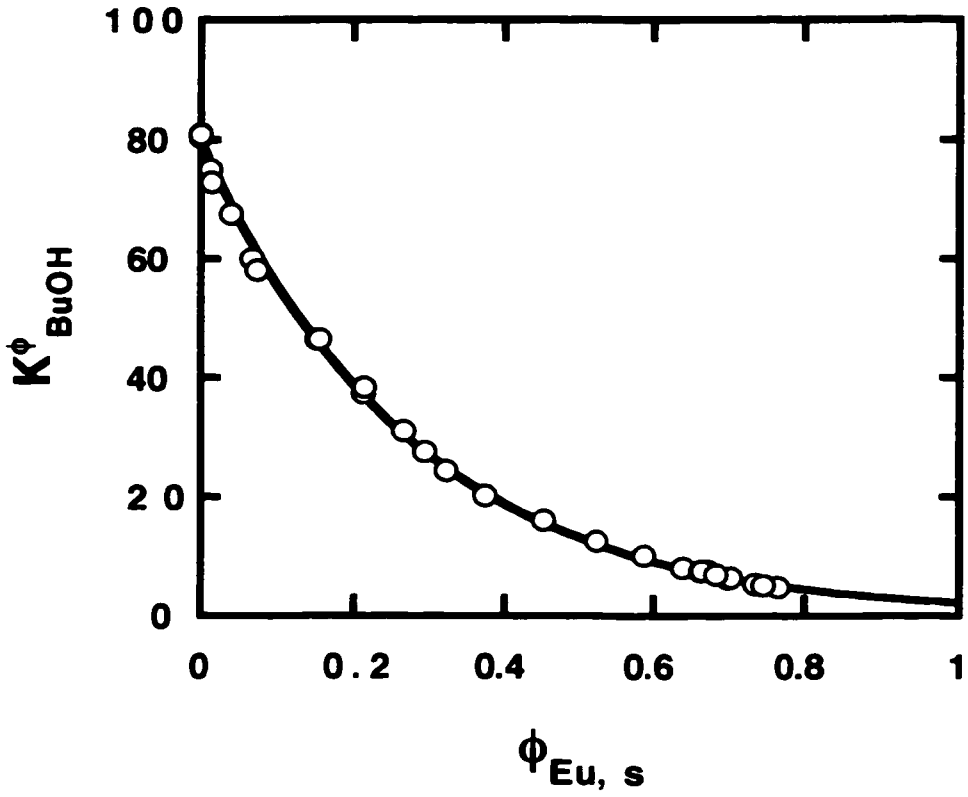

Figure 4.8. Replot of the data from Figure 4.7 according to equation 4.11. The points, which correspond to those in Figure 4.7, were calculated using equation 4.4 for K^{ϕ}_{BuOH}. The activity coefficient $\gamma^{T}_{BuOH,m}$ is equal to 1.0_0. The line was calculated from equation 4.11 using α_{BuOH} and β_{BuOH} from Figure 4.7. The value of $\phi_{Eu,s}$ is from equation 4.1 using $C_{C_{18}}\overline{V}_{C_{18}} = 2.3 \cdot 10^2$.

$\overline{V}_{Eu}$ = 167.5 mL/mol along with $C_{C_{18},s}\overline{V}_{C_{18}}$ = 2.3 · 10^2 mL/kg, from above. Then, K^{ϕ}_{BuOH} can be calculated from equation 4.11. Shown in Figure 4.8 is a plot of K^{ϕ}_{BuOH} *versus* $\phi_{Eu,s}$, in which $\gamma^{T}_{BuOH,m} = 1.0_0$ is assumed. The line in Figure 4.8 was calculated from equation 4.11 using the values of α_{BuOH} and β_{BuOH} which were obtained from the curve in Figure 4.7, as described above. This line is a good fit of the points (R^2 = 0.9988), showing that K^{ϕ}_{BuOH} decreases exponentially with increasing $\phi_{Eu,s}$, from a value $K^{\phi}_{BuOH} = \alpha_{BuOH}$ = 80.6 ± 0.3 for partition between C_{18} and water, to a value $K^{\phi}_{BuOH} = \alpha_{BuOH} \cdot \exp(\beta_{BuOH})$ = 2.16 ± 0.07 for partition between eucalyptol and water.

This exponential decrease of K^{ϕ}_{BuOH} with $\phi_{Eu,s}$ is consistent with the view that the C_{18} chains and eucalyptol form an ideal solution and that the interaction forces among C_{18}, eucalyptol and 1-butanol are dispersive in nature, as discussed in Section 4.2.2. For 1-butanol, this implies that its hydroxyl group is located near the interface between the C_{18}/eucalyptol stationary phase and the aqueous phase, and that only the butyl chain is "dissolved" in the C_{18}/eucalyptol phase. Thus, it would be expected that the K^{ϕ}_{BuOH} values measured in this system would not be equal to those for partitioning of BuOH between water and either bulk liquid Eu or bulk liquid octadecane.

4.3.5. Effect of 1-Butanol on Eucalyptol Sorption.

Shown in Figure 4.9 is a plot of $C_{Eu,s}$ *versus* $C_{BuOH,s}$ for the case in which $C_{Eu,m}$ was held constant at the trace concentration of $1.0_0 \cdot 10^{-5}$ mol/L (*i.e.*, $\phi_{Eu,m} = 1.68 \cdot 10^{-6}$)

and $C_{BuOH,m}$ was varied. The highest concentration of 1-butanol in the mobile phase is 0.300 mol/L which corresponds to $\phi_{BuOH,m} = 0.0275$. Since $\gamma^{T}_{Eu,m}$ cannot, *a priori*, be assumed to be equal to 1.0_0 over this range of mobile phase BuOH concentrations, cloud point experiments were performed for eucalyptol in pure water and in 0.300 mol/L 1-butanol in water. From cloud point experiments we found that solubility of eucalyptol in 0.300 mol/L 1-butanol solution is the same as its solubility in water. Thus, $\gamma^{T}_{Eu,m} = 1.0_0$ for $C_{BuOH,m} = 0.300$ mol/L. Since this was the highest concentration of 1-butanol in the mobile phase, $\gamma^{T}_{Eu,m} = 1.0_0$ for all the solutions in the Figure 4.10.

The line in Figure 4.9 which represents a nonlinear least squares fitting of equation 4.14 to the data points, gives $R^2 = 0.9981$, $A_{Eu} = 0.0094 \pm 0.0008$, $\alpha_{Eu} = (1.01 \pm 0.09)\cdot 10^4$, $\beta_{Eu} = -6.0 \pm 0.3$, and $S_{Eu} = 2.3 \pm 0.2$. From S_{Eu} and $\overline{V}_{BuOH} = 91.5$ mL/mol the value $C_{C_{18},s}\overline{V}_{C_{18}} = (2.1 \pm 0.2)\cdot 10^2$ mL C_{18}/kg is obtained. The agreement between this last value and the value $(2.3 \pm 0.1)\cdot 10^2$ mL C_{18}/kg reported above from the measurement of the effect of eucalyptol on the sorption of 1-butanol, constitutes strong support for the validity of equations 4.11 and 4.14.

Presented in Figure 4.10 is a plot of K^{ϕ}_{Eu} *versus* $\phi_{BuOH,s}$. The line in Figure 4.10 was calculated from equation 4.11 using the values of α_{Eu} and β_{Eu} which were obtained from Figure 4.9. The fit in Figure 4.10 is good ($R^2 = 0.9987$), with K^{ϕ}_{Eu} decreasing exponentially from $(1.01 \pm 0.09)\cdot 10^4$ for partition between C_{18} and water, to 26 ± 6 for partition between 1-butanol and water. Comparison of these two values reveals that C_{18}

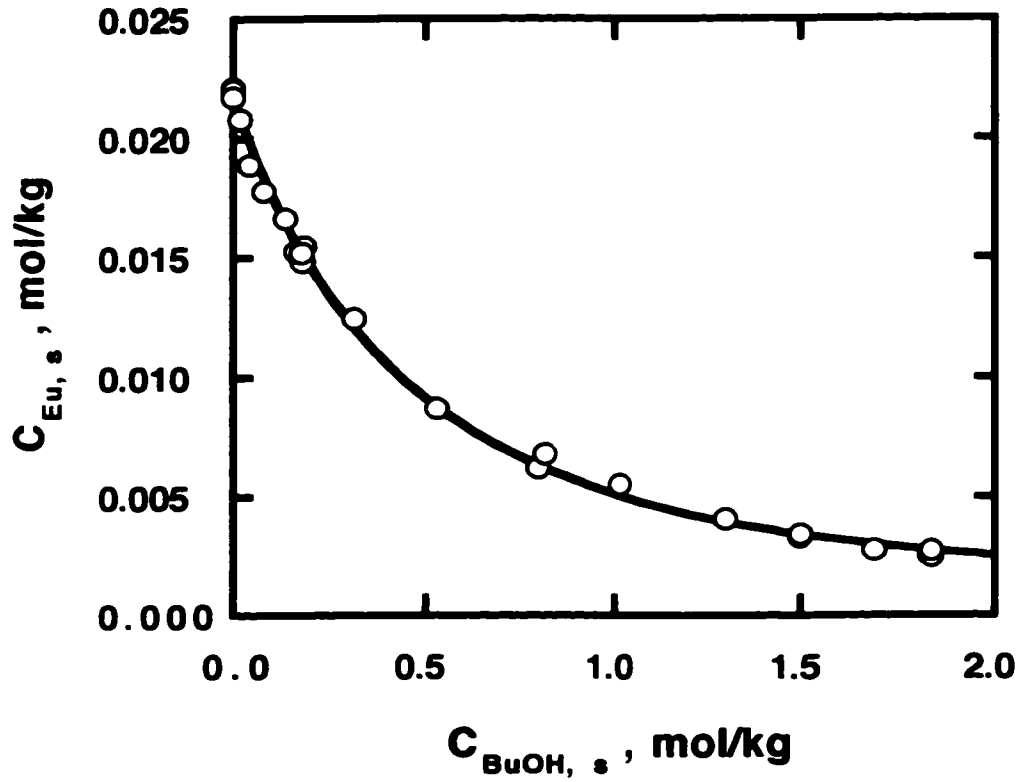

Figure 4.9. Sorbed concentration of eucalyptol versus sorbed concentration of 1-butanol from solutions in which eucalyptol concentration was kept constant at $1 \cdot 10^{-5}$ mol/L while 1-butanol concentrations were varied from 0 to 0.3 mol/L. The line is the fit to the data points by equation 4.14 with i = eucalyptol, solv = 1-butanol, and $\gamma^{T}_{Eu,m} = 1.0_0$.

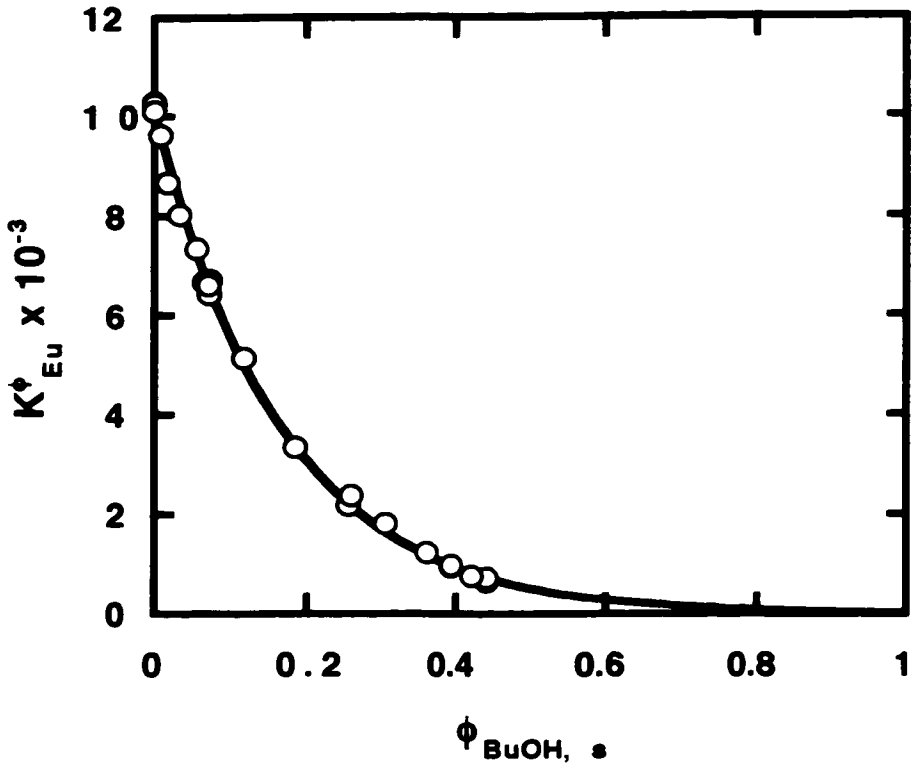

Figure 4.10. Replot of the data from Figure 4.9 according to equation 4.11. The points, which correspond to those in Figure 4.9, were calculated using equation 4.4 for K^{ϕ}_{Eu}. The activity coefficient $\gamma^{T}_{Eu,m}$ is equal to 1.0_0. The line was calculated from equation 4.11 using α_{Eu} and β_{Eu} from Figure 4.9. The value of $\phi_{BuOH,s}$ is from equation 4.1 using $C_{C_{18}}\overline{V}_{C_{18}} = 2.3 \cdot 10^2$.

is a very much stronger solvent for eucalyptol than 1-butanol is. The exponential decrease of K^{ϕ}_{Eu} with increasing $\phi_{BuOH,s}$ is further evidence that the C_{18} chains, the eucalyptol and the hydrocarbon tail of 1-butanol form an ideal solution.

Comparison of $\alpha_{BuOH} = 80$ with $\alpha_{Eu} = 1.0 \cdot 10^4$ for partition into pure C_{18} shows that the distribution of eucalyptol from water into C_{18} is more favored than the distribution of 1-butanol from water into C_{18}. Also, comparison of $\alpha_{BuOH} \cdot \exp(\beta_{BuOH}) = 2.16$ with $\alpha_{Eu} \cdot \exp(\beta_{Eu}) = 26$ for partition into *solvent* alone reveals that the distribution of eucalyptol into 1-butanol from water is more favored than the distribution of 1-butanol into eucalyptol from water. In all of these comparisons, the caveat regarding the fundamental difference between partitioning into a solvent-modified bonded-stationary phase, and into a bulk solvent, must be born in mind. For instance, here the distribution of eucalyptol into 1-butanol refers to the dissolving of eucalyptol in the butyl chains, not in bulk-liquid 1-butanol.

4.4. Sorption Model

The sorbed concentration of 1-butanol as solvent, shown in Figures 4.10 and 4.11, extends from trace levels up to about 60% of the monolayer concentration, $C_{BuOH,s,max}$. The 1-butanol-modified C_{18} stationary phase exhibits ideal solution behavior over this entire sorbed concentration range. This suggests that only the butyl group from 1-butanol enters the ODS phase where it serves merely to dilute the C_{18} chains and to be diluted by them. For eucalyptol as solvent, the sorbed concentration shown in Figures 4.8 and 4.9 extends from trace levels up to the equivalent of about nine "monolayers". In view of the

fact that the eucalyptol-modified C_{18} stationary phase also exhibits the properties of an ideal solution, it would appear that over the whole sorbed concentration range, the sorbed eucalyptol solvent is dissolved in the C_{18} chains where it, too, serves merely to dilute them and to be diluted by them. The use of the term "monolayer" is therefore inappropriate for eucalyptol solvent in this case, since there is no difference in the character of the sorption process at low and at high eucalyptol. This interpretation is consistent with the fit of equation 4.23 to the eucalyptol isotherm, with extensive "association" (*i.e.*, **p** small in equation 4.23).

The average value for $\mathbf{C}_{C_{18},s}\overline{\mathbf{V}}_{C_{18}}$ obtained from the two simultaneous sorption experiments (Figures 4.8 and 4.10) is $(2.2 \pm 0.3)\cdot 10^2$ mL/kg. Dividing this by 0.49 mol C_{18}/kg, for the 10.5% w/w carbon loading of the ODS-3 sorbent, yields an experimental value of $(4.5 \pm 0.6)\cdot 10^2$ mL/mol for $\overline{\mathbf{V}}_{C_{18}}$, the molar volume of bound C_{18}. This number is only about 40% higher than the literature value of 328 mL/mol for the molar volume of liquid *n*-octadecane[18]. A somewhat higher molar volume for bound C_{18} than for the pure liquid is reasonable because the bound phase is expected to have a density that is somewhat less than that of the pure liquid[19].

It is worth noting here that in surface chemistry a molecule which is present at the interface between a nonpolar bulk liquid and an aqueous solvent, with its tail group in the organic phase and its head group in the aqueous phase, is traditionally said to be "adsorbed" at the liquid-liquid interface[20]. Thus, a more traditional way to describe the sorption of 1-butanol between the ODS phase and the mobile phase is to say that it is adsorbed at their interface. On the other hand, eucalyptol which appears to be fully embedded within the C_{18} chains, might traditionally be said to be "partitioned" into a

bulk phase, though even with partitioning, it is necessary to recognize that the small thickness of the bonded phase (about 20 Å) can lead to competition for space among sorbed species that do not exhibit strong associative interactions[4].

The effects of simultaneous sorption of 1-butanol and eucalyptol on one another, as seen in Figures 4.8, 4.9, 4.10 and 4.11 are due exclusively to processes taking place in the stationary phase, which is at equilibrium with a water-rich mobile phase containing the organic modifier solvent. This illustrates the fact that mobile phase eluent strength is not synonymous with mobile phase solvent strength. That is, a change in *eluent strength of the mobile phase* with composition can arise from a mobile-phase induced change in *solvent strength of the stationary phase* as well as from the well-known change in *solvent strength of the mobile phase*. In Chapter 5, below, is described the study that distinguishes the contributions of stationary phase strength changes from mobile phase strength changes over a wide range of $\mathbf{C_{solv,m}}$, where the organic modifier (i.e., solv) is 1-propanol.

Practically speaking, water-rich mobile phases are not often used in RPLC because of the long sample retention times involved when conventional organic modifiers such as methanol and acetonitrile are used. Recently, however, there has been a growth of interest in using water-rich mobile phases. The problem of long retention has been reduced by using packings with very low phase ratios[21], by using elevated temperature[22,23] or by adding surfactants[24]. An alternative approach is illustrated by the present work in which low concentrations of a strongly sorbed, non-UV absorbing component like eucalyptol has been used as an organic modifier solvent.

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5. Influence of 1-Propanol on the Sorption of 1-Hexanol*

5.1. Introduction

In the experiments discussed in this Chapter 1-hexanol is a sample component and 1-propanol is an organic modifier solvent in the mobile phase. The experiments are designed to measure both the mobile phase contribution and the stationary phase contribution of organic modifier to the retention of 1-hexanol. The stationary phase contribution is brought about by sorption of 1-propanol into the stationary phase. The mobile phase contribution to changes in retention of 1-hexanol arises from changes in solvent strength in the mobile phase only; i.e. if no sorption of 1-propanol occurred into the stationary phase. The mobile phase influence of 1-propanol on 1-hexanol was measured by determining activity coefficients of 1-hexanol in mixtures of 1-propanol and water from solubility measurements by the cloud point method. The stationary phase influence was measured by studying simultaneous sorption of 1-hexanol and 1-propanol in the stationary phase using the column equilibration method. The relationship between the mobile and stationary phase contributions and the overall effect that 1-propanol organic modifier has on the sorption of 1-hexanol are discussed in detail in Section 5.2 of this Chapter.

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5.1.1. Simultaneous Sorption Studies

Phenomena related to the simultaneous sorption of two species on a C_{18} bonded phase have been the subject of previous studies in this research group[1,2,3]. Several different effects were discovered. In the simultaneous sorption of bipolar analytes, that possess a polar head and a hydrophobic tail and are sorbed at the interface between mobile phase and stationary phase (references 4 and 5, and see section 5.1.2 below), competition for space occurs[1]. In the simultaneous sorption of 1-butanol and naphthalene sulfonate the competition for space was linear and mutually reciprocal[1], and in the simultaneous sorption of 1-butanol and tetra-*n*-butylammonium ion (TBA)[2] the former exhibited a linear competition for space with the latter. That is, the amount of sample sorbed from a mobile phase, in which its concentration was kept constant, decreased linearly with the increase in sorption of the organic modifier. The absolute mobile phase concentration of the organic modifier was always kept low, so that the mobile phase solvent strength was constant and the effect observed was due only to the changes in the stationary phase.

In the study of simultaneous sorption of two cations, the nearly spherical shaped tetra-*n*-butylammonium (TBA) and the oval shaped surface-active (4-nitrobenzyl) trimethylammonium (NBTA)[3], it was found that, aside from electrostatic repulsion, sorbed TBA competed with NBTA for space, which decreased the sorption of NBTA, and caused the unfolding of collapsed bonded alkyl chains, which created more space in the stationary phase for NBTA and thus increased its sorption, depending on the ionic strength of the mobile phase. The credibility of the chain-unfolding effect of sorbed TBA ion was supported in a study of the effect of sorbed TBA on the sorption of 1-butanol.

