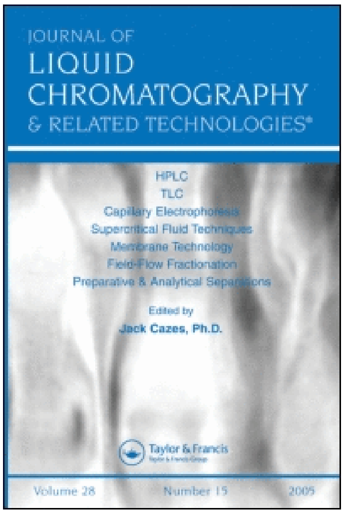

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SELECTIVITY OF PHENYLHEXYL-BONDED SILICA GEL FOR LIQUID CHROMATOGRAPHY

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ABSTRACT

The selectivity of a computer-designed phenylhexyl-bonded silica gel was studied from the chromatographic behavior of alkylbenzenes and polycyclic aromatic hydrocarbons. The retention capacity of this phenyl phase for alkylbenzenes was equivalent to a pentyl-bonded phase, and that for polycyclic aromatic hydrocarbons was equivalent to an octyl-bonded phase. The phenylhexyl-bonded silica gel demonstrated the selectivity for the separation of antibacterial drugs, even though the separation time was the same as that on an octadecyl-bonded silica gel.

INTRODUCTION

Reversed-phase liquid chromatography is the most commonly used separation method in liquid chromatography. More than 50 percent of all the applications in liquid chromatography are performed with reversed-phase liquid chromatography.

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One reason for this is the development of high performance packing materials made from silica gels. The theoretical plate number of alkyl group-modified silica gels is very high compared with organic polymer-based packing materials, even though the chemical stability of the latter products is excellent compared to silica gel-based packing materials. The disadvantages of silica gel-based bonded-phases are the presence of acidic and active silanol groups, a limited range of pH stability (2.5 ~ 7.5) and high levels of metal contaminations. These disadvantages cause the adsorption of basic drugs, resulting in tailing peaks or complete disappearance. At high pH, the silica gel itself dissolves, resulting in a loss of bonded phases. However, the recent development of silica gel-based bonded-phases improved the chemical stability and extended the range of pH (1.5 ~ 10.0).[1-7]

The separation power of liquid chromatography is due to the unlimited selection of mobile phases compared to gas chromatography, which has a variety of stationary phases. However, the optimal utilization of the power of liquid chromatography requires the new development of stationary phases. A popular selective phase in reversed-phase liquid chromatography is the so-called phenyl phase. Mono-, di- and triphenyl-bonded phases have been developed; their phenyl groups are generally bound with silica gels via one or two or three methylene groups. Thirty kinds of phenyl-modified porous glasses and silica gels were prepared, and the selectivity was studied from the chromatographic behavior of carbamazepine and diphenylhydantoin[8]. A phenylbutyl phase was developed, and the selectivity was studied from the chromatographic behavior of polycyclic aromatic hydrocarbons. The phenylbutyl phase demonstrated slightly greater steric selectivity than an octyl phase and an octadecyl phase.[9]

The phenyl phases have different and unique selectivities compared to the alkyl (pentyl, octyl and octadecyl)-bonded phases. However, the phenyl-bonded silica gels were generally unstable in buffer solutions due to a few methylene unit, and their retention capacity is poor compared with the popular octyl and octadecyl-bonded phases.[10] One condition obtained for octyl- and octadecyl-bonded phases could not be directly applied to a phenyl-bonded phase, and the eluent had to be basically modified for a phenyl-bonded phase. That is, the column selection is difficult between a phenyl phase and an octyl- or an octadecyl-bonded phase.

In this study, the selectivity and stability of phenyl phases with 1 ~ 12 methylene groups were analyzed by calculating logP values based on Rekkers fragmental constants.[11] BlogP[12] and CAChe-logP[13] values of alkylbenzenes and alkanes were also used for the analysis of hydrophobicity of phenylalkyl phases. The retention properties of phenylpropyl- and phenylhexyl-bonded silica gels were compared from the retention factors of alkylbenzenes and polycyclic aromatic hydrocarbons. These phenyl phases were used for drug analysis.

