ADSORPTION CHARACTERISTICS OF REVERSED-PHASE LIQUID CHROMATOGRAPHY WITH VARIOUS ALKYL BONDED PHASES

KANJI MIYABE AND MOTOYUKI SUZUKI**
Kurita water Industries Ltd., Atsugi 243-01

Key Words: Reversed Phase Chromatography, Alkyl Chain Length, Adsorption Equilibrium, Mass Transfer Rate, Thermodynamic Property, Surface Diffusion, Silica Gel, Methanol Water Mixture, Aromatic Derivative

The effect of chain length of alkyl bonded phases on adsorption characteristics, especially mass transfer phenomena and thermodynamic parameters, in reversed-phase liquid chromatography was studied by the chromatographic method and moment analysis. Adsorption equilibrium constant, isosteric heat of adsorption and activation energy of surface diffusion increased with an increase in length of alkyl ligands in short chain regions. On the contrary, surface diffusion coefficient increased with a decrease in alkyl chain length. Change of all the parameters decreased when the carbon number of the alkyl ligands was about 8 and above. Almost the same adsorption characteristics were observed when C₈ and C₁₈ packing materials were used as an adsorbent. It is concluded that only parts of long alkyl chains contribute to adsorption phenomena in reversed-phase liquid chromatography. The contribution of surface diffusion to intraparticle diffusion was significant and increased with an increase in alkyl chain length.

Introduction

Reversed-phase liquid chromatography has been frequently employed for both analytical and preparative separations. For analytical purpose, it is estimated that about 70-80% of separations have been carried out by using a reversed-phase technique. In the field of reversed-phase mode, several kinds of packing materials having different alkyl chains are used as the stationary phase^{21, 23}. Extensive studies were carried out on the effect of alkyl chain length on retention behaviors.

Two different results have been reported for the influence of alkyl chain length on capacity factor and separation factor. It was suggested by several investigators that capacity factor and separation factor continue to increase with an increase in alkyl chain length^{7, 10, 11, 16}. The other result was that capacity factor and separation factor were each a constant value in a chain length region longer than a "critical length" and that carbon number of the critical chain length was from about 6 to 14, irrespective of composition of the mobile phase^{2, 14}.

Two different models were proposed for the retention mechanism of reversed-phase liquid chromatography. Berendsen and de Galan² suggested a kind of adsorption model, in which only a part of long alkyl chains contributed adsorption phenomena, on the basis of the experimental results described above. On the other hand, Roumeliotis and Unger²² investigated correlations of capacity factor normalized by the surface area of silica gel and hydrophobic surface area of alkyl chains under various reversed-phase chromatographic conditions. A linear rela-

tionship was observed for some kinds of reversed-phase packing materials other than trimethylsilyl-silica gel (TMS). From this experimental result, they concluded that the entire length of alkyl chains contributed to hydrophobic interaction between adsorbates and ligands. A similar assumption was also presented by Hennion *et al.*⁷. Though a number of works have been made on the effect of alkyl chain length on retention behaviors, the adsorption mechanism of reversed-phase mode has not been elucidated sufficiently.

Various experimental results have been presented for the influence of alkyl chain length on column efficiency in reversed-phase mode. Karch *et al.*¹⁰ and Hennion *et al.*⁷ reported that column efficiency was not affected by alkyl chain length. Hennion *et al.*, however, suggested that an increase in column efficiency was accompanied by an increase in chain length when carbon number was less than 4. They assumed that silanol groups influenced chromatographic separations. Knox and Pryde¹² made kinetic studies on chromatographic separation by using both octadecylsilyl-silica gel (ODS) and a kind of reversed-phase packing material which had short alkyl chains. They suggested that chemical modification of the surface of silica gel with n-alkyl chains improved the mass transfer rate in packing materials and that the rate in the short chain adsorbent was faster than that in ODS.