Sorbed TBA changed the sorption of 1-butanol by competing for space, creating more space for the sorption of 1-butanol by opening alkyl chains, and changing the sorption strength of the stationary phase by changing the total contact area between the C_4 group of 1-butanol molecule and the surrounding C_{18} chains[2].

A different effect from competition for space can occur when the sample and the organic modifier sorb in different sorption planes within the stationary phase[6]. This was seen in Chapter 4, above. When 1-butanol and eucalyptol are sorbed simultaneously, they influence the distribution coefficient of one another by decreasing the sorption strength of the stationary phase by virtue of diluting it. The effect is similar to bulk partitioning, and an exponential dependence of the distribution coefficient of one compound on the volume fraction of the other one in the stationary phase is observed.

5.1.2. Behavior of Alcohols Sorbed at Interfaces

In a recent study of simultaneous sorption of phenylethanol and phenylpropanol from 1:1 v/v water/methanol solution onto C_{18} bonded phases it was discovered that competitive sorption models that took into account the interactions of these compounds in the stationary phase improved the fit to the experimental data[7]. In a study of chromatographic retention of steroids on a C_{18} bonded phase from 35% acetonitrile and 65% water v/v mobile phase, strong electrostatic and hydrogen bond interactions of the sorbed analytes in the stationary phase were shown to be responsible for co-elution of a number of steroids that had hydroxyl and carbonyl groups[8].

Sorption of alcohols at the air-liquid, liquid-liquid and mercury-liquid solution interfaces has been extensively studied. Sorption of long chain alcohols at alkane-water

interfaces was shown to obey the Shoefield-Rideal equation (a two-dimensional non-ideal gas equation):

$$\pi(A - A_0) = ikT \qquad (5.1)$$

where π is the two-dimensional interfacial pressure (N/m); A is the interfacial area (m^2); A_0 is the area occupied per molecule at the interface (m^2); k is Boltzmann constant ($1.381 \cdot 10^{-23}$ J/K); T is temperature (K); and i is an interaction parameter that is equal to 1 if there are no interactions, >1 for repulsive interactions and <1 for attractive interactions[9,10]. In these studies equation 5.1 with $i = 1$ was found to describe the experimental data, suggesting that no interactions were occurring between the adsorbed molecules. Equation 5.1 with $i = 1$ leads directly to a Langmuir sorption isotherm for the adsorbed alcohols. Both of these studies also compared fitting of experimental data to the Shofield-Rideal equation with the fits based on solution models of the interfacial layer. No improvement was found by using solution models.

In Langmuir-Blodgett studies of the sorption of C_8 - C_{18} *n*-alkyl alcohols at benzene-water[11] and oil-water[12] interfaces an inflection point is observed on the curves of interfacial pressure *versus* area. This signifies the occurrence of a phase transition within these alcoholic films with an onset at about $40 \cdot 10^{-20}$ m^2 per molecule, and therefore confirms the existence of intermolecular attraction at high surface coverages.

The nonlinear relationship between the interfacial pressure π and the concentration of alcohol in an aqueous solution which was observed for *n*-hexanol, *n*-heptanol and *n*-octanol in a study of alcohol sorption at air-solution interfaces was explained by intermolecular interactions between the sorbed alcohols[13]. In a different study the relative sorption of *n*-octyl and *n*-hexyl alcohols at the aqueous solution-air

interface was calculated based on measurements of the surface tension[14]. Sorption isotherms which take into account the interaction between the adsorbed molecules describe the experimental results considerably better than the isotherm equation based on the concept of the "ideal surface solution"[14].

Interactions were also observed in films of *n*-alkanols at the mercury/aqueous solution interfaces[15,16,17]. For the sorption of 1-butanol[15,16] and other lower alcohols[16,17] a Frumkin isotherm was used:

$$\mathbf{K_{ads}C_{i,m}} = \frac{\theta_i}{1-\theta_i}\mathbf{exp(-2a\theta_i)} \qquad (5.2)$$

where $\mathbf{K_{ads}}$ is the sorption equilibrium constant (L/mol); θ_i is the fractional surface coverage compared to a complete monolayer; and **a** accounts for the interactions within the sorbed monolayer; **a** >0 implies attractive interactions between alcohol molecules. It was observed that **a** is positive for all the alcohols studied and increases with the increase of the alcohol chain length.

The above studies suggest that alcohols sorbed at the interfaces might[7,8,11,12,13,14,15,16,17] or might not[9,10] experience intermolecular interactions.

5.2. Theoretical model

In this section, first the mobile and the stationary phase contributions of the 1-propanol organic modifier to the sorption of 1-hexanol sample are discussed. The mobile phase contribution is treated in terms of transfer activity coefficient (Chapter 4, Section 4.2.2). The stationary phase contribution is modeled in terms of three processes: (i) competition for space; (ii) decrease of space required per mole of alcohol in the stationary

phase with the increase in its sorbed concentration; (iii) change of free-energy of sorption with increasing concentration of sorbed 1-propanol.

The latter part of the Section deals with 1-hexanol and 1-propanol sorption isotherms. The isotherms are derived on the basis of the same theoretical approach as the one used to treat the stationary phase effect of 1-propanol on the sorption of 1-hexanol.

5.2.1. Mobile Phase Contribution to the Influence of 1-Propanol on the Sorption of 1-Hexanol

The influence of the mobile phase strength on retention of 1-hexanol is accounted for by the change in the transfer activity coefficient (Sections 2.2.6 and 4.2.2):

$$\mu^{o}_{HexOH,m} = \mu^{o}_{HexOH,H_2O} + RT\ln \gamma^{T}_{HexOH,m} \qquad (5.3)$$

where $\mu^{o}_{HexOH,m}$ and μ^{o}_{HexOH,H_2O} are the standard chemical potentials of 1-hexanol at infinite dilution either in a mixed solvent (*m*, in this case a mixture of 1-propanol and water) or in water, respectively, and $\gamma^{T}_{HexOH,m}$ is the transfer activity coefficient for the transfer of 1-hexanol from infinite dilution in water to infinite dilution in the water/solvent mixture. By this definition

$$\gamma^{T}_{HexOH,m} = \frac{\gamma^{PS}_{HexOH,m}}{\gamma^{PS}_{HexOH,H_2O}} \qquad (5.4)$$

where $\gamma^{PS}_{HexOH,m}$ and γ^{PS}_{HexOH,H_2O} are the pure solute standard state activity coefficients of 1-hexanol in the mobile phase and in water, respectively, at infinite dilution. If the mole-fraction solubility of a solute in a solvent mixture, $X_{i,m}$, is ≤ 0.01, solute activity

coefficient with respect to pure solute standard state can be approximated as the inverse of the mole-fraction solubility[18]. For 1-hexanol in a mixture of 1-propanol and water

$$\gamma^{PS}_{HexOH,m} = \frac{1}{X_{HexOH,m}} \tag{5.5}$$

and in water

$$\gamma^{PS}_{HexOH,H_2O} = \frac{1}{X_{HexOH,H_2O}} \tag{5.6}$$

The combination of equations 5.4, 5.5 and 5.6 yields

$$\gamma^{T}_{i,HexOH} = \frac{X_{HexOH,H_2O}}{X_{HexOH,m}} \tag{5.7}$$

In the study of the influence of 1-propanol on the sorption of 1-hexanol the concentration of 1-hexanol both in water and in the propanol/water mobile phase was kept constant at $3.00 \cdot 10^{-4}$ mol/L (i.e. $X_{i,m} = 5.40 \cdot 10^{-5}$). It can therefore be assumed that 1-hexanol is at infinite dilution, so that its activity, with respect to an extrapolated infinite dilution standard state in water, is equal to its concentration (i.e. $\gamma^{ID}_{HexOH,H_2O} = \gamma^{ID}_{HexOH,m} = 1.0_0$). Therefore, in 1-propanol/water mixtures 1-hexanol activity is

$$a^{ID}_{HexOH,m} = C_{HexOH,m}\gamma^{ID}_{HexOH,m}\gamma^{T}_{HexOH,m} \approx C_{HexOH,m}\gamma^{T}_{HexOH,m} \tag{5.8}$$

The distribution coefficient between the stationary and mobile phases for 1-hexanol is

$$K_{HexOH} = \frac{C_{HexOH,s}}{a^{ID}_{HexOH,m}} \approx \frac{C_{HexOH,s}}{C_{HexOH,m}\gamma^{T}_{HexOH,m}}, \quad L/kg \tag{5.9}$$

where K_{HexOH} is the 1-hexanol distribution coefficient and $C_{HexOH,s}$ is the experimentally determined moles of 1-hexanol sorbed in the stationary phase per kilogram of dry packing. A decrease in $\gamma^{T}_{HexOH,m}$ corresponds to a decrease in the sorbed

concentration of 1-hexanol and, in terms of the mobile phase contribution to chromatographic retention, to an increase in solvent strength of the mobile phase.

5.2.2. Stationary Phase Contribution to the Influence of 1-Propanol on the Sorption of 1-Hexanol

Since both 1-propanol and 1-hexanol are sorbed at the interface between the C_{18} bonded chains and the mobile phase, it is expected that competition for space will occur[1,2]. It is therefore convenient to express the sorbed concentration of 1-hexanol in the stationary phase in the units of moles sorbed per square meter of packing $C_{HexOH,s}/A_s$ where A_s is the *available* area per kg of dry packing, in m^2/kg. A new distribution coefficient for 1-hexanol $K_{D,HexOH}$ can be defined in these units:

$$K_{D,HexOH} = \frac{K_{HexOH}}{A_{sp}} = \frac{C_{HexOH,s}}{C_{HexOH,m}\gamma^{T}_{HexOH}A_s} \qquad (5.10)$$

When 1-propanol is sorbed, the amount of space in the stationary phase available for the sorption of 1-hexanol A_s is less than the specific surface area of packing A_{sp}, in m^2/kg, by the amount of space taken up by sorbed 1-propanol:

$$A_s = A_{sp} - \overline{A}_{PrOH,s}C_{PrOH,s} \qquad (5.11)$$

where $\overline{A}_{PrOH,s}$ is the area in the stationary phase occupied per mol of sorbed 1-propanol, in m^2/mol, and $C_{PrOH,s}$ is the concentration of sorbed 1-propanol in the stationary phase in mol/kg of dry packing. In this treatment it is important that the area occupied by sorbed 1-hexanol itself is small, i.e. $\overline{A}_{HexOH,s}C_{HexOH,s} << A_{sp}$. Substituting the expression for A_s from equation 5.11 into equation 5.10 yields:

$$\frac{C_{HexOH,s}}{\gamma^{T}_{HexOH,m}} = K_{D,HexOH} C_{HexOH,m} A_{sp} - K_{D,HexOH} C_{HexOH,m} \overline{A}_{PrOH,s} C_{PrOH,s} \qquad (5.12)$$

If both $K_{D,HexOH}$ and $\overline{A}_{PrOH,s}$ are constants, independent of $C_{PrOH,s}$, then equation 5.12 predicts a linear decrease of $C_{HexOH,s} / \gamma^{T}_{HexOH,m}$ for increasing $C_{PrOH,s}$ and constant $C_{HexOH,m}$.

Both the space occupied by a sorbed 1-propanol molecule and the free-energy of sorption of 1-hexanol may be perturbed by the presence of sorbed 1-propanol. As previously discussed[2], these two effects can most simply be incorporated by assuming that both the area occupied per mol and the free energy of sorption per mole change linearly with $C_{PrOH,s}$.

For the space effect:

$$\overline{A}_{PrOH,s} = \overline{A}^{o}_{PrOH,s} + k_{2,PrOH} \overline{A}^{o}_{PrOH,s} C_{PrOH,s} =$$

$$\overline{A}^{o}_{PrOH,s} (1 + k_{2,PrOH} C_{PrOH,s}) \qquad (5.13)$$

where $\overline{A}^{o}_{PrOH,s}$ is the area occupied by a mole of sorbed 1-propanol in the absence of perturbation by sorbed 1-propanol, and $k_{2,PrOH}$ is the fractional change in $\overline{A}^{o}_{PrOH,s}$ per mole of 1-propanol sorbed.

For the free energy effect:

$$\Delta G^{PrOH}_{HexOH} = \Delta G^{o}_{HexOH} - k^{PrOH}_{1,HexOH} C_{PrOH,s} \qquad (5.14)$$

where ΔG^{o}_{HexOH} is the free energy for the sorption of 1-hexanol into C_{18} in the absence of sorbed 1-propanol; $k^{PrOH}_{1,HexOH}$ is the free-energy difference for the sorption of 1-hexanol per unit concentration of sorbed 1-propanol; and ΔG^{PrOH}_{HexOH} is the free energy for the

sorption of 1-hexanol in the presence of sorbed 1-propanol. From equation 5.14 the value for the distribution coefficient is:

$$K_{D,HexOH} = K_{D,HexOH,o} \cdot \exp\left(\frac{k_{1,HexOH}^{PrOH} C_{PrOH,s}}{RT}\right) \tag{5.15}$$

where $K_{D,HexOH,o}$ is for the absence of perturbation and $K_{D,HexOH}$ is in the presence of perturbation caused by the sorbed 1-propanol.

Substituting from equations 5.13 and 5.15 into equation 5.12 yields the following relationship between $C_{HexOH,s} / \gamma_{HexOH,m}^{T}$ and $C_{PrOH,s}$:

$$\frac{C_{HexOH,s}}{\gamma_{HexOH,m}^{T}} = K_{D,HexOH,o} \cdot \exp\left(\frac{k_{1,HexOH}^{PrOH} C_{PrOH,s}}{RT}\right) C_{HexOH,m} A_{sp} - K_{D,HexOH} \cdot$$

$$\cdot \exp\left(\frac{k_{1,HexOH}^{PrOH} C_{PrOH,s}}{RT}\right) C_{HexOH,m} \bar{A}_{PrOH,s}^{o} \left(1 + k_{2,PrOH} C_{PrOH,s}\right) C_{PrOH,s} \tag{5.16}$$

Equation 5.16 can be rearranged to give an expression for the distribution coefficient K_{HexOH} in L/kg:

$$K_{HexOH} \equiv \frac{C_{HexOH,s}}{C_{HexOH,m}} = K_{D,HexOH,o} A_{sp} \gamma_{HexOH,m}^{T} \cdot \exp\left(\frac{k_{1,HexOH}^{PrOH} C_{PrOH,s}}{RT}\right) \cdot$$

$$\left(1 - \frac{\bar{A}_{PrOH,s}^{o}}{A_{sp}} C_{PrOH,s} - \frac{k_{2,PrOH} \bar{A}_{PrOH,s}^{o}}{A_{sp}} C_{PrOH,s}^{2}\right) \tag{5.17}$$

Equation 5.17 serves to define both the stationary phase effect, i.e. $\exp\left(\frac{k_{1,HexOH}^{PrOH} C_{PrOH,s}}{RT}\right) \cdot \left(1 - \frac{\bar{A}_{PrOH,s}^{o}}{A_{sp}} C_{PrOH,s} - \frac{k_{2,PrOH} \bar{A}_{PrOH,s}^{o}}{A_{sp}} C_{PrOH,s}^{2}\right)$, and the mobile phase effect, i.e. $\gamma_{HexOH,m}^{T}$, of the 1-propanol organic modifier on the sorption of

1-hexanol sample. The distribution coefficient K_{HexOH} is directly proportional to the product of the mobile and stationary phase effects. In the absence of 1-propanol, K_{HexOH} is equal to $K_{D,HexOH,o}A_{sp}$.

5.2.3. Sorption Isotherms of 1-Hexanol and 1-Propanol

The distribution coefficient for an alcohol ROH between the stationary phase and the mobile phase is:

$$K_{ROH} = \frac{C_{ROH,s}}{a^{ID}_{ROH,m}} \quad , \quad L/kg \tag{5.18}$$

where $C_{ROH,s}$ is sorbed concentration in mol ROH per kg of dry packing and $a^{ID}_{ROH,m}$ is the activity of ROH in the mobile phase with reference to an extrapolated infinite dilution standard state for ROH in water. The use of $a^{ID}_{ROH,m}$ in place of concentration in the mobile phase, $C_{ROH,m}$ (mol/L), accounts for the fact that the solvent strength of the mobile phase varies with the concentration of ROH. The relationship for activity is:

$$a^{ID}_{ROH,m} = C_{ROH,m}\gamma^{ID}_{ROH,m} \tag{5.19}$$

where $\gamma^{ID}_{ROH,m}$ is the activity coefficient for ROH in ROH/H_2O solvent.

Analogously to equation 5.10, the sorption equilibrium constant is expressed as:

$$K_{D,ROH} \equiv \frac{C_{ROH,s}}{a^{ID}_{ROH,m}A_s} = \frac{K_{ROH}}{A_s} \quad , \quad L/m^2 \tag{5.20}$$

As before, the unoccupied space is related to the specific surface area of sorbent A_{sp} by the expression:

$$A_s = A_{sp} - \overline{A}_{ROH,s}C_{ROH,s} \tag{5.21}$$

where $\bar{A}_{ROH,s}$ is the space occupied per mole of sorbed ROH. Substituting for A_s from equation 5.21 into equation 5.20 and solving for $C_{ROH,s}$ gives:

$$C_{ROH,s} = \frac{K_{D,ROH} A_{sp} a^{ID}_{ROH,m}}{1 + K_{D,ROH} \bar{A}_{ROH,s} a^{ID}_{ROH,m}} \tag{5.22}$$

If both $K_{D,ROH}$ and $\bar{A}_{ROH,s}$ are constants, independent of $C_{ROH,s}$, then equation 5.22 represents a Langmuir sorption isotherm. This is the case for ROH = HexOH in the present study, but not for ROH = PrOH.

If the space occupied by a sorbed ROH molecule and the free energy of sorption change due to perturbations caused by sorbed ROH then, as in equations 5.13 and 5.15:

$$\bar{A}_{ROH,s} = \bar{A}^{o}_{ROH,s}\left(1 + k_{2,ROH} C_{ROH,s}\right) \tag{5.23}$$

$$K_{D,ROH} = K_{D,ROH,o} \cdot \exp\left(\frac{k_{1,ROH} C_{ROH,s}}{RT}\right) \tag{5.24}$$

Substitution from equations 5.23 and 5.24 into equation 5.22 yields the following expression for the sorption isotherm:

$$C_{ROH,s} = \frac{K_{D,ROH,o} \cdot \exp\left(\frac{k_{1,ROH} C_{ROH,s}}{RT}\right) \cdot A_{sp} a^{ID}_{ROH,m}}{1 + K_{D,ROH,o} \cdot \exp\left(\frac{k_{1,ROH} C_{ROH,s}}{RT}\right) \cdot \bar{A}^{o}_{ROH,s} a^{ID}_{ROH,m}\left(1 + k_{2,ROH} C_{ROH,s}\right)} \tag{5.25}$$

5.3. Results and Discussion

Three types of experiments were performed: (i) Individual sorption isotherms for 1-propanol and 1-hexanol have been measured between the Partisil-10 ODS3 packing and aqueous solution; (ii) measurements of the influence of 1-propanol on retention of 1-

hexanol by simultaneous sorption of 1-hexanol and 1-propanol from aqueous solutions on Partisil-10 ODS3 packing, with 1-hexanol present at constant low mobile phase concentration of $3.00 \cdot 10^{-4}$ mol/L and 1-propanol mobile phase concentration varied from 0-30% volumetric; and (iii) measurement of 1-hexanol solubility in water and water/1-propanol mixtures by the cloud point technique.