EXPERIMENTAL

The computer used for the calculation was a Macintosh 8100/100 running the CAChe™ program including ProjectLeader™ from Sony-Tektronix (Tokyo). RlogP values of substitutes were calculated by a method based on that proposed by Rekker.[11] The addition of 0.175, the fragmental constant of hydrogen H, to the logP values of substitutes gives the RlogP values of the related molecules. BlogP values were calculated by the MOPAC-BlogP program from the CAChe™ program,[12] and CAChe-logP values[13] were calculated by ProjectLeader™ of the CAChe program. However, the fragmental constant of proton was not available for BlogP and CAChe-logP; therefore, the logP values of alkylbenzenes and alkanes were used as the relative values. The van der Waals volumes were calculated by the MOPAC-BlogP program provided by Sony-Tektronix. The molecules were first optimized by molecular mechanics calculation and optimized again by MOPAC, and then their van der Waals volumes and logP values were obtained.[12] Properties for the calculation were selected according to the manual from CAChe Scientific.

The liquid chromatograph was a model HP1090 from Hewlett-Packard (Palo Alto, CA, USA). The phenylhexyl (C6Ph)-, phenylpropyl (C3Ph)-, octadecyl (C18)-, octyl (C8)- and pentyl (C5)-bonded silica gels were obtained from Phenomenex (Torrance, CA, USA). According to the manufacturer, these packing materials passed the stability test and the inertness tests of pyridine, benzoic acid and 8-hydroxyquinoline.[14] The column size was 15 cm x 4.6 mm I.D. The chemicals used were from Aldrich (Milwaukee, WI, USA) and ChemService (West Chester, PA, USA). The HPLC-grade acetonitrile was from Fisher Scientific (Pittsburgh, PA, USA) and the HPLC grade water was from Merck (Darmstadt, Germany).

The retention factors of 17 polycyclic aromatic hydrocarbons and 10 alkylbenzenes listed in Table 1 were measured by reversed-phase liquid chromatography at 40°C in 70 and 80% aqueous acetonitrile. The selective separation of antibacterial drugs was examined on the C3Ph-, C6Ph-, C8-, and C18-bonded phases.

RESULTS AND DISCUSSION

The analysis of the properties of bonded-phases of silica gels from their molecular mass and logP values permitted designing new phenyl-bonded phases for improving the separation power of reversed-phase liquid chromatography. However, the larger molecular mass of aromatic rings such as pyrene indicated that the synthesis of an inert surface is difficult due to the difficulty of the end-capping.

Table 1

van der Waals Volumes and Logk Values of Polycyclic Aromatic Hydrocarbons and Alkybenzenes in 70% Aqueous Acetonitrile

Compound	VWV*[1]	C5[*2]	C8[*2]	C18[2]	C3Ph[*2]	C6Ph[*2]
Benzene	83.789	0.209	0.281	0.340	0.167	0.230
Naphthalene	127.598	0.410	0.486	0.582	0.305	0.470
Acenaththylene	144.969	0.450	0.540	0.663	0.364	0.521
Acenaphthene	150.675	---	0.629	0.814	0.447	0.616
Fluorene	161.639	0.527	0.629	0.794	0.436	0.602
Phenanthrene	171.286	0.538	0.660	0.854	0.468	0.655
Anthracene	171.486	0.562	0.688	0.894	0.490	0.679
Pyrene	187.603	0.614	0.777	1.038	0.553	0.778
Fluoranthene	188.723	0.599	0.747	0.989	0.535	0.746
Chrysene	214.558	0.673	0.842	1.160	0.635	0.869
Benzo(a)anthracene	215.641	0.692	0.860	1.177	0.647	0.878
Benzo(k)fluoranthene	232.418	0.741	0.959	1.347	0.726	0.982
Benzo(b)fluoranthene	232.462	0.741	0.943	1.325	0.714	0.970
Benzo(g,h,i)perylene	247.198	0.822	1.067	---	0.799	1.118
Dibenzo(a,h)anthracene	259.435	0.803	1.032	1.479	0.806	1.071
Indeno(1,2,3-c,d)pyrene	248.683	0.822	1.067	---	0.803	1.102
Toluene	100.456	0.312	0.396	0.489	0.253	0.326
Ethylbenzene	117.067	0.417	0.513	0.628	0.348	0.428
Propylbenzene	133.897	0.537	0.644	0.800	0.449	0.538
Butylbenzene	150.713	0.653	0.776	0.963	0.548	0.758
Pentylbenzene	167.564	0.772	0.908	1.134	0.646	0.758
Hexylbenzene	184.315	0.894	1.043	1.310	0.747	0.871
Heptylbenzene	201.171	1.019	1.181	1.491	0.851	0.985
Octylbenzene	217.884	1.144	1.318	1.673	0.947	1.095
Nonylbenzene	234.706	1.271	1.457	---	1.046	1.204
Decylbenzene	251.540	1.400	---	---	1.146	1.313