In order to elucidate a chromatographic separation mechanism, it is required to study adsorption phenomena in more detail from the standpoint of various properties such as mass transfer rate and thermodynamics other than retention. This paper is concerned with the effect of alkyl chain length of bonded phases on adsorption phenomena of reversed-phase liquid chromatography. Pulse response

* Received June 10, 1994. Correspondence concerning this article should be addressed to K. Miyabe.

**M. Suzuki is at Institute of Industrial Science, University of Tokyo, Tokyo 106

Table 1. Properties of columns and experimental conditions

Column	TMS	C4	C8	ODS
Average particle diameter, d_p [μm]	45	45	45	45
Particle density, ρ_p [g/cm^3]	0.74	0.73	0.75	0.86
True density [g/cm^3]	1.94	1.85	1.65	1.60
Porosity, ϵ_p [—]	0.62	0.61	0.55	0.46
Carbon content [wt%]	4.1	6.7	9.9	17.1
Mass of adsorbent [g]	1.8	1.8	1.8	2.1
Alkyl ligand [g]	0.095	0.15	0.21	0.36
Silica support [g]	1.70	1.65	1.59	1.74
Ligand amount [mmol/g]	1.2	0.93	0.83	0.71
Ligand density [nm^{-2}]	2.7	2.2	2.0	1.9
Column size [mm]	150 $\times$ 6 I. D.			
Void fraction, ϵ [—]	0.44	0.42	0.42	0.43
Peclet number, Pe [—]	0.65	0.93	0.62	1.02
Tortuosity factor, k^2 [—]	4.1	4.6	4.4	4.5
Column temperature [K]	288–308			
Mobile phase	Methanol/water : 70/30 (vol.)			
Superficial velocity, u_o [cm/s]	0.05–0.12			
Sample material	Benzene, Ethylbenzene, <i>n</i> -Butylbenzene, <i>n</i> -Hexylbenzene, Naphthalene, Anthracene			

measurements were made by using several packing materials having different alkyl chains. Not only adsorption equilibrium, but also mass transfer rates and thermodynamic properties were analyzed by the method of moment analysis²⁴⁾.

1 Experimental

1.1 Apparatus

A high performance liquid chromatograph (LC-6A, Shimadzu) was employed. Small volumes of sample solutions were introduced into the fluid flow by use of a sample injector. The column was kept at constant temperature in a thermostated water bath. The concentration of the sample in effluent was monitored by an ultraviolet detector.

1.2 Columns and reagents

The properties of columns (YMC) used are shown in Table 1. Each packing material was synthesized with various *n*-alkyldimethylsilane ligands. The carbon number of alkyl chains was 1, 4, 8, and 18. End capping of the packing materials, except for C₁₈, was not carried out. Ligand density ranged from 1.9 to 2.7 nm². It appeared that about 40–60% of silanol groups reacted with alkyl ligands. The void volume of the columns was determined by using uracil as an inert substance.

1.3 Procedure

Experimental conditions are also shown in Table 1. Pulse response experiments were carried out with varying temperature and flow rate of a carrier solvent under the conditions that the amount of sample substances adsorbed was negligibly small. Chromatographic peaks experimentally observed were analyzed by the method of moment. Details of the moment analysis method were described in previous papers^{17, 18, 20)}.

Fig. 1 Adsorption equilibrium constant as a function of alkyl chain length of stationary phases
Keys refer to Table 2

Fig. 2 Correlation of logarithm of adsorption equilibrium constant with surface area of adsorbates

2 Results and Discussion

2.1 First moment analysis

The first moment is expressed as^{17, 18, 20)}:

$$\mu_1 = (z / u_o) [\epsilon + (1 - \epsilon)(\epsilon_p + \rho_p K)] \quad (1)$$

Figure 1 shows correlations of adsorption equilibrium constants at 298 K with carbon number of alkyl

Fig. 3 Isosteric heat of adsorption as a function of alkyl chain length of stationary phases
Keys refer to Table 2

chains. Though the value of K increased with an increase in alkyl chain length, the slope of the curves gradually decreased when the carbon number of alkyl chains was about 8 or more. The overall tendency of the curves in Fig. 1 was similar to that of other studies^{2, 14}.

