Study on the Retention Mechanism
in
Reversed-Phase Liquid Chromatography

December, 1991

DOCTOR OF ENGINEERING

Kazue Tani

TOYOHASHI UNIVERSITY OF TECHNOLOGY

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逆相液体クロマトグラフィーにおける保持機構に関する研究

逆相液体クロマトグラフィー（ＲＰＬＣ）はあらゆる分野に普及し、分離分析の手段として広く使用されている。しかしながら、その普及率にもかかわらず溶質の保持については詳細な解明がなされていない。これまで、溶質の分配過程すなわち保持機構はソルボホビック理論によってモデル化され、ＲＰＬＣの一般的理解にこの理論は重要な貢献をしてきた。また、多くの実験結果との一致を示し移動相の特性に関連して起こる現象の取り扱いに対しては妥当な理論とみなされている。しかしながら、この理論は炭化水素の理想的固定相表面を想定したものであるため固定相の溶質保持への寄与については充分な説明を与えず、ＲＰＬＣの保持機構を完全に記述するには至っていない。

この研究の目的は、ＲＰＬＣにおける保持機構と固定相の働きを明らかにすることである。保持機構の考察では、溶質と固定相との相互作用を明らかにするため、溶質の誘導体骨格と置換基による溶質保持への影響を熱力学的な観点より論述する。また、固定相の働きを明らかにするため、移動相と固定相との界面での溶媒の吸着による溶質保持への寄与の考察から、吸着された有機溶媒量を表面過剰量として見積ることとした。さらに、この方法を用いて、担体表面に炭化水素鎖が化学結合された固定相では移動相溶媒によって大きく表面状態が変化し溶質保持に関しても異なった働きをすることを示す。

本論文の構成は次の通りである。第Ⅰ部では、ソルボホビック理論の問題点などを取り上げ本研究の背景を述べる。第Ⅱ部は、カルボニル誘導体の保持挙動の熱力学的解明に関する４つの章から成り、１章では、カルボニル誘導体の異性体間の溶出順序の違いを誘導体骨格構造に関連づけて述べる。２章では、２、４－ジニトロフェニルヒドラゾン誘導体の保持挙動へのニトロ置換基の位置の効果を熱力学的な保持挙動の比較によって示す。３章では、芳香族アルデヒド誘導体を用いて誘導体骨格と置換基の保持への影響を検討する。４章では、固定相の炭化水素鎖とカルボニル誘導体の炭化水素鎖との相互作用を熱力学的な見地から検討し、移動相の会合状態の違いが固定相炭化水素鎖の構造に影響を及ぼすことを述べる。第Ⅲ部は、表面過剰量を用いる固定相状態の解釈に関する５つの章からなる。１章では、表面過剰量をクロマトグラフデータから求める式を誘導する。２章では、種々の細孔をもつシリカゲルの表面過剰量を測定し、細孔の大きさよりもシリカゲル自体の違いが表面過剰量に影響することを示す。３章では、モノメリック相とポリメリック相の表面過剰量を比較し、メタノール－ジクロロメタン移動相では違いが生じることを記載する。４章では、シリカゲルとそれを化学結合したものとの表面過剰量とピリジンの保持値を比較し、様々な化学結合充填剤の性質の違いにシリカゲル自体も影響することを示す。５章では、これまでの表面過剰量の測定結果から、メタノール－水とアセトニトリル－水移動相でその量に大きな違いが示されたが、直鎖アルキルベンゼンの熱力学的保持挙動からその違いは固定相炭化水素鎖の状態が移動相で異なるためであることを示す。最後に、第Ⅳ部において本論文の結論と今後の展望を述べる。

Study on the Retention Mechanism in Reversed-Phase Liquid Chromatography

The spectacular ascent of "reversed phase" chromatography is one of the most interesting phenomena in the brief history of high performance liquid chromatography. It is estimated that more than 80 % of modern liquid chromatographic separations use the reversed-phase liquid chromatography (RPLC) mode. However, there is a lack of understanding of the details of solute retention despite the enormous popularity of RPLC. The most rigorous treatment to date is the solvophobic theory. The basic premise of the theory is reasonable and agreement with experiment is generally good, but the description is incomplete in which it does not provide a sufficiently detailed explanation of the dependence of solute retention on the stationary phase variables.

This dissertation research is composed of two related studies areas on the description of solute retention mechanism. These two studies are discussed in separate parts of the dissertation. Prior to discussion the background of the studies is presented in Part I.

First, in Part II, the influence of the derivative skeleton structure and substituted groups on solute retention is investigated from the thermodynamic point of view in order to elucidate the solute-stationary phase interaction. The difference of the two kinds of carbonyl derivative isomers in elution order is explored with respect to the derivative skeleton structure. The effects of the position and species of substituted groups on solute retention are examined by comparing the thermodaynamic retention behaviours. The interaction between the stationary phase and alkyl chains of the carbonyl derivatives is discussed from the thermodynamic point of view.

Secondly, in Part III, the amount of adsorbed modifier is determined as the surface excess amount in order to consider the contribution of adsorption at the mobile phase-stationary phase interface to solute retention. The effect of the pore size on the surface excess amount is examined in order to determine whether it can be used as a measure of the comparison of the chemically modified surfaces. This result is reasonable to be used as a measure of the comparison of the chemically modified surfaces. The comparisons of the monomeric and polymeric phases on the surface excess amount are examined in order to relate the difference between the two phases to their surface excess amount. The measurings of the surface excess amount on the native and modified surfaces are made to characterize the surface properties of unmodified and chemically modified silicas. The surface state of the stationary phase in acetonitrile-water and methanol-water systems is discussed from the thermodynamic point of view because the surface excess isotherms reflect the difference in the miscible state between the two systems.

Finally, in Part IV, the results obtained from each chapter are related to one another. It is indicated that the thermodynamic parameters obtained from the capacity factors give information about the dependence of solute retention on the stationary phase.

PREFACE

Reversed-phase liquid chromatography(RPLC) is playing an increasingly important role in high performance liquid chromatography. This is essentially due to the development of chemically bonded phases, which are much easier to use than mechanically held packings. But, it is generally acknowledged that there is a lack of understanding of the details of solute retention despite the enormous popularity of RPLC.

The solute distribution process, i.e., the retention mechanism, is modeled by invoking solvophobic interaction. The basic premise of the theory is reasonable and agreement with experiment is generally good, but, the description is incomplete in which it does not provide a sufficiently detailed explanation of the dependence of solute retention on the stationary phase.

This dissertation describes works focussed on the contribution of the stationary phase to solute retention in RPLC. Studies areas: effect of the derivative skeleton structure on solute retention; surface state of the packing materials.

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PART I

INTRODUCTION

Part I

Introduction

Reversed-phase liquid chromatography (RPLC), which may be broadly classified as involving the distribution of nonpolar or moderately polar solute between a polar mobile phase and a relatively nonpolar stationary phase, has developed into a highly advantageous and successful separation technique. Indeed, it is estimated that more than 80 % of modern liquid chromatographic separations are operated in the RPLC mode.

It is generally acknowledged (1-4) that there is a lack of understanding of the details of solute retention despite the enormous popularity of RPLC. Such an understanding is crucial for informed control and manipulation of separations, which ultimately require a sufficiently detailed description of how retention depends on the various kinds of mobile and stationary phases.

The nature of the solute distribution process in RPLC, i.e., the retention mechanism, has been a topic of much study, discussion and speculation (1-2, 4-9). The most rigorous treatment to date is the solvophobic theory put forth by Horvath and co-workers (6, 9). It focuses on the important role of the mobile phase, as was recognized at an earlier stage by Locke (5). Solute distribution is modeled by invoking "solvophobic" interactions, i.e., exclusion of the less polar solute molecule from the polar mobile phase with subsequent sorption by the nonpolar stationary phase. The basic premise of the theory is reasonable and agreement with experiment is generally good, but, as has been pointed out (2), the description is incomplete in which it does not provide a sufficiently detailed explanation of the dependence of solute retention on the stationary phase variables.

Therefore, the study of the stationary phase for use in RPLC is a matter of great interest. The most prevalent type of modern RPLC column packing is prepared by reacting uniformly small (5-10 μm in diameter) and porous (typical average pore diameter of < 30 nm) silica gel particles with, e.g., dimethyl octadecylchlorosilane or its alkyl counterpart, generally followed by reaction with trimethylchlorosilane to eliminate accessible, residual silanols ("end capping"). The bulk of this surface modification, resulting in a monolayer of alkyl chains, takes place within the high surface area of pores, which, to first approximation, may be taken as cylindrically shaped (10). So-called polymeric phase is formed by reaction with, e.g., dichloro- or trichlorosilanes. The actual topology on the molecular level is not known, but the modified surface is believed to be rather uniform and nonpolar. A variety of techniques for probing chemically modified surfaces have been employed, including fluorescence (11), infrared (12-15) and nuclear magnetic resonance (15-20) spectrometry. Information obtained by these techniques has provided important insights into surface-ligand structures and interactions, bonded layer solvation and segmental and total chain mobility.

In particular, the structure and the composition of the chemically modified surface in the presence of different solvents have been subjects of much study and discussion (1,2,8-10, 21-23) because of their directly bearing on the solute distribution process. What is clear at this point is that the extent to which these alkyl-modified surfaces can be swollen depends on the alkyl chain length and the nature of the solvent. The picture is that of a "breathing" surface which adjusts itself to maintain a relatively nonpolar character.

In this study, first, the influence of the derivative skeleton structure and substituted groups on solute retention is investigated from the thermodynamic point of view in order to elucidate the solute-stationary phase interaction. The difference of the two kinds of carbonyl derivative isomers in elution order is explored with respect to the derivative skeleton structure. The effects of the position and species of substituted groups on solute retention are examined by comparing the thermodaynamic retention behaviours. The interaction between the stationary phase and alkyl chains of the carbonyl derivatives is discussed from the thermodynamic point of view. Secondly, in order to consider the contribution of adsorption at the mobile phase-stationary phase interface to solute retention, the amount of adsorbed modifier is determined as the surface excess amount. The effect of the pore size and the comparisons of the monomeric and polymeric phases and of the native and modified surfaces on the surface excess amount are examined, finally the surface state of the stationary phase is discussed from the thermodynamical point of view. In the second part, the capacity factor is related to Gibbs free energy equation in order to consider thermodynamic aspects of solute retention.

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PART II

THERMODYNAMIC ELUCIDATION OF RETENTION BEHAVIOUR OF CARBONYL DERIVATIVES

Introduction

Thermodynamics in HPLC

In liquid chromatography, temperature often serves only a minor role in the development of separation methods. Instead, solute retention is usually controlled by easily adjusted parameters such as mobile phase composition and flow rate. In contrast to gas chromatography, the allowable range for column temperatures in liquid chromatography, is restricted by the properties of the mobile phase. Increased viscosity and freezing are limiting factors at low temperatures; at high temperatures boiling is a limiting consideration. Separations are commonly carried out under ambient conditions, although greater reproducibility in retention and quantitation result when isothermal conditions are established. The use of elevated column temperature has been reported as a way of increasing separation efficiency, particularly for large molecular weight solutes. Elevated column temperatures have also been used to take advantage for solutes having limited solubility in the mobile phase.

Thermodynamic aspects of solute retention have been studied in some detail. In general, solute retention is inversely related to temperature, i.e., retention decreases with increasing temperature. One of the first thermodynamic investigations was made by Knox and Vasvari (1). Knox plotted ln k' vs 1/T for a group of compounds separated on Permaphase ETH and ODS columns.

The resultant van't Hoff plots gave absolute enthalpies and relative entropies of transfer for the solutes. Since this early work, Guiochon and Horvath (2, 3) have worked in advancing our understanding of separation mechanisms through various thermodynamic considerations.

Solute retention is usually expressed in terms of the capacity factor, k', which is defined as (number of moles of solute in stationary phase)/(number of moles of solute in mobile phase). The capacity factor is proportional to the equilibrium constant K and can be written.

$$k' = \phi K \tag{1}$$

The constant of proportionality (ϕ) is the phase ratio (volume of stationary phase)/(volume of mobile phase). Gibbs free energy is related to the equilibrium constant in equation 2 and

$$\Delta G^0 = -RT\ln K \tag{2}$$

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 \tag{3}$$

equation 3 is the familiar relationship among standard enthalpies, entropies and free energy. R is the gas constant and T is the absolute temperature K. By combining equations 1-3, the capacity factor can be expressed in terms of standard enthalpies and entropies of transfer from mobile phase to stationary phase as shown as equation 4. If ΔH^0 and ΔS^0 are

independent of temperature over the temperature interval of

$$\ln k' = -\Delta H^0/RT + \Delta S^0/R + \ln \phi \tag{4}$$

interest, a plot of ln k' vs. 1/T should be linear. While the slope of this line gives standard enthalpies of transfer, standard entropies of transfer are calculated from the intercept and are thus dependent on the value of the phase ratio. It is important to note that ΔH^0 values are independent of the phase ratio. In addition, any uncertainity in the phase ratio affects ΔS^0 values equally.

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II-1

Effect of Derivative Skeleton Structure on Retention Behaviour of Carbonyl Derivatives

Introduction

Reversed-phase liquid chromatography (RPLC) is a rapidly growing separation technique that is successfully employed in a diverse array of analytical and preparative schemes. Despite the popularity and wide applicability of RPLC, a complete description of the solute retention mechanism has not yet been formulated. It is known that a major factor in determining the selectivity of a reversed-phase system is the degree of aqueous character of the mobile phase, and solute distribution has been modeled by invoking a solvophobic interaction, i.e., an exclusion of the more or less hydrophobic solute molecule from the polar solvent with subsequent sorption of the solute by the hydrocarbonaceous bonded phase where it is held relatively weak dispersion forces(1). While there is generally good agreement between theory and experiment when the solvophobic interaction model is applied, this description is incomplete in that it does not produce a sufficiently detailed explanation of the role of the bonded phase in solute distribution and does not explain the observation that the carbon chain length of the bonded alkyl ligand affects the selectivity, as well as the capacity factors, in reversed-phase systems(2-5).

The awareness of the role of temperature in RPLC has increased in recent years. Using a totally aqueous mobile phase and surfaces with chemically attached midrange hydrocarbons, unique changes in solute retention were observed as a function of increasing temperature(6). The separation of polycyclic aromatic hydrocarbons has been studied thermodynamically(7).

In this study, the author reports the thermodynamic behaviour of two kinds of carbonyl derivatives in RPLC using aqueous methanol as the eluent, and consider the effect of the derivative skeleton structure on retention. The point of this work is initiated by the following experimental fact: when two derivative types of aldehydes and their isomeric ketones, which are 2,4-dinitrophenylhydrazones(2,4-DNPH) and 1-dimethylamino-naphthalene-5-sulfonylhydrazones(dansylhydrazone), were separated by RPLC with aqueous methanol eluents, those of isomers were eluted in the reverse order to each other.

Experimental

Apparatus

The liquid chromatograph consisted of a TRI ROTAR pump, a VL-611 variable-loop injector and an UVIDEC 100-II variable-wavelength UV detector or a FP-110 fluorescent detector (Jasco, Tokyo, Japan). A constant-temperature water-bath (Shimizu Rikagaku, Tokyo, Japan) was used to maintain the column temperature, which was measured by an alumel-chromel thermocouple. The chromatograms were recorded on a Chromatopac C-

R1A (Shimadzu Seisakusho, Tokyo, Japan).

Chemicals and Materials

All the reagents used were obtained from Tokyo Kasei (Tokyo, Japan). A C_{18} bonded phase was prepared as follows. Prior to reaction, the silica (Develosil 60-5, Nomura Chemical, Seto, Japan) was dried at 220 °C for 4h. Dry silica (4g) was suspended in 100 ml of dry toluene, and 8 mmol of chlorodimethyloctadecyl-silane and 5 ml of pyridine were added to the slurry in succession. The mixture was then refluxed. After 12h it was filtered, washed with chloroform and methanol, and dried at 110 °C. The samples used were aliphatic saturated aldehydes from methanal to heptanal and their isomeric ketones, which were converted to the corresponding 2,4-DNPHs and dansylhydrazones, respectively. 2,4-DNPHs were prepared as follows: 2,4-dinitrophenylhydrazine was dissolved in 10% sulphuric acid and a carbonyl compound was then added. The precipitated 2,4-DNPH was filtered off and recrystallized from ethanol. In order to avoid overlapping of peaks, two mixtures of 2,4-DNPHs were prepared, i.e., a first mixture would contain derivatives from aldehydes and 3-ketones, a second mixture from 2-ketones. Dansylhydrazones were prepared as follows: dansylhydrazine was dissolved in methanol and carbonyl compound was then added. The mixture was heated at 80 °C for 20 min and water was added after cooling. The aqueous mixture was extracted with dichloromethane. In order to avoid overlapping of peaks, three mixtures of dansylhydrazones in dichloromethane were prepared, i.e., a first mixture would contain derivatives of aldehyde from propanal to heptanal, a second mixture from 2-ketones and a third mixture from 3-ketones, methanal and ethanal.

Procedures

The prepared bonded material, which is a spherical octadecylsilica containing 16.7% of carbon, was slurry-packed into a 150 x 4.6 mm I.D. stainless-steel tube. The mobile phases were mixtures of methanol-water(70, 75 and 80%(v/v)) at a flow-rate of 0.7 ml/min. The retention time of N,N-dimethyl-5-naphthylamine-1-sulphonic acid was taken as the column dead volume marker, t_o, and the capacity factor, k', was evaluated from the retention time of the solute, t_R, by the relationship $k' = (t_R - t_o)/t_o$.

van't Hoff plots

For each solute the retention is given by the following equation

$$\ln k' = -\Delta H^0/RT + \Delta S^0/R + \ln \phi \qquad (4)$$

where ΔH^0 is the partial molar enthalpy change for the transfer of the solute from the infinite dilution standard state in the mobile phase to the infinite dilution standard state in the stationary phase, ΔS^0 is the associated change in standard entropy, R the gas constant and ϕ the phase ratio. If ΔH^0 and ΔS^0 are independent of temperature over the temperature range of interest, a plot of ln k' vs 1/T will be linear. While the slope of this line gives standard enthalpies of transfer, standard entropies of transfer are calculated from the intercept and they are dependent on the value of the phase ratio. Enthalpies and entropies of the transfer were calculated by using linear regression analysis of van't Hoff plots.

Results and Discussion

For the two kinds of carbonyl derivatives and each mobile phase composition all the experimental plots of ln k' vs 1/T are linear over the temperature range between 30 and 70 °C. Some experimental results are shown in Figures 1 and 2. The slopes and intercepts of these lines are regarded as ΔH^0 and ΔS^0, respectively, since R is the gas constant and the phase ratio is (V_s/V_m) assumed to be approximately constant over this condition. The results are listed in Table I and II, for the respective derivatives of aldehydes and their isomeric ketones. In Figure 1, the similar retention behaviours are observed among 2,4-DNPH of aldehyde and isomeric ketone, and 2,4-DNPHs of aldehydes are slightly faster eluted than those of other isomers. In contrast to the data in Figure 1, dansylhydrazones of aldehydes are more strongly retained than those of other isomers, and methanal dansylhydrazone shows abnormally strong retention as can be seen in Figure 2. Comparison of Table I with II reveals that the values of ΔH^0 and ΔS^0 are similar to each other among 2,4-DNPHs isomers and distinct from one another among dansylhydrazones

TABLE I

THE VALUES OF $-\Delta H^0/R$ AND $\Delta S^0/R + \ln \phi$ IN AQUEOUS 75% METHANOL

2,4-DNPH		$-\Delta H^0/R$	$\Delta S^0/R + \ln \phi$
Methnal	(1)	1.10	-2.67
Ethanal	(2)	1.25	-2.88
Propanal	(3-1)	1.44	-3.17
Propanone	(3-2)	1.48	-3.26
Butanal	(4-1)	1.62	-3.45
Butanone	(4-2)	1.69	-3.57
Pentanal	(5-1)	1.85	-3.81
2-Pentanone	(5-2)	1.86	-3.77
3-Pentanone	(5-3)	1.88	-3.80
Hexanal	(6-1)	2.11	-4.23
2-Hexanone	(6-2)	2.09	-4.14
3-Hexanone	(6-3)	2.06	-4.02
Heptanal	(7-1)	2.36	-4.62
2-Heptanone	(7-2)	2.33	-4.49
3-Heptanone	(7-3)	2.27	-4.31

TABLE II

THE VALUES OF $-\Delta H^0/R$ AND $\Delta S^0/R + \ln \phi$ IN AQUEOUS 70% METHANOL

Dansylhydrazones		$-\Delta H^0/R$	$\Delta S^0/R + \ln \phi$
Methnal	(1)	1.23	-2.63
Ethanal	(2)	0.97	-2.22
Propanal	(3-1)	1.08	-2.29
Propanone	(3-2)	1.00	-2.13
Butanal	(4-1)	1.21	-2.42
Butanone	(4-2)	1.16	-2.35
Pentanal	(5-1)	1.44	-2.77
2-Pentanone	(5-2)	1.30	-2.46
3-Pentanone	(5-3)	1.27	-2.37
Hexanal	(6-1)	1.68	-3.15
2-Hexanone	(6-2)	1.52	-2.81
3-Hexanone	(6-3)	1.43	-2.56
Heptanal	(7-1)	1.93	-3.53
2-Heptanone	(7-2)	1.77	-3.18
3-Heptanone	(7-3)	1.66	-2.88

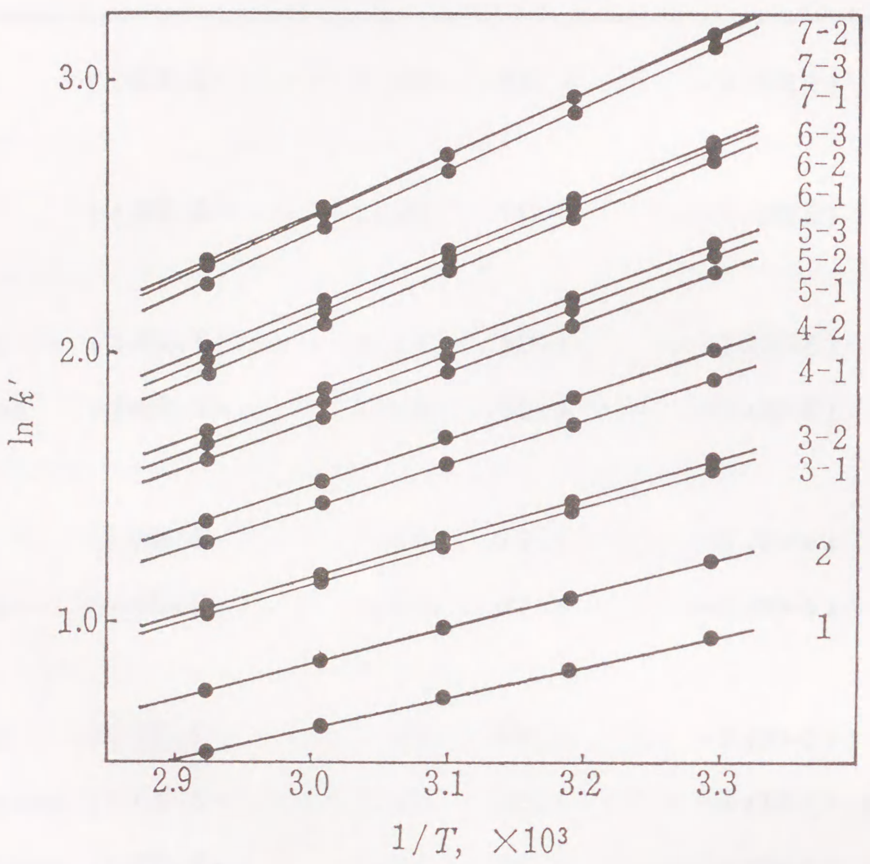

Figure 1. Logarithm of capacity factor k' versus reciprocal of temperature, 1/T(K)

Samples : 2,4-DNPH; Mobile phase : 75% methanol.