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*[1] van der Waals volume Å^3/mole.
*[2] see the details in the text.

The logP values of bonded phases indicate the retention capacity and the chemical stability. The larger logP values mean stronger hydrophobicity, and lead to the strong retention capacity related to the hydrophobicity of analytes. Such results obtained by computational chemical calculation were used to synthesize an alkyl-bonded phase with a weak hydrophobicity and high pH stability.

Table 2

LogP Values of Bonded Groups

Commercial Name	Structure	RlogP*[1]	RlogP*[2]	ClogP*[3]
Phenyl	-Ph	1.89	2.432	2.531
	$-CH_2Ph$	2.42	2.778	2.841
	$-(CH_2)_2Ph$	2.95	3.189	3.130
	$-(CH_2)_3Ph$	3.48	3.639	3.504
	$-(CH_2)_6Ph$	5.07	4.872	4.521
	$-(CH_2)_8Ph$	6.13	5.764	5.205
	$-(CH_2)_{14}Ph$	9.31	8.160	7.233
Methyl or C1	$-CH_3$	0.70	---	---
	$-(CH_3)_2$	1.40	---	---
	$-(CH_3)_3$	2.10	---	---
Butyl or C4	$-CH_2CH_2CH_2CH_3$	2.29	2.885	2.158
Octyl or C8	$-(CH_2)_7CH_3$	4.41	5.139	3.555
Octadecyl or C18	$-(CH_2)_{17}CH_3$	9.71	10.585	7.100
Cyano	$-(CH_2)_3CN$	0.52	---	---
	$-N(CH_3)(CH_2)_2CN$	-1.46	---	---
Amino	$-(CH_2)_3NH_2$	0.16	0.098	0.577
	$-(CH_2)_4NH_2$	0.69	0.743	0.909

---

*[1] RlogP values [7] of substitutes, *[2] BlogP values [8] of alkanes, alkylbenzenes and alkylamines, *[3] CAChe logP values[9] of alkanes, alkylbenzenes and alkylamines.

The candidate was pentyl-bonded phase. Up to butyl-bonded phase was not stable in high pH solutions, but the stability of the pentyl-bonded phase was equivalent to that of the octyl- and octadecyl-bonded phases. In addition, the chemical stability was improved using a second reaction, end-capping.

The calculated logP values of different groups based on Rekker's fragmental constants[11] are summarized in Table 2. The BlogP and CAChe-logP values of alkylbenzenes and alkanes are also summarized in Table 2. The logP values of chemically unstable phases such as propylamino, propylcyano, and butyl-groups are small. The instability can be understood in light of the alkyl chain-length effect to hydrogen bonding of alkanols. Up to four methylene units affect the hydrogen bonding capability of the hydroxy group, and the longer alkyl-chain did not further affect the hydrogen bonding capability.[15] This phenomenon can be observed on the miscibility of alkanols with water.

The relation between the number of methylene units, x, and the logP values of alkanes and alkylbenzenes is given by following equations:

$$\mathrm{RlogP(alkanes)} = 0.530(x) + 0.345 \qquad r^2 = 1.000$$
$$\mathrm{RlogP(alkylbenzenes)} = 0.526(x) + 2.110 \qquad r^2 = 1.000$$
$$\mathrm{BlogP(alkanes)} = 0.550(x) + 0.685 \qquad r^2 = 0.998$$
$$\mathrm{BlogP(alkylbenzenes)} = 0.411(x) + 2.406 \qquad r^2 = 0.999$$
$$\mathrm{CAChe\text{-}logP(alkanes)} = 0.353(x) + 0.746 \qquad r^2 = 0.999$$
$$\mathrm{CAChe\text{-}logP(alkylbenzenes)} = 0.339(x) + 2.487 \qquad r^2 = 0.999$$