Figure 2 shows correlations of K with surface area of adsorbates. The logarithm of K increased linearly by introduction of a methylene group or a phenyl ring to an adsorbate molecule. The increment in K accompanied by an increase in molecular size of adsorbates for a packing material having a long alkyl chain is larger than that for one having a short chain. This situation may result from steric interactions between alkyl chains and adsorbate molecules. Only planar interactions seem to be possible when TMS packing material is used as a stationary phase. On the other hand, the entire surface of adsorbates may contribute to the interaction with ODS.

Isosteric heat of adsorption, Q_{st} , was determined by the van't Hoff equation.

$$d \ln K / d (1/T) = -Q_{st} / R_g \quad (2)$$

Resulting values of Q_{st} are shown in **Fig. 3**. The values of Q_{st} were found to range from 8 kJ/mol to 18 kJ/mol. They were of the same order of magnitude compared with experimental results previously reported^{4-6, 8, 9, 13, 16, 25}.

Figure 3 shows plots of Q_{st} and carbon number of alkyl chains. The trend of the curves in Fig. 3 was similar to that in Fig. 1. It seems that strength of interaction between adsorbate molecules and alkyl chains increased with an increase in alkyl chain length or in molecular size of adsorbates.

The logarithm of K_0 in **Table 2** indicates entropy change arising from adsorption of each adsorbate. The adsorption process is accompanied by a reduction of entropy in each adsorption system. The magnitude of

Table 2. Experimental results

Key	Adsorbate	Column	K_0 [cm ³ /g]	Ds_0 [cm ² /s]
○	Benzene	TMS	3.1×10^{-2}	3.0×10^{-3}
		C4	3.2×10^{-2}	7.4×10^{-3}
		C8	5.8×10^{-2}	8.2×10^{-3}
		ODS	1.4×10^{-1}	7.0×10^{-3}
△	Naphthalene	TMS	3.3×10^{-2}	3.0×10^{-3}
		C4	3.5×10^{-2}	4.3×10^{-3}
		C8	4.5×10^{-2}	5.8×10^{-3}
		ODS	8.9×10^{-2}	4.8×10^{-3}
▽	Anthracene	TMS	4.0×10^{-2}	1.6×10^{-3}
		C4	3.4×10^{-2}	2.0×10^{-2}
		C8	2.6×10^{-2}	4.5×10^{-2}
		ODS	2.9×10^{-2}	5.5×10^{-2}
□	Ethylbenzene	TMS	3.9×10^{-2}	4.0×10^{-3}
		C4	3.7×10^{-2}	5.9×10^{-3}
		C8	5.3×10^{-2}	1.0×10^{-2}
		ODS	1.2×10^{-1}	1.4×10^{-2}
◇	<i>n</i> -Butylbenzene	TMS	3.4×10^{-2}	1.9×10^{-3}
		C4	3.2×10^{-2}	2.1×10^{-3}
		C8	3.6×10^{-2}	1.9×10^{-2}
		ODS	8.8×10^{-2}	4.0×10^{-2}
⬡	<i>n</i> -Hexylbenzene	TMS	2.5×10^{-2}	3.6×10^{-3}
		C4	2.0×10^{-2}	1.1×10^{-2}
		C8	2.0×10^{-2}	6.1×10^{-2}
		ODS	4.1×10^{-2}	8.0×10^{-2}