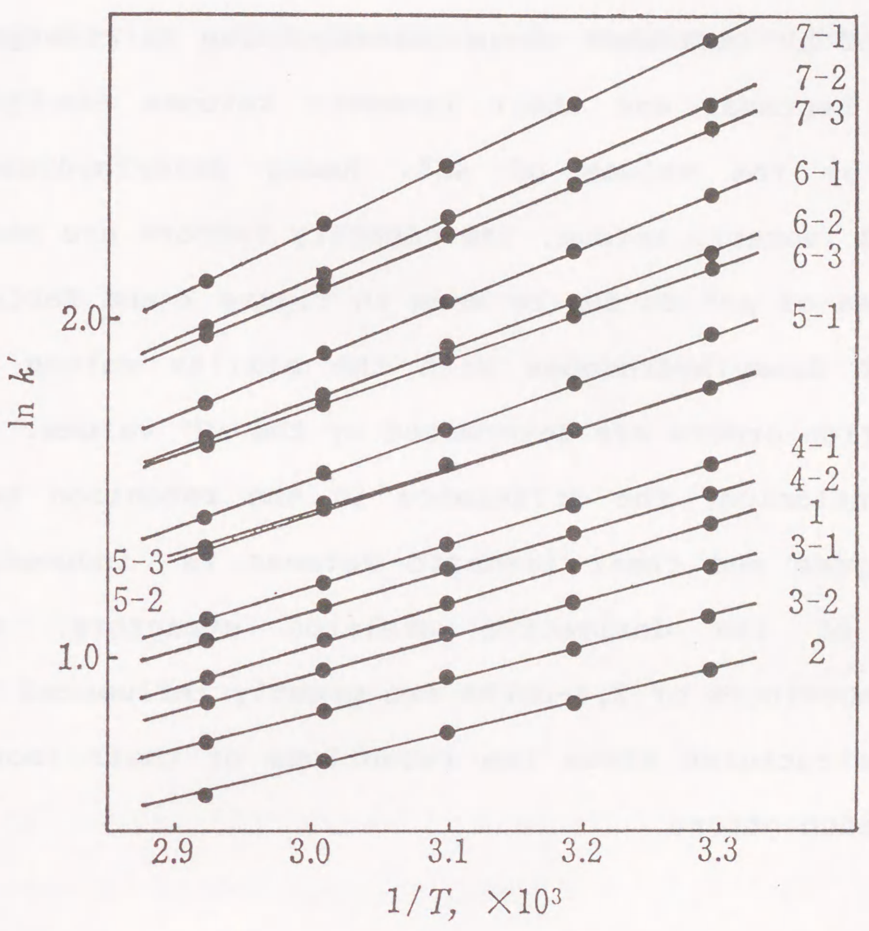

Figure 2. Logarithm of capacity factor k' versus reciprocal of temperature, 1/T(K)

Samples : Dansylhydrazones; Mobile phase : 70% methanol.

isomers. It can be seen from Figure 1 and Table I that the capacity factors of 2,4-DNPHs corresponding to aldehydes from methanal to pentanal and their isomeric ketones are controlled by the values of ΔH^0 and that those corresponding to aldehydes from hexanal to heptanal and their isomeric ketones are gradually influenced by the values of ΔS^0. Among dansylhydrazones of aldehyde and isomeric ketone, the capacity factors are controlled by the values of ΔH^0 as can be seen in Figure 2 and Table II. In the case of dansylhydrazones with the similar values of ΔH^0, their retention orders are determined by the ΔS^0 values.

In conclusion, the difference in the retention behaviour among aldehydes and their isomeric ketones is induced by the difference of the derivative skeleton structure, and the retention behaviours of 2,4-DNPHs are greatly influenced by their derivative structures since the retentions of their isomers are similar to each other.

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II-2

Effect of Nitro Group on Retention Behaviour of Carbonyl Derivatives

Introduction

Derivatization methods in RPLC are aimed at giving a high response in detection, an improved or enhanced selectivity in separation and an elimination of interference from co-existed materials. The separation of the derivatives is influenced by derivative skeleton structures. It is important to investigate the structure-retention relationship in order to completely describe the solute retention mechanism in RPLC. Several studies concerning the solute retention have been carried out by using substituted benzenes(1), isomeric alkylbenzenes(2), barbiturates (3) and isomeric benzamids(4) as sample solutes.

In this section, previous work has been extended. The retention behaviours of 2,4-dinitrophenylhydrazones are found to be greatly influenced by the derivative skeleton(5). The thermodynamic behaviours of phenylhydrazones(PH), o- and p-nitrophenylhydrazones(o-, p-NPH) and 2,4-dinitrophenylhydrazones (2,4-DNPH) corresponding to aldehydes from methanal to heptanal and their isomeric ketones will be described in order to investigate the influence of nitro group on retention behaviour of derivatives.

Experimental

Apparatus

The liquid chromatograph consisted of a TRI ROTAR pump, a VL-611 variable-loop injector and an UVIDEC 100-II variable-wavelength UV detector (Jasco, Tokyo, Japan). A constant-temperature water-bath (Shimizu Rikagaku, Tokyo, Japan) was used to maintain the column temperature, which was measured by an alumel-chromel thermocouple. The chromatograms were recorded on a Chromatopac C-R1A (Shimadzu Seisakusho, Tokyo, Japan). Nuclear magnetic resonance spectrometry of the derivatives was carried out by FT-NMR-FX-90Q (JEOL, Tokyo, Japan).

Chemicals and Materials

All the reagents used were obtained from Tokyo Kasei (Tokyo, Japan). 2,4-DNPHs which were prepared in the previous work (5) were used. O- and p-NPHs were prepared as follows: o- and p-nitrophenylhydrazines were dissolved in 50 % acetic acid and a carbonyl compound was added to the solution. The precipitated o- or p-NPH was filtered off and recrystallized from aqueous ethanol. Unprecipitated o-NPH was extracted by dichloromethane from the aqueous mixture. PH was prepared as follows: phenylhydrazine hydrochloride was dissolved in water and the carbonyl compound was then added. PH in the aqueous mixture was extracted by dichloromethane. Some mixtures of the derivatives were prepared in order to avoid overlapping of peaks.

Procedures

The prepared bonded material, which is a spherical octadecylsilica containing 16.7% of carbon, was slurry-packed into a 150 x 4.6 mm I.D. stainless-steel tube. The mobile phases were mixtures of methanol-water(70, 75 and 80%(v/v)) at a flow-rate of 0.7 ml/min. The retention time of N,N-dimethyl-5-naphthylamine-1-sulphonic acid was taken as the column dead volume marker, t_0, and the capacity factor, k', was evaluated from the retention time of the solute, t_R, by the relationship $k' = (t_R - t_0)/t_0$.

van't Hoff plots

For each solute the retention is given by the following equation

$$\ln k' = -\Delta H^0/RT + \Delta S^0/R + \ln \phi \qquad (4)$$

where ΔH^0 is the partial molar enthalpy change for the transfer of the solute from the infinite dilution standard state in the mobile phase to the infinite dilution standard state in the stationary phase, ΔS^0 is the associated change in standard entropy, R the gas constant and ϕ the phase ratio. If ΔH^0 and ΔS^0 are independent of temperature over the temperature range of interest, a plot of ln k' vs 1/T will be linear. While the slope of this line gives standard enthalpies of transfer, standard entropies of transfer are calculated from the intercept and they are dependent on the value of the phase ratio. Enthalpies and entropies of the transfer were calculated by using linear regression analysis for van't Hoff plots.

Results and Discussion

All the experimental plots of ln k' vs 1/T are linear over the temperature range between 30 and 70 °C with aqueous 75 % methanol as a eluent. The results of four derivatives of heptanal and its isomers are shown in Figure 3 as an example. Those of four derivatives of other aldehydes and their isomeric ketones are similar to Figure 3. It can be seen from Figure 3 that o-NPHs are more strongly retained than other derivatives, and that aldehyde derivatives are more strongly retained than other isomeric ketones in PHs and p-NPHs and the reverse fact is found for 2,4-DNPHs and o-NPHs show similar retention values to each other. The large chromatographic retention of o-NPHs is ascribed that intramolecular hydrogen bond can be formed between the hydrazone -NH- and the oxygen of the nitro group. The formation of the hydrogen bond, which is observed by the shift of the signal of the proton of the hydrazone to low magnetic field on NMR, causes o-NPHs to be fixed in a planar conformation by an intramolecular six-membered ring, and hence inhibits to decrease the number of hydrophobic sites. The earlier elution of 2,4-DNPHs than o-NPHs can be explained that para substituted nitro group produces an increase in solute-solvent association. But, the para substituted nitro group with 2,4-DNPH, which is not the same as p-NPH, is difficult to have a coplanar structure to the

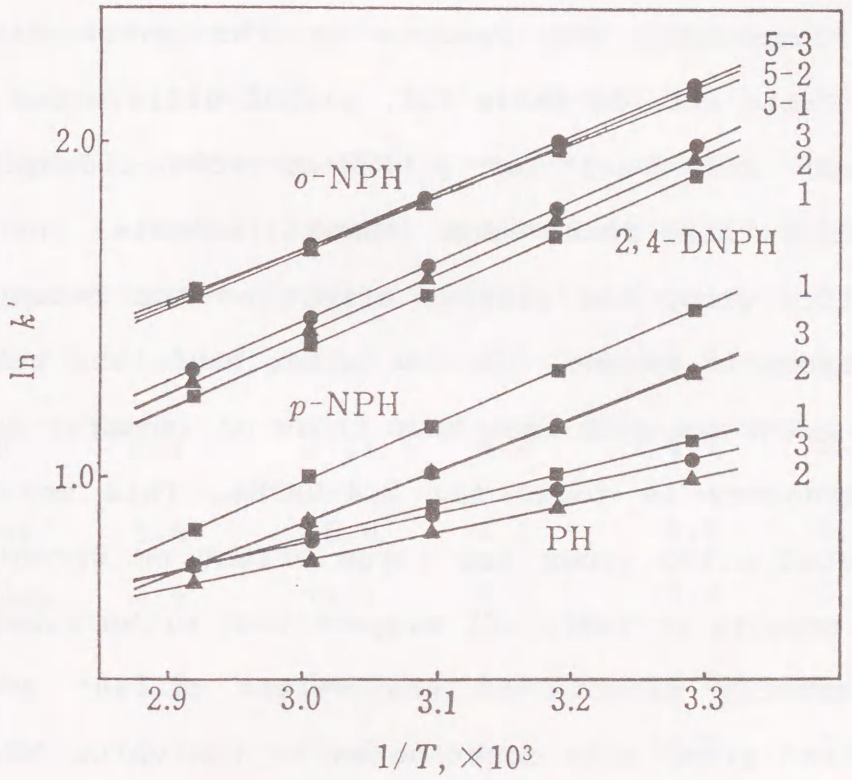

Figure 3. Logarithm of capacity factor(k') versus reciprocal of temperature(1/T)

■: aldehyde, ▲: 2-ketone, ●: 3-ketone; PH : Phenylhydrazones, p-NPH : p-nitrophenylhydrazones, o-NPH : o-nitrophenylhydrazones, 2,4-DNPH : 2,4-dinitrophenylhydrazones; 5-1 : Pentanal, 5-2 : 2-Pentanone, 5-3 : 3-Pentanone.

benzene ring.

The effect of nitro group can be interpreted by subtracting ΔH^0 and $(\Delta S^0/R + \ln \phi)$ of PH from those of other derivatives for each set of compounds. The results of the subtraction are summarized in Table III. In Table III, slight differences in the values of $-\Delta\Delta H^0$ and $-\Delta\Delta S^0$ of p-NPHs between aldehydes and isomeric ketones are found and these indicate that para substituted nitro group has similar effect on the retention of aldehyde and isomeric ketone. On the other hand, the values of aldehydes for o-NPH are much less than those of isomeric ketones. The similar tendency is found for 2,4-DNPHs. This means that ortho substituted nitro group has large effect on retention of ketones. The results in Table III suggest that ortho substituted nitro group greatly affects to the values of ΔH^0 and para substituted nitro group also contributes to the value of ΔS^0 of ketone derivative. The values for 2,4-DNPHs are not equal to the sum of their values for para and ortho positions because para substituted nitro group of 2,4-DNPH is not the same as p-NPH.

In conclusion, the retention behaviours of phenylhydrazones are influenced by the substituted position of nitro group. At the para position, nitro group has similar effect on aldehydes and isomeric ketones to resulting in great retention of aldehydes, while nitro group has great effect on ketone at the ortho position to resulting in similar retentions of aldehydes and isomeric ketones.

TABLE III

EFFECT OF SUBSTITUTED NITRO GROUPS ON THE VALUES OF $\Delta\Delta H^0$ AND $\Delta\Delta S^0$ IN AQUEOUS 75% METHANOL

	p-NPH		o-NPH		2,4-DNPH	
	$-\Delta\Delta H^0$ (kJ/mol)	$-\Delta\Delta S^0$ (J/molK)	$-\Delta\Delta H^0$ (kJ/mol)	$-\Delta\Delta S^0$ (J/molK)	$-\Delta\Delta H^0$ (kJ/mol)	$-\Delta\Delta S^0$ (J/molK)
Methanal	4.3	12.5	3.2	5.0	5.0	12.5
Ethanal	5.1	14.1	4.2	6.7	5.7	12.5
Propanal	5.6	15.0	4.2	5.8	6.1	13.3
Propanone	4.8	14.1	5.7	10.0	7.3	16.6
Butanal	5.9	16.6	4.3	5.8	6.4	14.1
Butanone	5.7	16.6	6.7	13.3	7.6	17.5
Pentanal	6.1	17.5	4.6	6.7	6.4	15.0
2-Pentanone	5.8	16.6	6.8	13.3	7.7	17.5
3-Pentanone	5.9	16.6	6.7	12.5	7.8	18.3
Hexanal	6.3	17.5	4.9	7.5	6.5	15.0
2-Hexanone	6.1	17.5	7.1	14.1	7.8	18.3
3-Hexanone	6.0	17.5	6.9	13.3	7.9	18.3
Heptanal	6.4	18.3	5.0	7.5	6.5	15.0
2-Heptanone	6.3	18.3	7.7	15.0	7.9	17.5
3-Heptanone	6.1	17.5	7.3	14.1	7.9	18.3

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II-3

Effect of Substituent Groups on Retention Behaviour of Aromatic Aldehyde Derivatives

Introduction

The retention behaviours of a solute are reflected by all of interactions which are presumed to be present in RPLC mechanism, although some of those may compensate each other, and it gives an important information for the interpretation of the retention mechanism. Various compounds, particularly homologous series and structural isomers, have been used as solutes (1-6). Homologous series are said to be useful because they allow differentiation between the non-specific contribution to retention by the aliphatic chain and the specific contribution of the remaining structure. It is clear that the retention behaviour of homologous series is characterized by this specific contribution.

The author has an interest in the contribution of the interactions of the molecular residue of homologous series with the mobile phase to retention, and recently she investigated it by using derivatized compounds as sample probes(7-10). One call the molecular residue "the derivative skeleton", i.e., the skeleton of the parent compound.

In this section she reports the retention behaviour of aromatic aldehyde derivatives in RPLC using aqueous methanol and acetonitrile as the eluent, and considers the effect of the derivative skeleton to their retention. The derivatives used are phenylhydrazones (PHs), p-nitrophenylhydrazones (p-NPHs) and 2,4-dinitrophenylhydrazones (2,4-DNPHs) of the aromatic aldehydes which are benzaldehyde (B), chlorobenzaldehyde (-Cl), hydroxybenzaldehyde (-OH), methylbenzaldehyde ($-CH_3$) and nitrobenzaldehyde ($-NO_2$). Also this describes the influence of these substituent groups on their retention. Unless otherwise noted, substituent groups are referred to those of benzaldehyde. Parent group is assumed as benzaldehyde.

Experimental

Apparatus

The liquid chromatograph consisted of a TRI ROTAR pump, a VL-611 variable-loop injector and an UVIDEC 100-II variable-wavelength UV detector set at 280 nm(Jasco, Tokyo, Japan). A constant-temperature water-bath(Model ESP-47, Toyo, Tokyo, Japan) was used to maintain the column temperature, which was measured by an alumel-chromel thermocouple. The chromatograms were recorded on a Chromatopac C-R1A (Shimadzu Seisakusho, Tokyo, Japan).

Chemicals and Materials

All the reagents used were obtained from Tokyo Kasei (Tokyo, Japan). PHs, p-NPHs and 2,4-DNPHs of aromatic aldehydes were prepared in similar manner in the previous work (10). Some

mixtures of the derivatives were prepared considering the elution order to avoid overlapping of those peaks.

Procedures

The prepared bonded material, which is a spherical octadecylsilica containing 15.9% of carbon, was slurry-packed into a 150 x 4.6 mm I.D. stainless-steel tube. The mobile phases consisted of aqueous 75 % (v/v) methanol and aqueous 70 % (v/v) acetonitrile at a flow-rate of 0.7 ml/min. The retention time of N,N-dimethyl-5-naphthylamine-1-sulphonic acid was taken as the column dead volume marker, t_0, and the capacity factor, k', was evaluated from the retention time of the solute, t_R, by the relationship $k' = (t_R - t_0)/t_0$.

van't Hoff plots

For each solute the retention is given by the following equation

$$\ln k' = -\Delta H^0/RT + \Delta S^0/R + \ln \phi \qquad (4)$$

where ΔH^0 is the partial molar enthalpy change for the transfer of the solute from the infinite dilution standard state in the mobile phase to the infinite dilution standard state in the stationary phase, ΔS^0 is the associated change in standard entropy, R the gas constant and ϕ the phase ratio. If ΔH^0 and ΔS^0 are independent of temperature over the temperature range of interest, a plot of ln k' vs 1/T will be linear. While the slope of this line gives standard enthalpies of transfer, standard entropies of transfer can be calculated from the intercept of the plot and they are dependent on the phase ratio value. Enthalpies and entropies of the transfer were calculated by using linear regression analysis for van't Hoff plots.

Results and Discussion

The effect of the derivative skeleton

In order to investigate the effect of the derivative skeleton on the retention, one can compare the capacity factors of three kinds of derivatives obtained from various aromatic aldehydes. The capacity factors determined at 30 °C are illustrated in Figures 4 and 5, where the respective mobile phases were aqueous 75 % (v/v) methanol and aqueous 70 % (v/v) acetonitrile.

The retention behaviours of the derivatives observed are greatly affected by the derivative skeletons, although a difference exists in the retention order between 75 % methanol and 70 % acetonitrile mobile phase. In the case of 75 % methanol mobile phase, the derivatives are retained in the order PH < p-NPH < 2,4-DNPH, and this corresponds to the increase of the size of the derivative skeleton. In the case of 70 % acetonitrile mobile phase, the p-NPH derivative is eluted more rapidly than the others and the elution order is p-NPH < PH < 2,4-DNPH. This may suggest the existence of specific interactions between the nitro group and acetonitrile in the mobile phase(11).

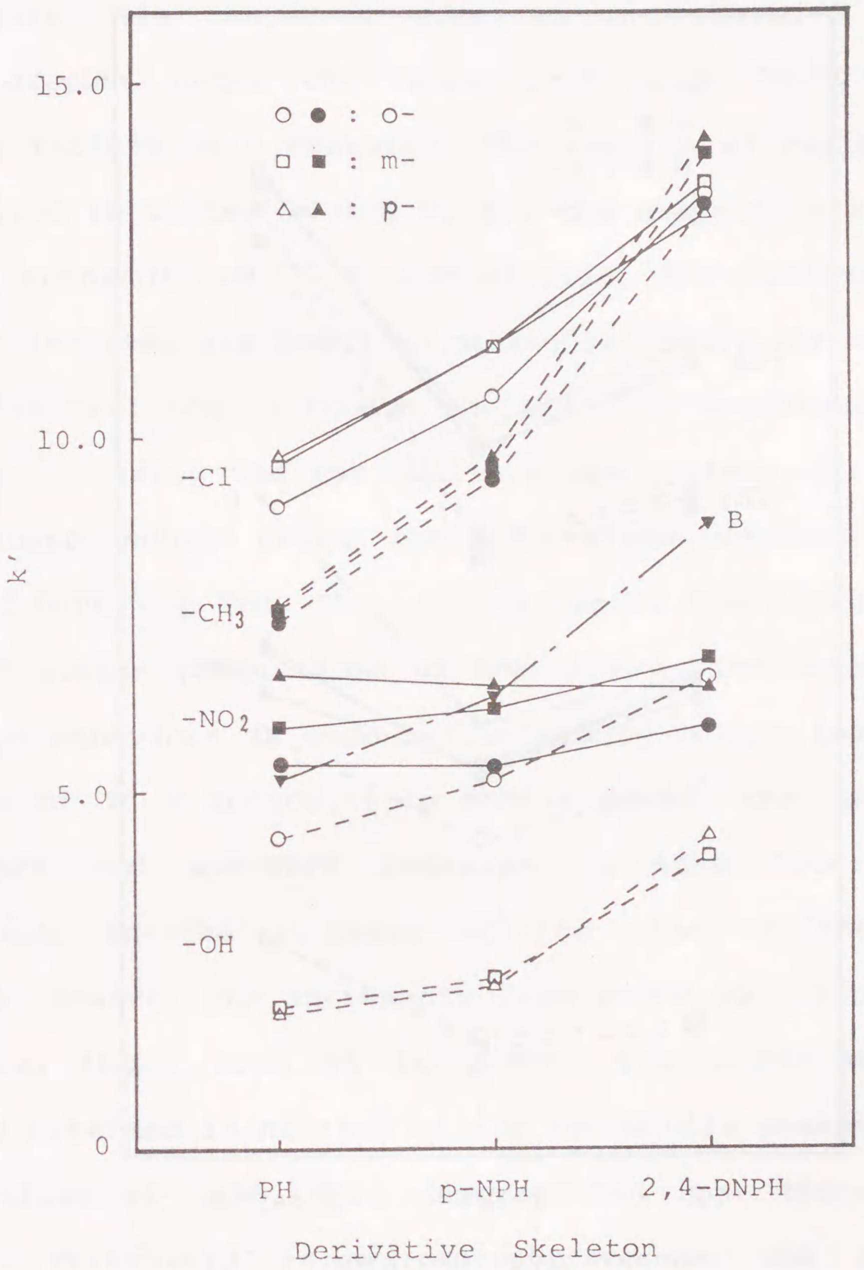


Figure 4. Retention behaviour of three kinds of derivatives in aqueous 75% methanol

B : Benzaldehyde; -OH : hydroxybenzaldehyde; $-NO_2$: nitrobenzaldehyde; $-CH_3$: methylbenzaldehyde; -Cl : chlorobenzaldehyde.

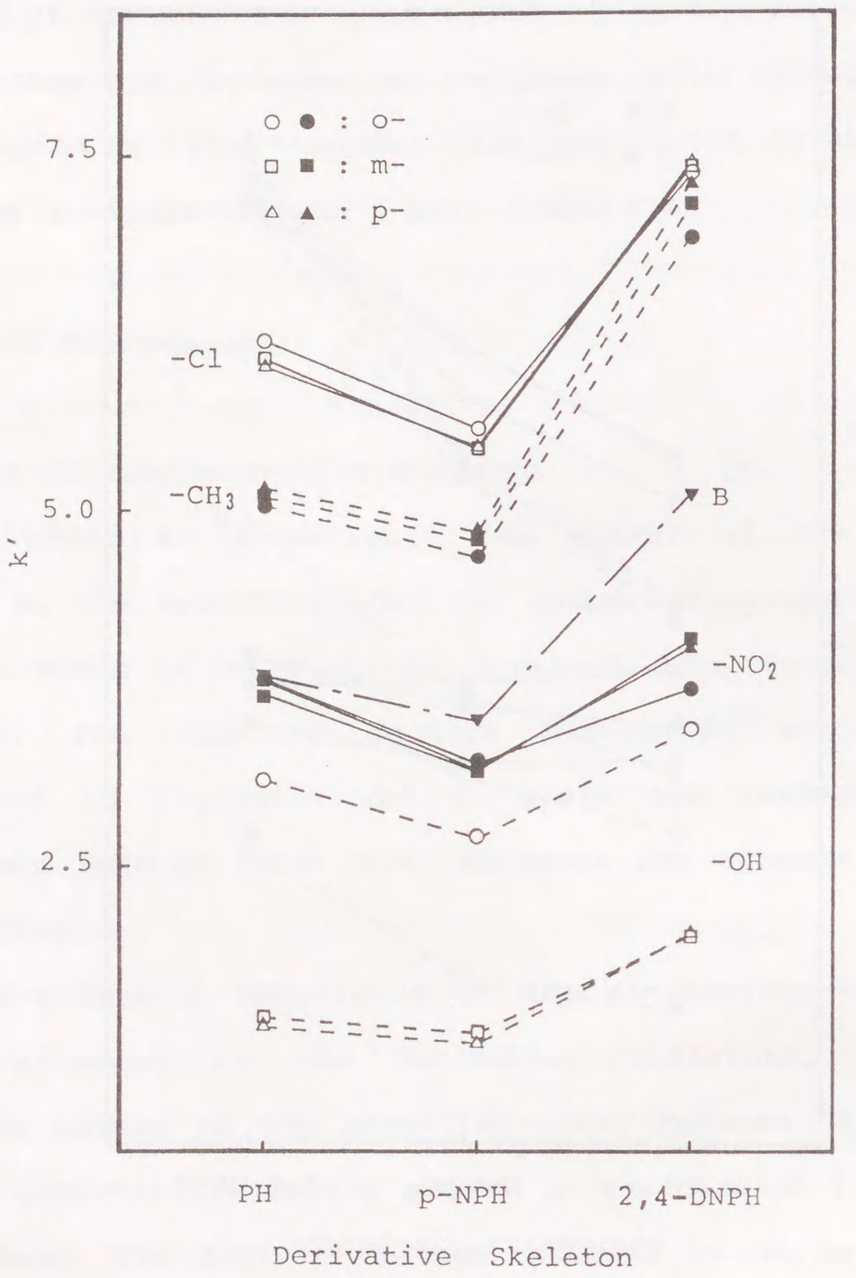


Figure 5. Retention behaviour of three kinds of derivatives in aqueous 70% acetonitrile Details as in Figure 4.