The analysis of the relative hydrophobicity of bonded-phases of silica gels from their molecular mass and logP values indicated that the phenylhexyl-bonded phase should theoretically lead to better stability and selectivity. The logP values of hexylbenzene were equivalent to nonane by RlogP, octane by BlogP and undecane by CAChe-logP. The logP values of propylbenzene were equivalent to hexane by RlogP, pentane by BlogP and octane by CAChe-logP. These calculation systems for logP values indicated the different relation between the logP values of hexylbenzene and the related alkanes; however, the phenylhexyl phase should be more hydrophobic than the octyl phase.

Their logk values measured in aqueous 80% acetonitrile at 40• C were first used for the analysis of hydrophobicity of bonded phases using van der Waals volumes of analytes as the standard. The relation between the logk values and the van der Waals volume of alkylbenzenes, shown in Figure 1, indicated that the retention capacity related to the hydrophobicity of C3Ph and C6Ph was less than that of the pentyl bonded-phase. The relation between the logk values measured in 80% aqueous acetonitrile and van der Waals volumes of alkylbenzenes were:

$$\log k\,(\mathrm{C5}) = 6.345 \times 10^{-3}\,(\mathrm{vwv}) - 4.777 \times 10^{-1} \qquad r^2 = 0.998$$
$$\log k\,(\mathrm{C8}) = 7.212 \times 10^{-3}\,(\mathrm{vwv}) - 5.104 \times 10^{-1} \qquad r^2 = 0.998$$
$$\log k\,(\mathrm{C18}) = 9.398 \times 10^{-3}\,(\mathrm{vwv}) - 6.941 \times 10^{-1} \qquad r^2 = 0.998$$
$$\log k\,(\mathrm{C3Ph}) = 4.603 \times 10^{-3}\,(\mathrm{vwv}) - 4.149 \times 10^{-1} \qquad r^2 = 1.000$$
$$\log k\,(\mathrm{C6Ph}) = 5.288 \times 10^{-3}\,(\mathrm{vwv}) - 3.975 \times 10^{-1} \qquad r^2 = 0.999$$

The relation between their slope and alkylchain length was:

$$\text{Alkyl chain length} = 4.321\mathrm{x(slope)} - 22.729 \qquad r^2 = 0.997 \quad (n = 3).$$

The estimated alkyl chain length related to the retention of alkylbenzenes on C3Ph and C6Ph was -2.8 and 0.12, respectively, from this equation. These values of the phenyl-bonded phase were smaller than those expected from their calculated logP values. The RlogP values of C3Ph and C6Ph were 3.468 and 5.046; about the same as that of the hexyl- and nonyl-groups.

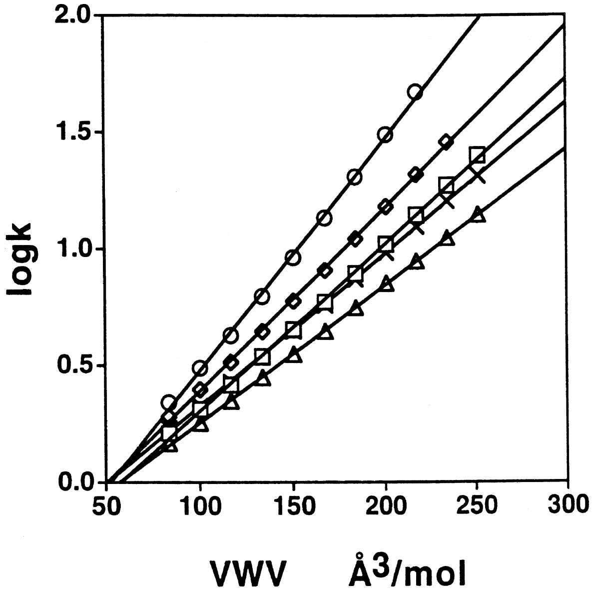

Figure 1. Selectivity of alkylbenzenes for different bonded phases. Columns: pentyl (C5)-, octyl (C8)-, octadecyl (C18)-, phenylpropyl (C3Ph)-, and phenylhexyl (C6Ph)-bonded phases; eluent: 80% aqueous acetonitrile, temperature: 40°C. Symbol: : C5, ◊: C8, O: C18, ΔC3Ph, X: C6Ph.