Table 3. Contributions of each rate process to second moments

Adsorbate	Column	$\mu'_2/(2z/u_0)$ [s]	δ_{ax} [%]	δ_f [%]	δ_d [%]
Benzene	TMS	0.13	33.8	21.2	45.0
	C4	0.19	36.1	21.5	42.5
	C8	0.28	33.1	24.3	42.7
	ODS	0.37	25.8	30.0	44.2
Naphthalene	TMS	0.21	29.6	24.2	46.1
	C4	0.37	30.0	25.7	44.4
	C8	0.82	31.7	29.3	39.0
	ODS	1.7	20.6	40.6	38.8
Anthracene	TMS	0.39	27.0	29.6	43.5
	C4	0.77	29.0	29.7	41.3
	C8	2.9	28.7	37.3	34.0
	ODS	11.3	19.5	55.1	25.4
Ethylbenzene	TMS	0.23	28.2	26.5	45.3
	C4	0.42	31.2	29.0	39.8
	C8	0.91	33.5	32.7	33.7
	ODS	1.8	23.9	44.3	31.8
<i>n</i> -Butylbenzene	TMS	0.45	26.9	32.3	40.8
	C4	1.4	29.5	34.6	35.9
	C8	4.3	32.1	42.8	25.1
	ODS	11.7	21.1	58.4	20.5
<i>n</i> -Hexylbenzene	TMS	1.2	22.1	37.1	40.8
	C4	4.6	29.5	41.8	28.6
	C8	22.2	31.2	50.8	18.0
	ODS	85.4	19.6	69.1	11.2

Temperature: 298K
 $u_0 = 0.12$ cm/s

entropy reduction increased with a decrease in length of alkyl chains or with an increase in molecular size of adsorbates.

2.2 Second moment analysis

Second moment analysis provided information about each mass transfer step involved in the adsorption processes. Second moment is expressed as^{17, 18, 20}:

$$\mu'_2 = (2z/u_0)(\delta_{ax} + \delta_f + \delta_d) \quad (3)$$

The contributions of each step to second moments are compared in **Table 3**. The contribution of δ_{ax} ranged from about 20 to 30%. Though the contributions of δ_f and δ_d

Table 4. Contributions of pore and surface diffusions to intraparticle diffusion

Adsorbate	Column	De [cm ² /s]	Dp [cm ² /s]	De/Dp [-]
Benzene	TMS	3.8×10^{-6}	1.2×10^{-6}	3.1
	C4	4.3×10^{-6}	1.1×10^{-6}	4.0
	C8	4.9×10^{-6}	1.0×10^{-6}	4.8
	ODS	5.7×10^{-6}	8.5×10^{-7}	6.7
Naphthalene	TMS	3.6×10^{-6}	9.5×10^{-7}	3.7
	C4	4.1×10^{-6}	8.2×10^{-7}	5.0
	C8	5.4×10^{-6}	7.9×10^{-7}	6.9
	ODS	7.4×10^{-6}	6.5×10^{-7}	11.3
Anthracene	TMS	4.1×10^{-6}	7.9×10^{-7}	5.2
	C4	4.6×10^{-6}	6.9×10^{-7}	6.6
	C8	7.0×10^{-6}	6.5×10^{-7}	10.9
	ODS	1.4×10^{-5}	5.5×10^{-7}	25.0
Ethylbenzene	TMS	4.0×10^{-6}	9.8×10^{-7}	4.1
	C4	5.3×10^{-6}	8.5×10^{-7}	6.2
	C8	7.2×10^{-6}	8.1×10^{-7}	8.8
	ODS	1.0×10^{-5}	6.7×10^{-7}	14.9
<i>n</i> -Butylbenzene	TMS	4.9×10^{-6}	8.2×10^{-7}	6.0
	C4	6.3×10^{-6}	7.1×10^{-7}	8.8
	C8	1.1×10^{-5}	6.7×10^{-7}	16.8
	ODS	1.8×10^{-5}	5.7×10^{-7}	32.3
<i>n</i> -Hexylbenzene	TMS	5.2×10^{-6}	7.2×10^{-7}	7.2
	C4	8.7×10^{-6}	6.3×10^{-7}	13.9
	C8	1.7×10^{-5}	5.9×10^{-7}	29.0
	ODS	3.7×10^{-5}	5.0×10^{-7}	72.9

Fig. 4 Surface diffusion coefficient as a function of alkyl chain length of stationary phases
Keys refer to Table 2

were of the same order of magnitude, the direction of change in the contributions was opposite. The contribution of δ_d was larger than that of δ_f under conditions when K was relatively small.