5.3.1. 1-Hexanol Isotherm

The1-hexanol isotherm was measured over the mobile phase composition range of $1 \cdot 10^{-4}$-$2 \cdot 10^{-3}$ mol/L because this range was of interest for selecting 1-hexanol mobile phase concentration for the study of the influence of 1-propanol on the sorption of 1-hexanol. At such low concentrations of 1-hexanol in the mobile phase we expect the activity coefficient of 1-hexanol $\gamma^{ID}_{HexOH,m}$ to be constant at 1.0_0. Therefore, the isotherm is plotted as concentration of 1-hexanol in the stationary phase, $C_{HexOH,s}$, *versus* the mobile phase concentration, $C_{HexOH,m}$. Figure 5.1 shows the fit of Langmuir isotherm equation (equation 5.22)

$$C_{HexOH,s} = \frac{K_{D,HexOH} A_{sp} C_{HexOH,m}}{1 + K_{D,HexOH} \overline{A}_{HexOH,s} C_{HexOH,m}} \qquad (5.26)$$

to the experimental data. Combining the two fitting parameters $K_{D,HexOH} A_{sp}$ and $K_{D,HexOH} \overline{A}_{HexOH,s}$ with the literature value of $A_{sp} = 3.0 \cdot 10^5$ m^2/kg[1,2,6] gives $K_{D,HexOH}$ = $(9.4 \pm 0.2) \cdot 10^{-4}$ L/mol and $\overline{A}_{HexOH,s} = (3.1 \pm 0.2) \cdot 10^5$ m^2/mol. Dividing $\overline{A}_{HexOH,s}$ by Avogadro's number gives $(51 \pm 3) \cdot 10^{-20}$ m^2/molecule (i.e. 51 $Å^2$/molecule) as the area occupied per molecule of 1-hexanol. This value is higher than 16 $Å^2$/molecule of 1-

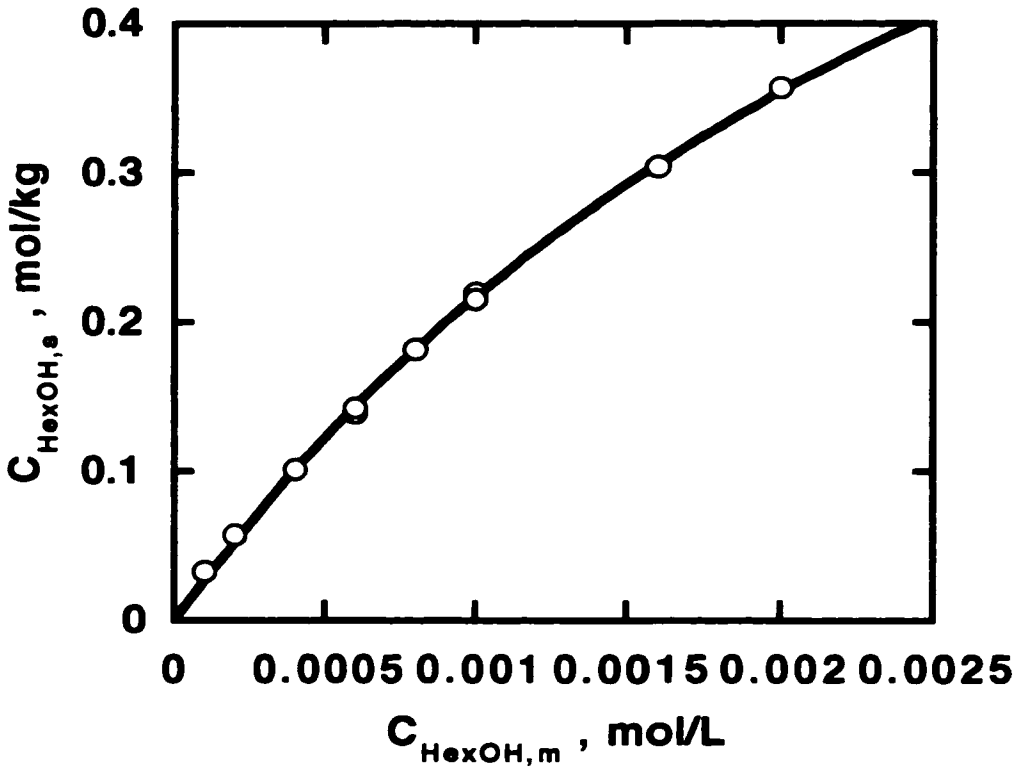

Figure 5.1. 1-Hexanol sorption isotherm on Partisil-10 ODS-3 stationary phase from aqueous mobile phase. Points are experimental and the line is from nonlinear least squares fitting with the Langmuir equation (equation 5.26). $R^2 = 0.9994$

butanol determined from the 1-butanol isotherm described in Chapter 4.

The highest experimental point in Figure 5.1 corresponds to about 35% of monolayer coverage. At this point there is evidently no effect of sorbed 1-hexanol on the values of $K_{D,HexOH}$ or $\bar{A}_{HexOH,s}$ (i.e. $k_{1,HexOH} = k_{2,HexOH} = 0$ in equation 5.25).

Based on the behavior shown in Figure 5.1 it was decided to employ the mobile phase concentration $C_{HexOH,m} = 3.00 \cdot 10^{-4}$ mol/L in studies of the effect of 1-propanol organic modifier on 1-hexanol sorption. This concentration is in the rectilinear region of 1-hexanol isotherm. The curvature of the isotherm is not serious at that point, and the concentration is high enough to provide good precision of the measurement of sorbed amount of 1-hexanol.

5.3.2. Holdup Volume

The holdup volume at various concentrations of 1-propanol in solution was measured as described in Section 3.4.6. The results are presented in Table 5.1 and Figure 5.2. It is instructive to compare ΔV_{HU} with $V_{PrOH,s}$, where ΔV_{HU} is the difference between the holdup volume when no 1-propanol was added to the mobile phase V^{o}_{HU} and the holdup volume $V_{HU,PrOH}$ in the presence of various mobile phase concentrations of 1-propanol:

$$\Delta V_{HU} = V^{o}_{HU} - V_{HU,PrOH} \qquad (5.27)$$

and $V_{PrOH,s}$ is the volume of sorbed 1-propanol that was obtained as follows from the 1-propanol isotherm measurement:

$$V_{PrOH,s} = \bar{V}_{PrOH} C_{PrOH,s} w \qquad (5.28)$$

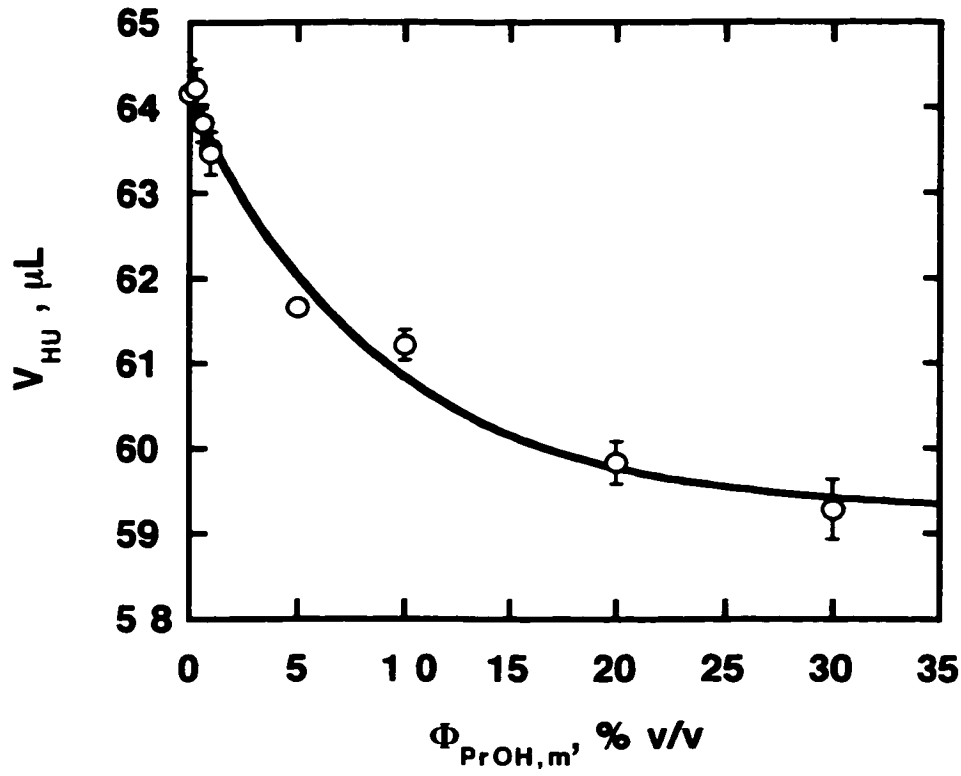

Figure 5.2. Plot of precolumn holdup volume, V_{HU}, *versus* volume percent of 1-propanol in the mobile phase, $\Phi_{PrOH,m}$, on $3.078 \cdot 10^{-5}$ kg of Partisil-10 ODS-3 in the precolumn.

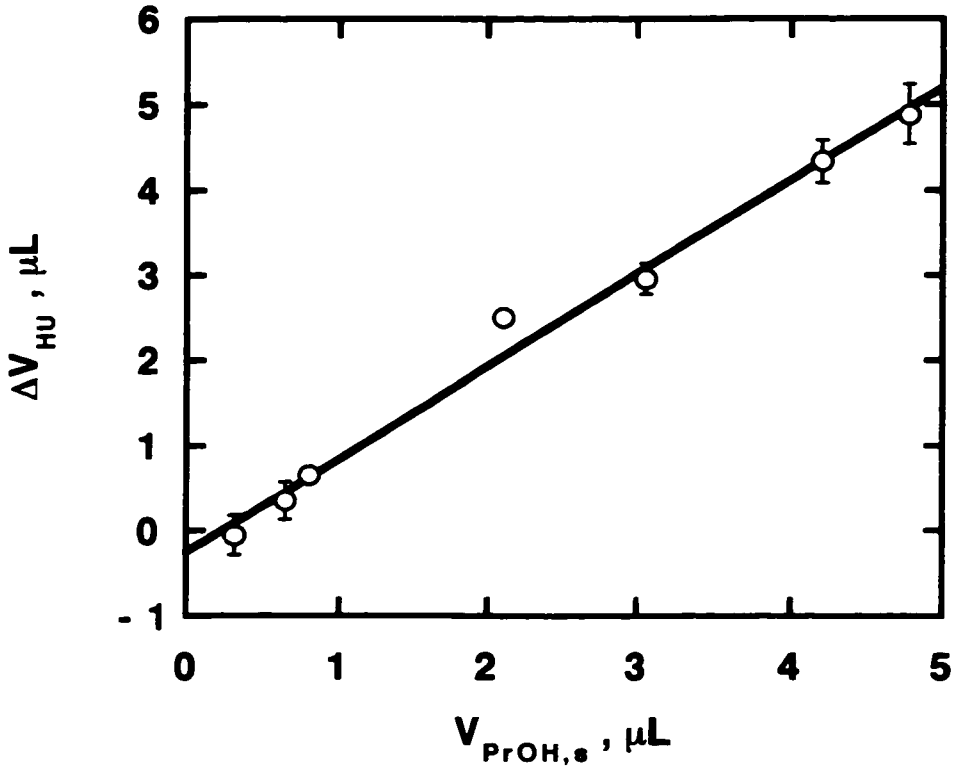

Figure 5.3. Holdup volume change (ΔV_{HU}) from equation 5.27 *versus* volume of 1-propanol sorbed ($V_{PrOH,s}$) from equation 5.28, on $3.078 \cdot 10^{-5}$ kg of Partisil-10 ODS-3 in the precolumn. Points are experimental with error bars of ± one standard deviation and the line is for linear regression with $R^2 = 0.989$.

Here $\overline{V}_{PrOH}$ is the molar volume of 1-propanol at 25°C in mL/mol[19] and **w** is the weight of packing in the precolumn in kg.

A correlation plot of ΔV_{HU} *versus* $V_{PrOH,s}$ is presented in Figure 5.3. There is a good correlation between ΔV_{HU} and $V_{PrOH,s}$ with $R^2 = 0.989$. The slope of 1.09 ± 0.05 is equal to 1.00 within two standard deviations, and the intercept of -0.25 ± 0.14 is equal to 0 within two standard deviations. The results agree with the data of McCormick and Karger[20] and are interpreted to mean that the partial molar volume of sorbed 1-propanol is equal to its molar volume as obtained from the density of pure liquid 1-propanol. This finding lends credibility to the use of equation 3.2 to iteratively calculate the holdup volume in the presence of organic modifier in Chapter 4. It also demonstrates that the entire 1-propanol molecule contributes to the decrease in void volume, not just the propyl chain.

5.3.3. 1-Propanol Isotherm

This was measured over the mobile phase concentration range of 0.1-30% v/v of 1-propanol because this was the concentration range used in the study of the influence of 1-propanol on the sorption of 1-hexanol. Activity of 1-propanol at these concentrations is not equal to concentration. Pure solute standard state activity coefficients for 1-propanol, $\gamma^{PS}_{PrOH,m}$, were calculated from vapor-liquid equilibrium data[21] using the Van Laar equation[22]. These activity coefficients were converted to extrapolated infinite dilution standard state in pure water activity coefficients, $\gamma^{ID}_{PrOH,m}$, using equation 4.19. The

activity of 1-propanol, $a^{ID}_{PrOH,m}$, was calculated as a product of molar concentration $C_{PrOH,m}$ and $\gamma^{ID}_{PrOH,m}$.

The data for the 1-propanol isotherm are presented in Table 5.1 and the plot in Figure 5.4. The fit by equation 5.25 is excellent with $R^2 = 0.9998$. Combining the fitting parameters with A_{sp} gives: $K_{D,PrOH,o}=(1.64 \pm 0.05) \cdot 10^{-5}$ L/m^2, $\bar{A}^{o}_{PrOH,s} = (1.87 \pm 0.08) \cdot 10^5$ m^2/mol, $k_{2,PrOH} = -0.32 \pm 0.06$ m$^2 \cdot$ kg/mol^2, and $k_{1,PrOH} = (-9.2 \pm 0.7) \cdot 10^2$ J$\cdot$ kg/mol^2. All four of these parameters are statistically significant.

The Langmuir component of the isotherm, obtained by putting the values of A_{sp}, $K_{D,PrOH}$ (= $K_{D,PrOH,o}$) and $\bar{A}_{PrOH,s}$ (= $\bar{A}^{o}_{PrOH,s}$) into equation 5.22, is shown as the dotted line in Figure 5.4. In the Langmuir monolayer the area occupied per 1-propanol molecule would be $(31 \pm 1) \cdot 10^{-20}$ m^2/molecule (i.e. 31 Å^2/molecule). This value lies between the areas occupied per molecule of sorbed 1-butanol (16 Å^2/molecule) and 1-hexanol (51 Å^2/molecule).

A negative value of $k_{1,PrOH}$ contributes to lower values of $C_{PrOH,s}$ as compared to values predicted by the Langmuir equation. A negative value of $k_{2,PrOH}$ opposes this trend, increasing $C_{PrOH,s}$ above the concentrations based upon Langmuir equation. At activities of 1-propanol in solution below 0.5 mol/L, the calculated Langmuir contribution lays above the fit of equation 5.25 to the data points. Therefore, at this concentration range the "free-energy" term overweighs the "area" term. At $a^{ID}_{PrOH,m} > 0.5$ mol/L there is an upward deviation of 1-propanol isotherm from the Langmuir component, indicating that the "area" term, represented by $k_{2,PrOH}$, overweighs the "free-energy" term, represented by $k_{1,PrOH}$.

Table 5.1. Data for 1-propanol isotherm on Partisil-10 ODS-3.

$\Phi_{Pr\,OH,m}$ %	$C_{Pr\,OH,m}$ ' mol/L	$\gamma^{PS}_{Pr\,OH,m}$	$\gamma^{ID}_{Pr\,OH,}$	$a^{ID}_{Pr\,OH,m}$ mol/L	$C_{Pr\,OH,s}$ ' mol/kg
0.200	0.02662	15.87	0.994	0.0265	0.118
0.200	0.02662	15.87	0.994	0.0265	0.114
0.300	0.03993	15.83	0.992	0.0396	0.161
0.300	0.03993	15.83	0.992	0.0396	0.161
0.400	0.05324	15.78	0.989	0.0527	0.214
0.400	0.05324	15.78	0.989	0.0527	0.219
0.500	0.06656	15.74	0.986	0.0656	0.264
0.500	0.06656	15.74	0.986	0.0656	0.258
0.600	0.07987	15.69	0.983	0.0785	0.290
0.600	0.07987	15.69	0.983	0.0785	0.295
0.700	0.09318	15.65	0.981	0.0914	0.328
0.700	0.09318	15.65	0.981	0.0914	0.329
0.800	0.1065	15.61	0.978	0.104	0.358
0.800	0.1065	15.61	0.978	0.104	0.360
1.00	0.1331	15.52	0.972	0.129	0.426
1.00	0.1331	15.52	0.972	0.129	0.422
1.00	0.1331	15.52	0.972	0.129	0.421
1.00	0.1331	15.52	0.972	0.129	0.419
1.00	0.1331	15.52	0.972	0.129	0.421
1.00	0.1331	15.52	0.972	0.129	0.419
5.00	0.6656	13.88	0.869	0.578	1.07
5.00	0.6656	13.88	0.869	0.578	1.06
10.0	1.331	12.02	0.753	1.00	1.45
10.0	1.331	12.02	0.753	1.00	1.49
20.0	2.662	8.98	0.563	1.50	1.92
20.0	2.662	8.98	0.563	1.50	1.93
30.0	3.993	6.64	0.416	1.66	2.11
30.0	3.993	6.64	0.416	1.66	2.12

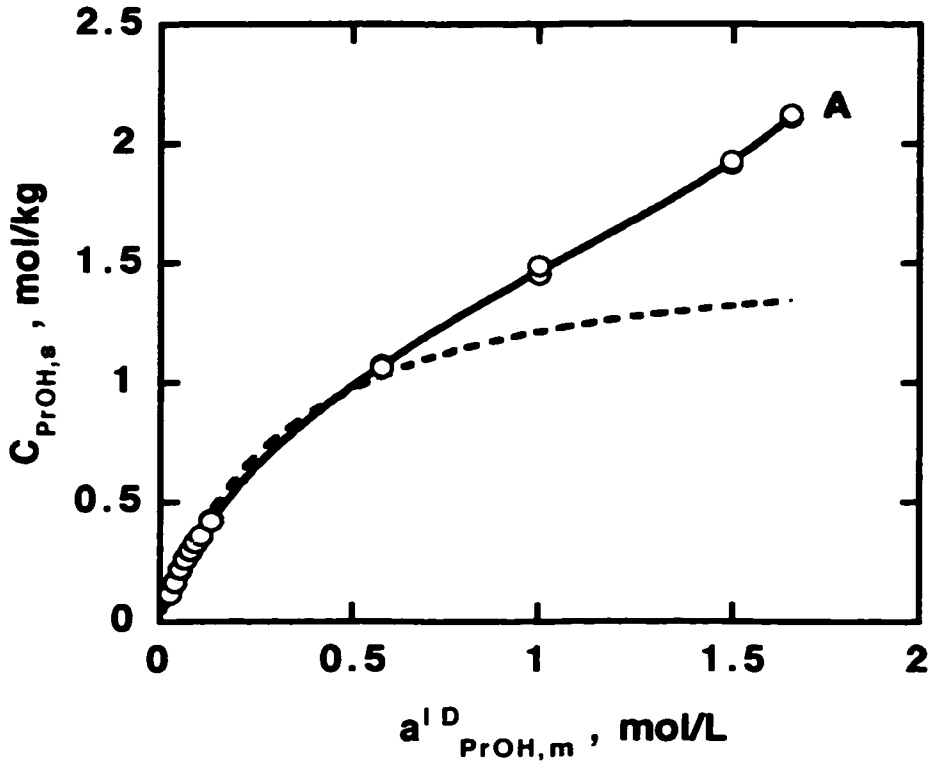

Figure 5.4. 1-Propanol sorption isotherm on Partisil-10 ODS-3 stationary phase from aqueous mobile phase. Points in curve A are experimental and the solid line is from nonlinear least squares fitting with equation 5.25. $\mathbf{R^2}$ = 0.9999. Dotted line is the Langmuir component where $\mathbf{k_{1,PrOH}} = \mathbf{k_{2,PrOH}} = 0$.

Sorption studies of a variety of alcohols that exhibit a deviation from Langmuir behavior show that in some cases the deviation can be explained by modifying the Langmuir equation with only an exponential free-energy term to give an isotherm based on the Frumkin, Fowler or "regular localized monolayer" model[7,15]. In other cases it is necessary also to include a term that takes into account a changing area occupied per sorbed molecule[9,10,12]. In the present case, this "area" term dominates the "free-energy" term. Concerning the physical significance of the observed behavior, it appears that 1-propanol causes a reorganization of the C_{18} chains, which leads to a reorientation of sorbed 1-propanol molecules. Alternatively, it may be that sorbed 1-propanol molecules experience attractive lateral interactions among themselves. The fact that the free-energy changes favor a decrease in the distribution coefficient, as reflected by $\exp\left(\frac{k_{1,PrOH} C_{PrOH,s}}{RT}\right)$, makes the latter explanation unlikely. This decrease of the distribution coefficient could be due to smaller contact of sorbed 1-propanol molecules with C_{18} chains[2].

5.3.4. Influence of 1-Propanol on the Sorption of 1-Hexanol

We have determined the solubility of 1-hexanol in aqueous solutions of 1-propanol at 25°C by the shake-flask and by the cloud point methods.

The results of shake-flask determination of 1-hexanol solubility in water are plotted in Figure 5.5 as the amount of 1-hexanol dissolved, $n_{HexOH,dissolv}$, *versus* the amount of 1-hexanol added to the flask, $n_{HexOH,added}$. The solutions corresponding to the three highest points in the plot had an undissolved excess of 1-hexanol, whereas the solution containing the three lowest amounts of 1-hexanol were completely clear. The

straight line in Figure 5.5 is the linear least-squares fit through the first three points. The fit is good with the $\mathbf{R^2} = 0.9989$, the slope 0.97 ± 0.03 and the intercept 0.06 ± 0.14. Since the slope is equal to one and the intercept is equal to zero within one standard deviation, the amount of 1-hexanol dissolved in the solutions is equal to the amount added. This is different from the results of shake-flask determination of eucalyptol solubility in water (Figure 4.1) and indicates that the 1-hexanol evaporation isn't significant in this case.