The retention capacity of the phenyl- phase was also smaller than expected from their carbon contents of these phenyl-bonded silica gels. The carbon contents of C6Ph, C3Ph, C5, C8, and C18 were 17.5, 17.5, 12.4, 13.1, and 17.8 %, respectively. A further study was performed in 70% aqueous acetonitrile.

The relation of the logk of alkylbenzenes and polycyclic aromatic hydrocarbons measured in 70% aqueous acetonitrile and their van der Waals volumes was given by the following equations.

For alkylbenzenes:

$\log k(\mathrm{C6Ph}) = 6.523 \times 10^{-3} - 3.296 \times 10^{-1}, \quad r^2 = 1.000$
$\log k(\mathrm{C8}) = 7.840 \times 10^{-3} - 3.960 \times 10^{-1}, \quad r^2 = 0.999$

For polycyclic aromatic hydrocarbons:

$\log k(\mathrm{C6Ph}) = 5.117 \times 10^{-3} - 2.032 \times 10^{-1}, \quad r^2 = 0.987$
$\log k(\mathrm{C8}) = 4.531 \times 10^{-3} - 9.604 \times 10^{-2}, \quad r^2 = 0.986.$

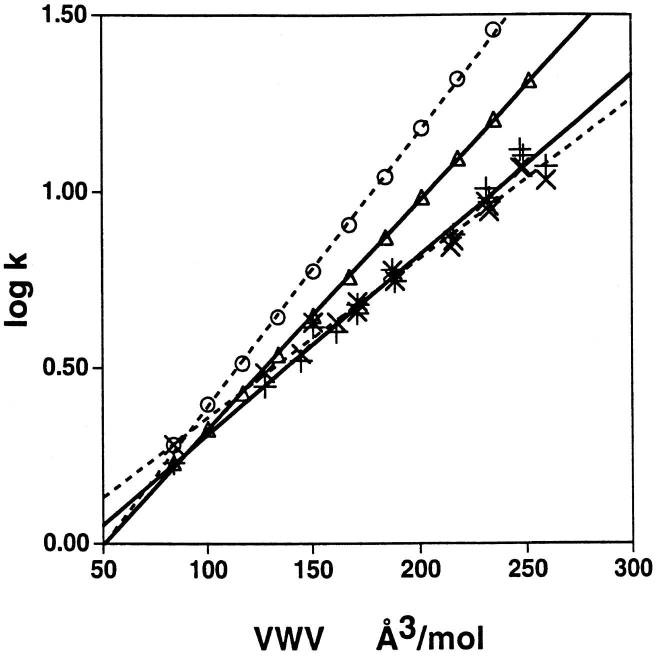

Figure 2. Selectivity of alkylbenzenes and polycyclic aromatic hydrocarbons for phenylhexyl (C6Ph)- and octyl (C8)-bonded phases; eluent: 70% aqueous acetonitrile; temperature: 40°C. symbol +: PAHs on C6Ph, Δ alkylbenzenes on C6Ph, X: polycyclic aromatic hydrocarbons on C8, O: alkylbenzenes on C8.

The retention capacity of C6Ph for alkylbenzenes in 70% aqueous acetonitrile was equivalent to that of C5; however, that for polycyclic aromatic hydrocarbons on C6Ph was equivalent to that of C8, as shown in Figure 2. This means that the slope-related logk and the van der Waals volumes of analytes were not directly related to their hydrophobic selectivity. The 10% difference of acetonitrile concentration significantly influenced the stationary phase. The phenyl-bonded phases should be saturated with acetonitrile more than would alkyl-bonded phases in 80% aqueous acetonitrile because acetonitrile is a dipole solvent. When the retention time of benzene was used as the standard, the slopes between the logk values and the van der Waals volumes indicated the different retention capacity of these bonded phases. The retention capacity for alkylbenzenes was 1.27 times greater than that for PAHs on the C6Ph phase. The retention capacity for alkylbenzenes was 1.73 times greater than that for PAHs on the C8 phase. This means that PAHs retained 1.36 times more on the C6Ph phase than on the C8-phase in 70% aqueous acetonitrile.