Intraparticle diffusivity is related to both pore diffusivity and surface diffusion coefficient as described in the following equation:

$$De = Dp + \rho_p KDs \quad (4)$$

The contributions of Dp and Ds to De were compared with each other in **Table 4**. The values of De were a few times or about one or two orders of magnitude larger than Dp .

Fig. 5 Activation energy of surface diffusion as a function of alkyl chain length of stationary phases
Keys refer to Table 2

The contribution of Ds to overall mass transfer in reversed-phase packing materials increased with an increase in alkyl chain length. It is concluded that surface diffusion is dominant for intraparticle diffusion.

Figure 4 shows the correlation of Ds with carbon number in alkyl chains. The value of Ds decreased with an increase in alkyl chain length. Almost the same values of Ds were observed when C_8 and C_{18} packing materials were used as adsorbents.

Activation energy of surface diffusion was calculated according to the Arrhenius equation.

$$d \ln Ds / d (1/T) = -Es / R_g \quad (5)$$

As shown in **Fig. 5**, the values of Es were from about 16 kJ/mol to 28 kJ/mol. Compared with the values of isosteric heat of adsorption previously measured, Es was found to be larger than Q_{st} irrespective of alkyl chain lengths. Similar results have also been reported in liquid phase adsorption^{1, 3, 15, 17, 19}. This condition may suggest that it must be easier for adsorbed molecules to desorb into the bulk phase than to migrate on the surface. This apparently unreasonable situation may result from the influence of the solvent. The authors quantitatively analyzed the solvent effect on adsorption equilibrium in reversed-phase liquid chromatography by applying the solvophobic theory¹⁹.

Figure 5 shows the correlation of Es with carbon number of alkyl chains. The trend of the curves in Fig. 5 was similar to that in Figs. 1 and 3. Because almost the same values of Q_{st} and Es were observed for each adsorbate when packing materials having long alkyl chains were used as the stationary phase, it seems that only parts of the long alkyl chains contribute to adsorption phenomena in reversed-phase liquid chromatography.

Conclusion

The effect of alkyl chain length of bonded phases on adsorption in reversed-phase liquid chromatography was studied from the viewpoint of not only adsorption equilibrium but also mass transfer rates and thermodynamic properties.

Almost the same trend was observed for adsorption equilibrium constant, isosteric heat of adsorption and activation energy of surface diffusion. Their values increased with an increase in alkyl chain length. The change in the values, however, gradually decreased when the carbon number of the alkyl chains was about 8 or more. In contrast to the changes, surface diffusion decreased with an increase in alkyl chain length. For all the properties, almost equal values were observed for C_8 and C_{18} packing materials. It was suggested that the strength of interaction between adsorbate molecules and alkyl chains increased with an increase in alkyl chain length or in molecular size of adsorbates and that only parts of the long alkyl chains contribute to adsorption phenomena in reversed-phase liquid chromatography.

The increment of adsorption equilibrium constant accompanied by an increase in molecular size of adsorbates with ODS was larger than that with TMS. It seems that steric interaction between an adsorbate and a stationary phase changes with alkyl chain length.

Surface diffusion has an important role on intraparticle diffusion. The contribution of surface diffusion to overall mass transfer in reversed-phase packing materials increased with increasing alkyl chain length.