In order to examine the dependence of retention on temperature, the compounds appeared in Figures 4 and 5 were chromatographed under the temperature range 30-70 °C and the capacity factors were measured. The results of van't Hoff plots are listed in Tables IV and V, for the respective mobile phases of 75 % methanol and 70 % acetonitrile. The $-\Delta H^0$ values of the three derivatives are found to be significantly influenced by the derivative skeleton, although the order of magnitude of $-\Delta H^0$ is different in using the two mobile phase systems. In the case of 75 % methanol mobile phase, the $-\Delta H^0$ values increase in the order PH < 2,4-DNPH < p-NPH. The $-\Delta H^0$ values of the p-NPH derivatives are much greater than those of the others, indicating that this retention behaviour is much influenced by column temperature. In the case of 70 % acetonitrile mobile phase, the $-\Delta H^0$ values of PH, p-NPH and 2,4-DNPH increase in this order, and this corresponds to the increase of the size of the derivative skeleton. However the increments from p-NPH to 2,4-DNPH are much less than those from PH to p-NPH. The p-NPH derivative is strongly retained in neither of the two mobile phases despite the large values of $-\Delta H^0$. This demonstrates that the entropy term makes a relatively large contribution to the retention in equation 4.

These results suggest that the retention behaviours of the three derivatives, except those of o-, p-nitrobenzaldehydes and m-, p-hydroxybenzaldehydes, depend mainly on the structure of the derivative skeleton and particularly the p-NPH derivatives show a characteristic behaviour under being influenced by the organic

TABLE IV

VALUES OF $-\Delta H^o$ (kJ/mol) OBTAINED AQUEOUS 75 % METHANOL

Parent aldehyde	PH	p-NPH	2,4-DNPH
o-Hydroxybenzaldehyde	11.1	16.2	15.8
m-Hydroxybenzaldehyde	7.9	12.8	14.8
p-Hydroxybenzaldehyde	9.3	13.0	15.4
o-Nitrobenzaldehyde	14.4	17.0	15.4
m-Nitrobenzaldehyde	16.2	19.5	18.6
p-Nitrobenzaldehyde	17.0	21.0	16.1
Benzaldehyde	11.7	16.5	15.4
o-Methylbenzaldehyde	12.6	17.9	16.9
m-Methylbenzaldehyde	13.0	18.1	17.5
p-Methylbenzaldehyde	12.7	17.9	17.0
o-Chlorobenzaldehyde	15.3	20.1	17.5
m-Chlorobenzaldehyde	16.0	20.6	18.9
p-Chlorobenzaldehyde	16.5	21.2	18.7

TABLE V

VALUES OF $-\Delta H^o$ (kJ/mol) OBTAINED AQUEOUS 70 % ACETONITRILE

Parent aldehyde	PH	p-NPH	2,4-DNPH
o-Hydroxybenzaldehyde	8.5	10.0	10.6
m-Hydroxybenzaldehyde	6.3	7.5	9.0
p-Hydroxybenzaldehyde	6.1	7.8	9.5
o-Nitrobenzaldehyde	9.8	10.9	10.8
m-Nitrobenzaldehyde	9.8	11.3	12.0
p-Nitrobenzaldehyde	10.4	11.7	12.3
Benzaldehyde	8.8	10.6	11.3
o-Methylbenzaldehyde	9.5	11.4	12.2
m-Methylbenzaldehyde	9.7	11.6	12.4
p-Methylbenzaldehyde	9.9	11.9	12.7
o-Chlorobenzaldehyde	10.5	12.3	12.4
m-Chlorobenzaldehyde	10.6	12.4	12.9
p-Chlorobenzaldehyde	10.7	12.6	13.2

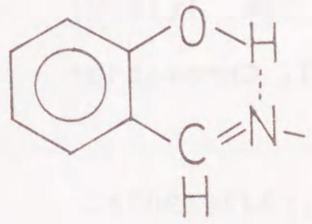
modifier in the mobile phase. This behaviour is resulted by the interaction between the nitro group and the organic solvent, and the mesomeric effect through the nitro group at the para-position may be supposed to play a primary role in this retention mechanism. The 2,4-DNPH derivatives also have a nitro group at the para-position, but it cannot be a coplanar structure to the aromatic ring as like that of the p-NPH derivatives. Because 2,4-DNPH is fixed at a planar conformation by an intramolecular six-membered ring formed by hydrogen bonding between the hydrazone -NH- and the oxygen of the nitro group at the ortho-position. Therefore, the 2,4-DNPH derivatives are little influenced by the organic modifier, in comparison with the p-NPH derivatives.

The effect of the substituent group

The effect of the substituent group was estimated on the basis of the benzaldehyde derivatives which have no substituent group. The benzaldehyde derivatives are more strongly retained than the derivatives with hydroxyl or nitro groups, except those with nitro groups in 75 % methanol, and more rapidly eluted than those with chloro or methyl groups as seen in Figures 4 and 5. This difference results from the differences in polarities of the substituent groups. The individual substituent groups, except the nitro group, exert different influences on the retention behaviours of the three derivatives under the control of the derivative skeleton.

As seen from Figures 4 and 5, the derivatives of o-hydroxybenzaldehyde display an higher affinity to the stationary

phase than those in the case of other isomers. This indicates that ortho isomer is less polar than the corresponding meta and para isomers. The results can be explained by the formation of an intramolecular hydrogen bond between the phenol O-H and the nitrogen of the hydrazone. The formation of the phenol-imine(12) shown in Scheme 1 would induce the decrease of the number of interaction sites available for association with the solvent. This decrease in solute-solvent association should then produce a decrease in solute polarity, resulting in larger retention. In the absence of such an intramolecular hydrogen bond, as in the derivatives of m- and p-hydroxybenzaldehyde, the effect of the hydroxyl group is for increase the $-\Delta H^0$ values of the 2,4-DNPH derivatives in 75 % methanol. Therefore, the $-\Delta H^0$ values do not reflect the influence of the derivative skeleton. With other substituent groups, their positions do not have much effect on the retention behaviour of the derivatives.



Scheme 1.

The derivatives having a nitro group are little influenced by structural changes of the derivative skeleton as shown in Figures 4 and 5, and are slightly retained despite the large values of $-\Delta H^0$. It seems that the nitro group contributes to entropic effect on the retention. Their retention behaviours probably are resulted from overlap or offset of the effect of interaction between the nitro group and the organic solvent because they have the same substituent group in the derivative skeleton.

The behaviours of the derivatives with chloro or methyl groups are controlled by the derivative skeleton, although the two derivatives are not retained in the same way. The derivatives with chloro substituents show similar retention behaviours to those of the benzaldehyde derivatives. In the case of the derivatives with methyl groups, 2,4-DNPH shows a large increase in their retention as seen from Figures 4 and 5.

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II-4

Effect of Eluent and Its Composition on Retention Behaviour of Carbonyl Derivatives

Introduction

It is known that a major factor in determining the selectivity of RPLC is the degree of aqueous character of the mobile phase, and solute distribution has been modeled by invoking a solvophobic interaction, i.e., an exclusion of more or less hydrophobic solute molecule from the polar solvent with subsequent sorption of the solute by the chemically bonded phase where it is held by relatively weak dispersion forces. While there is generally good agreement between theory and experimental facts when the solvophobic interaction model is applied, this description is incomplete in that it does not produce a sufficiently detailed explanation of the role of the bonded phase in solute distribution and does not explain the observation that the carbon chain length of the bonded alkyl ligand affects the selectivity, as well as the capacity, in RPLC (1-7). Not only the bonded phase but also the organic modifier plays an important role in retention. It was reported that remarkable change of the retention behaviour was caused by changing organic modifier in the mobile phase at the similar degree of hydrophobic interaction (8). Yonker et al. (9, 10) reported that the formation of the stationary phase was dependent on both the solvation of the bonded moiety and the residual silanols on the native surface, and the solvation significantly influenced the solute retention mechanism. Thermodynamic approaches were made to describe the solute retention mechanism (11-15).

The purpose of this work is to study the thermodynamic behaviour of two derivatives of aldehydes and their isomeric ketones as a function of eluent composition in order to investigate the influence of organic modifier on retention mechanism.

Experimental

Apparatus

The liquid chromatograph consisted of a TRI ROTAR pump, a VL-611 variable-loop injector and an UVIDEC 100-II variable-wavelength UV detector or a FP-110 fluorescent detector (Jasco, Tokyo, Japan). A constant-temperature water-bath (Shimizu Rikagaku, Tokyo, Japan) was used to maintain the column temperature, which was measured by an alumel-chromel thermocouple. The chromatograms were recorded on a Chromatopac C-R1A (Shimadzu Seisakusho, Tokyo, Japan).

Chemicals and Materials

All the reagents used were obtained from Tokyo Kasei (Tokyo, Japan). The samples used were aliphatic saturated aldehydes from methanal to heptanal and their isomeric ketones, which were converted to the corresponding 2,4-dinitrophenylhydrazones (2,4-DNPH) and 1-dimethylaminonaphthalene-5-sulfonylhydrazones (dansylhydrazone), respectively, in the previous work (16).

Procedures

The prepared bonded material, which is a spherical octadecylsilica containing 16.7% of carbon, was slurry-packed into a 150 x 4.6 mm I.D. stainless-steel tube. The mobile phases consisted of mixtures of methanol-water and acetonitrile-water(70, 75 and 80%(v/v)) at a flow-rate of 0.7 ml/min. The retention time of N,N-dimethyl-5-naphthylamine-1-sulphonic acid was taken as the column dead volume marker, t_o, and the capacity factor, k', was evaluated from the retention time of the solute, t_R, by the relationship $k' = (t_R - t_o)/t_o$.

van't Hoff plots

For each solute the retention is given by the following equation

$$\ln k' = -\Delta H^0/RT + \Delta S^0/R + \ln \phi \qquad (4)$$

where ΔH^0 is the partial molar enthalpy change for the transfer of the solute from the infinite dilution standard state in the mobile phase to the infinite dilution standard state in the stationary phase, ΔS^0 is the associated change in standard entropy, R the gas constant and ϕ the phase ratio. If ΔH^0 and ΔS^0 are independent of temperature over the temperature range of interest, a plot of ln k' vs 1/T will be linear. While the slope of this line gives standard enthalpies of transfer, standard entropies of transfer are calculated from the intercept and they are dependent on the value of the phase ratio. Enthalpies and entropies of the transfer were calculated by using linear regression analysis for van't Hoff plots.

Results and Discussion

All over the temperature range between 30 and 70 °C. The values of ΔH^0 and $(\Delta S^0/R + \ln \phi)$ were calculated through linear regression analysis for individual cases. Figure 6 shows the plots of ΔH^0 against mole fraction of organic modifier. The trends of ΔH^0 for 2- and 3-ketones are similar to those for aldehydes. Figure 6-B for an example shows the plots of ΔH^0 for dansylhydrazones of 2-ketones. Similarly, Figure 7 shows the plots of $(\Delta S^0/R + \ln \phi)$ against mole fraction of organic modifier. The trends of $(\Delta S^0/R + \ln \phi)$ are regarded as those of ΔS^0 since the phase ratio ϕ assumes to be approximately constant over these condition.

By comparing aqueous methanol and aqueous acetonitrile systems, one can find an interesting fact. In aqueous methanol system, the trends of ΔH^0 for dansylhydrazones are similar to those for 2,4-DNPHs as shown in Figure 6. In Figure 7, the values of $(\Delta S^0/R + \ln \phi)$ for 2,4-DNPHs increase linearly with increasing concentration of methanol, and those for dansylhydrazones increase steeply with increasing concentration from 75 % to 80 %

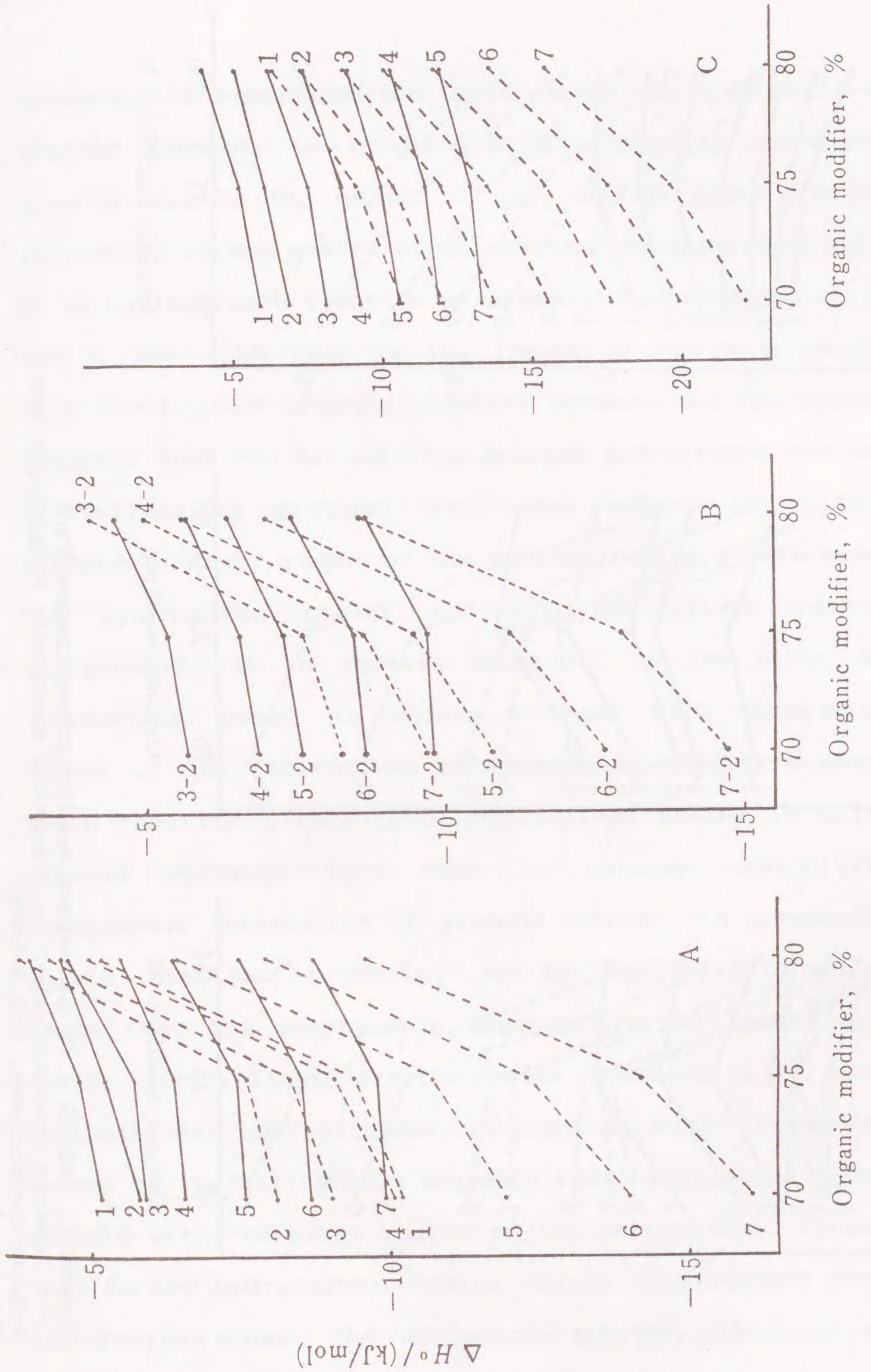

Figure 6. ΔH° as a function of mobile phase composition ——: Aqueous acetonitrile, ·······: Aqueous methanol; Samples: A and B=Dansylhydrazones, C=2,4-DNPH; 1=methanal; 2=ethanal; 3=propanal; 3-2=propanone; 4=butanal; 4-2=butanone; 5=pentanal; 5-2=2-pentanone; 6=hexanal; 6-2=2-hexanone; 7=heptanal; 7-2=2-heptanone.

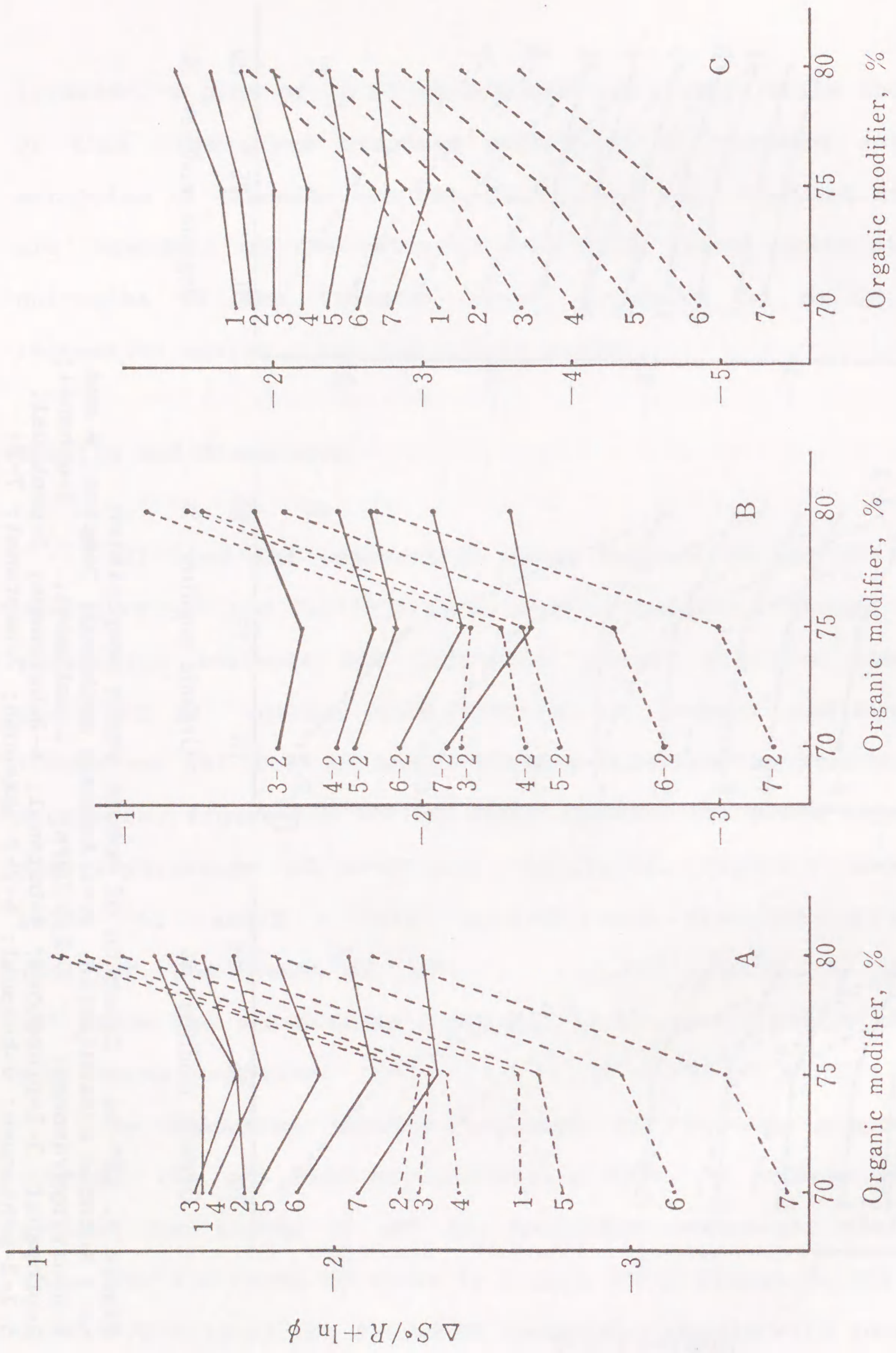

Figure 7. (ΔS°/R + ln φ) versus mobile phase composition
——: Aqueous acetonitrile, ------: Aqueous methanol; Samples: A and B=Dansylhydrazones, C=2,4-DNPH;
The sample numbers are the same as shown in Figure 6.

methanol. In aqueous acetonitrile system, both derivatives have a similar tendency to increase with increasing concentration of acetonitrile in the values of ΔH^0, and decrease gradually with increasing carbon number of derivatives in the trends of ($\Delta S^0/R$ + ln ϕ) against mole fraction of acetonitrile as shown in Figures 6 and 7. The difference in the trends of ($\Delta S^0/R$ + ln ϕ) against mole fraction of organic modifier between the two mobile phases suggests that the interaction between hydrocarbon chains and the derivatives is distinct from each other. In spite of the different carbon number of the derivative the interaction between the hydrocarbon chains and the derivatives seems to be homogeneous one in aqueous methanol. On the other hand, the interaction seems to become stronger with increasing carbon number of the derivatives in aqueous acetonitrile system. This means that the structure of hydrocarbon chains is different in aqueous methanol from that in aqueous acetonitrile. The homogeneous interaction in aqueous methanol is presumed that the solute retention is carried out by liquid-liquid partition. It seems that the solvophobic aggregations of bonded hydrocarbon chains form "liquid-droplet" like clusters (17). In aqueous acetonitrile, the stronger interaction with increasing carbon number of the derivatives suggests that hydrocarbon chains are in contact with the alkyl chains of the derivatives. It is expected that bonded hydrocarbon chains stands into aqueous acetonitrile as bristle brush. The difference of the structure of bonded hydrocarbon chains in the two mobile phase systems is ascribed to the difference of associated states of those mobile phase composers. The weak association between acetonitrile and water seems to promote the adsorption of a lot of acetonitrile on bonded hydrocarbon chains. The strong association between methanol and water caused from hydrogen bonding seems to keep the aggregation of bonded hydrocarbon chains each other.

In conclusion, thermodynamic retention behaviours of two derivatives reflect the distinct structure of bonded hydrocarbon chains between the two mobile phases. The structure of bonded hydrocarbon chains is found to be influenced by the association of organic modifiers and water.

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PART III

UNDERSTANDING OF STATIONARY PHASE STATE

BY USING SURFACE EXCESS AMOUNT

Introduction

Concept of Function of Stationary Phase in Reversed-Phase Liquid Chromatography

The popularity of reversed-phase separations, particularly with C_{18} bonded packing materials, can be attributed to the success of this technique in solving a diverse array of problems. It is a surprising fact, however, that a detailed understanding of the mechanisms of solute retention is lacking. The difficulty in describing the retention process in RPLC comes from the difficulty to know nature of the bonded phase itself.

The question of whether a bonded phase should be considered a surface or a thin liquid film has given to rise two theories of solute retention in RPLC: adsorption and partition. Adsorption means the interactions of the stationary phase with the solute or solvent molecules covering the external molecular layer of the "adsorbent". In the simplest case, adsorption is characterized by a monolayer in which the solute and solvent molecules are in competition to cover the external surface of the packing material. More often, the interfacial region between the adsorbent and the bulk mobile phase can be composed of several layers. The separation surface results from an arbitrary choice and it is generally selected in a position such that the Gibbs' surface excess for the solvent is zero. In a partition process, the sole role of the support is to create a large surface area where the stationary liquid phase will be deposited. The phenomena in the interfacial region are then generally neglected and the solute molecules partition into the two liquid phases. Each phase contains two components: the solvent and the solute in the mobile phase and the stationary liquid and the solute in the stationary phase. Considerable effort has been paid by workers trying to classify retention as one of the two categories (1, 2), but evidence for such a classification remains inconclusive. Clearly, before an accurate picture of solute retention can be built up, the microscopic properties of the anchored hydrocarbons must be elucidated. A number of models have been advanced. Halasz and co-workers (3) originally pictured the bonded chains as "bristles" that extended vertically from the silica surface. A different structure has been proposed by Hemetsberger (4) who notes that "the nonpolar ligands of a chemically bonded phase are subject to the same solvophobic interactions as the solutes," and envisions the bonded ligands as folded to minimize exposed surface area. Lochmüllar (1) has set forth an alternate arrangement in which groups of bonded chains associate to form pseudoliquid patches termed "liquid droplets". Gilpin has formulated a theory in which both folded and bristle states are possible to exist and transitions between two situations take place with changes in temperature or solvent (5, 6).