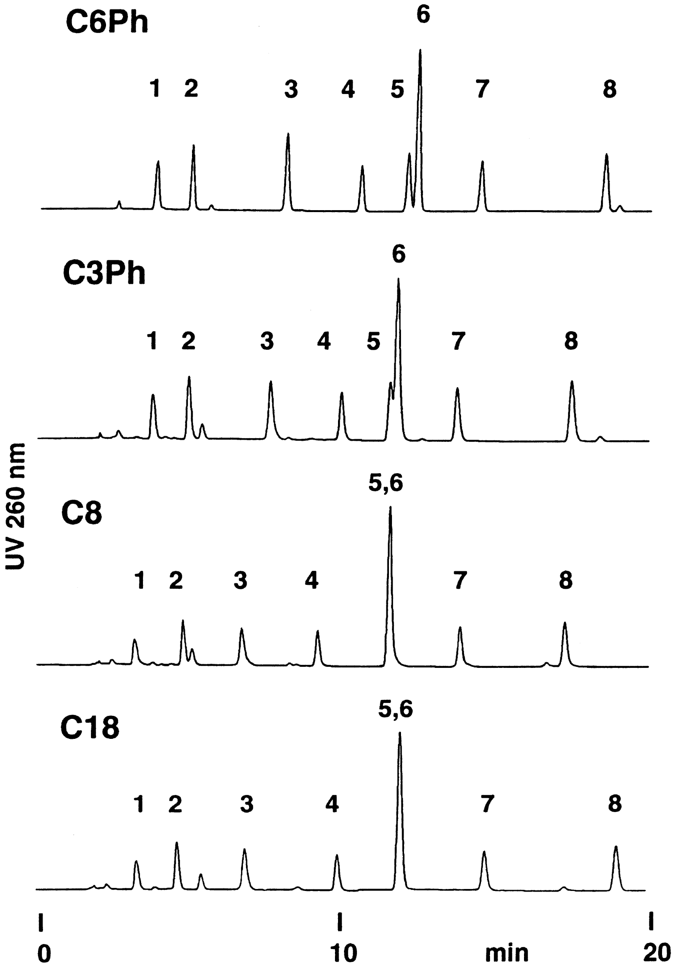

Figure 3. Separation of antibacterial drugs on phenyl and alkyl phases. Column: 5 μm phenylhexyl-, phenylpropyl-, octyl- and octadecyl-bonded silica gels, 15 cm x 4.6 mm I.D.; eluent: gradient from A (20 mM potassium phosphate buffer, pH 2.5) to B (acetonitrile). Start A:B (80:20), then 75:25 in 5 min and 55:45 in 15 min; flow rate: 1 mL/min at 40°C; detection: UV 260 nm. Peak 1: carbadox, 2: thiamphenicol, 3: furazolidone, 4: oxolinic acid, 5: sulfadimethoxine, 6: sulfaquinoxiline, 7: malidixic acid, 8: primodic acid.

The selectivity was applied for the separation of antibacterial drugs. The mixtures of carbadox, thiamphenicol, furazolidone, oxolinic acid, sulfadimethoxine, sulfaquinoxaline, malidixic acid, and piromidic acid were chromatographed using potassium dihydrogenphosphate and acetonitrile mixtures. The total elution time was about 20 min on the C3Ph, C6Ph, C8, and C18 phases in the same separation conditions as those shown in Figure 3. The perfect resolution between sulfadimethoxine and sulfaquinoxaline was achieved only on the C6Ph phase; the separation of these two compounds was not achieved on the C8 and C18 phases.

In conclusion, such selective retention capability of phenyl-bonded phases can expand the separation power in reversed-phase liquid chromatography. An eluent for a C8-bonded phase can be easily applied to a C6Ph phase for the selective separation. The above results indicate that a phenylhexadecyl-bonded phase will be compatible with a C18-bonded phase for improving the separation power in reversed-phase liquid chromatography. The chemically stable and selective bonded phases can be designed by computational chemistry for the designing drugs and new materials.

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