Nomenclature

A	= surface area	[cm ² /mol]
De	= intraparticle diffusion coefficient	[cm ² /s]
Dp	= pore diffusivity	[cm ² /s]
Ds	= surface diffusion coefficient	[cm ² /s]
Ds_0	= frequency factor	[cm ² /s]
E_s	= activation energy of surface diffusion	[kJ/mol]
K	= adsorption equilibrium constant	[cm ³ /g]
K_0	= adsorption equilibrium constant at $1/T = 0$	[cm ³ /g]
R_g	= gas constant	[-]
Q_{st}	= isosteric heat of adsorption	[kJ/mol]
T	= temperature	[K]
u_0	= superficial velocity	[cm/s]
z	= longitudinal position in bed	[cm]

δ_{ax}	= contribution of axial dispersion to μ_2'	[s]
δ_f	= contribution of fluid-to-particle mass transfer to μ_2'	[s]
δ_d	= contribution of intraparticle diffusion to μ_2'	[s]
ϵ	= void fraction in bed	[-]
ϵ_p	= porosity	[-]
μ_1	= first moment	[s]
μ_2'	= second moment	[s ²]
ρ_p	= particle density	[g/cm ³]

Literature Cited

- 1) Awum, F., S. Narayan and D. Ruthven: *Ind. Eng. Chem. Res.*, **27**, 1510-1515 (1988)
- 2) Berendsen, G. E. and L. de Galan: *J. Chromatogr.*, **196**, 21-37 (1980)
- 3) Ching, C.B., K. Hidajat and M. S. Uddin: *Sep. Sci. Technol.*, **24**, 581-597 (1989)
- 4) Colin, H., J. C. Diez-Masa, G. Guiochon, T. Czajkowska and I. Miedziak: *J. Chromatogr.*, **167**, 41-65 (1978)
- 5) Colin, H. and G. Guiochon: *J. Chromatogr.*, **141**, 289-312 (1977)
- 6) Cofin, H. and G. Guiochon: *J. Chromatogr.*, **158**, 183-205 (1978)
- 7) Hennion, M.C., C. Picard and M. Caude: *J. Chromatogr.*, **166**, 21-35 (1978)
- 8) Horvath, C. and W. Melander: *J. Chromatogr. Sci.*, **15**, 393-404 (1977)
- 9) Issaq, H. J. and M. Jaroniec: *J. Liquid Chromatogr.*, **12**, 2067-2082 (1989)
- 10) Karch, K., I. Sebastian and I. Halasz: *J. Chromatogr.*, **122**, 3-16 (1976)
- 11) Karch, K., I. Sebastian, I. Halasz and H. Engelhardt: *J. Chromatogr.*, **122**, 171-184 (1976)
- 12) Knox, J. H. and A. Pryde: *J. Chromatogr.*, **112**, 171-188 (1975)
- 13) Knox, J. H. and G. Vasvari: *J. Chromatogr.*, **83**, 181-194 (1973)
- 14) Lochmuller, C. H. and D. R. Wilder: *J. Chromatogr. Sci.*, **17**, 574-579 (1979)
- 15) Ma, Y. H., Y. S. Lin and H. L. Fleming: *AIChE Symp. Ser.*, **84**, 1-12 (1988)
- 16) Majors, R. E. and M. J. Hopper: *J. Chromatogr. Sci.*, **12**, 767-778 (1974)
- 17) Miyabe, K. and M. Suzuki: *AIChE J.*, **38**, 901-910 (1992)
- 18) Miyabe, K. and M. Suzuki: *to be published in AIChE J.* (1994a)
- 19) Miyabe, K. and M. Suzuki: *to be published in AIChE J.* (1994b)
- 20) Miyabe, K. and M. Suzuki: *Ind. Eng. Chem. Res.*, **33**, 1792-1802 (1994c)
- 21) Poole, C. F. and S. K. Poole: "Chromatography Today," Ch.4, Elsevier, Amsterdam (1991)
- 22) Roumeliotis, P. and K. K. Unger: *J. Chromatogr.*, **149**, 211-224 (1978)
- 23) Sander, L. C. and S. A. Wise: *CRC Critical Reviews in Analytical Chemistry*, **18**, 299-415 (1987)
- 24) Suzuki, M.: "Adsorption Engineering," Ch.6, Kodansha/Elsevier, Tokyo/Amsterdam (1990)
- 25) Unger, K.K.: "Porous Silica," p122, Elsevier, Amsterdam (1979)