It is seen that the last three points form a plateau. The average value of $\mathbf{n_{HexOH,dissolv}}$ for these points is $(5.72 \pm 0.04)\cdot 10^{-3}$ mol. The amount of water in the solutions is 5.54 mol. Thus the mole fraction of 1-hexanol is $(1.03 \pm 0.01) \cdot 10^{-3}$. This value is the solubility of 1-hexanol in water at 25 °C as determined by the shake-flask method. It is within experimental error of the value of $1.02\cdot 10^{-3}$ (0.568% w/w) obtained by linear interpolation of the reported 1-hexanol solubility values at 20 and 30 °C[23].

As described in Chapter 3, for every composition of 1-propanol/water solution the cloud point temperature was measured at several different concentrations of 1-hexanol in the mixture. The solubility of 1-hexanol at 25 °C was then obtained by interpolation from the plots of cloud point temperature *versus* mole fraction of 1-hexanol in solution, $\mathbf{X_{HexOH,m}}$. Such plots for all the studied 1-propanol/water mixtures are found in the Appendix B as Figures B1-B10. In order to calculate the 1-hexanol transfer activity coefficient from equation 5.7 a value of 1-hexanol solubility in water is required. Since solubilities at 0, 5, 10 and 15% v/v of 1-propanol were within experimental error of one another, $\mathbf{X_{HexOH,H_2O}}$ was taken to be average of 1-hexanol solubilities at these concentrations of 1-propanol and is equal to $(1.04 \pm 0.05) \cdot 10^{-3}$. This value is within experimental error of 1-hexanol solubility in water determined by the shake-flask method

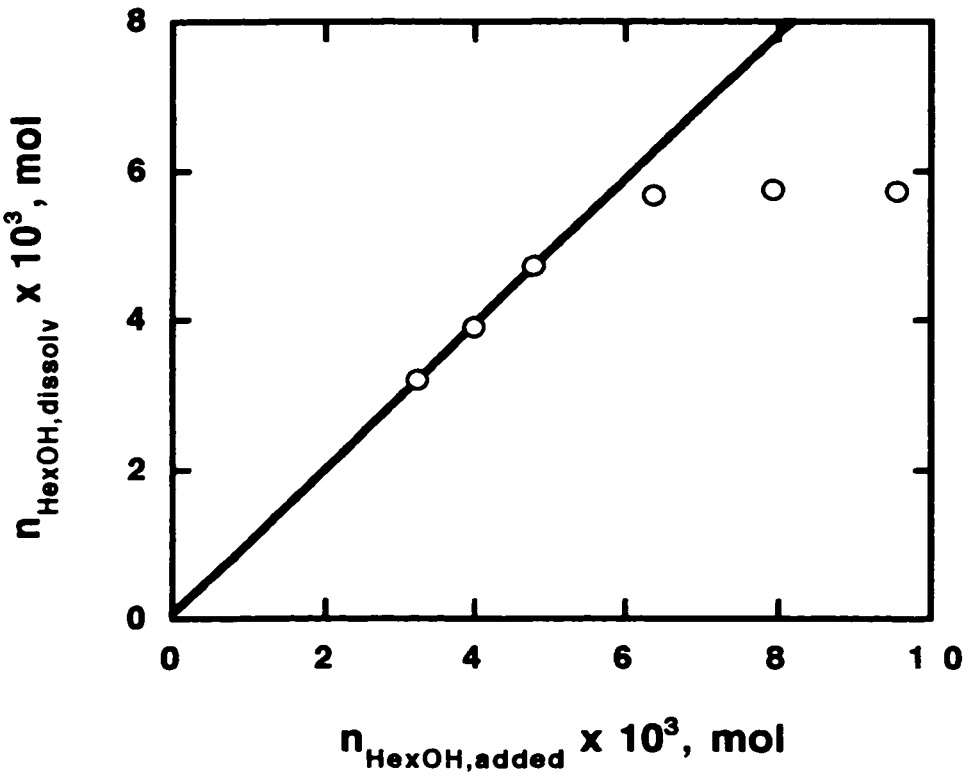

Figure 5.5. The results of shake-flask determination of 1-hexanol solubility in water. See details in text.

reported above.

In Figure 5.6 are plotted both 1-hexanol solubilities and 1-hexanol transfer activity coefficients *versus* true volumetric percent of 1-propanol in the mobile phase, $\Phi_{PrOH,m}$. The solubilities of 1-hexanol in the 1-propanol mixtures containing less than 15% 1-propanol v/v are the same as in water. In the mixtures containing greater than 15% v/v 1-propanol the solubility of 1-hexanol increases with increasing 1-propanol concentration. The general shape of the plot of 1-hexanol solubility *versus* volumetric percent of 1-propanol is similar to what has been reported for other systems[24,25].

In the absence of 1-propanol, the distribution coefficient $\mathbf{K_{HexOH}}$ is equal to $\mathbf{K_{D,HexOH}} \cdot \mathbf{A_{sp}}$, where $\mathbf{K_{D,HexOH}}$ is a constant for concentrations in the linear region of the 1-hexanol isotherm. Equation 5.17 shows that the change in $\mathbf{K_{HexOH}}$ caused by the presence of 1-propanol arises from both a **mobile phase effect** and a **stationary phase effect**. The mobile phase effect is expressed by the transfer activity coefficient $\gamma^{T}_{HexOH,m}$ obtained through solubility experiments in the mobile phase, in the absence of the ODS stationary phase. The stationary phase effect is then measured by sorption experiments. Non-linear least-squares fitting of equation 5.16 yields the solid line in Figure 5.7. The fit is good, with $\mathbf{R^2} = 0.9992$. Combining the fitting parameters with the constants $\mathbf{A_{sp}}$ and $\mathbf{C_{HexOH,m}}$ (=$3.00 \cdot 10^{-4}$ mol/L) yields the following: $\mathbf{K_{D,HexOH,o}} = (9.84 \pm 0.02) \cdot 10^{-4}$ L/m^2; $\mathbf{k_{2,PrOH}}$ = -0.28 ± 0.02 m^2·kg/mol^2, which agrees well with –0.32 ± 0.06 m^2·kg/mol^2 from the 1- propanol isotherm; and $\mathbf{k^{PrOH}_{1,HexOH}}$ = -0.002 ± 0.025, which is equal to zero, meaning that the exponential term should be omitted from equations 5.16 and 5.17. The value of $\overline{A}^{o}_{PrOH,s} = (2.96 \pm 0.07) \cdot 10^5$ m^2/mol (i.e. 49 Å^2/molecule) is higher

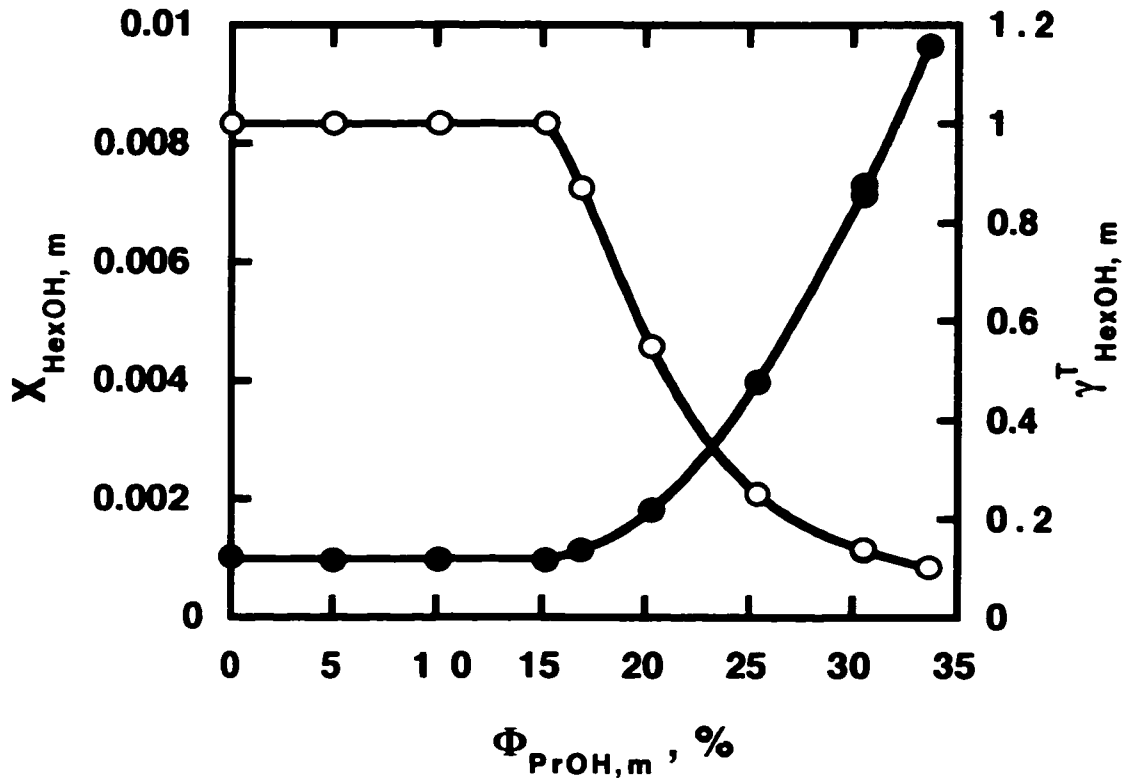

Figure 5.6. Plots of mole fraction solubility $X_{HexOH,m}$ (closed circles) and of transfer activity coefficient $\gamma^{T}_{HexOH,m}$ (open circles) of 1-hexanol *versus* volume percent of 1-propanol in the mobile phase.

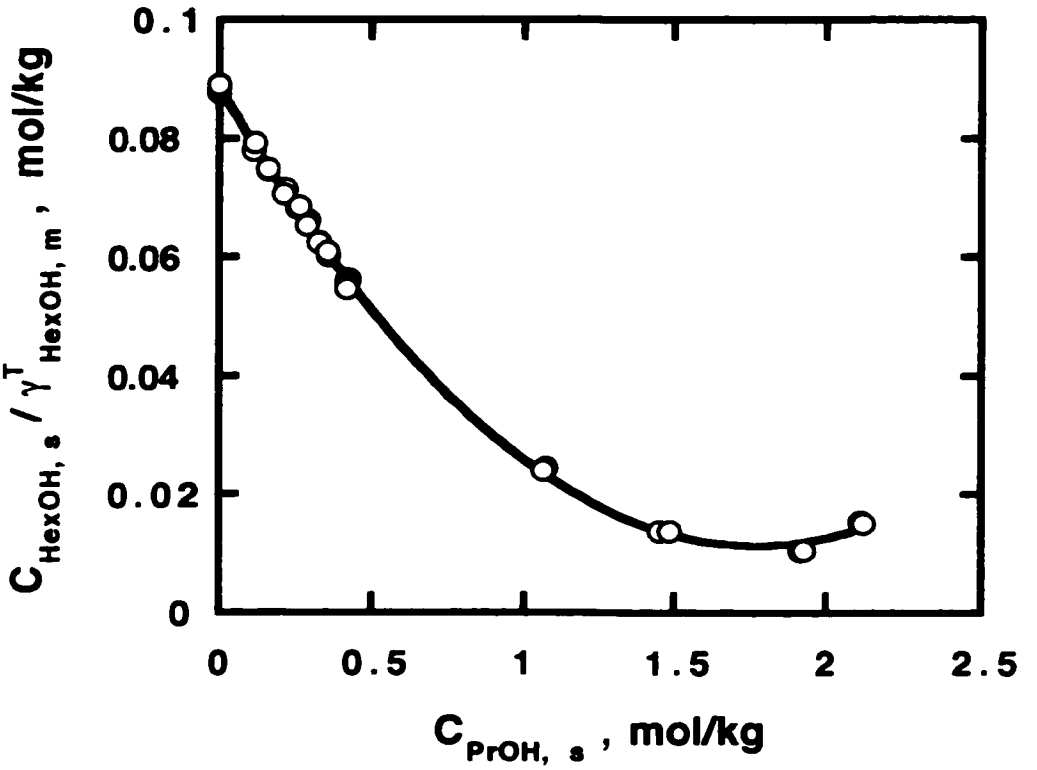

Figure 5.7. Plot of sorbed concentration of 1-hexanol, adjusted for mobile phase effect, ($C_{HexOH,s} / \gamma^{T}_{HexOH,m}$) *versus* sorbed concentration of 1-propanol ($C_{PrOH,s}$). Points are experimental and the line is from nonlinear least squares fitting of equation 5.16 to the data points. $R^2 = 0.9992$.

than the value of $\bar{A}^{o}_{Pr\,OH,s} = (1.87 \pm 0.08)\cdot 10^5$ m^2/mol (i.e. 31 Å^2/molecule) obtained from 1-propanol isotherm. However, 1-propanol isotherm includes one extra effect, namely the change in 1-propanol interaction with the stationary phase in the presence of sorbed 1-propanol. Although this effect is not important to describe the influence of 1-propanol on the sorption of 1-hexanol, it is important in 1-propanol isotherm, and contributes to a lower value of $\bar{A}^{o}_{Pr\,OH,s}$ obtained from 1-propanol isotherm.

Based on $k^{Pr\,OH}_{1,HexOH} = 0$ equation 5.16 simplifies to:

$$\frac{C_{HexOH,s}}{\gamma^{T}_{HexOH,m}} = K_{D,HexOH,o} C_{HexOH,m} A_{sp} -$$

$$K_{D,HexOH,o} C_{HexOH,m} \bar{A}^{o}_{Pr\,OH,s} (1 + k_{2,Pr\,OH} C_{Pr\,OH,s}) C_{Pr\,OH,s} \qquad (5.29)$$

The reason why nearly the same values of $k_{2,PrOH}$ and $\bar{A}^{o}_{Pr\,OH,s}$ are obtained from measurements of sorbed 1-hexanol in the present of 1-propanol and from measurements of sorbed 1-propanol in its isotherm is because $k_{2,PrOH}$ represents the change in space occupied by a sorbed 1-propanol molecule and because 1-hexanol is present at very low sorbed concentrations. The fraction of the total area per kg which is not occupied by sorbed alcohol molecules (A_s) is determined only by the amount of sorbed 1-propanol. The "extra" area which results from the decreasing value of $\bar{A}_{Pr\,OH,s}$ as $C_{PrOH,s}$ increases, is available equally for sorption of both 1-propanol and 1-hexanol molecules.

Furthermore, the fact that $k^{Pr\,OH}_{1,HexOH}$ is negligible means that 1-hexanol experiences a negligible contribution to its free-energy of sorption due to the presence of sorbed 1-propanol. Its enhanced sorption, seen as an upward deviation from a straight line in Figure 5.7 is due exclusively to the "extra" space made available by 1-propanol at

higher sorbed concentrations. Again, it favors the argument that the effect of high concentrations of 1-propanol on the stationary phase results more from reorganization of the C_{18} chains and reorientation of sorbed 1-propanol molecules than it does from lateral interactions among sorbed molecules, since the latter process might be expected to affect the free-energy of sorption of 1-hexanol as well as of 1-propanol.

Equation 5.17, with $k^{PrOH}_{1,HexOH} = 0$, shows explicitly how the stationary phase effect on K_{HexOH} depends on the sorbed concentration of 1-propanol and how it varies with $\gamma^{T}_{HexOH,m}$ in the mobile phase. For chromatographic practice it would be more instructive to see these effects plotted in terms of mobile phase concentration of 1-propanol, $\Phi_{PrOH,m}$. For the mobile phase effect, the relationship between $\gamma^{T}_{HexOH,m}$ and $\Phi_{PrOH,m}$, shown in Figure 5.5, is reproduced in Figure 5.8 as a dotted line. For the stationary phase effect, conversion of $C_{PrOH,s}$ to $\Phi_{PrOH,m}$ by the sequence $C_{PrOH,s} \rightarrow a^{ID}_{PrOH,m} \rightarrow C_{PrOH,m} \rightarrow \Phi_{PrOH,m}$, yields the dashed line. The open-circle points, through which is drawn the solid line, show the experimentally measured dependence of K_{HexOH} on $\Phi_{PrOH,m}$. The coincidence of the solid and the dashed line shows that up to about 15% v/v 1-propanol in the mobile phase, the effect of 1-propanol organic modifier on sorption of 1-hexanol is due completely to the presence of 1-propanol in the stationary phase. The effect is dramatic: by 15% 1-propanol the stationary phase effect has reduced K_{HexOH} to about 0.08 of its value in a pure aqueous mobile phase. Above about 15% 1-propanol both mobile and stationary phase effects contribute to the change in K_{HexOH}. At 30% 1-propanol the two effects contribute about equally (approximately 0.15) each, and

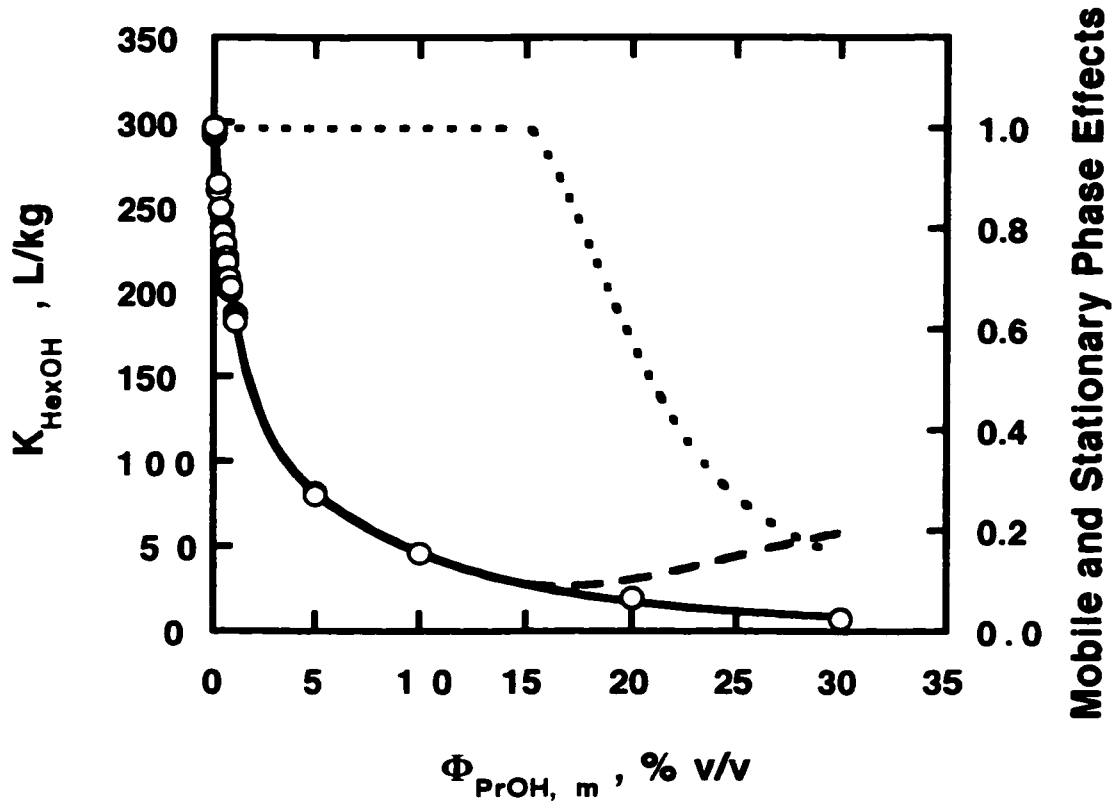

Figure 5.8. Mobile and stationary phase contributions of 1-propanol to the sorption of 1-hexanol on Partisil-10 ODS-3. Open circle experimental points and empirical-fit solid line show the dependence of K_{HexOH} on $\phi_{PrOH,m}$ (left-hand vertical axis). Dotted line shows the mobile phase effect and dashed line shows the stationary phase effect (right-hand vertical axis). The combined effect of 1-propanol on the sorption of 1-hexanol is the product of the mobile and stationary phase effects.

the observed value of $\mathbf{K_{HexOH}}$ has been reduced to about $(0.15)^2 = 0.02$ of its value in pure aqueous mobile phase.

In chromatography, both $\mathbf{K_{sample}}$ and the phase ratio of stationary to mobile phase in the column will affect sample retention volume. Since sorption of the organic modifier reduces the void volume, as seen in Figure 5.3, the resulting increase in phase ratio upon changing to higher $\Phi_{\mathbf{PrOH,m}}$ must also be taken into account when predicting the effect of $\Phi_{\mathbf{PrOH,m}}$ on retention volume.