There is much evidence that the solvation of the bonded alkyl chains themselves charges in response to varying composition of the mobile phase. Yonker et al. have shown that the bonded phase is preferentially solvated by the organic component of the mobile phase (7, 8). They have proposed the view

of the stationary phase being a ternary combination of bonded organic moiety, adsorbed solvent molecules and residual silanols on the silica surface. There have been several studies of the sorption by alkyl bonded phases of commonly used organic modifiers in reversed-phase liquid chromatography eluents. The salient findings may be qualitatively summarized as follows: the more compatible the solvent is with n-alkanes, the more it is sorbed by the bonded phase; more compatible solvents displace previously sorbed, less compatible solvents from the bonded phase.

The structure of the stationary phase in the presence of different solvents have been subjects of many studies and discussions. Solvents compatible with n-alkanes tend to swell the surface and promote to extend the bonded alkyl chains, while those which are incompatible tend to collapse the chains upon each other and bend toward the underlying surface. At the extremes, the former case (nonpolar solvents) presumably leads to make a stationary phase having a brush-like structure, where the extended chains are translationally restricted and oriented more or less normal to the surface, and/or where there is full penetration by the solvent and potentially full penetration by the solute. The latter (fairly polar solvents) induces a quasi-liquid-like layer of recumbent alkyl chains still translationally restricted and possibly oriented in some manner, where there is negligible solvent penetration. However, the possibility of solute penetration is not denied. The picture that emerges is that of a "breathing" surface which adjusts itself to maintain a relatively nonpolar character.

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III-1

Concept of Surface Excess Amount

Quantitative definitions for the adsorption of binary liquid mixtures at solid-liquid interfaces in terms of surface excess amounts were given by Everett(1). Riedo and Kovats(2) derived general relationships for calculating these surface excess amounts from chromatographic retention data. Here some important equations are summarized and approximations involved.

A sample of a binary liquid mixture containing an amount n° at a mole fraction x_2° is equilibrated with a mass m of solid. The final mole fraction x_2^{ℓ} is experimentally determined. The reduced surface excess of component 2, $n_2^{\sigma(n)}$ is then given by the equation

$$n_2^{\sigma(n)} = n^{\circ} x_2^{\circ} - n^{\circ} x_2^{\ell} = n_2 - n^{\circ} x_2^{\ell} \tag{5}$$

where n_2 is the total amount of component 2 in bulk liquid. The areal reduced surface excess, $\Gamma_2^{(n)}$, is

$$\Gamma_2^{(n)} = n_2^{\sigma(n)}/mS \tag{6}$$

Where S is the specific surface area of the solid. If adsorption from solution is represented in terms of liquid volumes and volume concentrations, $n_2^{\sigma(v)}$, equation 5 should be described as equation 7 where V° is the initial volume of liquid, and c_2^{ℓ} is the concentration of component 2 in bulk liquid.

$$n_2^{\sigma(v)} = n_2 - V^{\circ} C_2^{\ell} \qquad (7)$$

If we assume the molar volume of the liquid mixture, v_m, is a strictly linear function of the mole fractions (i.e., when the partial molar volumes, v_1 and v_2, of the components are constant over the concentration range considered), the following relationship should be obtained.

$$n_2^{\sigma(v)} = [1 + (v_1 - v_2)C_2^{\ell}]n_2^{\sigma(n)} = n_2^{\sigma(n)} \; v_1/v_m \qquad (8)$$

Adsorption isotherms obtained by chromatographic method

Let us consider a liquid solution flowing through a packed column with a uniform cross-sectional area. The equation of material balance for component 2 in the section at distance z from the column inlet can be written as follows, assuming one-dimensional plug flow under being absence of longitudinal diffusion :

$$(1/L)\frac{\partial n_2}{\partial t} = -F \frac{\partial C_2^{\ell}}{\partial z} \qquad (9)$$

where L is the column length and F represents the flow rate, which is presumed to be constant throughout the column.

Substituting equation 7 into equation 9 gives the material balance in terms of surface excess amount.

$$\frac{\partial}{\partial t}(n_2^{\sigma(v)} + V^{\circ} C_2^{\ell}) = -FL \frac{\partial C_2^{\ell}}{\partial z} \qquad (10)$$

For a binary liquid, the surface excess isotherm is a function of the concentration C_2^{ℓ}. Hence, the transport equation becomes

$$(V^{\circ} + dn_2^{\sigma(v)}/dC_2^{\ell})\frac{\partial C_2^{\ell}}{\partial t} = -FL \frac{\partial C_2^{\ell}}{\partial z} \qquad (11)$$

Equation 11 can be solved for the case when the sample enters the column in a very short time, resulting in an equation for the migration velocity u_x of a concentration C_2^{ℓ}.

$$\frac{\partial z}{\partial t} = u_x = FL/(V^{\circ} + dn_2^{\sigma(v)}/dC_2^{\ell}) \qquad (12)$$

From equation 12, an expression which describes the residence time t_R of the concentration C_2^{ℓ} in the column can be derived.

$$t_R = L/u_x = L(V^{\circ} + dn_2^{\sigma(v)}/dC_2^{\ell})/FL$$
$$Ft_R = V_R = V^{\circ} + dn_2^{\sigma(v)}/dC_2^{\ell} \qquad (13)$$

By the approximation of $V^{\circ} \simeq V_m$, the retention volume of the solvent disturbance peak provides the first derivative of the surface excess isotherm.

$$dn_2^{\sigma(v)}/dc_2^{\ell} = V_R - V_m \tag{14}$$

where V_m is the column hold-up volume. In this case, components 1 and 2 correspond to water and organic modifier, respectively. Experimental points ($V_R - V_m$) determined at given composition of the eluent plotted as a function of c_2^{ℓ} show parabolic curves. Therefore, the quadratic expression is fitted on the experimental points. The areal reduced surface excess of component 2, $\Gamma_2^{(n)}$, is given by equation 6 when $n_2^{\sigma(v)}$ is calculated by equation 8. The separation surface is resulted from an arbitrary choice and is generally in a position such that the Gibbs' surface excess for the solvent is zero. Therefore, the areal reduced surface excess of component 1, $\Gamma_1^{(n)}$, is

$$\Gamma_1^{(n)} = -\Gamma_2^{(n)} \tag{15}$$

In the case of using the retention volume of the labelled components, 1* or 2*, in the eluent instead of the perturbation of the concentration, c_2^{ℓ}, the areal reduced surface excess, $\Gamma_2^{(n)}$, is given by the combination of the results with equation 15.

$$\Gamma_2^{(n)} = (V_{R,2*} - V_{R,1*})x_2^{\ell} x_1^{\ell}/Sv_m \tag{16}$$

where x_1^{ℓ} and x_2^{ℓ} are the mole fraction of components 1 and 2 in the eluent. V_R is the retention volume, s is the surface area of silica and v_m is the mean molar volume of the eluent at the

$$v_m = v_1 x_1^{\ell} + v_2 x_2^{\ell} \tag{17}$$

composition x_1^{ℓ}, and v_1 and v_2 are the partial molar volume of components 1 and 2.

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III-2

Effect of Pore Size on Surface Excess Amount

Introduction

The study of chemically modified surfaces for use in liquid chromatography is a matter of great interest. A variety of techniques for probing chemically modified surfaces have been employed, including fluorescence (1), infrared (2-5), and nuclear magnetic (5-10) resonance spectrometry. Information obtained by these techniques has provided important insights into surface-ligand structures and interactions, bonded layer solvation and segmental or total chain mobility.

It is well known that the organic solvent is enriched in the bonded phase in RPLC. The amount of enriched modifier can be determined as the surface excess amount. The surface excess amount of a given component is defined as the difference between the amount of component actually present in the system and that which would be present in a reference system if the bulk concentrations in the adjoining phases were maintained up to a chosen geometrical dividing surface (11). Therefore, the surface excess amount can be used as a measure of the comparison of the chemically modified surface. The surface excess isotherm is accessible to direct experimental determination. General relationships for calculating the surface excess amount from chromatographic retention data were given by Riedo and

Kováts (12). Chromatographic methods to determine the surface excess isotherms were based on measurements of the retention volumes of concentration steps (frontal analysis) (13-15), labelled components of the eluent (16) and solvent disturbance peaks (17-19).

In order to make it possible to use the surface excess isotherm as a measure for the comparison of the chemically modified surfaces with various pore sizes, it is necessary to investigate the influence of pore size on the surface excess isotherm. This work explored whether the pore sizes of column packings influence to their surface excess isotherms or not. The silicas were used as packing materials in order to investigate the net effect of the pore size on the exclusion of the chemically bonded phase. The surface excess isotherms of organic modifier on these packings were evaluated in binary aqueous systems. The method was based on the measurements of the retention volumes of labelled components in the eluent.

Experimental

Apparatus

The liquid chromatograph consisted of a 880-PU pump (Jasco, Tokyo, Japan), a Rheodyne Model 7125 injector and a Shodex RI SE-51 differential refractive index detector (Showa Denko, Tokyo, Japan). A constant-temperature water-bath (Model TM108M, Toyo, Tokyo, Japan) was used to maintain the column temperature, which was measured by an alumel-chromel thermocouple. The chromatograms

were recorded on a Chromatopac CR1A (Shimadzu, Kyoto, Japan).

Chemicals and Materials

Liquid chromatographic grade solvents and deuterated compounds were obtained from Nacalai Tesque (Kyoto, Japan). Silicas with pore diameters ranging from 100 to 300 A were used as packing materials. Silicas used were Vydac 101TPB (300 A)(Separation Group, Hesperia, CA, U.S.A.), Shiseido's silica gel (120 A, 300 A) and Super Micro Bead silica gel(100 A, 150 A, 300 A)(Fuji-Davison Chemical, Kasugai, Japan). They were packed by means of the balanced slurry technique into 250 x 4.6 mm I.D. stainless-steel tubes in our laboratory. Data of the column packings are summarized in Table VI.

Procedures

The retention volumes of labelled components of the eluent were obtained from the flow-rate and retention time, t_R. The nominal flow-rate used throughout the study was 1 ml/min. The actual flow-rate was measured by collecting the column eluent in a 10-ml volumetric flask and recording the collection time. The sample concentration was chosen as low as possible while maintaining a suitable signal-to-noise ratio in refractive index detection. Eluents of appropriate compositions were prepared by weighing. The column materials were removed from each column after chromatographic measurements and washed with methanol, dried under reduced pressure and weighed.

TABLE VI

PHYSICAL PROPERTIES OF SILICA MATERIALS

silica	particle diameter (μm)	pore diameter (A)	area (m^2/g)	pore volume (ml/g)	pH
Super Micro Bead[a]					
100A	5.9	108	385	1.04	5.1
150A	5.7	167	181	1.08	5.7
300A	5.1	321	95	1.07	5.6
Shiseido's silica gel[b]					
120A	5	124	264	0.80	
300A	5	274	168	1.39	
Vydac 101TPB[b]	5	303	75	0.59	

[a] Data presented by manufacturer

[b] Data analyzed by Y. Shiojima of Shiseido

Surface excess amounts

Quantitative definitions for adsorption of binary liquid mixture at solid-liquid interface in terms of surface excess amounts were given by Everett (11). Riedo and Kováts (12) derived general relationships for calculating their surface excess amounts from chromatographic retention data.

The necessary expressions for calculating the adsorption isotherms are taken from ref 12. The surface concentration of component 2 is given by

$$\Gamma_2^{(n)} = (V_{R,2}* - V_{R,1}*)x_2^{\ell} x_1^{\ell} / Sv_m \qquad (16)$$

where $\Gamma_2^{(n)}$ is the areal reduced surface excess of component 2 and $\mathbf{x}_1^{\ell}$ and $\mathbf{x}_2^{\ell}$ are the mole fraction of components 1 and 2 in the eluent. $\mathbf{V}_R$ is the retention volume, S is the surface area of silica and $\mathbf{v}_m$ is the mean molar volume of the eluent at the

$$v_m = v_1 x_1^{\ell} + v_2 x_2^{\ell} \qquad (17)$$

composition $\mathbf{x}_1^{\ell}$, and $\mathbf{v}_1$ and $\mathbf{v}_2$ are the partial molar volume of components 1 and 2. The asterisk refers to labelled components of the element, 1* or 2*.

The layer model

When dealing with an actual system, it is not sufficient to obtain the surface excess isotherm, because it only represents the total change of the system and does not refer to the actual amount of a component present in the mobile phase and adsorbed phase. Therefore, the layer model(11) was used to calculate the composition of the adsorbed phase and determine the absolute amount adsorbed.

Assuming that the adsorbed phase consists of t layers of molecules on a plane smooth homogeneous surface, the mole fraction, x_2^s, of component 2 in the surface phase is given by :

$$x_2^s = \frac{t x_2^\ell + a_1^\circ \Gamma_2^{(n)}}{t - (a_2^\circ - a_1^\circ)\Gamma_2^{(n)}} \qquad (18)$$

where a_1° and a_2° are the molar cross-sectional areas of components 1 and 2, which can be calculated from the molar volumes, v_1 and v_2, using the equation of $a_i^\circ = 9200(v_i)^{2/3}$, $(i=1,2)$. Using these a_i° values and the experimental surface excess data, $\Gamma_2^{(n)}$, the minimum number of layers, t_{min}, was calculated by satisfying the criteria that the values of x_2^s calculated do not exceed unity, and x_2^s always increased with x_2^ℓ i.e., $(\partial x_2^s/\partial x_2^\ell) > 0$. The values of t_{min} are given in Table VII. x_2^s was calculated using equation 18 with values of t_{min}. In this study, components 1 and 2 correspond to water and organic modifier, respectively.

Results and Discussion

The method of calculation of adsorption isotherms is based

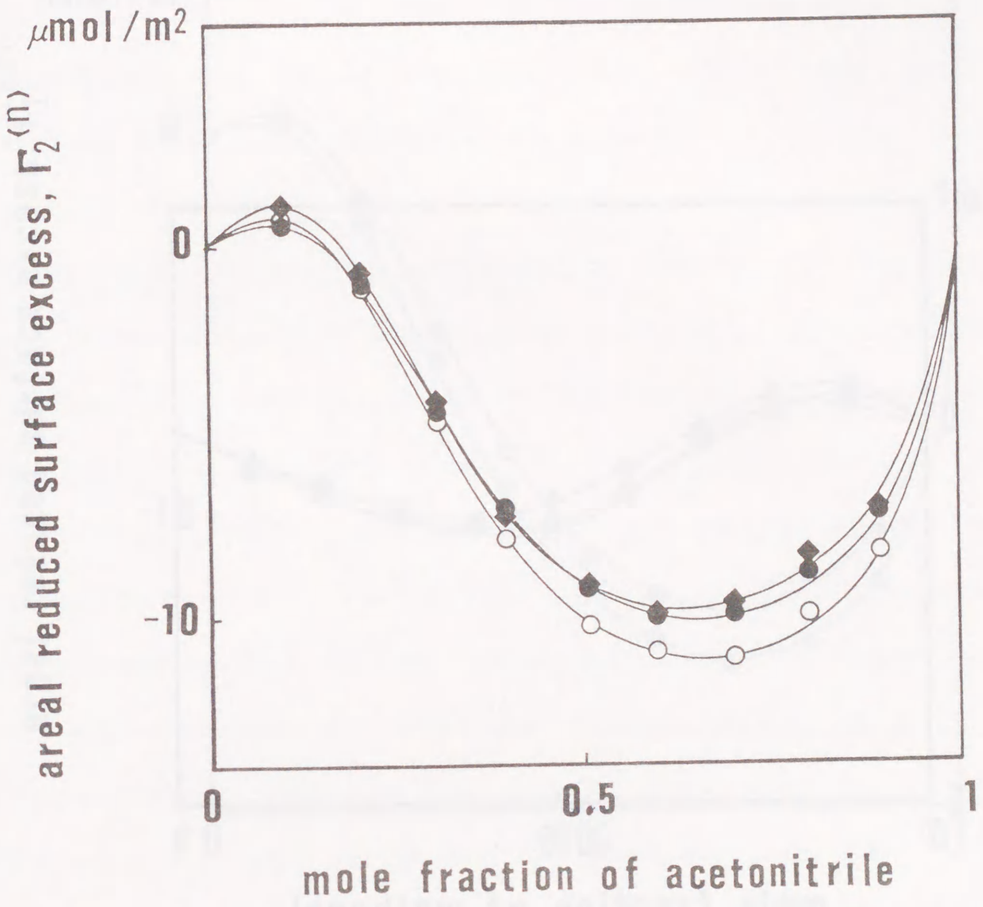

Figure 8. Surface excess amount of acetonitrile adsorbed at 30°C from an aqueous mixture on Super Micro Bead silica gel plotted against the mole fraction of acetonitrile Pore size; ● = 100A; ○ =150A; ◆ =300A.

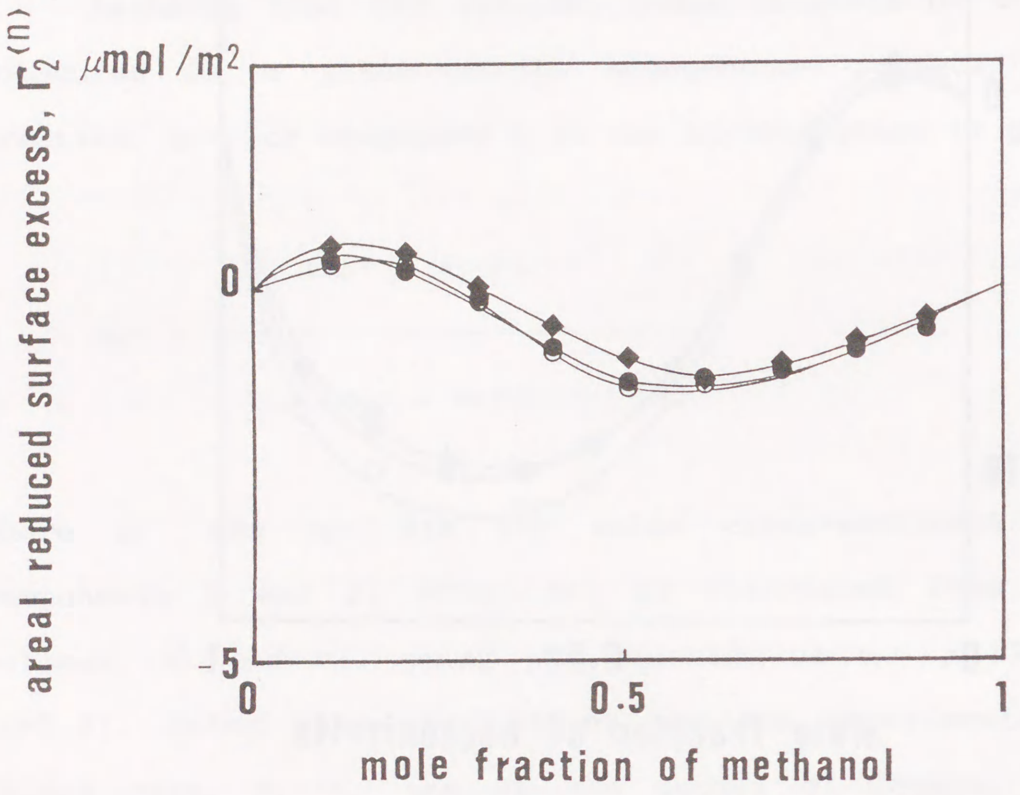

Figure 9. Surface excess amount of methanol adsorbed at 30°C from an aqueous mixture on Super Micro Bead silica gel plotted against the mole fraction of methanol Symbols as in Figure 8.

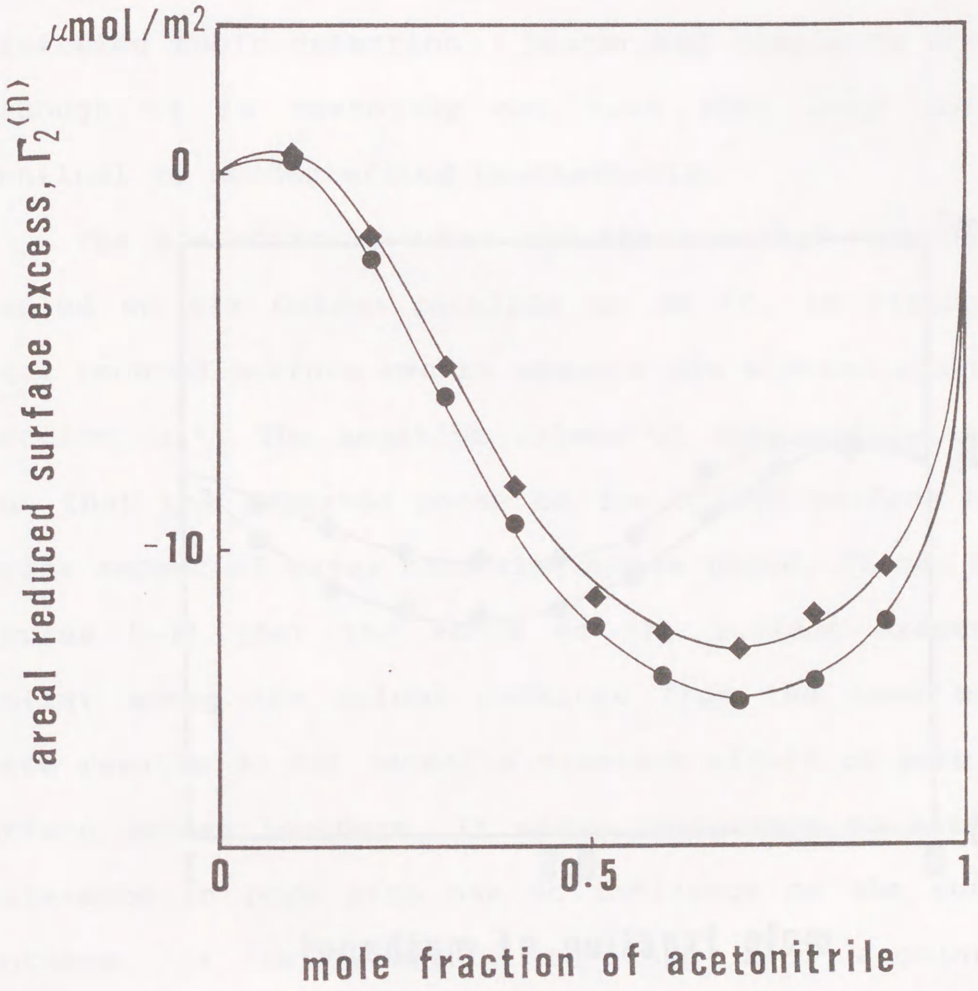

Figure 10. Surface excess amount of acetonitrile adsorbed at 30°C from an aqueous mixture on Shiseido's silica gel plotted against the mole fraction of acetonitrile Pore size; ●= 120A; ◆ =300A.

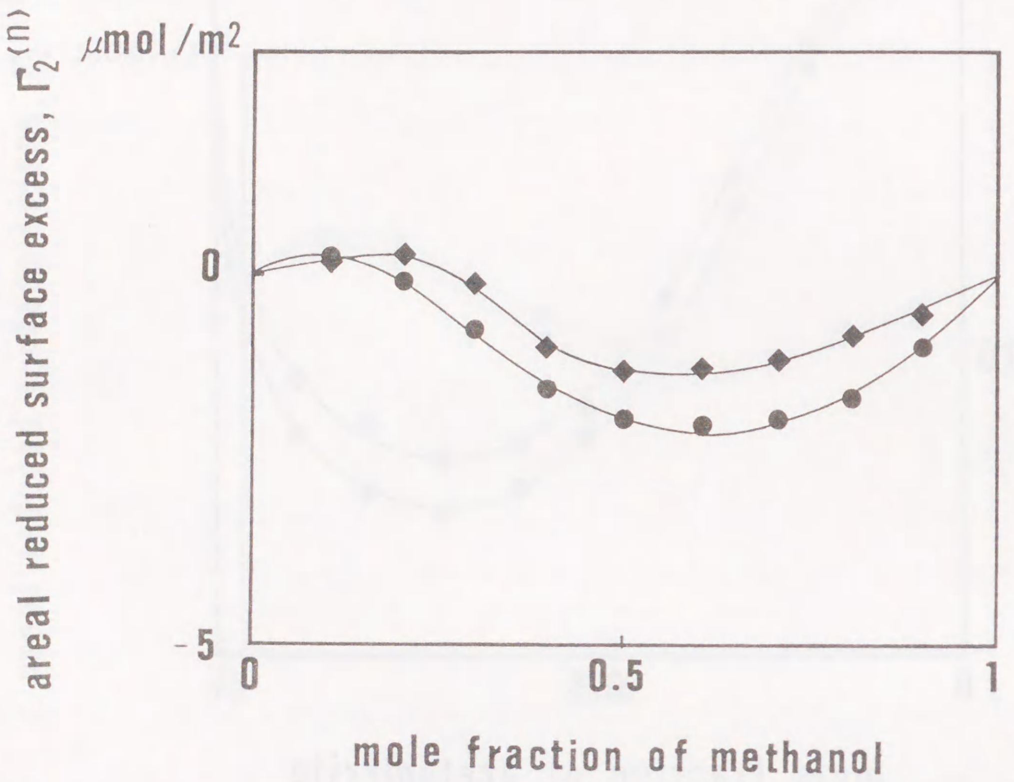

Figure 11. Surface excess amount of methanol adsorbed at 30°C from an aqueous mixture on Shiseido's silica gel plotted against the mole fraction of methanol Symbols as in Figure 10.

on the existence of solutes which are identical in every respect with components of the eluent except one property permitting their detection. Deuterated compounds should be used although it is certainly not true that they are completely identical to nondeuterated counterparts.

The acetonitrile-water and the methanol-water systems were studied on six column packings at 30 °C. In Figures 8-11, the areal reduced surface excess amounts are plotted against the mole fraction, x_2^{ℓ}. The negative values of the surface excess amount mean that the adsorbed phase on the silica surface holds a much larger amount of water than the mobile phase. It can be seen from Figures 8-11 that the value of the surface excess amount is similar among the column packings from the same manufacturer. These results do not reveal a distinct effect of pore size on the surface excess isotherm. It seems reasonable to assume that the difference in pore size has no influence on the surface excess isotherm. It is necessary to take into account the pore distribution and structure. However, the effect of pore distribution might be negligible because the pore size of the silica materials used in this study extends from 100 to 300 A. With regard to the pore structure, it is not apparent whether it influences the surface excess isotherm or not, because there is no information related to the pore structure. Figures 12 and 13 show the surface excess isotherms of the silica with pore size 300 A of the different manufacturers. The result suggests that the surface excess isotherm is influenced by the substrate rather than the pore size. It seems that the difference in the substrate

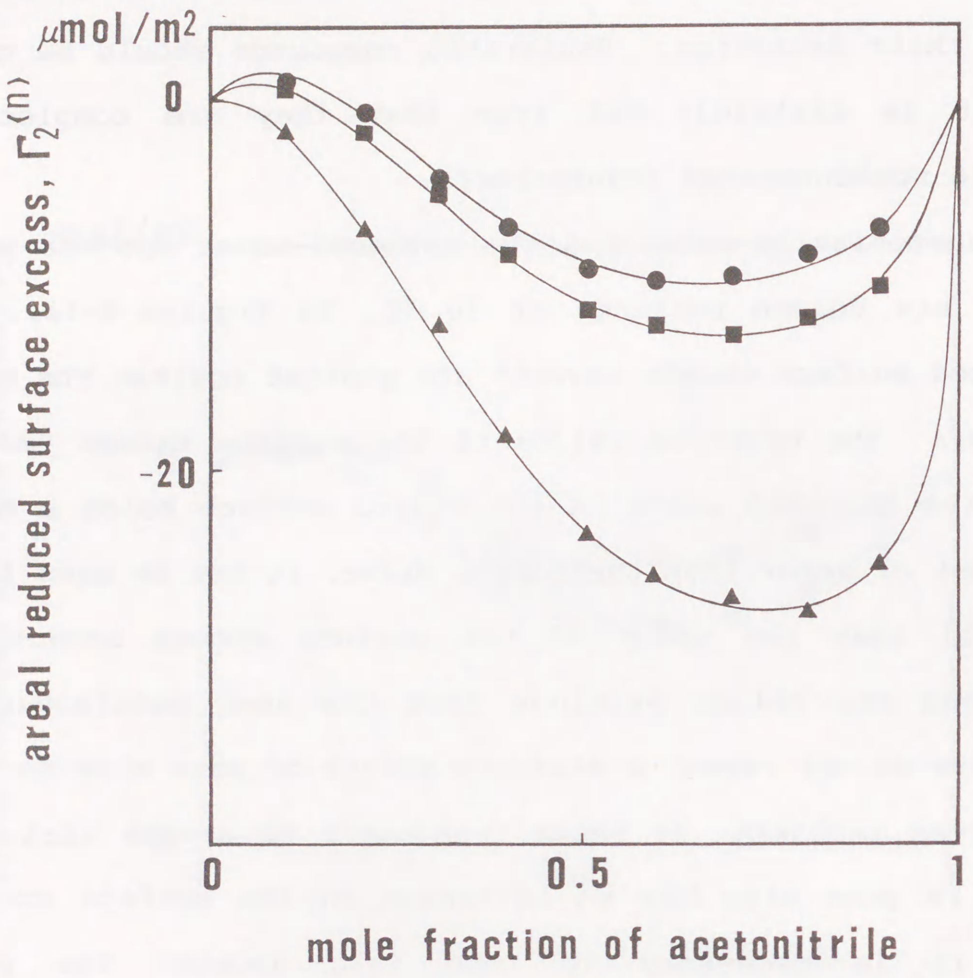

Figure 12. Surface excess amount of acetonitrile adsorbed at 30°C from an aqueous mixture on silicas with pore size 300A plotted against the mole fraction of acetonitrile

●: Super Micro Bead silica gel; ■: Shiseido's silica gel;

▲: Vydac 101TPB.

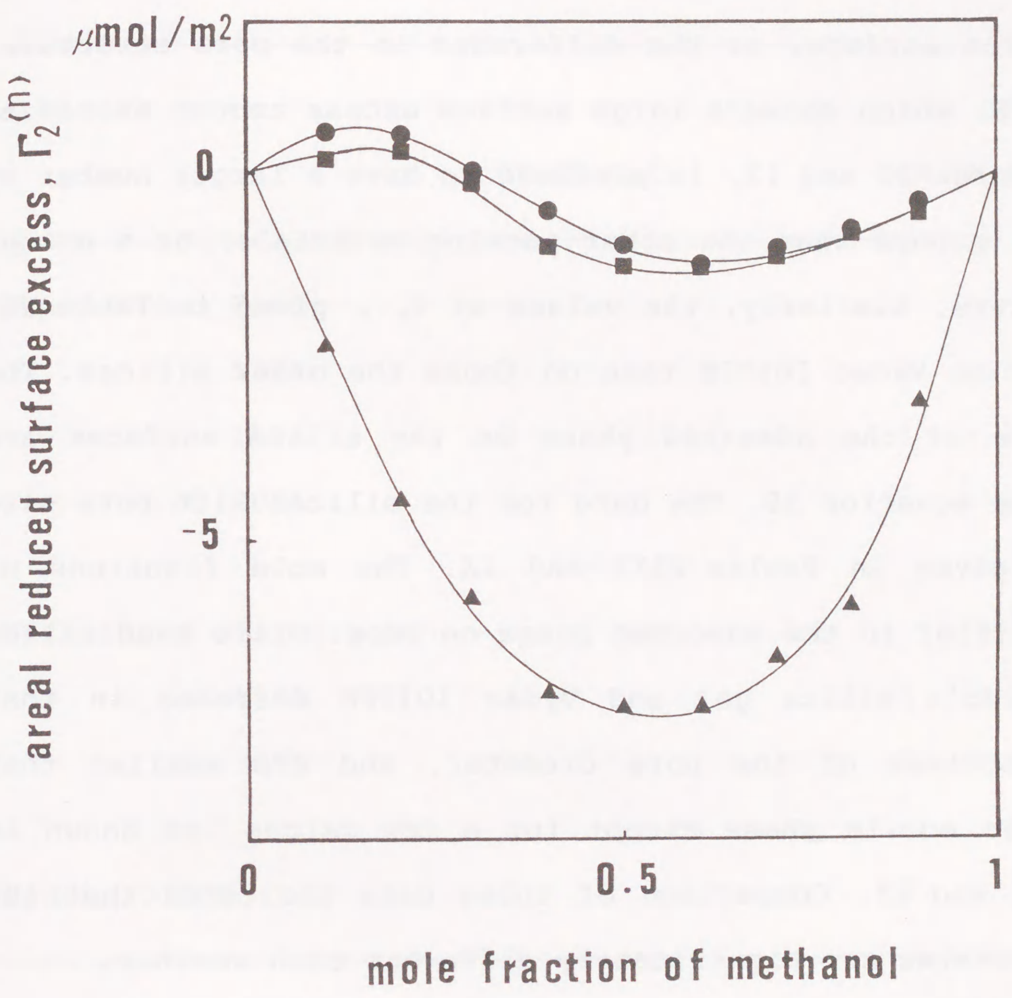

Figure 13. Surface excess amount of methanol adsorbed at 30°C from an aqueous mixture on silicas with pore size 300A plotted against the mole fraction of methanol Symbol as in Figure 12.

is based on the number of the silanol groups, which plays an important role in the adsorption of water from an aqueous mixture on the silica surface, or the difference in the pore structure. Vydac 101TPB, which shows a large surface excess amount as can be seen in Figures 12 and 13, is presumed to have a larger number of the silanol groups than the other packing materials, or a unique pore structure. Similarly, the values of t_{min} given in Table VII are greater on Vydac 101TPB than on those the other silicas. The compositions of the adsorbed phase on the silica surfaces are given by the equation 18. The data for the silicas with pore size 300 A are given in Tables VIII and IX. The mole fractions of organic modifier in the adsorbed phase on Super Micro Bead silica gel, Shiseido's silica gel and Vydac 101TPB decrease in that order, regardless of the pore diameter, and are smaller than those in the mobile phase except for a few values, as shown in Tables VIII and IX. Comparison of these data indicates that the silica substrates are significantly different each another.

Comparison of Figure 8 with Figure 9 or Figure 10 with Figure 11 indicates that the surface excess amount of water is much greater in aqueous acetonitrile than in aqueous methanol. In previous work (20), it has been found that acetonitrile was more preferentially adsorbed on the chemically modified surface than methanol in aqueous mixture. These results and the values of t_{min} given in Table VII indicate the difference in the miscible state between acetonitrile-water and methanol-water systems. In acetonitrile-water system, the weak association between acetonitrile and water seems to promote the adsorption of water

TABLE VII

VALUES OF tmin SATISFYING THE CRITERIA

silica	t_{min}	
	CH_3CN-H_2O	CH_3OH-H_2O
Super Micro Bead		
100A	3	1
150A	3	1
300A	3	1
Shiseido's silica gel		
120A	3	1
300A	3	1
Vydac 101TPB	5	2

TABLE VIII

COMPOSITION OF THE ADSORBED PHASE ON THE SILICAS WITH PORE SIZE 300A FOR THE ACETONITRILE(2)-WATER(1)SYSTEM

x_2^{ℓ}	x_2^{s}		
	Super Micro Bead	Shiseido's silica gel	Vydac 101TPB
0.839	0.643	0.544	0.432
0.798	0.524	0.435	0.327
0.697	0.407	0.334	0.263
0.595	0.317	0.265	0.201
0.506	0.260	0.213	0.160
0.397	0.210	0.185	0.133
0.305	0.198	0.176	0.128
0.205	0.186	0.162	0.105
0.099	0.124	0.110	0.074

TABLE IX

COMPOSITION OF THE ADSORBED PHASE ON THE SILICAS WITH PORE SIZE 300A FOR THE METHANOL(2)-WATER(1)SYSTEM

x_2^{ℓ}	x_2^{s}		
	Super Micro Bead	Shiseido's silica gel	Vydac 101TPB
0.898	0.856	0.842	0.746
0.805	0.734	0.724	0.545
0.706	0.611	0.299	0.433
0.603	0.493	0.493	0.320
0.500	0.420	0.393	0.233
0.398	0.357	0.319	0.151
0.297	0.298	0.286	0.103
0.202	0.237	0.222	0.056
0.103	0.139	0.115	0.028

on the silica surface or acetonitrile on the chemically modified surface because silica has a strong affinity for water through hydrogen bonding with silanol groups, and the chemically modified surface prefers an organic solvent more than water owing to non-polarity of bonded hydrocarbon chains. In aqueous methanol, the low surface excess amount might be due to keeping a strongly associated state of methanol and water as a result of strong hydrogen bonding.

In conclusion, the data obtained from this study suggest that the surface excess isotherm on the silica surface was influenced by the substrate itself rather than pore size. This means that the surface excess isotherm can be used as a measure of the comparison of the chemically modified surfaces. Further, the surface excess isotherm reflects the difference in the miscible state between acetonitrile-water and methanol-water systems. Therefore, the comparison of isotherms may be useful in studying the state of the binary mobile phases.

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III-3

Comparison of Monomeric and Polymeric Phases on Surface Excess Amount

Introduction

Recently, unique properties of polymeric C_{18} bonded phases have come into investigation. The enhanced selectivity exhibited by these phases to certain compound classes makes them an attractive alternative to conventional monomeric C_{18} bonded phases, especially for the separation of polycyclic aromatic hydrocarbons (PAHs). The separation of PAHs by RPLC has been studied extensively for both monomeric and polymeric C_{18} bonded phases(1-7). For selective separation of PAHs, polymeric C_{18} bonded phases are more suitable than monomeric C_{18} bonded phases because polymeric C_{18} bonded phases have higher capability in planarity recognition. Wise et al.(2) proposed a "slot model" for polymeric C_{18} bonded phases, since alkyl chains of polymeric C_{18} bonded phase is more rigid than monomeric C_{18} bonded phase and experimental data confirmed that their model is reasonable. Jinno et al.(5-7) have also studied PAH retention with various types of chemically bonded phases in RPLC. They found that polymeric C_{18} bonded phases have the unique ability to recognize PAH planarity well, especially for large PAHs. However, Wise's model is not intended to describe the actual morphology of the bonded phase. Rather, it is used to explain experimentally observed facts in retention that is arisen from differences in the molecular shape of the solute.

Theoretically, polymeric phases represent a more complex system than monomeric phases. Monomeric phases are often described in picturesque terms such as "bristles", "brushes" or "molecular fur". Polymeric phases are difficult to visualize because little is known about the extent of cross-linking and the degree of polymerization of the alkyl chains.

It is well known that the organic solvent is enriched in the bonded phase in RPLC. The amount of enriched modifier can be determined as the surface excess amount. General relationships for calculating the surface excess amount from chromatographic retention data were given by Riedo and Kováts (8). Chromatographic methods to determine the surface excess isotherms were based on measurements of the retention volumes of concentration steps(frontal analysis)(9-11), of peaks of labelled components of the eluent(12), and of solvent disturbance peaks(13-15).

The surface excess amount depends upon the solvent strength and the composition of the mobile phase. Furthermore, the surface excess isotherm is expected to be under the influence of the bonded phase, since bonded phase effects are clearly evident among commercial C_{18} columns that differ in absolute retention and selectivity. In this paper, an attempt is made to relate the differences between the monomeric and the polymeric C_{18} bonded phases to their surface excess isotherms. The surface excess isotherms of organic modifier on the monomeric and the polymeric

C_{18} bonded phases are evaluated in binary mobile phase solvents systems. The method is based on the measurements of the retention volumes of solvent disturbance peaks or peaks of labeled components of the eluent. The isotherm of the polymeric C_{18} bonded phase is compared to that of the monomeric C_{18} bonded phase.

Experimental

Apparatus

Two HPLC systems were utilized in this investigation. For aqueous mobile phases, a Tri Rotar pump(Jasco) equipped with a Shodex RI SE-51 differential refractive index detector (Showa Denko) was used. For dichloromethane-methanol mobile phase, an 880-PU pump(Jasco) coupled with a Shodex RI SE-II detector(Showa Denko) was used. Two constant-temperature water-baths(ESP-47 and TE-108M, Toyo) were used to control the column temperature, which was measured with an alumel-chromel thermocouple. The chromatograms were recorded on a Chromatopac CR1A (Shimadzu).

Chemicals and Materials

Liquid chromatographic grade solvents and deuterated compounds were obtained from Nacalai Tesque. Monomeric and polymeric C_{18} bonded phases (which were prepared as described below) were used as column materials (see Table X). Cosmosil 5SL silica (Nacalai Tesque) was used for the synthesis of monomeric and polymeric phases. Prior to silanizing reaction, the silica

Table X

CHARACTERIZATION OF THE ADSORBENTS

	Silica	Monomeric phase	Polymeric phase
surface area (m^2/g)	342	165	168
% carbon	-	19.5	18.5
surface coverage ($\mu mol/m^2$)		3.17	3.29
mean pore diameter (A)	102	91.9	91.2

was dried at 120 °C for 5 h under reduced pressure. In a monomeric phase synthesis, 10 g of dry silica was added to a flask containing 200 ml of dry toluene. Then 10 g of chlorodimethyloctadecylsilane and 10 g pyridine were added to the slurry in succession. The mixture was then refluxed for 6 h. After refluxing, it was filtered, washed with chloroform, and dried at 50 °C. The polymeric phase was prepared as described above except that trichlorooctadecylsilane was used instead of the monofunctional reagent. Endcapping was performed with trimethylchlorosilane as a reagent.

Procedures

The column materials were packed by means of a balanced slurry technique into 250 x 4.6 mm i.d. stainless steel tubes. The column hold-up volume, V_m, was determined by injections of small pulses of its deuterated compound into the pure organic solvent. The retention volume was obtained from the flow rate, F (1.0 mL/min), and retention time, t_R. The flow rate was measured by the volumetric flask method. The sample concentration was chosen as low as possible while maintaining suitable signal-to-noise ratios in RI detection. Eluents of appropriate compositions were prepared by weighing. In order to calculate the molar volumes and the component concentrations in prepared eluents, the density of the eluents was determined by using a pycnometer. After chromatographic measurements, the column materials were removed from each column, washed with methanol, dried under reduced pressure and weighed.

Surface excess amounts

Quantitative definitions for the adsorption of binary solvent mixtures at solid-liquid interfaces in terms of surface excess amounts were given by Everett(16). Riedo and Kováts(8) derived general relationships for calculating these surface excess amounts from chromatographic retention data. The practical equation 14 is obtained for calculating the surface excess isotherm after some important equations are derived from the material balance equation(17).

$$\frac{dn_2^{\sigma(v)}}{dc_2^{\ell}} = V_R - V_m \qquad (14)$$

where $n_2^{\sigma(v)}$ is surface excess of component 2 on volume basis, c_2^{ℓ} is concentration of component 2 in bulk liquid, V_R is the retention volume of the solvent disturbance peak, and V_m is the column holdup volume. Equation 1 states that the retention volume of the solvent disturbance peak provides the first derivative of the surface excess isotherm. In this case, components 1 and 2 correspond to water and organic modifier, respectively. Experimental points, ($V_R - V_m$), determined at given composition of the eluent plotted as a function of c_2^{ℓ} show parabolic curves. Therefore, the quadratic expression was fitted on the experimental points. If we assume the molar volume of the liquid mixture, v_m ($= v_1 x_1^{\ell} + v_2 x_2^{\ell}$, x_1^{ℓ} and x_2^{ℓ} are the mole fraction

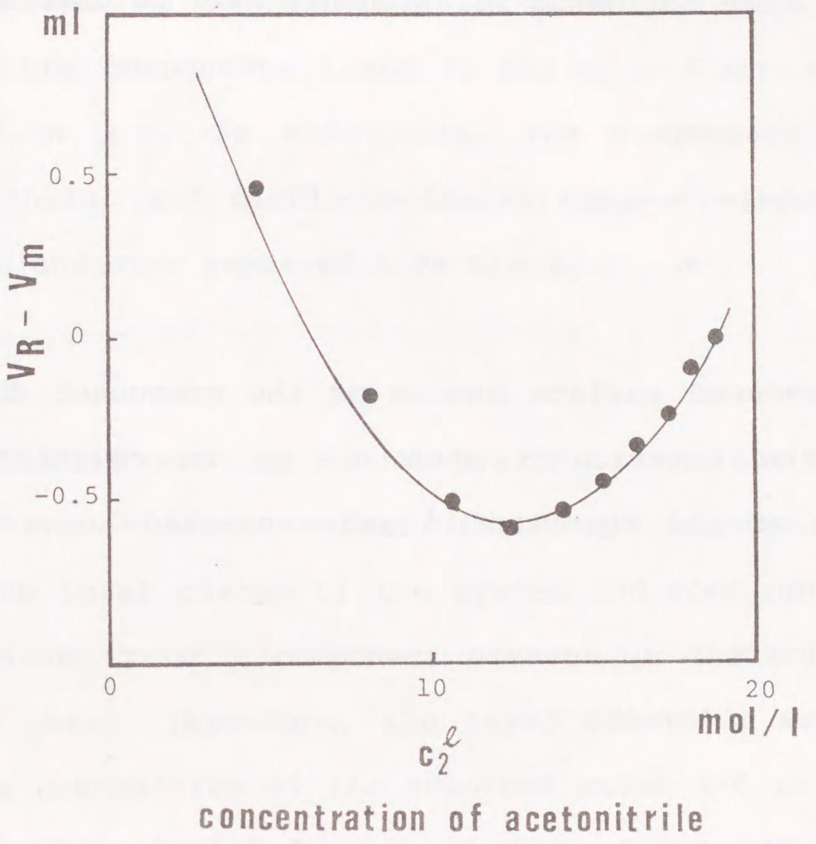

Figure 14. An example of the experimental points ($V_R - V_m$) and the regression curve with monomeric C_{18} phase
Eluent : water-acetonitrile.

of component 1 and 2), is a strictly linear function of the mole fractions (i.e., when the partial molar volumes, v_1 and v_2 of the components are constant over the concentration interval considered), the following relationship will be obtained :

$$n_2^{\sigma(v)} = \frac{v_1}{v_m} n_2^{\sigma(n)} \tag{8}$$

The areal reduced surface excess of the component 2, $\Gamma_2^{(n)}$, is given by the equation 3 when $n_2^{\sigma(v)}$ is calculated by the integration of the equation 14 and converted to $n_2^{\sigma(n)}$ by the equation 8.

$$\Gamma_2^{(n)} = n_2^{\sigma(n)}/S \tag{6}$$

where S is the total surface area of column packings. And the areal reduced surface excess of the component 1, $\Gamma_1^{(n)}$, is as follows :

$$\Gamma_1^{(n)} = -\Gamma_2^{(n)} \tag{15}$$

In the case of methanol-dichloromethane mobile phase, experimental points ($V_R - V_m$) do not fit to the regression curve well. Therefore, we calculate the surface excess amounts by using equation 16, derived by Riedo and Kováts (8).

$$\Gamma_2^{(n)} = (V_{R,2}* - V_{R,1}*)x_1^{\ell} x_2^{\ell}/Sv_m \tag{16}$$

where $V_{R,1}*$ and $V_{R,2}*$ are the retention volumes of the labeled components 1 and 2, respectively. x_1^{ℓ} and x_2^{ℓ} are the mole fractions of the components 1 and 2, and $v_m = v_1 x_1^{\ell} + v_2 x_2^{\ell}$ at the composition x_1^{ℓ}. In this case, the components 1 and 2 represent methanol and dichloromethane, respectively. All runs were at 30 °C and were repeated five times.

The layer model

When dealing with an actual system, it is not sufficient to obtain the surface excess isotherm, because it only represents the total change of the system and does not refer to the actual quantity of a component present in the mobile phase and adsorbed phase. Therefore, the layer model(17) was used to calculate the composition of the adsorbed phase and estimate the absolute adsorbed amount.