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6. Summary and Future Work

6.1. Summary

In the two studies discussed in Chapters 4 and 5 it was demonstrated that sorbed organic modifier influences the sorption of sample compounds. Depending upon the nature of sample and organic modifier, the effect is different. In one case (case 1), the interactions of sample with the stationary phase decrease exponentially with the increase in the volume fraction of sorbed organic modifier (equation 4.11). This type of dependence was observed for eucalyptol and 1-butanol. One reason why such a dependence was observed could be that sorption of these compounds occurs in different sorption planes within the stationary phase: 1-butanol was sorbed at the C_{18}/mobile phase interface, whereas eucalyptol is believed to be fully embedded within the C_{18} chains. A simple exponential dependence of volume fraction of sorbed sample upon the volume fraction of sorbed modifier, i.e. the ideal solution behavior in the stationary phase, is caused by the relatively nonpolar nature of the chemical moieties that interpenetrate the bonded chains, i.e. the alkyl part of 1-butanol and the eucalyptol molecule.

In the second case (case 2) the decrease of sample sorption is caused by the competition for space in the stationary phase between the sample and the organic modifier. It is due to the sorption both of the sample, 1-hexanol, and the organic modifier, 1-propanol, at the same location within the stationary phase, i.e. at the interface of C_{18} and mobile phase.

6.2. Implications

The influence of the sorption of the organic modifier on resolution and selectivity might be expected to be different for case 1 and case 2. Selectivity $\alpha_{i/j}$ between two sample compounds *i* and *j* is defined as

$$\alpha_{j/i} = \frac{k'_j}{k'_i} \tag{6.1}$$

where k'_i and k'_j are the retention factors of *i* and *j*. Conventionally compound j is always the one that has higher k', so the $\alpha_{j/i}$ is always larger than 1. A retention factor is a product of the distribution coefficient K_i times the phase ratio of the stationary to the mobile phase for the column. For the same column, the phase ratio cancels out and the selectivity becomes the ratio of the respective distribution coefficients. Resolution R_s is a parameter used to characterize the disengagement between two chromatographic peaks for compounds *i* and *j*. It can be approximated as:

$$R_s = \frac{\sqrt{N}}{4}\left(\frac{\alpha_{j/i} - 1}{\alpha_{j/i}}\right)\left(\frac{k'_j}{1 + k'_j}\right) \tag{6.2}$$

where N is the number of theoretical plates of the column and defines the efficiency of the column. For the same column, resolution will be proportional to the product of two parenthetic terms in equation 6.2.

6.2.1. Influence of Sorbed Organic Modifier on Selectivity and Resolution. Case 1 (1-Butanol and Eucalyptol)

Dependence of the distribution coefficient on the amount of sorbed organic modifier for case 1 is obtained by dividing the left and right sides of equation 4.14 by $C_{i,m}$ and substituting K_i for $C_{i,s} / C_{i,m}$:

$$\frac{K_i}{\gamma_{i,m}^T} = \frac{A_i}{C_{i,m}} (C_{solv,s} + S_{solv}) \exp\left(\beta_i \frac{C_{solv,s}}{C_{solv,s} + S_{solv}}\right) \qquad (6.3)$$

According to equation 6.3, changes in the distribution coefficient of sample compound caused by the stationary phase sorption of organic modifier are determined by β_i, which depends on both the nature of the sample and the nature of the organic modifier (equation 4.16), and by S_{solv}. Parameter S_{solv} (equation 4.15) depends on the properties of stationary phase and organic modifier only, and is the same for all sample compounds chromatographed under the same mobile and stationary phase conditions. Using equation 6.3, it is possible to model the influence of organic modifier on the retention, selectivity and resolution of sample compounds. For this purpose, 1-butanol was chosen as an organic modifier, since the value of parameter S_{solv} for it was previously determined in Chapter 4. It is also important that small mobile phase concentrations of 1-butanol produce significant stationary phase concentrations, so that by using 1-butanol, it is possible to affect the properties of the stationary phase without changing the transfer activity coefficients of sample compounds in the mobile phase. Next, the influence of the difference in $A_i / C_{i,m}$ and β_i on the changes in retention, selectivity and resolution caused by sorbed 1-butanol will be discussed.

First, three hypothetical sample compounds with $A_1 / C_{1,m} = A_2 / C_{2,m} = A_3 / C_{3,m} = 2$; $\beta_1 = -2$, $\beta_2 = -3$ and $\beta_3 = -4$ are considered. Plots of predicted dependence of $K_i / \gamma^T_{i,m}$ on $C_{BuOH,s}$ are presented in Figure 6.1. The distribution coefficients of sample compounds in the absence of 1-butanol are the same. However, when 1-butanol is sorbed in the stationary phase, the distribution coefficients of the three sample compounds no longer are the same, and the differences between $K_i / \gamma^T_{i,m}$ increase with the increase of 1-butanol stationary phase concentration. At the same time, the absolute value of retention decreases for all sample compounds. A plot of selectivity and resolution for compounds 1 and 2 is presented in Figure 6.2. The selectivity increases with the increase of the amount of sorbed 1-butanol, from a value of 1 (no separation) in its absence to a value of 1.6 at $C_{BuOH,s} = 2$ mol/kg. The influence of an increase in $\alpha_{1/2}$ on resolution overweighs the influence of a decrease of distribution coefficients on resolution. The resolution $R_{s,1/2}$ increases with the increase of sorbed 1-butanol.

It is of interest to see how the sorption of 1-butanol influences the distribution coefficients of compounds that are already well resolved in its absence. Two cases are possible. In the first one, $A_1 / C_{1,m} > A_2 / C_{2,m} > A_3 / C_{3,m}$ and $-\beta_1 > -\beta_2 > -\beta_3$. A simulation of the dependence of $K_i / \gamma^T_{i,m}$ on $C_{BuOH,s}$ for this case is presented in Figure 6.3. It is observed that initially the difference in $K_i / \gamma^T_{i,m}$ decreases with the increase in $C_{BuOH,s}$ until a certain critical concentration of 1-butanol in the stationary phase at which $K_1 / \gamma^T_{1,m} = K_2 / \gamma^T_{2,m} = K_3 / \gamma^T_{3,m}$. After that, the difference in $K_i / \gamma^T_{i,m}$ increases with the increase of $C_{BuOH,s}$. Again, the plot of selectivity and

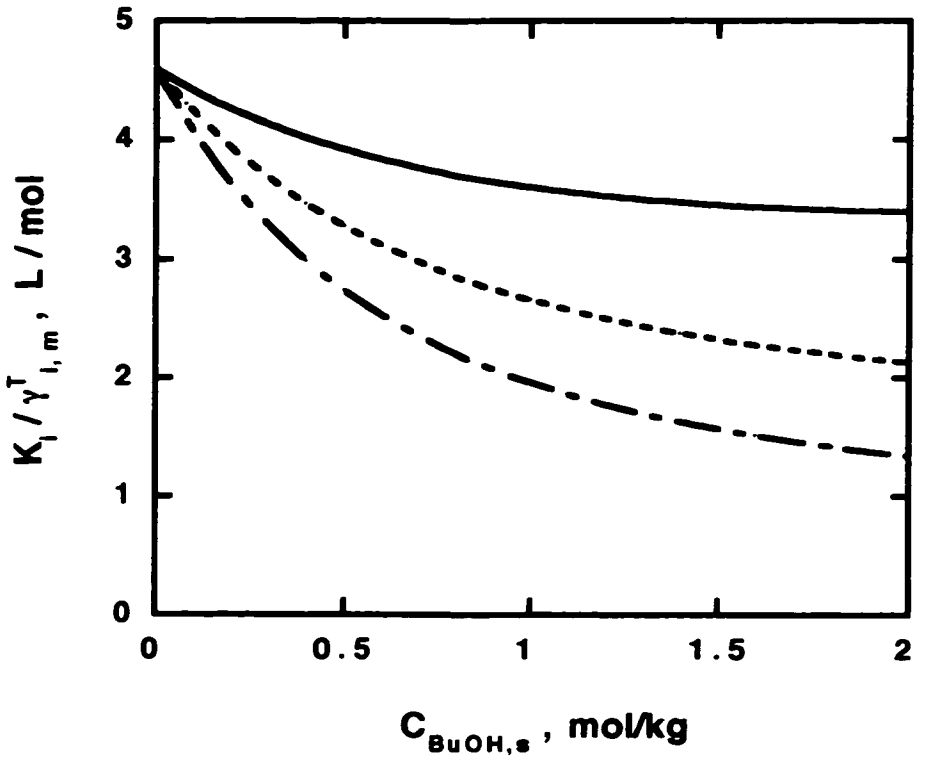

Figure 6.1. (Case 1) Influence of sorbed 1-butanol on $K_i / \gamma^T_{i,m}$ for three sample compounds with $A_1 / C_{1,m} = A_2 / C_{2,m} = A_3 / C_{3,m} = 2$; $\beta_1 = -2$, $\beta_2 = -3$ and $\beta_3 = -4$ and $S_{BuOH} = 2.3$. Solid line - compound one, dashed line – compound two, dash-dot line – compound three.

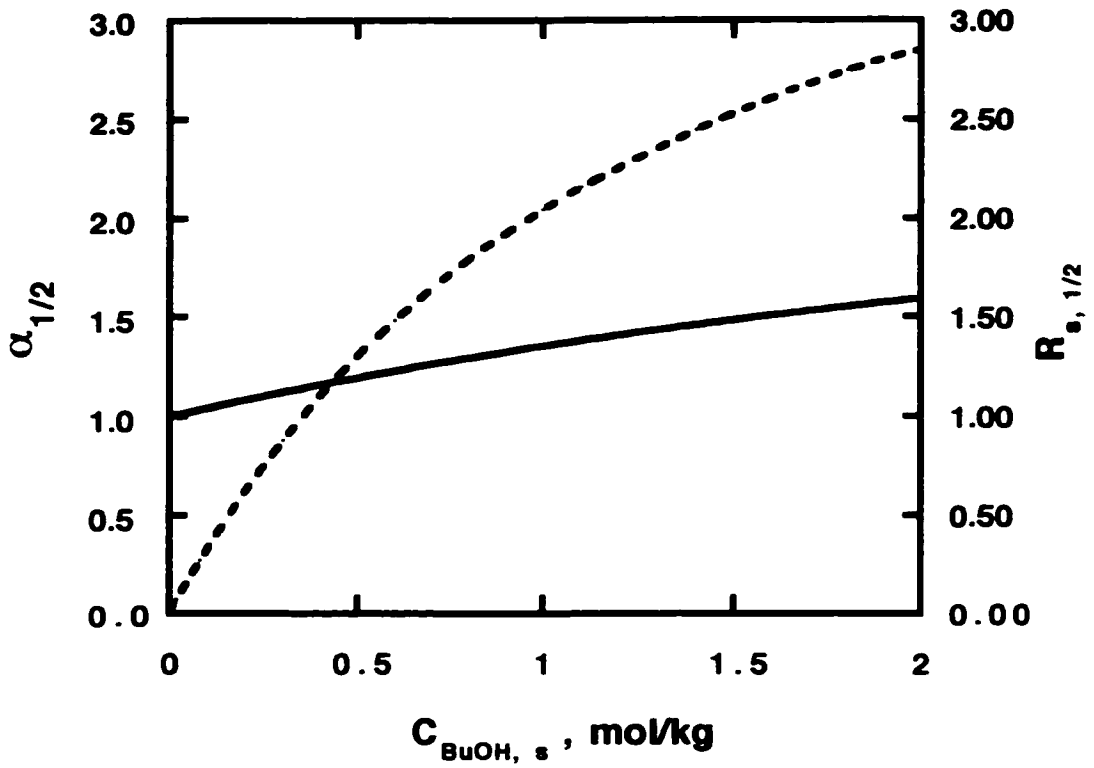

Figure 6.2. (Case 1) A plot of influence of sorbed 1-butanol on selectivity (solid line) and resolution (dashed line) of compounds 1 and 2 from Figure 6.2 (compound 1 is retained stronger than compound 2). The number of theoretical plates in equation 6.2 is 1600, phase ratio = 1.

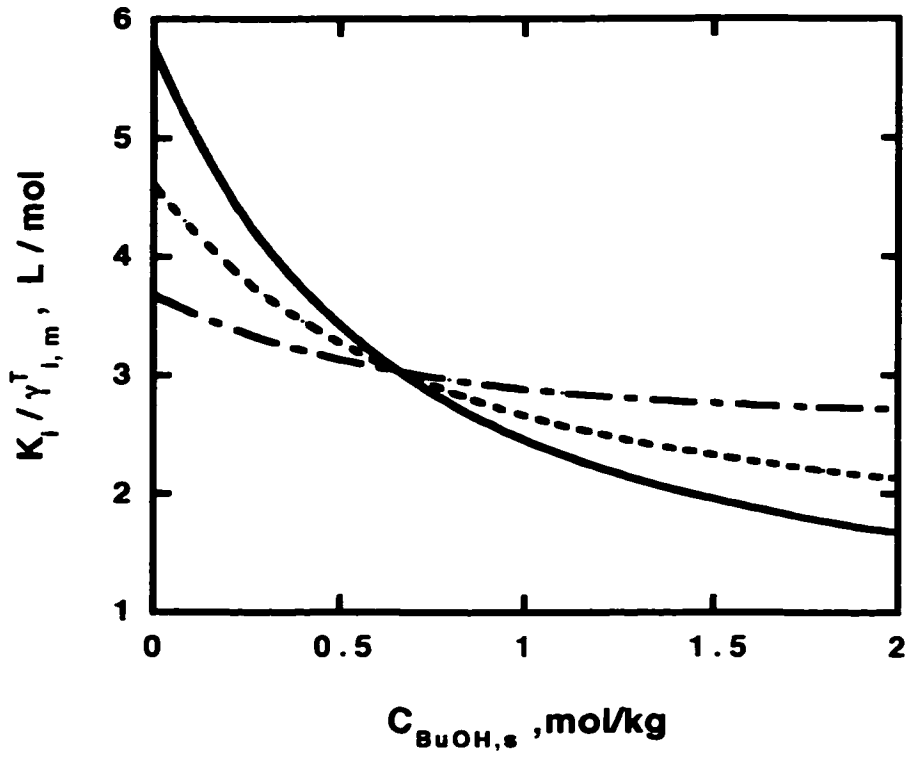

Figure 6.3. (Case 1) Plot of the influence of the sorbed 1-butanol on $K_i / \gamma^T_{i,m}$ of three "eucalyptol-like" compounds with $A_1 / C_{1,m} = 2.5$; $A_2 / C_{2,m} = 2.0$; $A_3 / C_{3,m} = 1.6$; $\beta_1 = -4$, $\beta_2 = -3$ and $\beta_3 = -2$; $S_{BuOH} = 2.3$. Solid line - compound one, dashed line – compound two, dash-dot line – compound three.

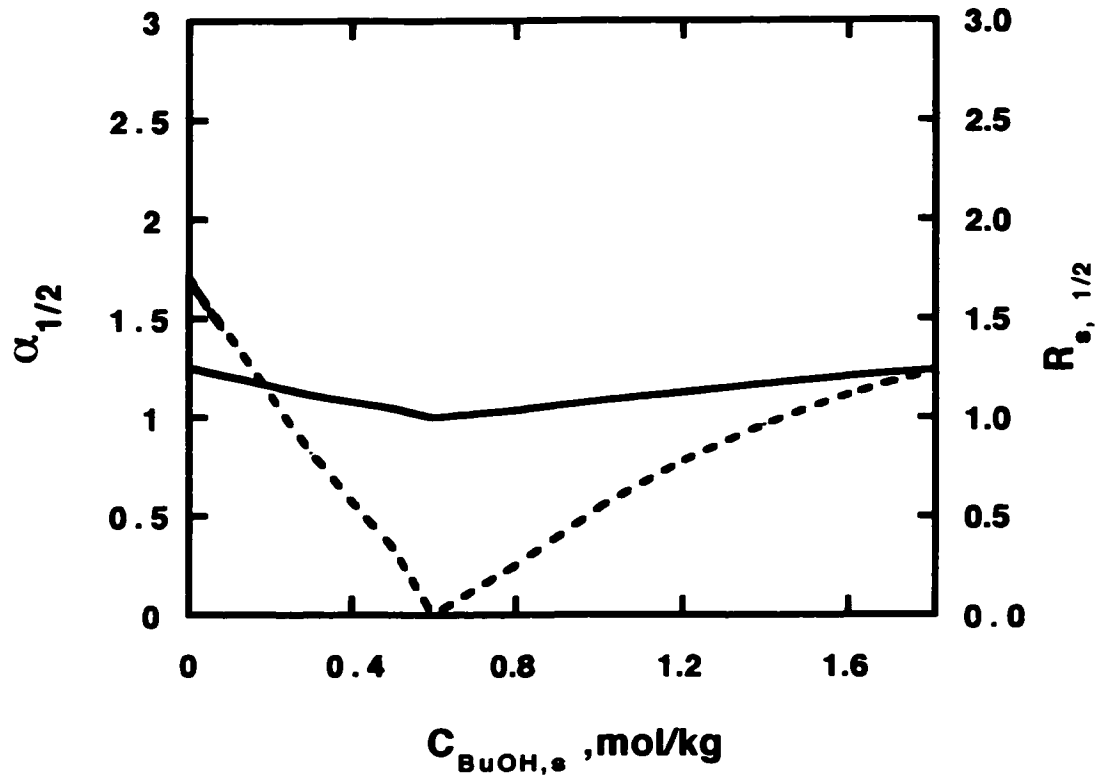

Figure 6.4. (Case 1) The influence of sorbed 1-butanol on selectivity (solid line) and resolution (dashed line) of compounds 1 and 2 from Figure 6.3. The number of theoretical plates in equation 6.2 is 1600, phase ratio = 1.

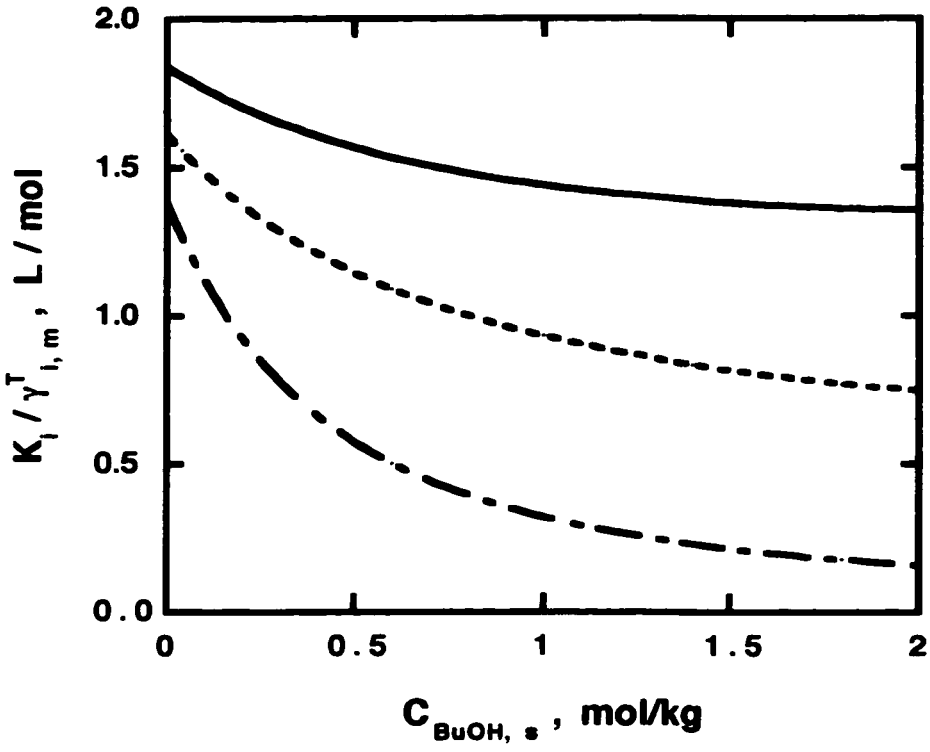

Figure 6.5. The influence of sorbed 1-butanol on $K_i / \gamma^T_{i,m}$ of three sample compounds with $A_1 / C_{1,m} = 0.8$; $A_2 / C_{2,m} = 0.7$; $A_3 / C_{3,m} = 0.6$; $\beta_1 = -2$, $\beta_2 = -3$ and $\beta_3 = -6$; $S_{BuOH} = 2.3$. Solid line - compound one, dashed line – compound two, dash-dot line – compound three.