Assuming that the adsorbed phase consists of t layers of molecules on a plane smooth homogeneous surface, the mole fraction, x_2^s, of component 2 in the surface phase is given by

$$x_2^s = \frac{t x_2^{\ell} + a_1^{\circ}\Gamma_2^{(n)}}{t - (a_2^{\circ} - a_1^{\circ})\Gamma_2^{(n)}} \tag{18}$$

where a_1° and a_2° are the molar cross-sectional areas of components 1 and 2, which can be calculated from the molar volumes, v_1 and v_2, using the equation of $a_i^\circ = 9200(v_i)^{2/3}$, $(i=1,2)$. This equation is based on the assumption that a molecule is a rigid sphere and has a face-centered cubic structure. Assuming this, $a_i^\circ = 2\sqrt{3}r^2 N$ and $r=(v_i/4\sqrt{2}N)^{1/3}$ are obtained, where r is a molecular radius and $N=6.0 \times 10^{23}$. Using these a_i° values and the experimental surface excess data, $\Gamma_2^{(n)}$, the minimum number of layers, t_{min}, was estimated by satisfying the criteria that values of x_2^s calculated do not exceed unity, and x_2^s always increased with x_2^ℓ i.e. $(\partial x_2^s/\partial x_2^\ell) > 0$. The values of t_{min} are given in Table XI. x_2^s is calculated by the equation 18 with t_{min} values, except that t=2 is used for the monomeric phase in the water-methanol system in order to be compared with the polymeric phase.

The reduced surface excess in the surface phase model can be expressed in the following way.

$$n_2^{\sigma(n)} = (x_2^s - x_2^\ell)n^s \qquad (19)$$

Hence, the total amount of component in the surface phase, n^s, was calculated by equation 19 and the amounts of the components 1 and 2, n_1^s and n_2^s, were obtained. Results were converted to units per area. These results are given in Table XII-XIV.

Results and Discussion

Aqueous mobile phase

The water-acetonitrile and water-methanol systems were

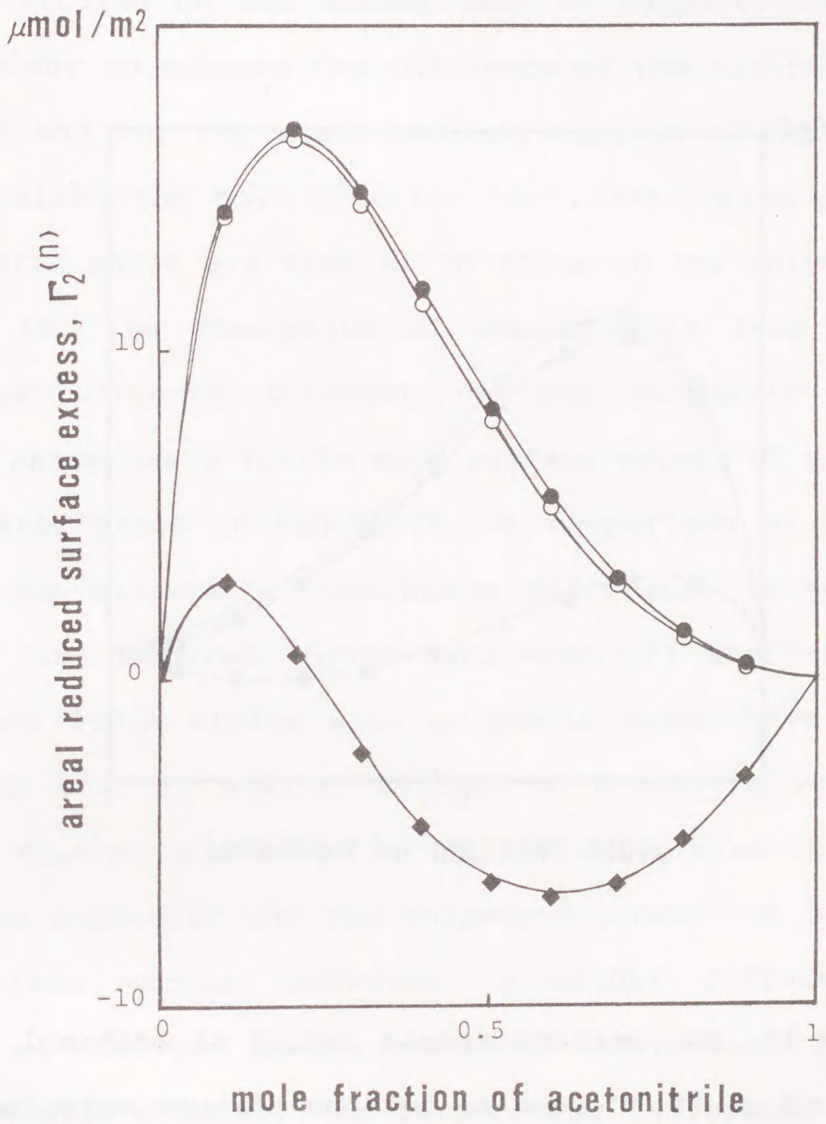

Figure 15. The surface excess amount of acetonitrile adsorbed at 30 °C from an aqueous mixture onto (●) monomeric phase, (○) polymeric phase and (◆) silica plotted against the mole fraction of acetonitrile

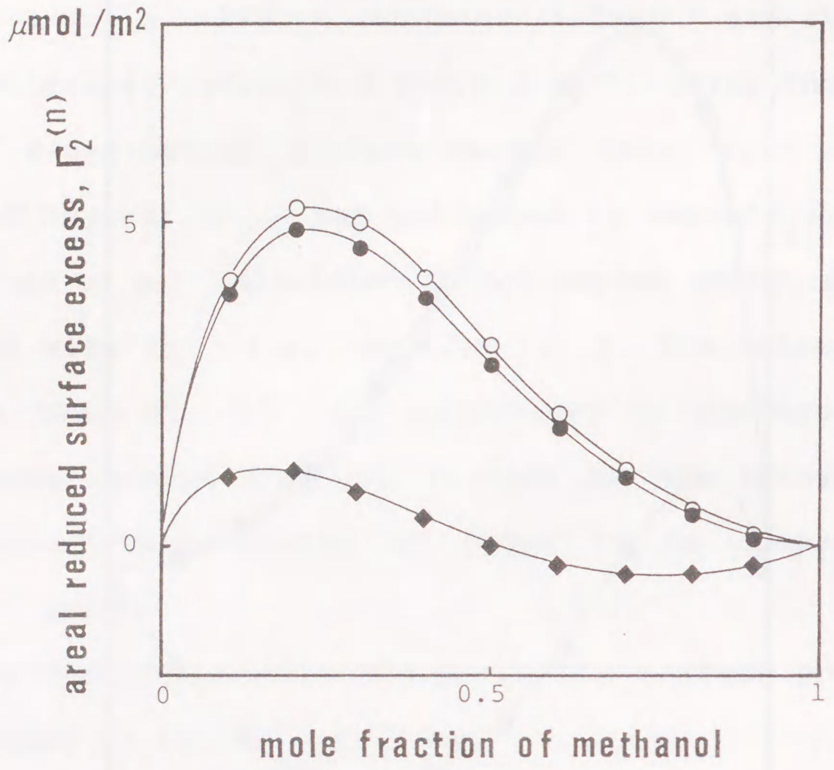

Figure 16. The surface excess amount of methanol adsorbed at 30 °C from an aqueous mixture onto (●) monomeric phase, (○) polymeric phase and (◆) silica plotted against the mole fraction of methanol

studied on the monomeric and polymeric phase at 30 °C. Similarly, they were studied on the silica used as supports of C_{18} bonded phase in order to observe the influence of the silica surface. In Figures 15 and 16, the areal reduced surface excess amounts are plotted against the mole fraction, x_2^{ℓ}. Adsorption isotherms on the monomeric phase are similar to those on the polymeric phase. In Figure 15, the adsorption of acetonitrile from the aqueous mixture is slightly stronger on the monomeric phase. The polymeric phase has a little more surface excess of methanol than the monomeric phase in Figure 16. On comparison of isotherms in each aqueous mixture a remarkable difference between the two phases is not observed. Sander and Wise (2) have reported that the 60- and 100-A silica with polymeric phase have selectivity toward PAHs that is similar to that of monomeric and oligomeric phases in aqueous acetonitrile. If the difference in selectivity between the monomeric and the polymeric phases can be related to their surface excess isotherms, a slight difference of the isotherms on the two phases described above seems to agree with Sander and Wise's report. An interesting difference is that the monomeric phase adsorbs acetonitrile more than the polymeric phase does, and the latter adsorbs more methanol than the former, so that the value of t_{min} in aqueous methanol is different between the two phases, as shown in Table XI. The two phases seem to be somewhat different in selectivity toward the solvent.

Comparison of Figure 15 with Figure 16 indicates that acetonitrile is more preferentially adsorbed on the two phases than methanol is in the aqueous system. This means the amount of

Table XI

THE VALUES OF t_{min} SATISFYING THE CRITERIA

	t_{min}	
	Monomeric phase	Polymeric phase
CH_3CN/H_2O	4	4
$MeOH/H_2O$	1	2
$CH_2Cl_2/MeOH$	2	2

Table XII

COMPOSITION OF ADSORBED PHASE AND THE ABSOLUTE ADSORBED AMOUNT FOR WATER(1)-ACETONITRILE(2) SYSTEM USING THE LAYER MODEL

	Monomeric phase			Polymeric phase		
x_2^ℓ	x_2^s	n_2^s *	n_1^s *	x_2^s	n_2^s *	n_1^s *
0.893	0.907	28.96	2.97	0.903	30.25	3.25
0.789	0.840	28.64	5.46	0.834	28.57	5.69
0.697	0.787	27.42	7.42	0.779	27.14	7.70
0.595	0.751	26.70	8.85	0.741	26.41	9.23
0.506	0.734	26.38	9.56	0.722	26.11	10.06
0.397	0.725	26.17	9.93	0.711	25.90	10.53
0.305	0.713	25.94	10.44	0.700	25.62	10.98
0.205	0.650	24.56	13.22	0.638	24.30	13.79
0.099	0.423	18.63	25.41	0.418	18.44	25.67

* Measurements in $\mu mol/m^2$

Table XIII

COMPOSITION OF ADSORBED PHASE AND THE ABSOLUTE ADSORBED AMOUNT FOR WATER(1)-METHANOL(2) SYSTEM USING THE LAYER MODEL

	Monomeric phase			Polymeric phase		
x_2^l	x_2^s	n_2^s *	n_1^s *	x_2^s	n_2^s *	n_1^s *
0.898	0.904	17.03	1.80	0.907	17.13	1.76
0.805	0.827	16.24	3.40	0.832	16.73	3.38
0.706	0.755	15.41	5.00	0.763	15.66	4.87
0.603	0.688	14.60	6.62	0.700	14.69	6.30
0.500	0.628	13.67	8.10	0.642	13.80	7.70
0.397	0.567	12.70	9.69	0.583	12.99	9.28
0.298	0.496	11.57	11.76	0.514	11.84	11.20
0.202	0.400	9.84	14.77	0.416	10.13	14.22
0.103	0.247	6.65	20.28	0.257	6.88	19.89

* Measurements in μmol/m^2

enriched organic solvent is dependent on its solvent strength. It can be seen from Tables XI through XIV that the value of t_{min} in aqueous acetonitrile is twice or more that in the other mobile phase, and the absolute adsorbed amount of acetonitrile is much larger than that of the others. This suggests that acetonitrile permeates well into crossed and overlapped alkyl chains. Therefore, the alkyl chains in aqueous acetonitrile are presumed to be more mobile than those in the other mixture. In aqueous methanol, a small adsorbed amount seems to bring about strong interaction between water and methanol on the basis of isotherms in this study, though the association of water with methanol is complicated(18). According to Sander et al.(19), the methanol molecules could behave as a surfactant, orientating themselves so that the hydrophobic methyl groups intercalate among the alkyl chains, and the polar hydroxyl groups project into the bulk mobile phase. The hydroxyl groups would be available for hydrogen bonding to water as well as to methanol in the bulk mobile phase, and hence the adsorbed amount of methanol is small as a whole.

On the other hand, the isotherms of the silica in these two aqueous mixtures exhibit a pronounced difference as seen in Figures 15 and 16. The silica adsorbs a much larger amount of water in aqueous acetonitrile. The isotherm of the silica in aqueous methanol is flat. This means that water is not selectively adsorbed on the silica, because water associates with methanol, and methanol as well as water has a good affinity for the silanol group on the silica. The isotherms of the silica in the two aqueous mixtures reflect the difference in the

Table XIV

COMPOSITION OF ADSORBED PHASE AND THE ABSOLUTE ADSORBED AMOUNT FOR METHANOL(1)-DICHLOROMETHANE(2) SYSTEM USING THE LAYER MODEL

	Monomeric phase			Polymeric phase		
x_2^ℓ	x_2^s	n_2^s *	n_1^s *	x_2^s	n_2^s *	n_1^s *
0.901	0.893	12.84	1.54	0.792	11.21	2.95
0.792	0.855	12.00	2.03	0.753	10.93	3.58
0.698	0.816	11.52	2.60	0.724	10.50	4.00
0.601	0.780	11.14	3.14	0.677	9.94	4.74
0.505	0.704	10.28	4.33	0.652	9.68	5.17
0.401	0.600	9.03	6.02	0.599	8.51	6.71
0.298	0.469	7.36	8.33	0.418	6.63	9.23
0.201	0.333	5.46	10.93	0.284	4.74	11.95
0.101	0.173	2.97	14.17	0.151	2.62	14.76

* Measurements in $\mu mol/m^2$

miscible state between water-acetonitrile and water-methanol.

Methanol-dichloromethane system

The methanol-dichloromethane system was studied on the monomeric, polymeric phases and the silica itself at 30 ºC. This system has frequently been used as an eluent for separation of large PAHs(6, 7, 20). Jinno et al.(7) have reported that the retention order of the four PAHs used as probes changes drastically between the monomeric and the polymeric phases, regardless of the pore sizes. This report is expected to make a difference between the two phases in the surface excess isotherms. In Figure 17, the areal reduced surface excess amounts are plotted against the mole fraction, x_2^ℓ. The surface excess is less than in the two aqueous mixtures. Comparison of the monomeric and polymeric phases reveals a significant difference in the surface excess amounts. The monomeric phase adsorbs a larger amount of dichloromethane than the polymeric phase. In other words, the polymeric phase holds a larger amount of methanol than the monomeric phase. The polymeric phase seems to have a stronger affinity for methanol than the monomeric phase. As shown in Figure 17, the silica preferentially adsorbs methanol, whose surface excess amount is comparable to that of water in aqueous acetonitrile. The selective adsorption of methanol on the silica is expected to yield a large adsorbed amount of dichloromethane on the bonded phases; in aqueous acetonitrile, water is selectively adsorbed on the silica and acetonitrile is largely adsorbed on the bonded phases. In

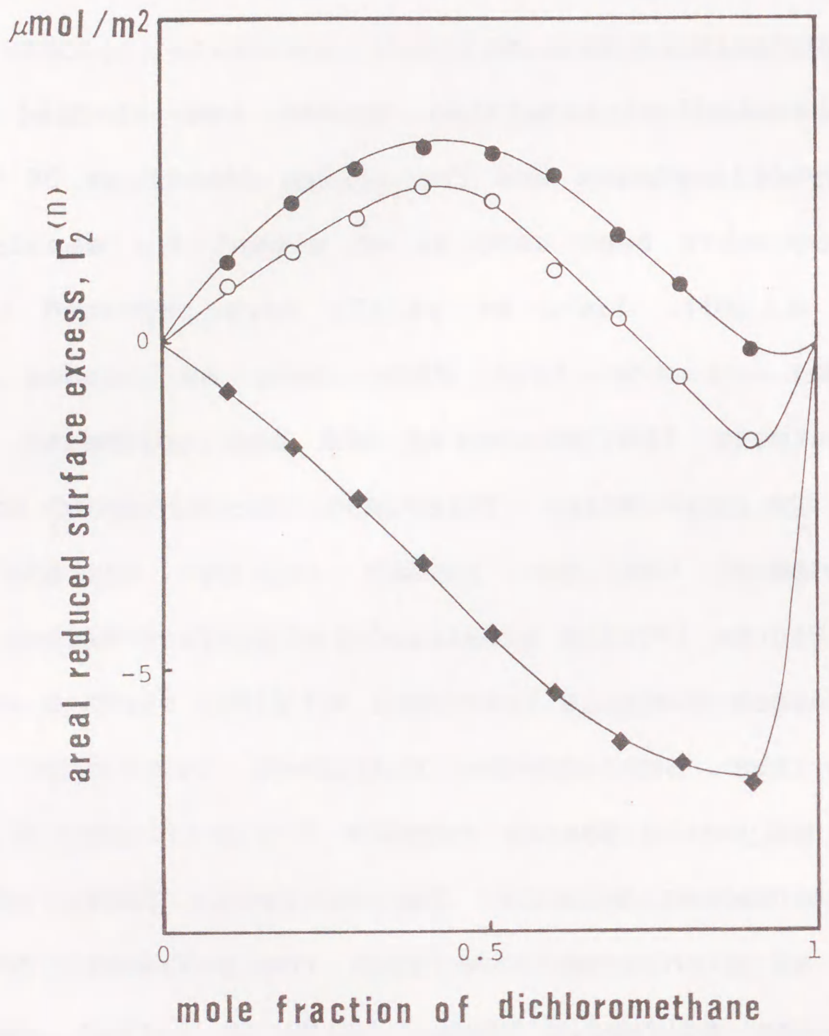

Figure 17. The surface excess amount of dichloromethane adsorbed at 30 °C from a methanol mixture onto (●) monomeric phase, (○) polymeric phase, and (◆)silica plotted against the mole fraction of dichloromethane

practice, however, dichloromethane is poorly adsorbed on the bonded phases, as shown in Figure 17. This result seems to be under the influence of the silica, that is, it seems reasonable to assume that the silica is exposed to the mobile phase. It is envisioned as a result of the alkyl chain aggregation. This means that the structure of the bonded phases becomes more rigid. As seen in Table XIV, the polymeric phase adsorbs much more methanol than the monomeric phase does. This suggests that the alkyl chains of the polymeric phase aggregate more tightly than those of the monomeric phase. Therefore, it seems that the polymeric phase becomes more rigid and shows a good selectivity toward PAHs. The cause of alkyl chain aggregation must be considered in detail by further experiment.

In conclusion, the isotherms obtained from two aqueous mixtures gave poor information about differences in structure between the monomeric and the polymeric phases. However, the significant difference between the two phases in methanol-dichloromethane indicates that the structure of the polymeric phase, which is more rigid than in the aqueous mixture, is more tight than that of the monomeric phase. Therefore, the polymeric phase shows a high selectivity toward PAHs. From an alternative point of view, the comparison of isotherms of the silica in three mobile phase systems may give a clue to studying the state of the binary mobile phase.

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III-4

Comparison of Native and Modified Surfaces on Surface Excess Amount

Introduction

The vast variety of bonded alkyl phases has brought about the growth of reversed-phase liquid chromatography. For the preparation of the bonded alkyl phases, especially C_{18} bonded phase, various methods have been used and also various end-capping reactions were applied. The C_{18} bonded phases from several manufacturers often provide different chromatographic properties. It is accepted (1-6) that their chromatographic properties are ascribed to differences in the nature of the silica support making up the bonded phase materials. Silanol groups on the surface of silica-based packings are said to play a key role in undesirable retention processes (1-8).

In this paper, author has attempted to characterize the surface properties of unmodified and chemically modified silicas by measuring their surface excess isotherms and the retention of pyridine with aqueous acetonitrile mobile phase. Four kinds of silicas were used as native surfaces and were chemically modified by using the conventional preparation method of the monomeric C_{18} phase. The chromatographic method to determine the surface excess isotherms is based on measurements of the retention volumes of labelled components of the eluent constituents. Pyridine was used in aqueous acetonitrile containing trifluoroacetic acid to assess the contribution of ion exchange sites on native and modified surfaces to its retention. Phenol was used to normalize the pyridine retention to eliminate the contribution of the differences in hydrophobic properties of packing materials to the values.

Experimental

Apparatus

Two HPLC systems were utilized in this investigation. For measuring the retention of pyridine, a Tri Rotar pump equipped with a UVIDEC-100-11 detector (Jasco) was used. For determination of the surface excess isotherms, an 880-PU pump (Jasco) coupled with a Shodex RI SE-51 differential refractive index detector (Showa Denko) was used. Two constant-temperature water baths (model ESP-47 and TE-108M, Toyo) were used to control the column temperature, which was measured with an alumel-chromel thermocouple. The chromatograms were recorded on a Chromatopac CR1A (Shimadzu).

Chemicals and Materials

Liquid chromatographic grade solvents, deuterated compounds and trifluoroacetic acid were obtained from Nacalai Tesque. Monomeric C_{18} bonded phases were prepared as follows: prior to the reaction, the silica was dried at 220 °C for 4h. Dry silica (4g) was suspended in 100ml of dry toluene, and 5ml of

chlorodimethyloctadecylsilane and 5ml of pyridine were added to the slurry in succession. The mixture was then refluxed for 6h after which it was filtered, washed with chloroform and methanol, and dried at 110 °C. The silicas used were Vydac 101TPB(Vy-101, Separations Group), Shiseido's silica(S-300, Shiseido), Super Micro Bead Silica Gel(F-300, Fuji-Davison) and YMC-GEL SIL-300-S(YMC-300, YMC). Details about the characteristics of the column packings are summarized in Table XV.

Procedures

The column materials were packed by means of a balanced slurry technique into 250 X 4.6 mm i.d. stainless steel tubes. The column hold-up volume was determined by injecting small pulses of deuterated acetonitrile in pure acetonitrile mobile phase. The retention volume was obtained from the flow rate which was measured by the volumetric flask method and retention time. The sample concentration was chosen as low as possible while maintaining suitable signal-to-noise ratio in RI detection. Eluents of appropriate compositions were prepared by weighing. A mixture of pyridine and phenol was eluted with 60 % (v/v) aqueous acetonitrile pH controlled by trifluoroacetic acid.

Surface excess amounts

Quantitative definitions for the adsorption of binary liquid mixtures at solid-liquid interfaces in terms of surface excess amounts were given by Everett (9). Riedo and Kovats (10) derived general relationships for calculating these surface

Table XV

Characteristics of the adsorbents

	surface area (m^2/g)	% carbon	surface coverage ($\mu mol/m^2$)	mean pore diameter (A)
Vy-101	67	-	-	382
Vy-C_{18}	50	4.9	3.25	352
S-300	168	-	-	274
S-C_{18}	123	10.2	2.91	260
F-300	82	-	-	398
F-C_{18}	70	5.5	3.03	376
YMC-300	107	-	-	352
YMC-C_{18}	82	6.7	2.85	328

excess amounts from chromatographic retention data. The necessary expressions for calculating points on the adsorption isotherms are taken from ref 10. The surface concentration of component 2 is given by

$$\Gamma_2^{(n)} = (V_{R,2}* - V_{R,1}*)x_2^{\ell}x_1^{\ell}/Sv_m \qquad (16)$$

where $\Gamma_2^{(n)}$ is the areal reduced surface excess of component 2 and x_1^{ℓ} and x_2^{ℓ} are the mole fraction of components 1 and 2 in the eluent. The symbol V_R is the retention volume, S is the surface area of silica, and v_m is the mean molar volume of the eluent at

$$v_m = v_1 x_1^{\ell} + v_2 x_2^{\ell} \qquad (17)$$

the composition x_1^{ℓ}, and v_1 and v_2 are the partial molar volume of components 1 and 2. The asterisk refers to labelled components of the element, 1* or 2*. And the areal reduced surface excess of component 1 is as follows :

$$\Gamma_1^{(n)} = -\Gamma_2^{(n)} \qquad (15)$$

The layer model

When dealing with an actual system, it is not sufficient to obtain the surface excess isotherm, because it only represents the total change of the system and does not refer to the actual quantity of a component present in the mobile phase and adsorbed phase. Therefore, the layer model(9) was used to calculate the composition of the adsorbed phase and estimate the absolute adsorbed amount.

Assuming that the adsorbed phase consists of t layers of molecules on a plane smooth homogeneous surface, the mole fraction, x_2^s, of component 2 in the surface phase is given by :

$$x_2^s = \frac{t x_2^\ell + a_1^\circ \Gamma_2^{(n)}}{t - (a_2^\circ - a_1^\circ)\Gamma_2^{(n)}} \qquad (18)$$

where a_1° and a_2° are the molar cross-sectional areas of components 1 and 2, which can be calculated from the molar volumes, v_1 and v_2, using the equation of $a_i^\circ = 9200(v_i)^{2/3}$, (i=1,2). Using these a_i° values and the experimental surface excess data, $\Gamma_2^{(n)}$, the minimum number of layers, t_{min}, was estimated by satisfying the criteria that x_2^s values calculated do not exceed unity, and x_2^s always increased with x_2^ℓ i.e. $(\partial x_2^s/\partial x_2^\ell) > 0$. x_2^s is calculated by the equation 18 by inserting t_{min} value.