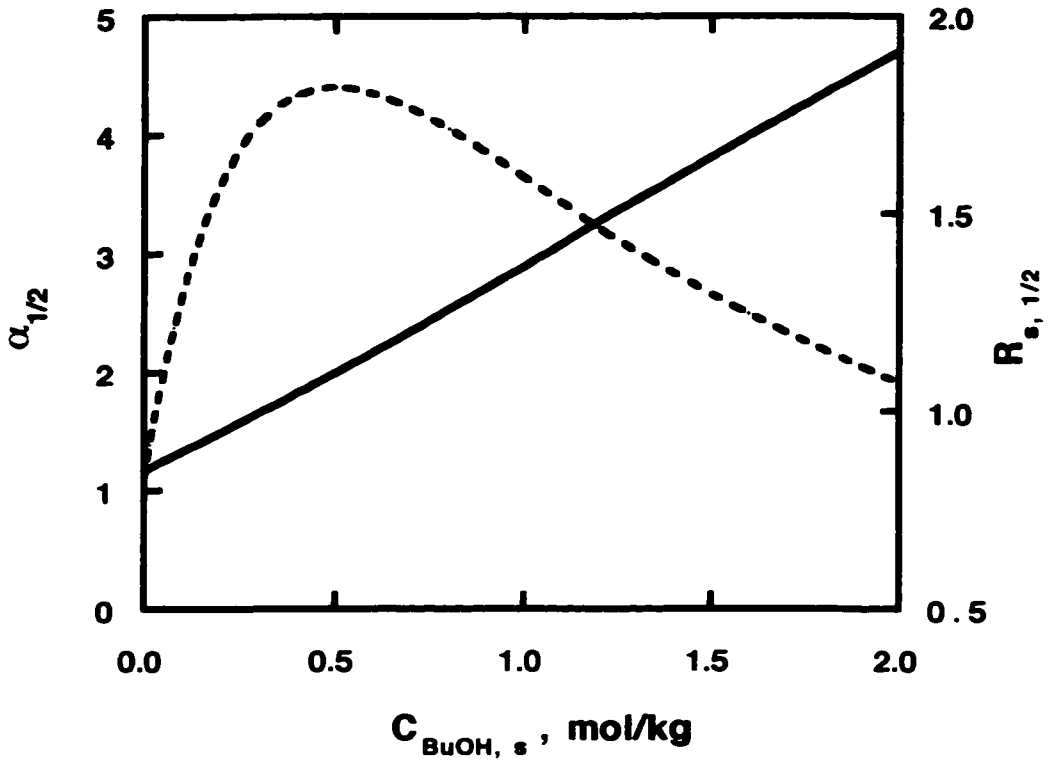

Figure 6.6. The influence of sorbed 1-butanol on selectivity (solid line) and resolution (dashed line) of compounds 2 and 3 from Figure 6.5. The number of theoretical plates in equation 6.2 is 1600, phase ratio = 1.

resolution for compounds 1 and 2 was constructed (Figure 6.4). Selectivity decreases to the value of 1 at the concentration of 1-butanol at which the three distribution coefficients are equal, and then increases (with peaks in the reverse order) with the increase of 1-butanol concentration in the stationary phase. Resolution follows selectivity. It is also somewhat influenced by the decrease of the absolute value of distribution coefficients as well. The value of $\alpha_{1/2}$ at $C_{BuOH,s}$ = 0 mol/kg is the same as the value of $\alpha_{2/1}$ at $C_{BuOH,s}$ = 1.8 mol/kg, but $R_{s,2/1}$ at $C_{BuOH,s}$ = 1.8 mol/kg is smaller than $R_{s,1/2}$ at $C_{BuOH,s}$ = 0 mol/kg due to the decreased value of the distribution coefficients.

When $A_1 / C_{1,m} > A_2 / C_{2,m} > A_3 / C_{3,m}$, but $-\beta_1 < -\beta_2 < -\beta_3$ the difference in $K_i / \gamma^T_{i,m}$ increases with the increase of $C_{BuOH,s}$ for all concentrations of 1-butanol in the stationary phase (Figure 6.5), similarly to Figure 6.3. However, in Figure 6.5 the values of $A_i / C_{i,m}$ were chosen to be smaller than in Figure 6.3 in order to demonstrate the adverse effect of the decrease of distribution coefficients on the resolution. Resolution and selectivity for the compounds 1 and 2 are plotted in Figure 6.6. As seen from the Figure, the resolution $R_{s,1/2}$ goes through a maximum at $C_{BuOH,s} \cong 0.6$ mol/kg. The increase of resolution with the amount of sorbed 1-butanol at $C_{BuOH,s} < 0.6$ mol/kg follows the increase in selectivity, and the decrease of resolution at $C_{BuOH,s}$ above 0.6 mol/kg is due to the decline of retention.

6.2.2. Influence of Sorbed Organic Modifier on Selectivity and Resolution. Case 2 (1-Hexanol and 1-Propanol)

Case 2 dependence of sorbed sample on the sorbed modifier is described by equation 5.17 in general. When 1-hexanol is a sample and 1-propanol is a standard, then

the exponential term in equation 5.17 becomes equal to 1, and the equation 5.29 is valid. If both left and right sides of equation 5.29 are divided by $C_{HexOH,m}$, and the equation is written in general form where subscript 'i' represents sample and subscript 'solv' represents solvent, we obtain:

$$\frac{C_{i,s}}{C_{i,m}\gamma^{T}_{i,m}} = K_{D,i}A_{sp} - K_{D,i}\bar{A}^{o}_{solv,s}C_{solv,s} + K_{D,i}\bar{A}^{o}_{solv,s}k_{2,solv}C^{2}_{solv,s} \qquad (6.4)$$

where all terms have the same meaning as in Chapter 5. If two compounds have the same $K_{D,i}$, the sorption of organic modifier will not help to separate them, as all the other parameters on the right hand side of equation 6.4 are independent of the solute nature, and the sorption of organic modifier will influence them all in the same way. If equation 6.4 applies also to the influence of 1-propanol on another alcohol in addition to 1-hexanol, it could be used to predict the influence of 1-propanol on the selectivity and resolution between 1-hexanol and this other alcohol. The plot of $K_i / \gamma^{T}_{i,m}$ for 1-hexanol and an alcohol with the $K_{D,i}$ two times larger than that of 1-hexanol is presented in Figure 6.7. The absolute value of retention of both alcohols decreases with the increase of 1-propanol concentration in the stationary phase. However, the ratio of $K_1 / \gamma^{T}_{1,m}$ to $K_2 / \gamma^{T}_{2,m}$ is unchanged. Therefore, the selectivity $\alpha_{1/2}$ will not change with the increase of $C_{PrOH,s}$. Resolution will decrease with the decrease in retention. Equation 6.2 can be used to find out what minimal retention (k') is required to give a satisfactory resolution for a given column (number of theoretical plates) and selectivity.

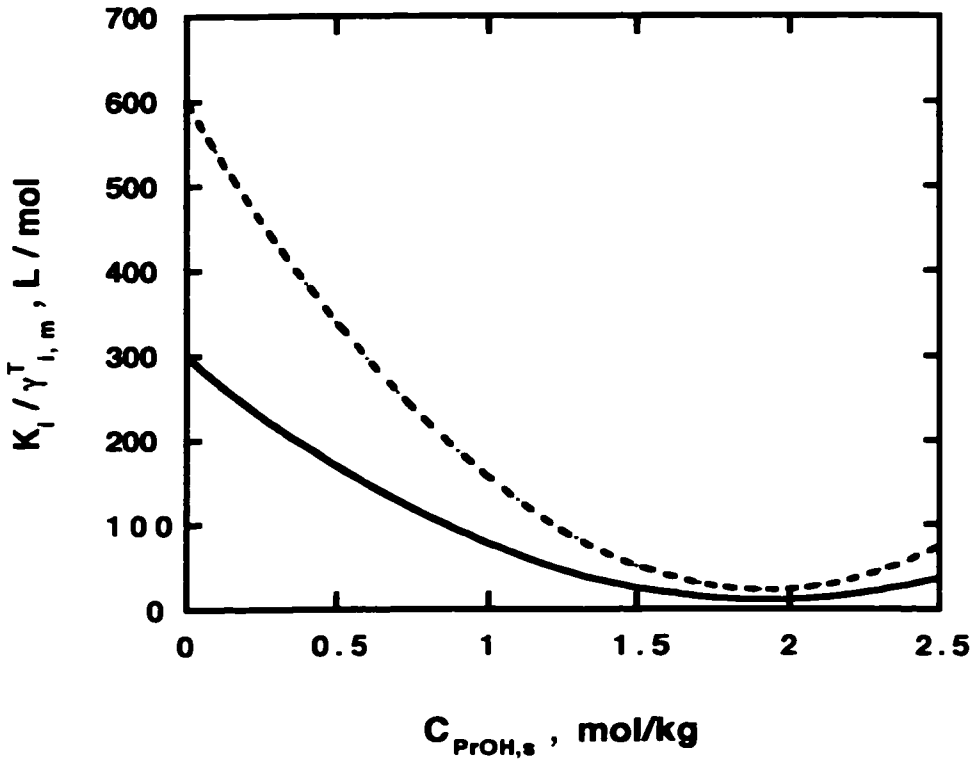

Figure 6.7. (Case 2) The influence of sorbed 1-propanol on $K_i / \gamma^T_{i,m}$ of 1-hexanol (solid line) and of another alcohol obeying equation 6.4 (dashed line). $K_{D,HexOH}$ = 0.001; $K_{D,alcohol}$ = 0.002. Values of A_{sp}, $\overline{A}^o_{Pr\,OH,s}$ and $k_{2,PrOH}$ are the same as in chapter 5.

If the exponential term in equation 5.17 is not equal to one, then the sorption of organic modifier will influence the resolution of a pair of sample compounds for which coefficients $\mathbf{k}_{1,i}^{PrOH}$ aren't the same.

6.2.3. Influence of Sorbed Organic Modifier on Selectivity and Resolution of a Case 1 and a Case 2 Compound.

In a different study done by our research group it was shown that 1-butanol competes for space with tetra-*n*-butylammonium (TBA) ions sorbed by Partisil 10 ODS3[1]. It could be predicted how retention of a "eucalyptol-like" and a "TBA-like" compound would be influenced by 1-butanol sorption. The influence of 1-butanol on the $\mathbf{K}_i / \gamma_{i,m}^T$ of a "TBA-like" sample is described by the first two terms on the right-hand side of equation 6.4, and the influence of 1-butanol on the $\mathbf{K}_i / \gamma_{i,m}^T$ of "eucalyptol-like" compound is described by equation 6.3. A mathematical simulation for the case when hypothetical "TBA-like" and "eucalyptol-like" compounds have the same value of $\mathbf{K}_i / \gamma_{i,m}^T$ in the absence of 1-butanol organic modifier is presented in the Figure 6.8. When 1-butanol sorbs in the stationary phase, the distribution of both compounds decreases. However, the distribution coefficient **K** of a "TBA-like" compound decreases linearly with 1-butanol concentration (mol/L) in the stationary phase, whereas the volume fraction distribution coefficient $\mathbf{K}^{\phi}$ of "eucalyptol-like" compound decreases exponentially with the volume fraction of 1-butanol in the stationary phase. In Figure 6.8 both of these dependencies were converted to the same set of coordinates ($\mathbf{K}_i / \gamma_{i,m}^T$ on the vertical axis and $\mathbf{C}_{BuOH,s}$ on the horizontal axis). It is seen that the sorption of 1-

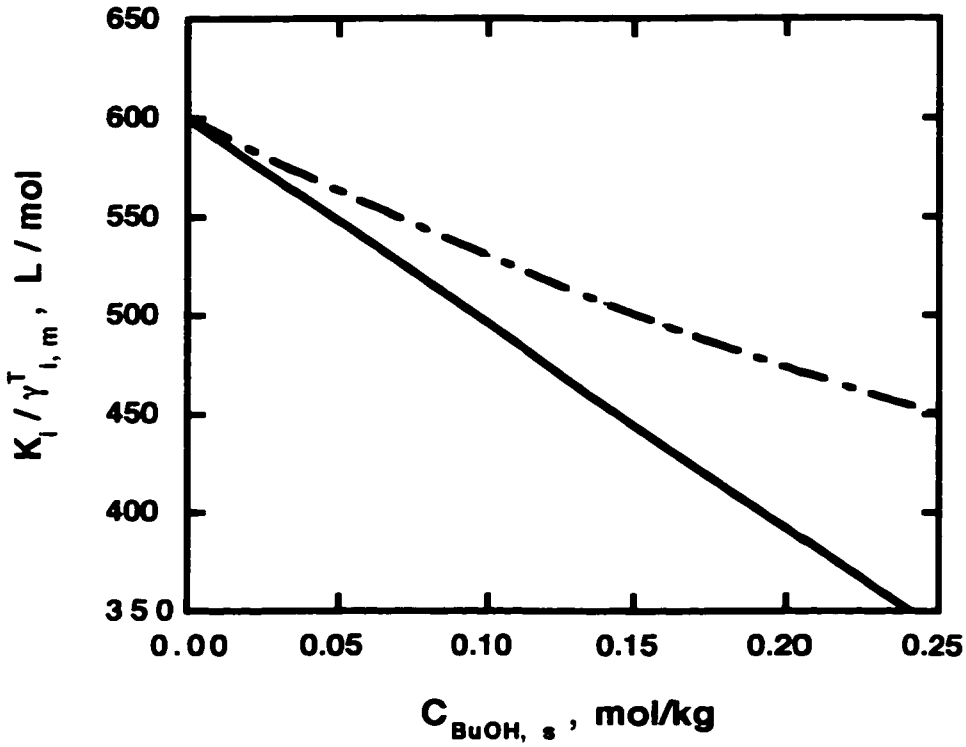

Figure 6.8. (Case 1 + Case 2) The influence of 1-butanol on the sorption of a "eucalyptol-like" (dashed line) and a "TBA-like" (solid line) samples. For sample 1: $A_1 / C_{1,m} = 261$; $\beta = -4$; $S_{BuOH} = =2.3$. For compound 2: $K_2 / \gamma^T_{2,m} = 0.002$. Specific surface area $A_{sp} = 3 \cdot 10^5$ m^2/g, $\overline{A}^o_{BuOH} = 5.2 \cdot 10^5$ m^2/mol[1].

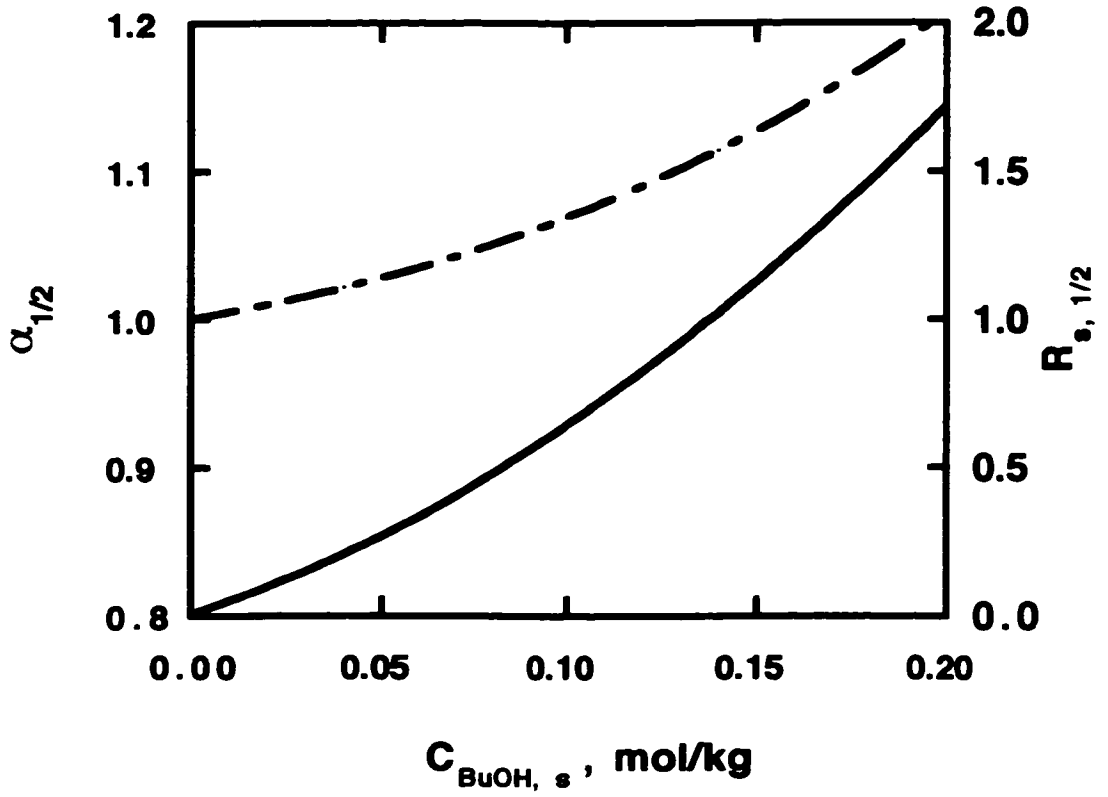

Figure 6.9. (Case 1 + Case 2) The influence of sorbed 1-butanol on selectivity (dashed line) and resolution (solid line) of a "eucalyptol-like" and a "TBA-like" compounds from Figure 6.8. The number of theoretical plates in equation 6.2 is 1600, phase ratio = 1.

butanol makes the separation of these sample compounds possible. In fact, both selectivity and resolution increase as the concentration of 1-butanol in the stationary phase increases (Figure 6.9). However, if these compounds are well separated in the absence of 1-butanol in the stationary phase, it is possible that the sorption of 1-butanol could degrade selectivity (similar to the effect in Figure 6.3 at low concentration of 1-butanol in the stationary phase, i.e. before the cross-over point).

6.3. Directions for Future work

As a result of work presented in these thesis, as well as some other studies[1,2] it can be stated with a reasonable degree of confidence that bipolar linear molecules such as straight chain alcohols compete with one another for space in the C_{18} stationary phase. It is not clear, however, why eucalyptol and 1-butanol influence the sorption of each other by the virtue of diluting the alkyl stationary phase, whereas 1-butanol competes for space with sorbed TBA. Charge on the TBA ion might be responsible for this difference, but that would have to be established by studying the influence of 1-butanol on the sample that has structure similar to TBA but no charge. From a more general perspective, it would be of interest to establish guidelines as to what properties of sample compounds and organic modifiers determine what type of influence the sorption of organic modifier will have on sample retention.

The column equilibration technique provides a means for the study of such interactions. It might be worth exploring somewhat different experimental pathways that would allow the study of larger number of compounds at the same time. Retention could be studied by column chromatography, and the amount of sorbed modifier could be

determined by column equilibration technique. This method would have a disadvantage of not giving an exact concentration of sample in the stationary phase. The advantage would be the possibility of studying several sample compounds at once. In such experiments, one could choose a number of sample compounds with similar retention, but different structure, and see directly the influence of sorbed modifier on selectivity. Care has to be taken so that the methods of void volume determination for elution chromatography and column equilibration techniques are equivalent. It is possible to use deuterated water as an unretained component in elution chromatography, but one must ensure that the void volume obtained in such way is equivalent to the void volume obtained in the column equilibration experiment. Extensive literature exists on the topic[3,4,5] and will aid in the proper choice of void volume determination method.

Exploration of different kinds of organic modifiers, particularly the ones that have a high distribution coefficient into the stationary phase, could provide a whole new means for manipulating selectivity in reversed phase liquid chromatography. An interesting pioneering work in this field was done almost two decades ago[6], but little further investigation followed. A number of stationary phase interactions were defined in this paper, but they were not quantitated, and it certainly would be useful to do that.

From a different perspective, this work needs to be extended to the study of different types of stationary phases. Specifically, the study of the influence of bonding density on the stationary phase interactions would be important from both theoretical and practical points of view. One could also investigate sorbed organic modifier/sorbed sample interactions on stationary phases with such different bonding chemistry as horizontal polymerization. It would be valuable to investigate the role of chain length in

determining whether competition for space or a eucalyptol/butanol-like interaction take place between sorbed sample and sorbed organic modifier. Using chains longer than eighteen carbon atoms for such study would be of interest.

References for Chapter 6.

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Appendix A

Tables of Data for Figures in This Thesis

Table A.1. Data for Figure 3.1 - eucalyptol and 1-butanol loading experiment #1.

time, min	h_{Eu} / h_{Std}	h_{BuOH} / h_{Std}
5.0	1.245	0.185
10.0	2.041	0.190
15.0	1.990	0.180
20.0	2.050	0.190
45.0	2.090	0.180
60.0	2.100	0.184
100.0	2.070	0.182

Table A.2. Data for Figure 3.2 – eucalyptol and 1-butanol loading experiment #2.

time, min	h_{Eu} / h_{Std}	h_{BuOH} / h_{Std}
5.0	3.92	0.0448
10.0	3.89	0.0445
15.0	3.87	0.0446
20.0	3.87	0.0448
30.0	3.86	0.0440
40.0	3.94	0.0452

Table A.3. Data for Figure 3.3 – 1-hexanol loading experiment.

time, min	h_{HexOH}/h_{Std}
3.0	1.147
3.0	1.159
5.0	1.139
5.0	1.161
8.0	1.150
8.0	1.159
12.0	1.156
15.0	1.166
20.0	1.156
30.0	1.160
60.0	1.139

Table A.4. Data for Figure 3.4. - eucalyptol and 1-butanol elution experiment.

Fraction #	h_{Eu} / h_{Std}	h_{BuOH} / h_{Std}
1	0.331	0.410
2	0.345	0.102
3	0.069	0.000
4	0.0050	0.000
5	0.000	0.000
6	0.000	0.000
7	0.000	0.000

Table A.5. Data for Figure 3.5 - 1-hexanol and 1-propanol elution experiment.

Fraction #	h_{HexOH} / h_{Std}	h_{PrOH} / h_{Std}
1	2.315	3.820
2	0.290	0.039
3	0.017	0.000
4	0.007	0.000
5	0.003	0.000
6	0.002	0.000
7	0.000	0.000

Table A.6. Data for Figure 4.1 - the determination of eucalyptol solubility in water by shake flask method.

V_{Euc}, μL	$C_{Euc, added} \times 10^3$, μL	$C_{Euc, dissolv} \times 10^3$, μL
0.000	0.000	0.000
0.334	10.0	9.83
0.417	12.5	11.9
0.501	15.0	13.4
0.584	17.5	15.8
0.668	20.0	17.1
0.751	22.5	18.5
0.835	25.0	18.9
0.918	27.5	18.8

Table A.7. Data for Figure 4.3 – the cloud point determination of eucalyptol solubility in water and in aqueous 0.300 mol/L 1-butanol solution.

$C_{BuOH,m}$, mol/L	T, °C	1/T, K^{-1}	% Eu, w/w	ln $X_{Euc,m}$
0	29.5	0.00330	0.2143	-8.294
0	30.8	0.00329	0.2169	-8.282
0	25.8	0.00334	0.2381	-8.189
0	26.5	0.00334	0.2429	-8.169
0	24.9	0.00335	0.2466	-8.153
0	19.9	0.00341	0.2809	-8.023
0.300	25.5	0.00335	0.2410	-8.170
0.300	25.3	0.00335	0.2398	-8.175

Table A.8. Data for Figure 4.6 - the eucalyptol isotherm.

$C_{Euc,m}$, mol/L	$C_{Euc,s}$, mol/kg
1.00e-05	0.0219
2.00e-05	0.0381
3.00e-05	0.0516
4.00e-05	0.0652
6.00e-05	0.0853
8.00e-05	0.105
1.00e-04	0.122
1.00e-04	0.122
2.00e-4	0.189
4.00e-4	0.282
6.00e-4	0.356
8.00e-4	0.415
1.00e-3	0.466
1.00e-3	0.471
1.00e-3	0.461
1.00e-3	0.465
1.00e-3	0.465
2.00e-3	0.640
3.00e-3	0.790
4.00e-3	0.919
5.00e-3	1.07
6.00e-3	1.22
8.00e-3	1.55
8.15e-3	1.57
8.17e-3	1.55
9.78e-3	1.90
11.6e-3	2.29
11.7e-3	2.28

Table A.9. Data for Figures 4.7 and 4.8 – the influence of sorbed eucalyptol on the sorption of 1-butanol.

$C_{Euc,m}$, mol/L	$C_{Euc,s}$, mol/kg	$C_{BuOH,s}$, mol/kg	$\phi_{Euc,s}$	$\phi_{BuOH,s} \times 10^{-3}$	K^{ϕ}_{BuOH}
0	0	0.0190	0	7.38	80.7
0	0	0.0190	0	7.38	80.6
0	0	0.0189	0	7.36	80.4
0	0	0.0191	0	7.41	81.0
0	0	0.0190	0	7.38	80.6
0	0	0.0191	0	7.40	80.9
0	0	0.0190	0	7.36	80.4
0	0	0.0190	0	7.36	80.4
0	0	0.0191	0	7.40	80.9
1.00×10^{-5}	0.0187	0.0179	0.0131	6.86	75.0
1.00×10^{-5}	0.0213	0.0174	0.0149	6.66	72.8
4.00×10^{-5}	0.0588	0.0166	0.0401	6.18	67.5
1.00×10^{-4}	0.102	0.0151	0.0677	5.48	59.9
1.00×10^{-4}	0.113	0.0147	0.0742	5.31	58.0
4.00×10^{-4}	0.252	0.0129	0.152	4.25	46.5
4.00×10^{-4}	0.258	0.0129	0.155	4.25	46.4
8.00×10^{-4}	0.380	0.0112	0.213	3.43	37.4
8.00×10^{-4}	0.382	0.0115	0.214	3.51	38.3
1.20×10^{-3}	0.510	0.0099	0.267	2.8	31
1.60×10^{-3}	0.585	0.0092	0.294	2.5	28

Table A.9. continued.

$C_{Euc,m}$, mol/L	$C_{Euc,s}$, mol/kg	$C_{BuOH,s}$, mol/kg	$\phi_{Euc,s}$	$\phi_{BuOH,s} \times 10^{-3}$	K^{ϕ}_{BuOH}
2.00×10^{-3}	0.668	0.0085	0.323	2.2	24
3.00×10^{-3}	0.836	0.0076	0.374	1.8	20
6.00×10^{-3}	1.15	0.0068	0.451	1.5	16
8.00×10^{-3}	1.52	0.0062	0.521	1.2	13
0.0100	2.00	0.0057	0.587	0.92	10
0.0120	2.47	0.0053	0.638	0.74	8.1
0.0120	2.74	0.0052	0.662	0.69	7.5
0.0120	2.86	0.0054	0.672	0.69	7.5
0.0120	2.77	0.0053	0.664	0.69	7.6
0.0120	3.00	0.0051	0.682	0.63	6.9
0.0140	3.23	0.0049	0.700	0.58	6.3
0.0140	3.31	0.0050	0.703	0.59	6.4
0.0160	3.88	0.0047	0.735	0.49	5.3
0.0180	4.56	0.0048	0.765	0.44	4.8
0.0180	4.11	0.0047	0.746	0.47	5.1

Table A.10. Data for Figures 4.9 and 4.10 – the influence of sorbed 1-butanol on the sorption of eucalyptol.

$C_{BuOH,m}$, mol/L	$C_{BuOH,s}$, mol/kg	$C_{Euc,s}$, mol/L	$\phi_{BuOH,s}$	$\phi_{Euc,m} \times 10^2$	$K^{\phi}_{Euc} \times 10^3$
0.00	0	0.0219	0	1.71	10.2
0.00	0	0.0219	0	1.71	10.2
0.00	0	0.0219	0	1.71	10.2
0.00	0	0.0218	0	1.70	10.2
0.00	0	0.0221	0	1.72	10.3
0.00	0	0.0219	0	1.71	10.2
0.00	0	0.0220	0	1.72	10.2
0.00	0	0.0217	0	1.69	10.1
7.65×10^{-4}	0.0179	0.0208	0.00757	1.61	9.62
2.19×10^{-3}	0.0419	0.0189	0.0176	1.45	8.67
4.37×10^{-3}	0.0801	0.0178	0.0331	1.35	8.03
7.45×10^{-3}	0.138	0.0166	0.0556	1.23	7.34
1.00×10^{-2}	0.165	0.0153	0.0662	1.12	6.67
1.00×10^{-2}	0.181	0.0152	0.0718	1.11	6.60
1.09×10^{-2}	0.185	0.0155	0.0736	1.13	6.70
1.09×10^{-2}	0.182	0.0148	0.0724	1.08	6.44
2.00×10^{-2}	0.313	0.0124	0.119	0.862	5.14
4.00×10^{-2}	0.528	0.00870	0.185	0.559	3.34
7.00×10^{-2}	0.793	0.00618	0.255	0.363	2.17
7.00×10^{-2}	0.811	0.00678	0.259	0.397	2.37

Table A.10. continued.

$C_{BuOH,m}$, mol/L	$C_{BuOH,s}$, mol/kg	$C_{Euc,s}$, mol/L	$\phi_{BuOH,s}$	$\phi_{Euc,m} \times 10^2$	$K^{\phi}_{Euc} \times 10^3$
0.100	1.02	0.00551	0.305	0.302	1.81
0.150	1.30	0.00406	0.360	0.205	1.23
0.200	1.50	0.00331	0.3931	0.159	0.949
0.200	1.50	0.00341	0.394	0.164	0.977
0.250	1.69	0.00276	0.422	0.126	0.754
0.300	1.83	0.00255	0.442	0.113	0.672
0.300	1.84	0.00250	0.443	0.111	0.660
0.300	1.84	0.00272	0.443	0.120	0.718

Table A.11. Data for Figure 5.1 - 1-hexanol sorption isotherm. The effective weight of stationary phase in the precolumn is $2.636 \cdot 10^{-5}$ kg.

$C_{HexOH,m}$, mol/L	$n_{HexOH,s} \cdot 10^6$, mol	$C_{HexOH,s}$, mol/kg
$1.00 \cdot 10^{-4}$	0.887	0.0336
$1.00 \cdot 10^{-4}$	0.881	0.0334
$2.00 \cdot 10^{-4}$	1.53	0.0579
$4.00 \cdot 10^{-4}$	2.67	0.101
$6.00 \cdot 10^{-4}$	3.67	0.139
$6.00 \cdot 10^{-4}$	3.76	0.143
$8.00 \cdot 10^{-4}$	4.80	0.182
$1.00 \cdot 10^{-3}$	5.79	0.220
$1.00 \cdot 10^{-3}$	5.68	0.215
$1.60 \cdot 10^{-3}$	8.02	0.304
$2.00 \cdot 10^{-3}$	9.41	0.357

Table A.12. Data for Figure 5.2 - the determination of holdup volume V_{HU} of precolumn #2 at varying true volume percent of 1-propanol $\Phi_{PrOH,m}$ in the mobile phase.

$\Phi_{PrOH,m}$, v/v %	V_{HU} ± one std. deviation, µL
0	64.2 ± 0.4
1	63.5 ± 0.0
5	61.7 ± 0.0
10	61.2 ± 0.2
20	59.8 ± 0.3
30	59.3 ± 0.4
0	64.2 ± 0.4
0.3	64.2 ± 0.2
0.6	63.8 ± 0.2
1	63.5 ± 0.3
0	64.2 ± 0.4
1	63.5 ± 0.0
5	61.7 ± 0.0
10	61.2 ± 0.2
20	59.8 ± 0.3
30	59.3 ± 0.4

Table A.13. Data for Figure 5.3 - the plot of the change of holdup volume ΔV_{HU}, caused by the sorption of 1-propanol, *versus* the volume of sorbed 1-propanol $V_{PrOH,s}$. ΔV_{HU} is given with an error of ± one standard deviation.

$\Phi_{PrOH,m}$, % v/v	ΔV_{HU}, μL	$V_{PrOH,s}$, μL
1.00	0.65 ± 0.03	0.80
5.00	2.50 ± 0.04	2.11
10.00	3.0 ± 0.2	3.06
20.00	4.3 ± 0.3	4.21
30.00	4.9 ± 0.4	4.78
0.300	-0.1 ± 0.2	0.31
0.600	0.4 ± 0.2	0.64

Table A.14. Data for Figure 5.5 – plots of 1-hexanol mol fraction solubility $X_{HexOH,m}$ and transfer activity coefficient γ^{T}_{HexOHm} *versus* true volume percent of 1-propanol in the mobile phase. $X_{HexOH,m}$ and γ^{T}_{HexOHm} are given with an error of ± one standard deviation.

$\Phi_{HexOH,m}$ %	$X_{HexOH,m} \times 10^3$	γ^{T}_{HexOHm}
0	1.03 ± 0.01	1.00 ± 0.01
5.02	0.98 ± 0.01	1.00 ± 0.01
10.08	0.99 ± 0.02	1.00 ± 0.02
15.17	0.98 ± 0.02	1.00 ± 0.02
16.87	1.15 ± 0.03	0.87 ± 0.02
20.28	1.82 ± 0.01	0.548 ± 0.003
25.4	3.98 ± 0.07	0.251 ± 0.004
30.53	7.31 ± 0.04	0.137 ± 0.001
30.53	7.14 ± 0.08	0.140 ± 0.002
33.62	9.65 ± 0.05	0.103 ± 0.001

Table A.15. Data for Figure 5.6 – plot of sorbed concentration of 1-hexanol, adjusted for the mobile phase effect, versus sorbed concentration of 1-propanol.

$\Phi_{PrOH,m}$, %	$C_{PrOH,s}$, mol/kg	$C_{HexOH,s} / \gamma^{T}_{HexOH,m}$, mol/kg
0	0	0.0883
0	0	0.0887
0	0	0.0888
0	0	0.0877
0	0	0.0888
0	0	0.0892
0	0	0.0883
0	0	0.0884
0	0	0.0891
0.2	0.114	0.0779
0.2	0.118	0.0792
0.3	0.161	0.0746
0.3	0.161	0.0748
0.4	0.219	0.0714
0.4	0.214	0.0705
0.5	0.258	0.0681
0.5	0.264	0.0685
0.6	0.295	0.0662
0.6	0.290	0.0653
0.7	0.329	0.0625

Table A.15. continued.

$\Phi_{PrOH,m}$, %	$C_{PrOH,s}$, mol/kg	$C_{HexOH,s} / \gamma^{T}_{HexOH,m}$, mol/kg
0.7	0.328	0.0624
0.8	0.360	0.0602
0.8	0.358	0.0609
1	0.421	0.0562
1	0.420	0.0556
1	0.426	0.0562
1	0.422	0.0556
1	0.421	0.0550
1	0.419	0.0547
5	1.07	0.0244
5	1.06	0.0240
10	1.45	0.0138
10	1.49	0.0138
20	1.92	0.0104
20	1.93	0.0105
30	2.11	0.0153
30	2.12	0.0150

Table A.16. Data for Figure 5.7 – plots of K_{HexOH} and mobile and stationary phase contributions of 1-propanol to the sorption of 1-hexanol on Partisil-10 ODS-3.

$\Phi_{Pr\,OH,m}$, %	K_{HexOH}, L/kg	Mobile Phase Effect ($\gamma^{T}_{HexOH,m}$)	Stationary Phase Effect (Eq. 5.29)
0.000	294	1.00	1.00
0.000	296	1.00	1.00
0.000	296	1.00	1.00
0.000	292	1.00	1.00
0.000	296	1.00	1.00
0.000	297	1.00	1.00
0.000	294	1.00	1.00
0.000	295	1.00	1.00
0.000	297	1.00	1.00
0.200	260	1.00	0.880
0.200	264	1.00	0.894
0.300	249	1.00	0.842
0.300	249	1.00	0.845
0.400	238	1.00	0.806
0.400	235	1.00	0.796
0.500	227	1.00	0.769
0.500	228	1.00	0.773
0.600	221	1.00	0.747
0.600	218	1.00	0.737
0.700	208	1.00	0.706

Table A.16. continued.

$\Phi_{PrOH,m}$, %	K_{HexOH}, L/kg	Mobile Phase Effect ($\gamma^{T}_{HexOH,m}$)	Stationary Phase Effect (**Eq. 5.29**)
0.700	208	1.00	0.705
0.800	201	1.00	0.680
0.800	203	1.00	0.687
1.00	187	1.00	0.635
1.00	185	1.00	0.628
1.00	187	1.00	0.635
1.00	185	1.00	0.628
1.00	183	1.00	0.621
1.00	182	1.00	0.618
5.00	81.5	1.00	0.276
5.00	80.0	1.00	0.271
10.0	45.9	1.00	0.155
10.0	45.9	1.00	0.156
20.0	19.2	0.573	0.117
20.0	19.5	0.573	0.119
30.0	7.49	0.150	0.173
30.0	7.33	0.150	0.169

Table A.17. Data for Figures B.1 – B.9 – the plots of cloud point temperature t_{cloud} (given with ± one standard deviation) *versus* 1-hexanol solubility at that temperature, in the 1-propanol/water mixtures with $\Phi_{Pr\,OH,m}$ true volume percent of 1-propanol.

$\Phi_{Pr\,OH,m}$, mol/L	1-Hexanol Solubility $X_{HexOH,m}$	t_{cloud}, °C
0.00	0.001085	21.6 ± 0.4
0.00	0.000995	30.6 ± 0.8
0.00	0.001040	24.4 ± 0.5
5.02	0.001003	22.5 ± 0.5
5.02	0.001051	18 ± 1
5.02	0.001076	16 ± 1
10.08	0.000985	25 ± 2
10.08	0.001052	19 ± 2
15.17	0.001120	34.4 ± 0.4
15.17	0.001036	30.8 ± 0.4
15.17	0.000954	27 ± 1
15.17	0.001194	37.8 ± 0.5
16.87	0.001298	31.4 ± 0.5
16.87	0.001262	29.4 ± 0.5
16.87	0.001181	26.4 ± 1
16.87	0.001138	24.7 ± 1
20.28	0.001664	21.6 ± 0.1
20.28	0.001957	28.2 ± 0.3
25.40	0.003656	21.2 ± 0.8
25.40	0.003894	23.6 ± 0.8
25.40	0.004323	30 ± 1
30.53	0.00762	28.6 ± 0.2
30.53	0.00701	24.0 ± 0.3
30.53	0.00733	26.4 ± 0.1
33.62	0.00962	24.8 ± 0.5
33.62	0.00992	27.65 ± 0.5

Appendix B

Plots of Cloud Point Temperature versus 1-Hexanol Solubility in Aqueous Solutions Containing Varying Concentrations of 1-Propanol (used in Section 5.3.4 for Figure 5.6).

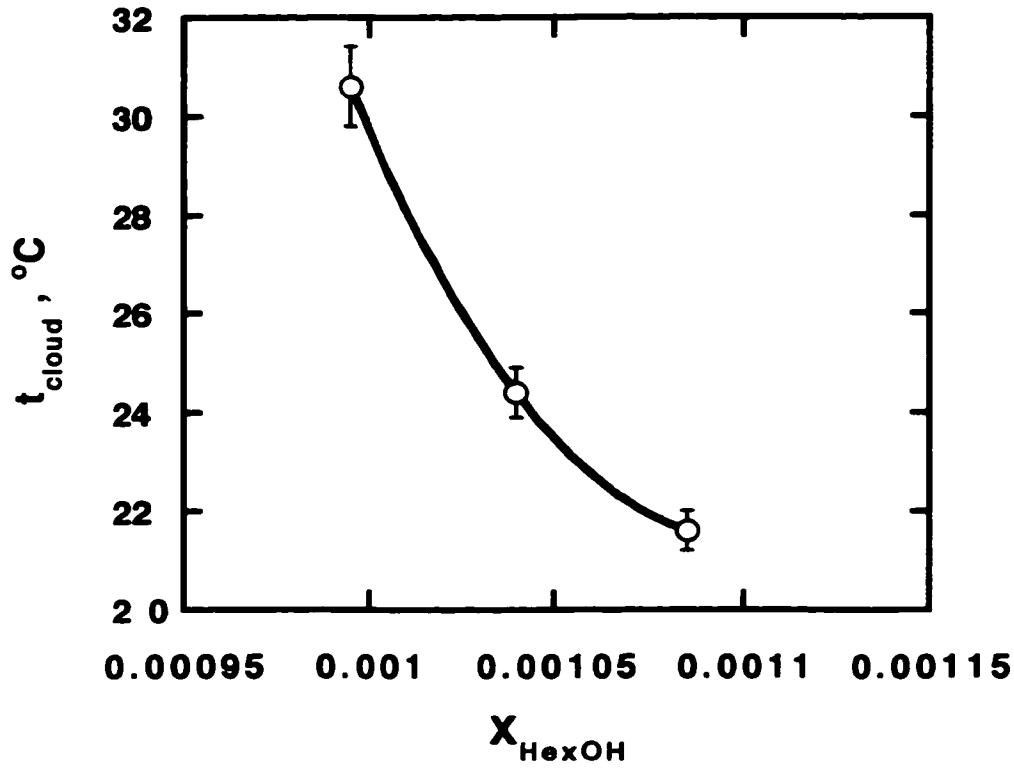

Figure B.1. The plot of cloud point temperature *versus* 1-hexanol mole fraction solubility at that temperature in aqueous mobile phase. Error bars are ± one standard deviation of t_{cloud}.