Results and Discussion

To observe the changes on the surface of unmodified and chemically modified silicas, the surface excess isotherms were determined in aqueous acetonitrile at 30 °C. In Figures 18 and 19, the areal reduced surface excess amount are plotted against

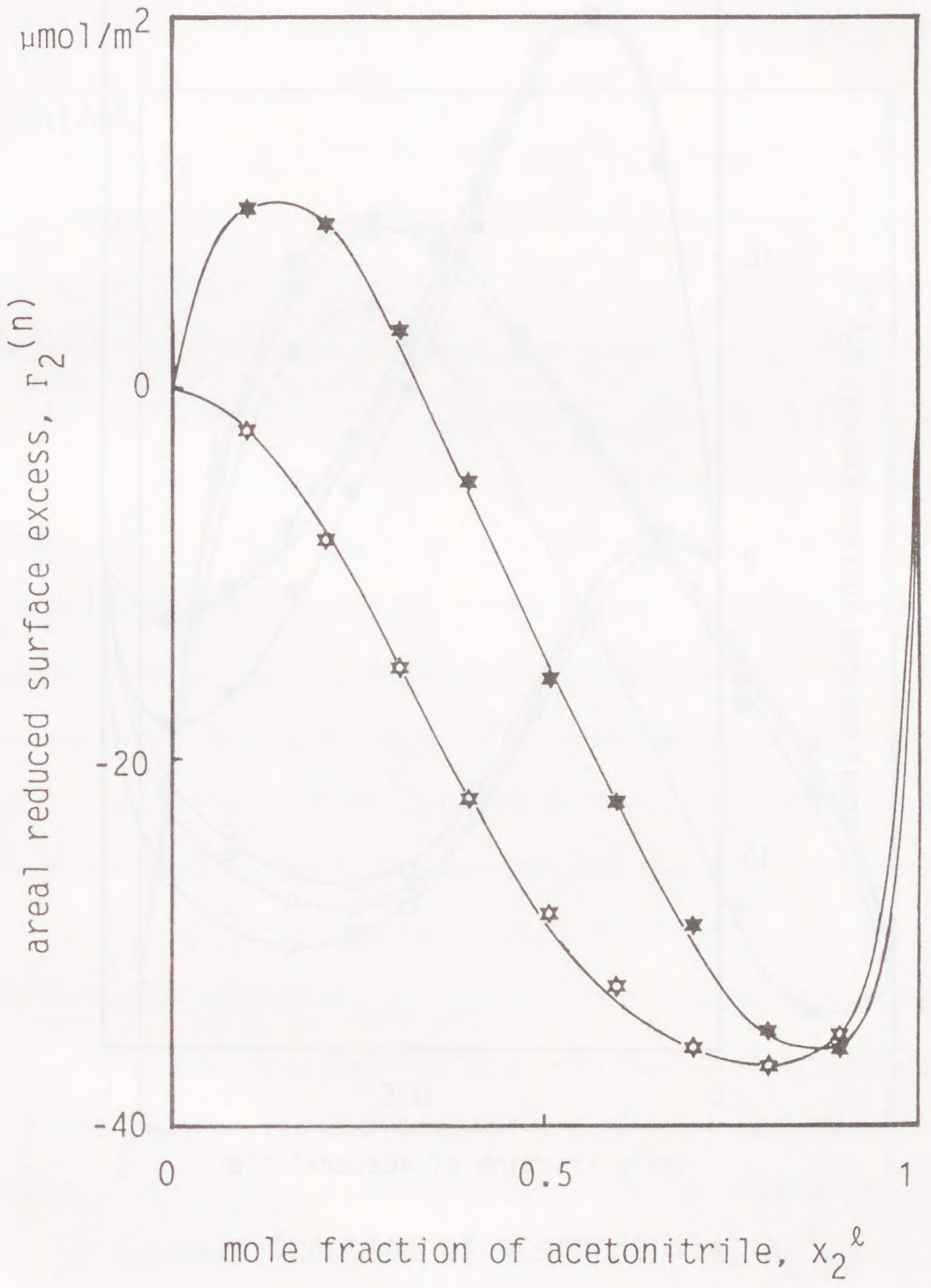

Figure 18. The surface excess amount of acetonitrile adsorbed at 30 °C from an aqueous mixture onto (✡) Vy-101 and (✱) Vy-C_{18} plotted against the mole fraction of acetonitrile

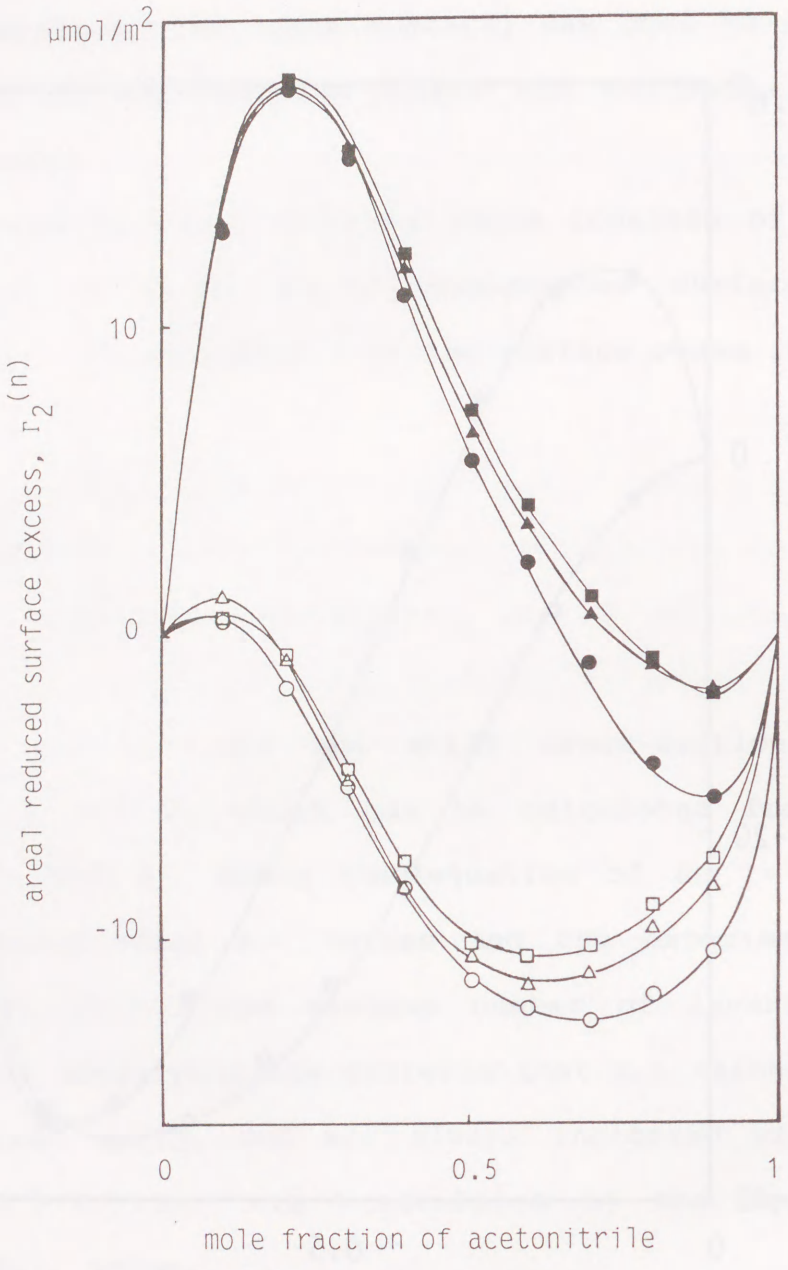

Figure 19. The surface excess amount of acetonitrile adsorbed at 30 °C from an aqueous mixture onto (○) S-300, (●) S-C_{18}, (△) F-300, (▲) F-C_{18}, (□) YMC-300 and (■) YMC-C_{18} plotted against the mole fraction of acetonitrile

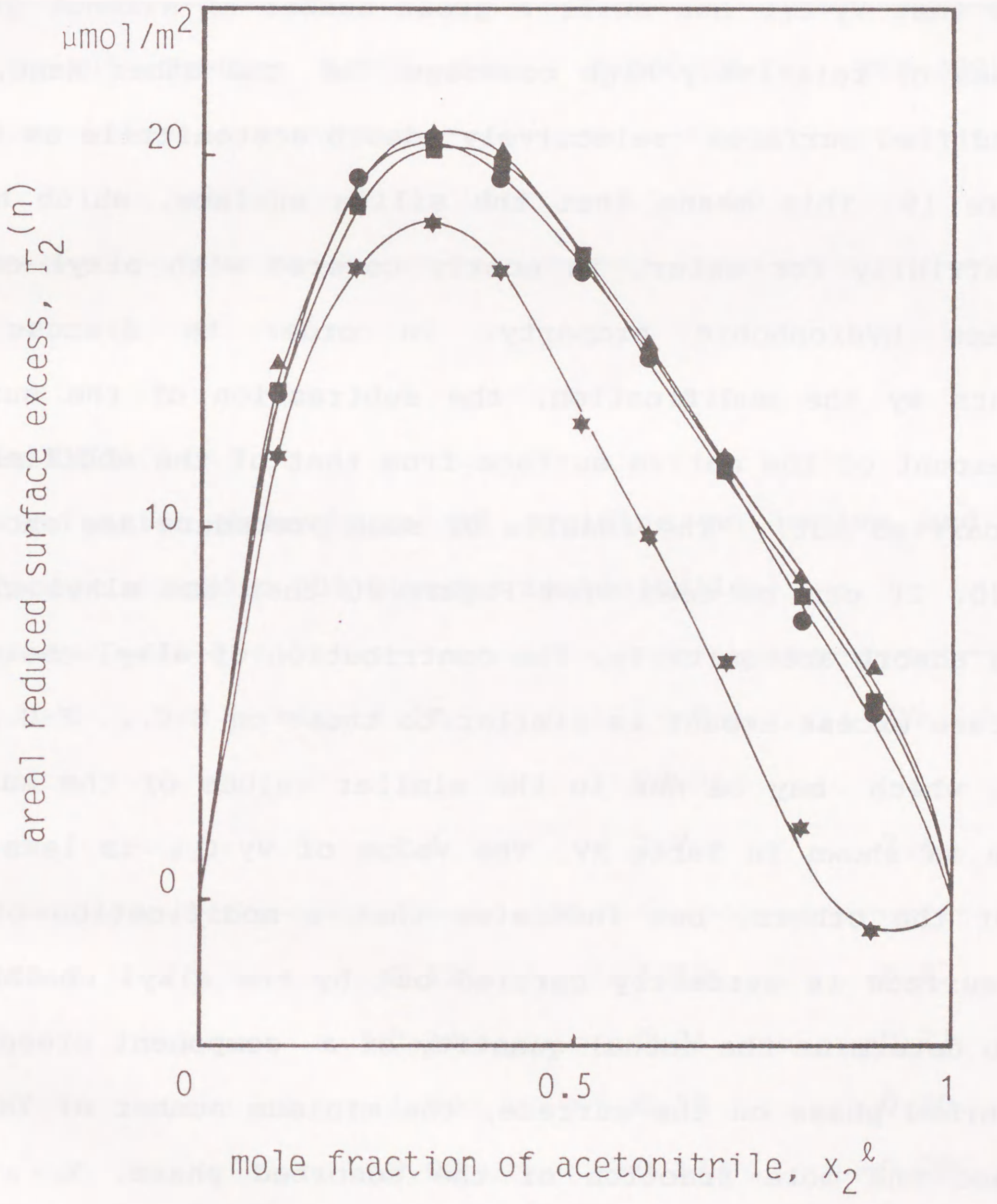

Figure 20. The increments by the modification on the surface excess amount for acetonitrile adsorbed at 30 °C from an aqueous mixture

Symbols as in Figure 18 and 19.

the mole fraction, x_2^ℓ. Vy-C_{18}, as well as Vy-101, adsorbs a large quantity of water as seen in Figure 18. This result suggests that Vy-C_{18} has still a great number of silanol groups regardless of relatively high coverage. On the other hand, the other modified surfaces selectively adsorb acetonitrile as found in Figure 19. This means that the silica surface, which has a strong affinity for water, is mostly covered with alkyl chains which are hydrophobic property. In order to discuss the increments by the modification, the subtraction of the surface excess amount of the native surface from that of the modified one can be carried out. The results of such procedure are shown in Figure 20. It can be seen from Figure 20 that the alkyl chains strongly adsorb acetonitrile. The contribution of alkyl chains to the surface excess amount is similar to those on S-C_{18}, F-C_{18} and YMC-C_{18}, which may be due to the similar values of the surface coverage as shown in Table XV. The value of Vy-C_{18} is less than those of the others, but indicates that a modification of the native surface is evidently carried out by the alkyl chains. In order to determine the actual quantity of a component present in the adsorbed phase on the surface, the minimum number of layers, t_{min}, and the mole fraction of the adsorbed phase, x_2^s, were calculated by using the layer model. These results are shown in Table XVI and Figure 21. The number of adsorbed layers and the amount of acetonitrile in the adsorbed phase are increased by the modification as indicated in Table XVI and Figure 21. The modified surface should be greatly depended upon the native one in the concern of the surface excess amount, since the surface

Table XVI

The values of t_{min} satisfied the criteria

	Vy-101	Vy-C_{18}	S-300	S-C_{18}	F-300	F-C_{18}	YMC-300	YMC-C_{18}
t_{min}	6	9	3	6	4	6	3	5

Table XVII

The retention behaviours of pyridine on native and modified surfaces in acidic 60% aqueous acetonitrile

	$k'_{py} = (V_{R,pyridine} - V_{R,phenol})/V_{R,phenol}$		
		PH	
	3.4	2.7	2.4
Vy-101	0.13	-0.03	-0.03
Vy-C_{18}	-0.14	-0.21	-0.21
S-300	1.00	0.28	0.20
S-C_{18}	0.09	-0.17	-0.20
F-300	1.92	0.45	0.29
F-C_{18}	0.95	0.30	0.12
YMC-300	1.10	0.32	0.23
YMC-C_{18}	1.16	0.39	0.10

excess amount of the modified surface is predicted to a summation of those of the alkyl chains and the native one.

Silanol groups on the surface are commonly known to be either isolated or hydrogen bonded. The majority is weakly acidic, although a small proportion is strong acidic and is ionized even at low pH. It has been reported (2,3,7,11) that the small portion of silanol groups causes undesired adsorption of basic compounds. To demonstrate the change of the highly acidic silanol groups before and after the modification of silicas, the retention of pyridine was measured with acidic aqueous acetonitrile mobile phase. Table XVII demonstrates the results for native and modified surfaces using k'_{py} which is normalized by phenol's retention. As can be seen in Table XVII, Vy-101 and Vy-C_{18} cannot retain protonated pyridine. It is speculated that Vy-101 has a great number of ordinary silanol groups which are undissociated at pH below 3, and that the majority of the residual silanol groups of Vy-C_{18} are probably "buried" silanol groups (2). The other native surfaces retain protonated pyridine, but cannot induce any tailing under this condition except F-300. It seems that the siloxane bridges play an important role in the retention of protonated pyridine although it is not clear what interaction is induced with protonated one. The peak of pyridine is more broadening on F-C_{18} than on F-300. This suggests that the ion exchange sites which are responsible for the adsorption of pyridine cannot be modified by chemically bonded reaction. S-C_{18}, prepared from S-300 which has no ion exchange sites at these pH, cannot retain protonated pyridine since the

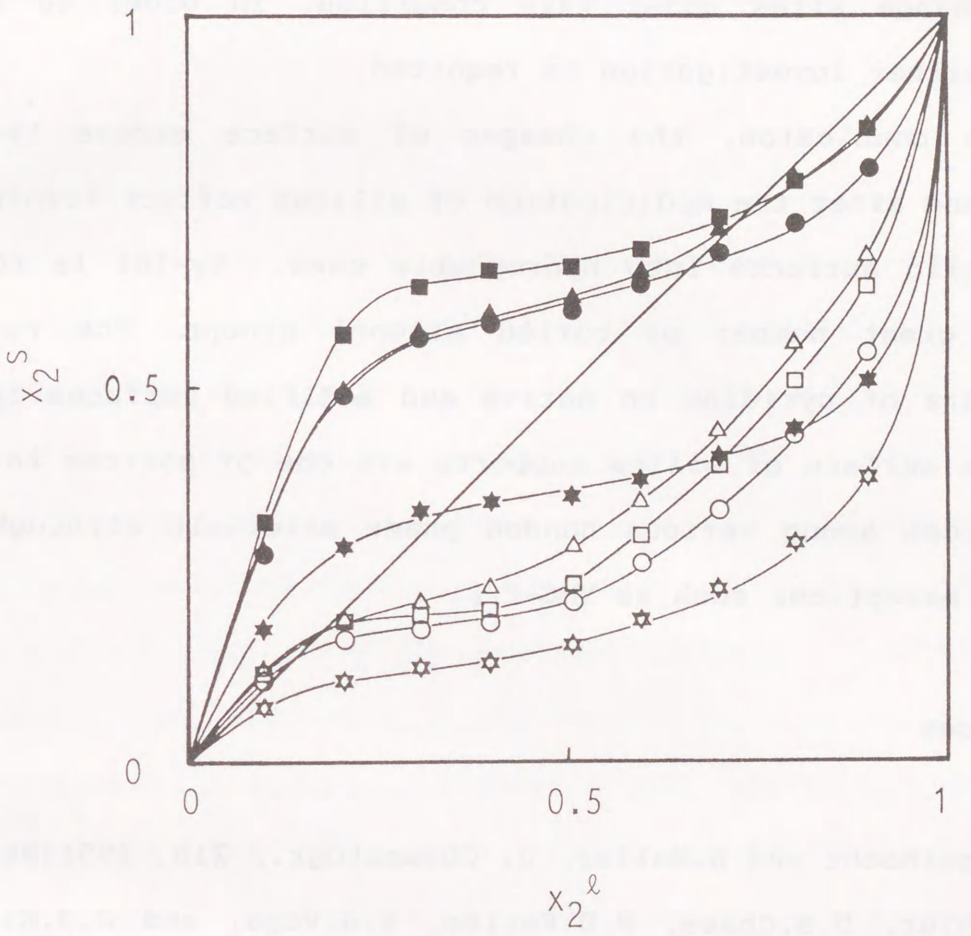

Figure 21. **The composition of the adsorbed phase at 30 °C from an aqueous mixture onto (✡) Vy-101, (✱) Vy-C_{18}, (○) S-300, (●) S-C_{18}, (△) F-300, (▲) F-C_{18}, (□) YMC-300 and (■) YMC-C_{18} plotted against the mole fraction of acetonitrile**

retention sites of pyridine may be covered with alkyl chains. The most striking result is that YMC-C$_{18}$ shows some tailing for the pyridine peak in spite of being made from YMC-300 which has no ion exchange sites under this condition. In order to explain this, further investigation is required.

In conclusion, the changes of surface excess isotherms before and after the modification of silicas reflect turning from hydrophilic surfaces into hydrophobic ones. Vy-101 is found to have a great number of buried silanol groups. The retention behaviours of pyridine on native and modified surfaces indicate that the surface of silica supports are one of sources to induce differences among various bonded phase materials although there is some exceptions such as YMC-C$_{18}$.

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III-5

Surface State of Chemically Modified Silicas in Aqueous Acetonitrile and Methanol

Introduction

It is well known that the organic solvent in the mobile phase is enriched in the stationary bonded phase in reversed-phase liquid chromatography. The enrichment modifier can be determined as the surface excess amount. The surface excess amount of a given component of the mobile phase is defined as the difference between the amount of component actually present in the system, and that being present in a reference system if the bulk concentrations in the adjoining phases were maintained up to a geometrically dividing surface(1). In previous work(2-4), author has found that the surface excess amount differs between acetonitrile-water and methanol-water systems, reflecting the difference of the associated states in these two eluents. Very recent investigation by Gilpin et al.(5) has also indicated that for methanol-water the difference between the compositions of the mobile and surface phases is small, but for acetonitrile-water this difference is significant. In the acetonitrile-water system, the weak association between acetonitrile and water seems to promote the adsorption of water on the silica surface and acetonitrile on the chemically modified surface because the silica matrix has a strong affinity for water through hydrogen bonding with silanol groups, and the chemically modified surface prefers an organic solvent owing to the non-polarity of the bonded hydrocarbon moieties. In the methanol-water system, the low surface excess amount might be due to a strongly associated state of methanol and water caused by strong hydrogen bonding. The concepts stated above are supported by the investigation of Katz et al.(6,7). This difference of the associated states between the two eluents is assumed to be influenced by the motion of chemically bonded hydrocarbon chains on the silica surface.

Tchapla et al.(8) demonstrated that there is a break in the log k' vs n_c (the number of carbon atoms in the solute alkyl chain) graph for all available bonded phases. They studied the retention of different homologous series as a function of temperature in order to investigate whether there is a similar discontinuity in the plot of ΔH^0 vs n_c and ΔS^0 vs n_c and also, whether the discontinuity is related to the chain length of the stationary phase. They concluded that all the results support the idea that there is a penetration of the solute into the bonded layer. Therefore, the thermodynamic behaviour of homologous series may be expected to give information about the surface state of the chemically modified silica. The purpose of this work was to study the thermodynamic behaviour of a series n-alkylbenzenes as a function of eluent composition in order to investigate the influence of the aqueous organic eluent on the surface state of the chemically modified silica.

Experimental

Apparatus

The liquid chromatograph was constructed from a 880-PU pump (Jasco, Tokyo, Japan), a sample injector (Model-EIE005, Senshu Scientific Co., Tokyo, Japan) and a UVIDEC-100-II detector (Jasco). A Model ESP-47 water-bath (Toyo, Tokyo, Japan) was used to maintain the column temperature, which was measured with an alumel-chromel thermocouple. The chromatograms were recorded on a Chromatopac CR1A (Shimadzu, Kyoto, Japan).

Chemicals and Materials

Liquid chromatographic grade solvents, deuterated compounds and n-alkylbenzenes (the number of carbon atoms in the alkyl chains, n = 4,5,6,7,8,10) were obtained from Nacalai Tesque (Kyoto, Japan). Monomeric C_{18} phases (Vy-C_{18}, S-C_{18}, YMC-C_{18}) which were prepared as in previous work(4) were used as the packing materials. They were packed by means of a balanced slurry technique into 250 x 4.6 mm I.D. stainless-steel tubes in our laboratory. Information on the column packings is summarized in Table XVIII.

Procedures

The column holdup volume was determined by injecting a small pulse of deuterated analogue into the pure organic solvent with refractive index detection. The retention volume, V_R, was obtained from the flow-rate and measured retention time.

Table XVIII

Characteristics of the adsorbent

	surface area (m^2/g)	% carbon	surface coverage ($\mu mol/m^2$)	mean pore diameter(A)
Vy-C_{18}	50	4.9	3.25	352
S-C_{18}	123	10.2	2.91	260
YMC-C_{18}	82	6.7	2.85	328

The nominal flow-rate used throughout the study was 1 ml/min. The actual flow-rate was measured by collecting the column eluent in a 10-ml volumetric flask. The k' values were determined at column temperatures of 30, 40, 50 and 60 °C. Eluents of appropriate compositions were prepared by weighing.

van't Hoff plots

For each solute the retention is given by the following equation

$$\ln k' = -\Delta H^0/RT + \Delta S^0/R + \ln \phi \qquad (4)$$

where ΔH^0 is the partial molar enthalpy change for the transfer of the solute from the infinite dilution standard state in the mobile phase to the infinite dilution standard state in the stationary phase, ΔS^0 is the associated change in standard entropy, R is the gas constant and ϕ the phase ratio. If ΔH^0 and ΔS^0 are independent of temperature over the temperature range of interest, a plot of ln k' vs 1/T will be linear. The slope of this line gives standard enthalpies of transfer while standard entropies of transfer are calculated from the intercept and they are dependent on the value of the phase ratio. Enthalpies and entropies of the transfer were calculated through linear regression analysis of van't Hoff plots. Eluent compositions were varied from 0.5 to 0.8 mole fraction because the phase ratio is assumed to be approximately constant over this concentration range as a result of previous studies.