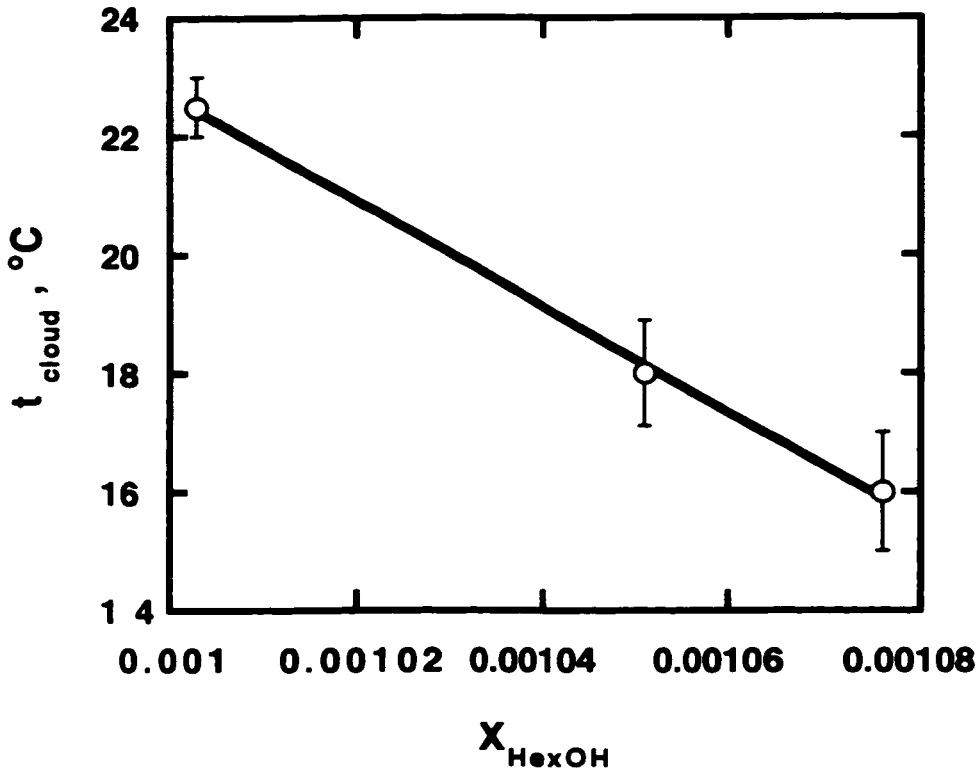

Figure B.2. The plot of cloud point temperature *versus* 1-hexanol mole fraction solubility at that temperature in 1-propanol/water mobile phase with true volume percent of 1-propanol $\Phi_{PrOH,m}$ = 5.02 %. Error bars are ± one standard deviation of t_{cloud}.

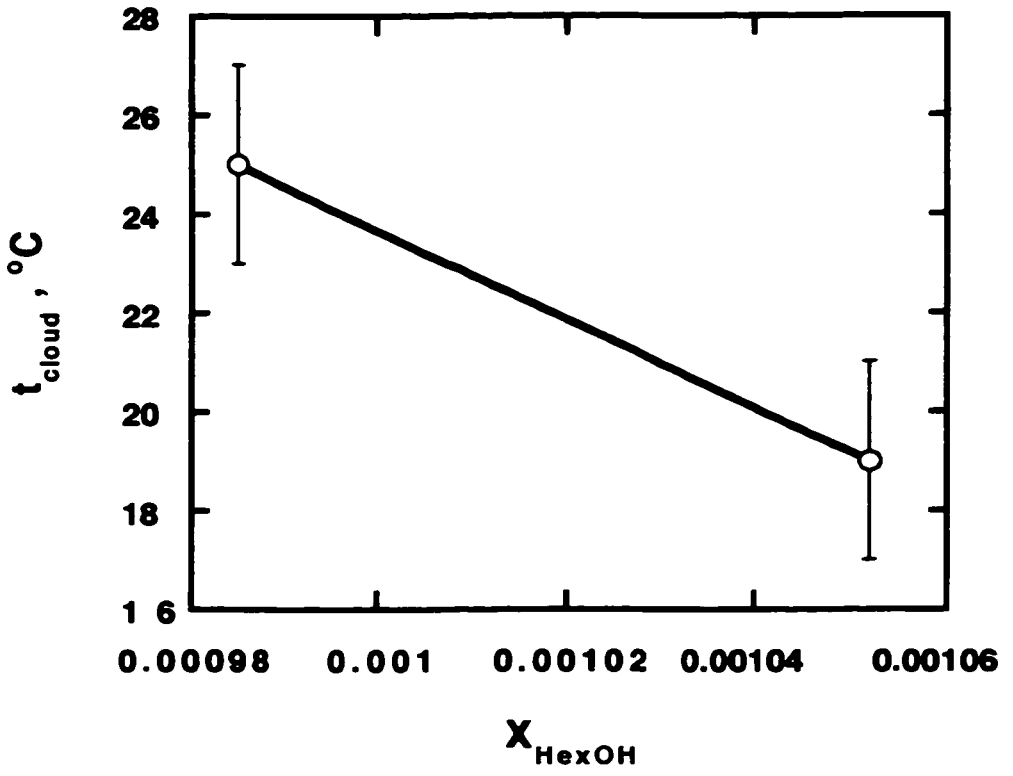

Figure B.3. The plot of cloud point temperature *versus* 1-hexanol mole fraction solubility at that temperature in 1-propanol/water mobile phase with true volume percent of 1-propanol $\Phi_{PrOH,m}$ = 10.08 %. Error bars are ± one standard deviation of t_{cloud}.

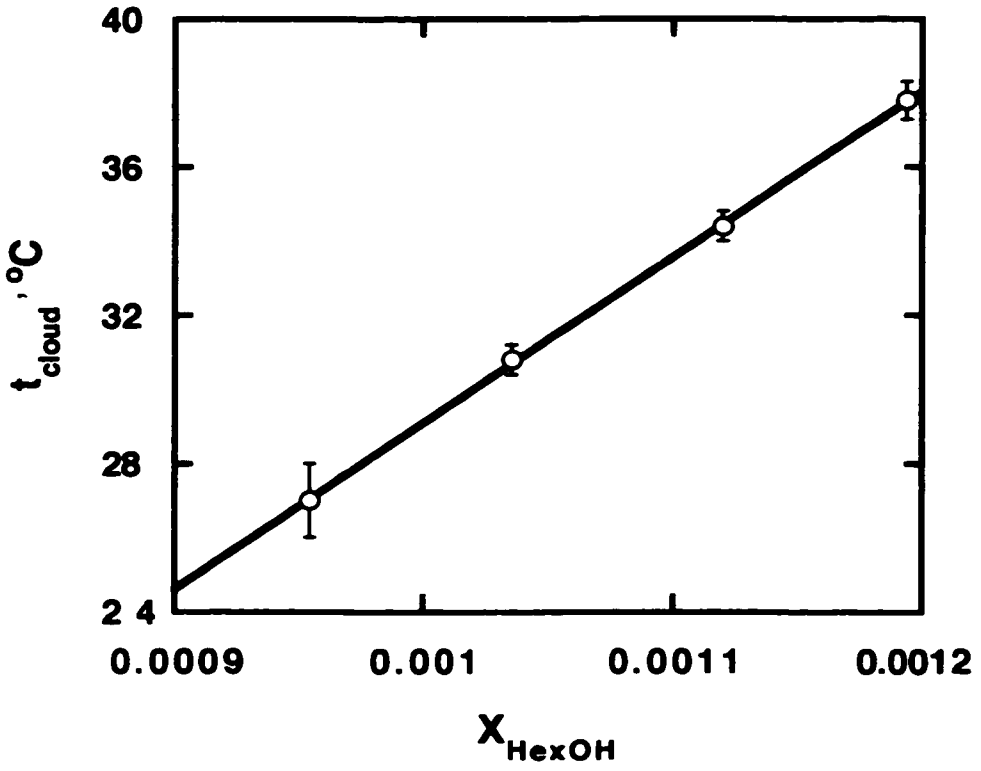

Figure B.4. The plot of cloud point temperature *versus* 1-hexanol mole fraction solubility at that temperature in 1-propanol/water mobile phase with true volume percent of 1-propanol $\Phi_{PrOH,m}$ = 15.17 %. Error bars are ± one standard deviation of t_{cloud}.

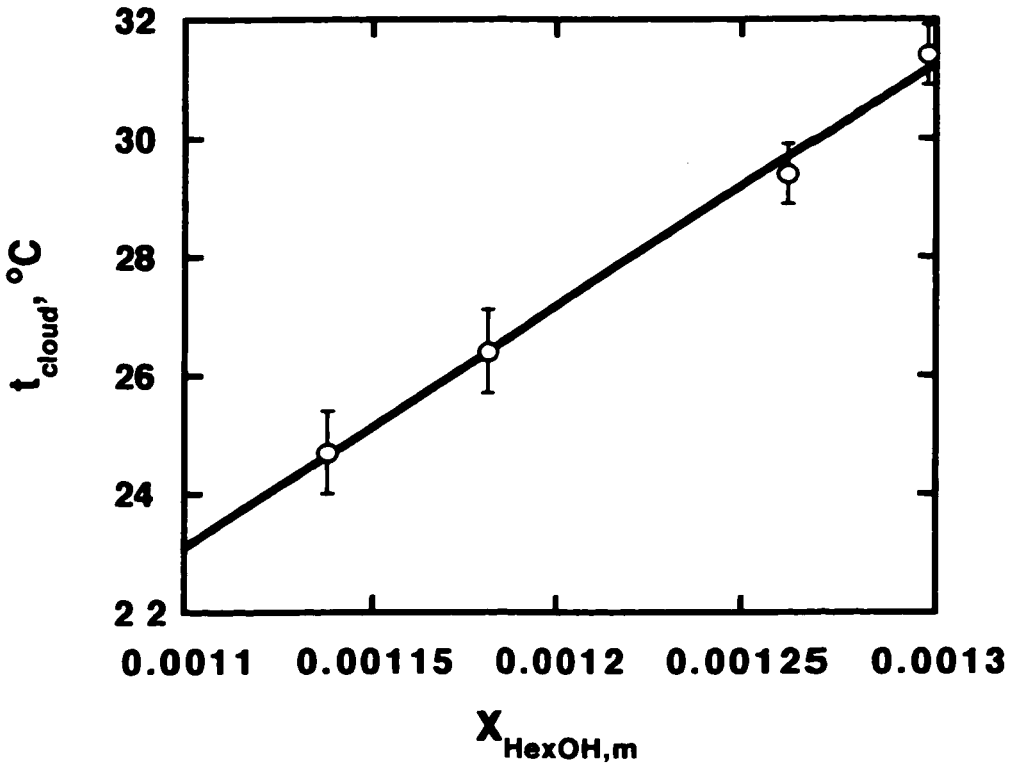

Figure B.5. The plot of cloud point temperature *versus* 1-hexanol mole fraction solubility at that temperature in 1-propanol/water mobile phase with true volume percent of 1-propanol $\Phi_{PrOH,m}$ = 16.87 %. Error bars are ± one standard deviation of t_{cloud}.

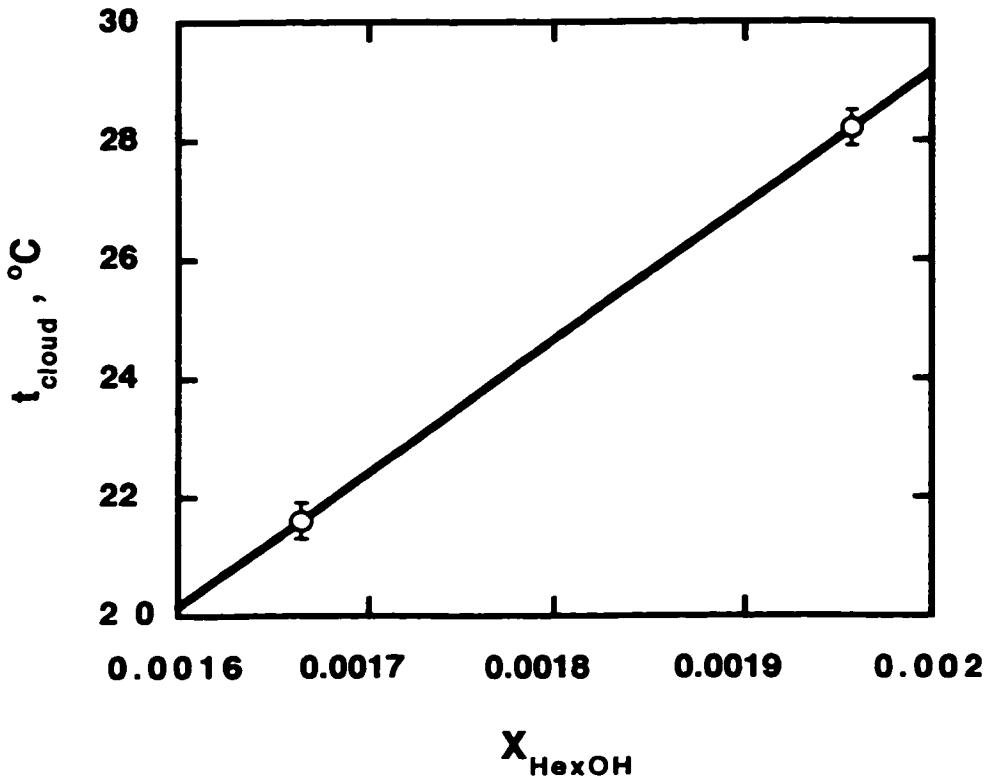

Figure B.6. The plot of cloud point temperature *versus* 1-hexanol mole fraction solubility at that temperature in 1-propanol/water mobile phase with true volume percent of 1-propanol $\Phi_{PrOH,m} = 20.28\%$. Error bars are ± one standard deviation of t_{cloud}.

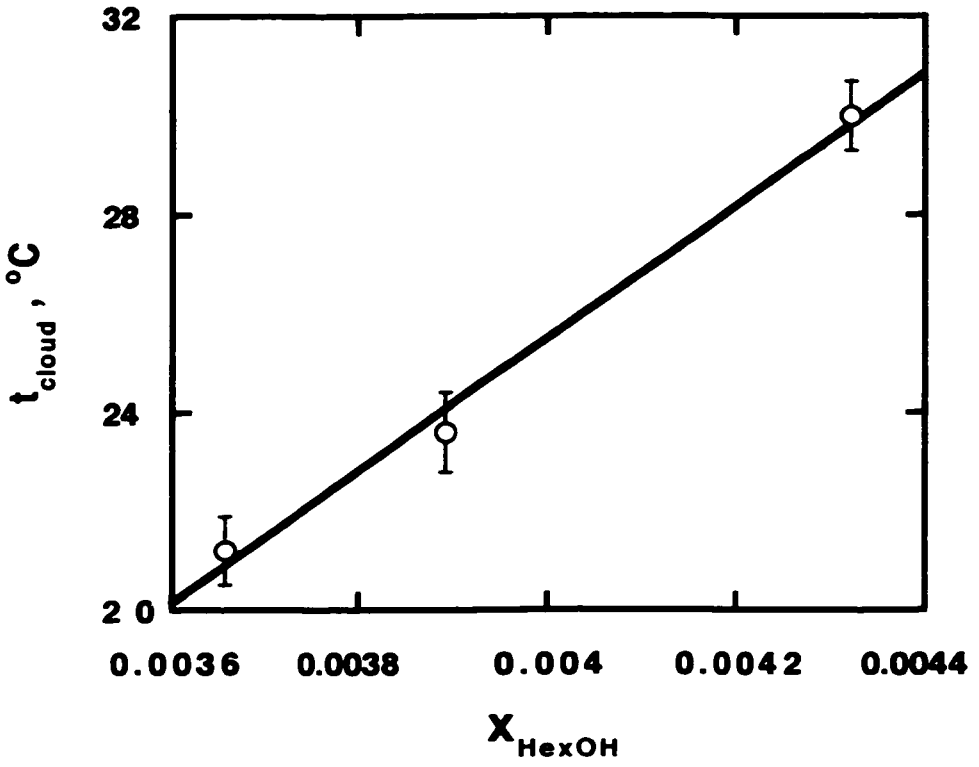

Figure B.7. The plot of cloud point temperature *versus* 1-hexanol mole fraction solubility at that temperature in 1-propanol/water mobile phase with true volume percent of 1-propanol $\Phi_{PrOH,m}$ = 25.40 %. Error bars are ± one standard deviation of t_{cloud}.

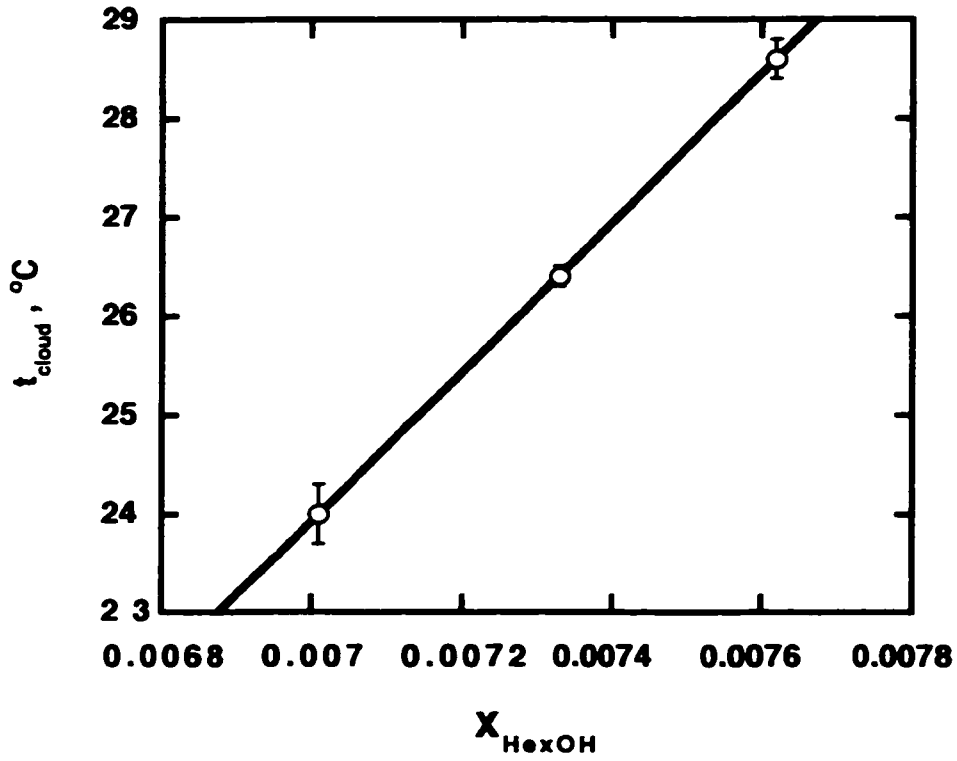

Figure B.8. The plot of cloud point temperature *versus* 1-hexanol mole fraction solubility at that temperature in 1-propanol/water mobile phase with true volume percent of 1-propanol $\Phi_{PrOH,m}$ = 30.53 %. Error bars are ± one standard deviation of t_{cloud}.

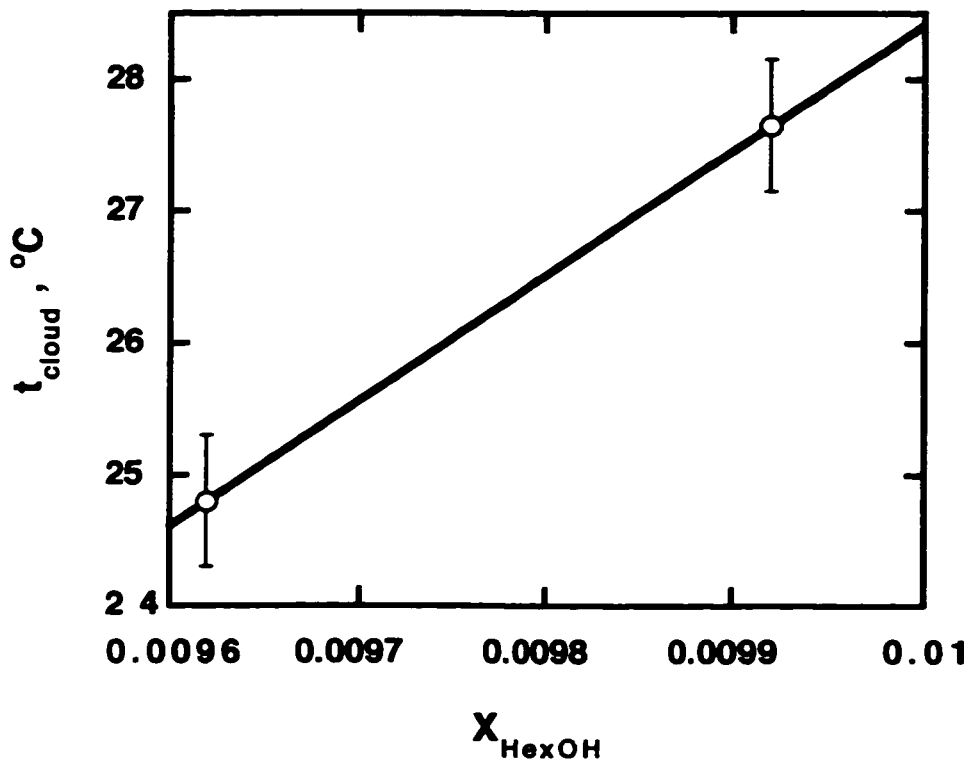

Figure B.9. The plot of cloud point temperature *versus* 1-hexanol mole fraction solubility at that temperature in 1-propanol/water mobile phase with true volume percent of 1-propanol $\Phi_{PrOH,m}$ = 33.62 %. Error bars are ± one standard deviation of t_{cloud}.