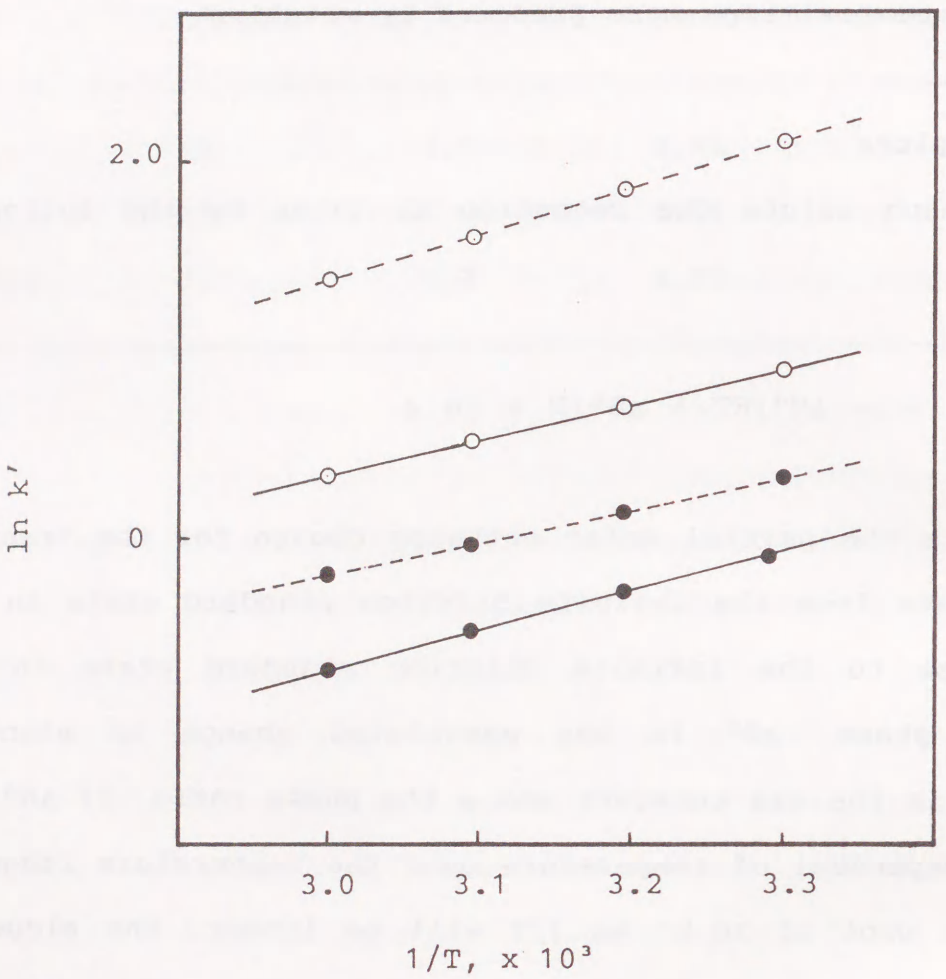

Figure 22. van't Hoff plots for octylbenzene

—— : CH_3CN/H_2O, ------ : CH_3OH/H_2O

mole fraction of organic modifier = 0.6 (○), 0.8 (●).

Results and Discussion

For the n-alkylbenzenes, each mobile phase composition and each bonded phase, all the experimental plots of ln k' vs 1/T are linear over the temperature range 30-60 °C. Some experimental results are shown in Figure 22. The contribution of the temperature to the retention behaviour of n-alkylbenzene becomes greater with increasing water concentration in the methanol-water system, where the capacity factors of the n-alkylbenzenes decrease fairly rapidly with an increase in temperature at high water concentration as can be seen in Figure 22. In acetonitrile-water system, the capacity factors of n-alkylbenzenes decrease more rapidly with increasing temperature in high acetonitrile concentrations than in low ones although the effect is small. With the other packing materials, a similar tendency has been found. ΔH^0 and $(\Delta S^0 + \ln \phi)$ were calculated through linear regression analysis for individual cases. The correlation coefficients were always greater than 0.996 in acetonitrile-water or 0.998 in methanol-water. Figure 23 A, B and C show the trends of change of ΔH^0 and Figure 24 A, B and C show the trends of change of ΔS^0 (because $\ln \phi$ is approximately constant) as a function of mole fraction of organic modifier.

By comparing acetonitrile-water and methanol-water systems, an interesting fact emerges. In each instance, both enthalpies and entropies of transfer increase with the mole fraction of methanol in the eluent, and decrease with increasing acetonitrile concentration in the eluent. These plots often

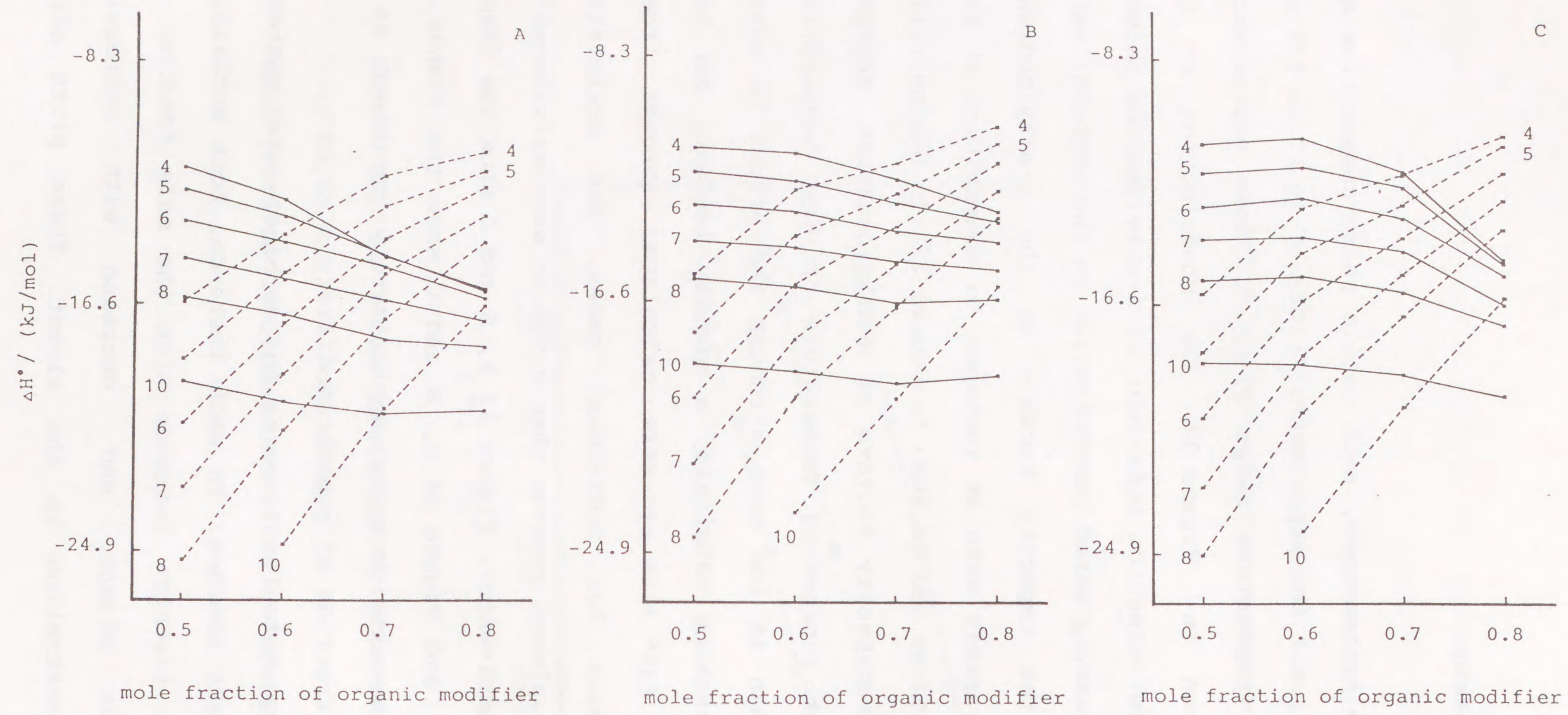

Figure 23. ΔH⁰ as a function of mobile phase composition

—— : CH_3CN/H_2O, ········ : CH_3OH/H_2O

4 = butylbenzene, 5 = pentylbenzene, 6 = hexylbenzene,

7 = heptylbenzene, 8 = octylbenzene, 10 = decylbenzene

A : Vy-C_{18}, B : S-C_{18}, C : YMC-C_{18}

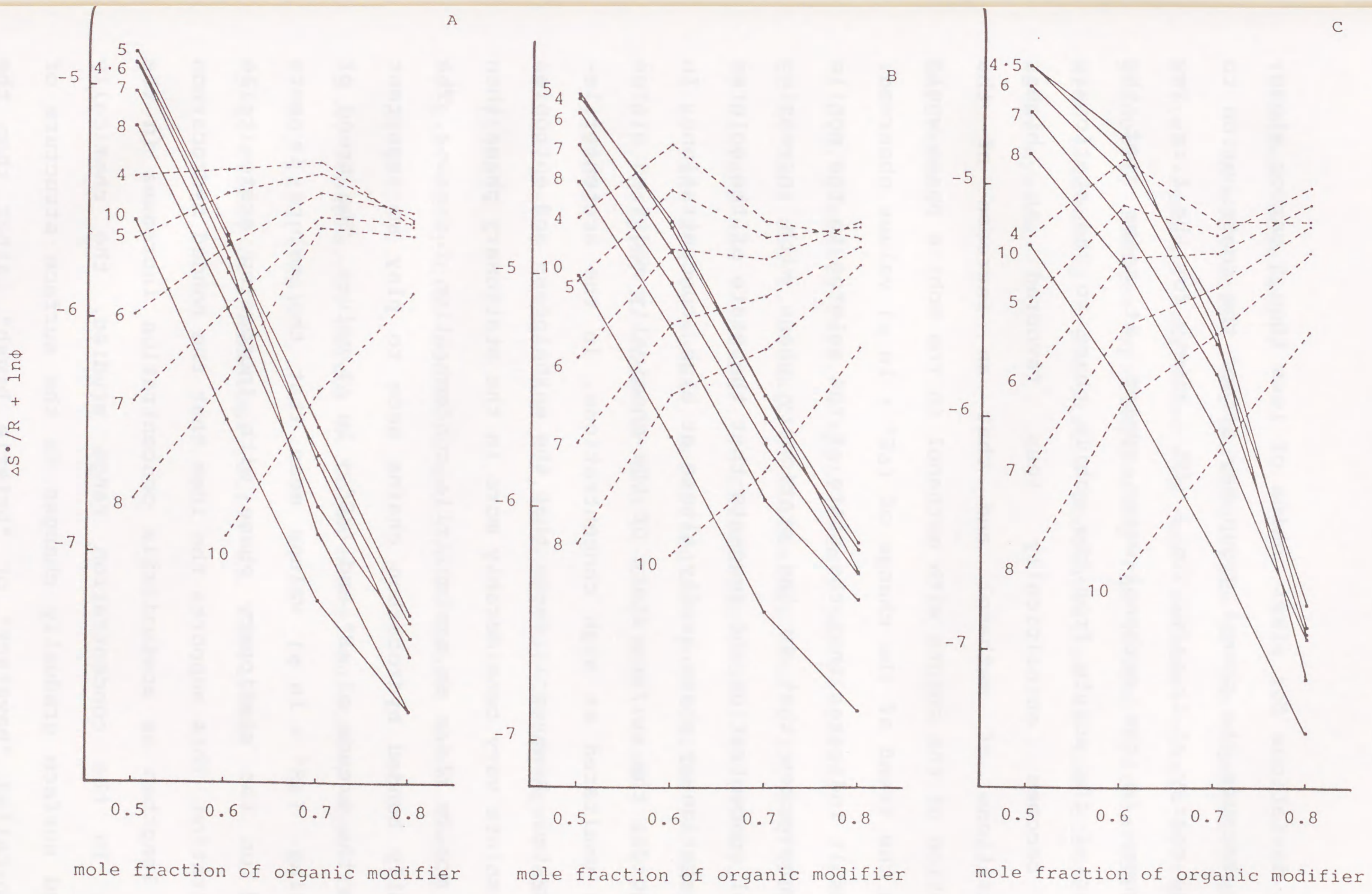

Figure 24. (ΔS⁰/R + ln φ) versus mobile phase composition.
The sample numbers and symbols are the same as shown
in Figure 23

exhibit deviations for alkyl chains of less than 5 carbon atoms. This is because the phenyl group may affect the contribution to the free energy of transfer for a $-CH_2-$ moiety for the first few homologues. In the methanol-water system, it seems that the transfer of the solute from the mobile phase to the stationary phase becomes enthalpically less favoured at higher concentrations of methanol and that an increase of the association of the solute with methanol in the mobile phase would explain the trend of the change of (ΔS^0 + ln ϕ) values observed. This result indicates that the state of the solutes in the mobile phase approaches that in the stationary phase with increasing methanol concentration and suggests that the state of the solutes in the stationary phase hardly alters at high concentrations. In other words, the surface state of the chemically modified silica remains unaltered at high concentrations. In the acetonitrile-water system, however it seems that the enthalpies and entropies of the solute vary considerably more in the stationary phase than in the mobile phase as acetonitrile concentration increases. The chemically bonded hydrocarbon chains seem to play an important role in the trends of ΔH^0 and (ΔS^0 + ln ϕ) values. The trend of decreasing (ΔS^0 + ln ϕ) values mean that the solute is more ordered on the stationary phase with increasing acetonitrile concentration. This supports the idea that the bonded hydrocarbon chains lengthen as acetonitrile concentration increases in the eluent. In the concentration range studied, the chemically modified surface gradually changes to the surface structure of the so-called "haystack" or "bristle brush" rather than the

"blanket" models(9).

In conclusion, the surface state of the chemically modified silica remains unaltered in the methanol-water system, and gradually changes by lengthening the bonded hydrocarbon chains with increasing acetonitrile concentration in the acetonitrile-water system.

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PART IV

CONCLUSION

Part IV

Conclusion

When two derivative types of aldehydes and their isomeric ketones, which are 2,4-dinitrophenylhydrazones (2,4-DNPHs) and 1-dimethylaminonaphthalene-5-sulfonylhydrazones (dansylhydrazones), were separated by RPLC with aqueous methanol eluents, those of isomers were eluted in the reverse order to each other. The difference in the retention behaviour among aldehydes and their isomeric ketones was induced by the difference of the derivative skeleton structure, and the retention behaviours of 2,4-DNPHs were found to be greatly influenced by the derivative skeleton structures (II-1). The derivative skeleton of 2,4-DNPH had two nitro groups at the para and ortho positions. It was observed that the oxygen of the nitro group at the ortho position formed intramolecular hydrogen bond to the hydrazone -NH- (II-2). The formation of the hydrogen bond caused the derivative structure to be fixed in a planar conformation by an intramolecular six-membered ring, and hence inhibited to decrease the number of hydrophobic sites. The influence of this formation was small on 2,4-DNPHs of aldehydes because the alkyl chains on only one side were presumably able to decrease the number of hydrophobic sites and, consequently, those of aldehydes were eluted earlier than those of their isomeric ketones, whose elution order was the reverse of that of dansylhydrazones. This influence was much greater on o-nitrophenylhydrazone (o-NPH) whose derivative skeleton had one nitro group at the only ortho position. On the other hand, the retention behaviour of p-nitrophenylhydrazone (p-NPH) suggested that para substituted nitro group produced an increase in solute-solvent association, and was under the great influence of the organic modifier in the mobile phase (II-3). The elution behaviours of the derivatives supported the solvophobic theory, but gave no information about the dependence of solute retention on the stationary phase. When the thermodynamic parameters, which obtained from the experimental plots of ln k' vs 1/T, were treated as a function of eluent composition, the variation of the different behaviour reflected the distinct structure of bonded hydrocarbon chains between the two mobile phases which were methanol and acetonitrile (II-4). It was found that the relationship between the thermodynamic parameters obtained from the capacity factors and the eluent composition gave information in more detail, though the capacity factors which were the only experimental data were obeyed alone by the solvophobic theory.

Furthermore, the general relationships for calculating the surface excess amount could be derived from chromatographic retention data, which were obtained the solvent disturbance peak or the retention volume of the labelled components in the eluent (III-1). The surface excess isotherms obtained from the chromatographic data could be used as a measure of the comparison of the chemically modified surfaces, and were found to reflect the difference in the miscible state between acetonitrile-water and methanol-water systems (III-2). In acetonitrile-water system,

the weak association between acetonitrile and water seemed to promote the adsorption of water on the silica surface or acetonitrile on the chemically modified surface because silica had a strong affinity for water through hydrogen bonding with silanol groups, and the chemically modified surface preferred an organic solvent more than water owing to non-polarity of bonded hydrocarbon chains. In aqueous methanol, the low surface excess amount might be due to keeping a strongly associated state of methanol and water as a result of strong hydrogen bonding. The isotherms obtained from two aqueous mixtures gave poor information about differences in structure between the monomeric and the polymeric phases. However, the significant difference between the two phases in methanol-dichloromethane indicated that the structure of the polymeric phase, which was more rigid than in the aqueous mixture, was more tight than that of the monomeric phase (III-3). The changes of surface excess isotherms before and after the modification of silicas reflected turning from hydrophilic surfaces into hydrophobic ones. The retention behaviours of pyridine on native and modified surfaces indicated that the surface of silica supports were one of sources to induce differences among various bonded phase materials although there was some exceptions (III-4). The thermodynamic behaviour of a series n-alkylbenzenes as a function of eluent composition suggested that the surface state of the chemically modified silica remained unaltered in the methanol-water system, and gradually changed by lengthening the bonded hydrocarbon chains with increasing acetonitrile concentration in the acetonitrile-water system (III-5), and supported that the difference between the two systems was ascribed in the miscible state which was reflected by the surface excess isotherm.

The results described above could be obtained by the chromatographic data without using a variety of techniques for probing chemically modified surfaces including fluorescence, infrared and nuclear magnetic resonance spectrometry. But the efficient use of these techniques will be necessary in order to deal with the direct influence of the state of the chemically modified surface on the capacity factor, which are generally obeyed by the solvophobic theory.

SUMMARY

SUMMARY

Part I. Introduction

The spectacular ascent of "reversed phase" chromatography is one of the most interesting phenomena in the brief history of high performance liquid chromatography. It is estimated that more than 80 % of modern liquid chromatographic separations use the reversed-phase liquid chromatography (RPLC) mode. However, there is a lack of understanding of the details of solute retention despite the enormous popularity of RPLC. The most rigorous treatment to date is the solvophobic theory. The basic premise of the theory is reasonable and agreement with experiment is generally good, but the description is incomplete in which it does not provide a sufficiently detailed explanation of the dependence of solute retention on the stationary phase variables.

This dissertation research is composed of two related studies areas on the description of solute retention mechanism. These two studies are discussed in separate parts of the dissertation.

Part II. Thermodynamic Elucidation of Retention Behaviour of Carbonyl Derivatives

II-1

When two derivative types of aldehydes and their isomeric ketones, which are 2,4-dinitrophenylhydrazones (2,4-DNPHs) and 1-dimethylaminonaphthalene-5-sulfonylhydrazones (dansylhydrazones), were separated by RPLC with aqueous methanol eluents, those of isomers were eluted in the reverse order to each other.

The difference in the retention behaviour among aldehydes and their isomeric ketones was induced by the difference of the derivative skeleton structure, and the retention behaviours of 2,4-DNPHs were greatly influenced by the derivative structure since the retention of their isomers are similar to each other.

II-2

Previous work has been extended. The retention behaviours of 2,4-DNPHs are found to be greatly influenced by the derivative skeleton. The thermodynamic behaviours of phenylhydrazones (PH), o- and p-nitrophenylhydrazones (o-, p-NPH) and 2,4-DNPHs corresponding to aldehydes from methanal to heptanal and their isomeric ketones were described in order to investigate the influence of nitro group on retention behaviour of derivatives.

The retention behaviours of phenylhydrazones were influenced by the substituted position of nitro group. At the para position, nitro group had similar effect on aldehydes and isomeric ketones to resulting in great retention of aldehydes, while nitro group had great effect on ketone at the ortho position to resulting in similar retentions of aldehydes and isomeric ketones.

II-3

The retention behaviours of three kinds of aromatic aldehyde derivatives, which are PHs, p-NPHs and 2,4-DNPHs of benzaldehyde, chlorobenzaldehyde, hydroxybenzaldehyde, methylbenzaldehyde and nitrobenzaldehyde, were investigated by RPLC using aqueous methanol and acetonitrile as the eluent. The effects of the derivative skeletons and these substituent groups were discussed on their retention.

The retention behaviours of the three derivatives depended mainly on the structure of the derivative skeleton and particularly the p-NPH derivatives showed a characteristic behaviour under being influenced by the organic modifier in the mobile phase. The individual substituent groups exerted different influences on the retention behaviours of the derivatives. The benzaldehyde derivatives were more strongly retained than the derivatives with hydroxyl or nitro groups, and more rapidly eluted than those with chloro or methyl groups.

II-4

The thermodynamic behaviour of two derivatives of aldehydes and their isomeric ketones, which are 2,4-DNPHs and dansylhydrazones, were treated as a function of eluent composition in order to investigate the influence of organic modifier on retention mechanism.

Thermodynamic retention behaviours of two derivatives reflected the distinct structure of bonded hydrocarbon chains between the two mobile phases, which are aqueous methanol and acetonitrile. The structure of bonded hydrocarbon chains was found to be influenced by the association of organic modifier and water.

Part III. Understanding of Stationary Phase State by Using Surface Excess Amount

III-1

It is well known that organic solvent is enriched in the bonded phase in RPLC. The amount of enriched modifier can be determined as the surface excess amount. The general relationships for calculating the surface excess amounts were derived from chromatographic retention data. Some important equations were summarized and approximations involved.

III-2

In order to make it possible to use the surface excess isotherm as a measure for the comparison of the chemically modified surfaces with various pore sizes, the influence of pore size on the surface excess isotherm was explored for the silicas with pore diameters ranging from 100 to 300 A.

The data obtained from this study suggested that the surface excess isotherm on the silica surface was influenced by the substrate itself rather than pore size. This meant that the surface excess isotherm could be used as a measure of the comparison of the chemically modified surfaces. Further, the surface excess isotherm reflected the difference in the miscible state between acetonitrile-water and methanol-water systems.

III-3

The differences between the monomeric and the polymeric bonded phases were attempted to relate to their surface excess

isotherms using acetonitrile-water, methanol-water and methanol-dichloromethane systems.

The isotherms obtained from two aqueous mixtures gave poor information about differences in structure between the monomeric and the polymeric phases. However, the significant difference between the two phases in methanol-dichloromethane indicated that the structure of the polymeric phase, which was more rigid than in the aqueous mixture, was more tight than that of the monomeric phase.

III-4

The attempt was made to characterize the surface properties of unmodified and chemically modified silicas by measuring their surface excess isotherms and the retention of pyridine with aqueous acetonitrile mobile phase.

The changes of surface excess isotherms before and after the modification of silicas reflected turning from hydrophilic surfaces into hydrophobic ones. One of silicas was found to have a great number of buried silanol groups. The retention behaviours of pyridine on native and modified surfaces indicated that the surface of silica supports were one of sources to induce differences among various bonded phase materials although there was some exceptions.

III-5

The thermodynamic behaviour of a series n-alkylbenzenes was explored as a function of eluent composition in order to investigate the influence of the aqueous organic eluent on the surface state of the chemically modified silicas.

The surface state of the chemically modified silica remained unaltered in the methanol-water system, and gradually changed by lengthening the bonded hydrocarbon chains with increasing acetonitrile concentration in the acetonitrile-water system.

Part IV. Conclusion

The results obtained from each chapter were related to one another. It was indicated that the capacity factors were obeyed alone by the solvophobic theory, but the thermodynamic parameters obtained from the capacity factors gave information about the dependence of solute retention on the stationary phase.

CURRICULUM VITAE

PUBLICATIONS

CURRICULUM VITAE

Kazue Tani

Present Address: Faculty of Engineering, Yamanashi University,
4-3-11, Takeda, Kofu, 400, Japan
Tel. 0552-52-1111 (ext. 5356)

Nationality: Japanese

Sex: Female

Born: October 28, 1953

Education: 1973-1977 Shinshu University
Bachelor of Science

Experience: 1977-present Instructor, Faculty of Engineering,
Yamanashi University

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