Journal of Chromatography, 142 (1977) 353–365

CHROM. 10,321

SOLVENT SELECTIVITY IN REVERSED-PHASE HIGH-PRESSURE LIQUID CHROMATOGRAPHY

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SUMMARY

The polarity and selectivity of several mobile phases used with reversed-phase high-pressure liquid chromatography columns were studied. Binary and ternary solvent mixtures were examined, using conventional isocratic and gradient elution, as well as two-variable ternary programs where solvent strength and selectivity are independently varied. Emphasis was on water, methanol, acetonitrile and tetrahydrofuran, but some experiments included dichloromethane, ethyl ether, dimethyl sulfoxide and dimethylformamide. Selectivity differences are shown to be enough to provide an important and easy-to-use means of improving resolution and controlling the separation.

Selectivity was studied for eleven functional groups: chloro, methoxy, ketone, aldehyde, phenol, carboxyl, methyl ester, amide, amine, nitro and nitrile. These groups were monosubstituted on benzene and two C_{10} homologs, butylbenzene and naphthalene.

Solvents are classified in terms of polarity and functional group selectivity. The classification is compared to that predicted by both classical solubility parameters and modern solubility concepts. The practical significance of reversed-phase solvent selectivity is discussed and compared with the wide use of stationary phase selectivity in gas chromatography.

INTRODUCTION

Liquid chromatography with the mobile phase more polar than the stationary phase has been termed "reversed-phase" (RP) chromatography since early in its use[1,2]. Although often employed for biochemically oriented open-column separations, it was not until the advent of packings with covalently bonded functional moieties in pellicular/superficially porous[3] and microparticulate/totally porous[4] particles that RP chromatography became significant in the practice of modern high-pressure liquid chromatography (HPLC). The popularity of RP, especially where the stationary phase is a hydrocarbon, has recently grown dramatically.

It is estimated that 60–80% of HPLC separations are accomplished using RP packings[5,6], a figure supported by our own survey of several laboratories using HPLC. It is interesting that different workers perceive the inherent advantages of the technique in entirely different ways. Thus Horváth *et al.* consider most workers as initially thinking of RP as primarily a tool for non-polar substances[5], whereas Karch *et al.* believe the main advantages lie in the separation of polar samples that are not eluted from silica columns[7]. The current literature testifies that RP HPLC is in fact used throughout a broad polarity range and in diverse applications. A notable example is the work of Twitchett and Moffat, who, as a result of an investigation of 30 compounds selected as representative of a wide variety of drug substances, claimed that drugs of any lipid solubility, molecular weight, chemical structure and acidity/basicity can be chromatographed using RP if an appropriate eluent (solvent composition and pH) is chosen[8].

The point is not that RP columns are always better than more polar packings, silica for example, but that the extent of their use and inherent advantages in many circumstances suggest the importance of understanding how best to use them. The reversed phases referred to in this paper comprise linear 8-carbon and 18-carbon hydrocarbon chemically bonded to silica particles.

Definitions of terms

The primary topic of this paper is solvent selectivity. Since the term has various meanings, we will do well to carefully define its use here. In the first definition, selectivity refers to one of the three fundamental chromatography parameters as described in the resolution expression[9]:

$$Rs = \text{f (selectivity) (retention) (efficiency)} \tag{1}$$

$$Rs = \frac{1}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k'}{1 + k'}\right)(N^{\frac{1}{2}}) \tag{2}$$

Selectivity is expressed in terms of relative retention, α, the net retention time ratio for two components:

$$\alpha = \frac{t_{r2} - t_0}{t_{r1} - t_0} = \frac{k_2'}{k_1'} \tag{3}$$

In this sense selectivity simply refers to the ability of the column to retain different solutes for different times, and thus make separation possible[10,11].

In this paper, however, the term selectivity primarily refers to the ability of a solvent to exhibit specific solute interactions which another solvent of approximately similar strength or polarity does not have. Solvent strength means the degree to which a solvent causes faster zone migration; the stronger the solvent the less the retention (smaller capacity factor, k'). Strength is often used synonymously with polarity. But whereas greater strength always decreases retention, greater polarity can have two opposite effects: decreasing retention in normal phase systems and increasing it in RP chromatography. In this paper we often use the terms interchangeably, but it is helpful to keep the distinction in mind.

To understand the term selectivity, recall that solvents have often been listed in order of chromatographic strength, an eluotropic series[12–14]. In the case of RP systems, increasing strength is decreasing polarity, starting with water and ending with hydrocarbons. Numerical values can be assigned to quantify strength, the Hildebrand solubility parameter being one example[15–19].

Although a polarity value is useful, it does not accurately reflect a solvent's eluting strength for all solutes. This is because strength is the sum total of three types of intermolecular interactions acting concurrently, *e.g.*, dispersion, orientation and hydrogen bonding[19–24]. Each solvent has these interactive components in a unique ratio. Thus two solvents of approximately equal polarity can have different interactive profiles. Solutes also have a profile; and when there is a good interactive match between the profiles of solvent and solute, the solvent strength is particularly high. This is the concept of selectivity as used here: the degree to which a solvent is chromatographically stronger for a particular solute by virtue of its ability to enter into specific intermolecular interactions to a greater degree than for other solutes. For example, we can speak of a solvent as being selective for alcohols over amines. This interactive selectivity can, when properly employed, cause a better separation of components in a sample, and then we can speak of the high selectivity of the system using the term in a general sense as in the first definition.

Stated somewhat differently, RP chromatography retention of a particular solute depends on solvent strength, but the latter is in turn the sum of several specific interactions. Polarity describes the gross solvent strength. Selectivity describes the fine structure of strength, the profile of polarity sub-parameters which is particularly powerful in separating solutes of similar polarity. In normal-phase adsorption chromatography, mobile phase selectivity has been well documented[25–28], although it is often difficult to predict or explain retention behavior. Selectivity has also been described in normal-phase liquid–liquid chromatography[9,10,29–33], although the practical difficulties of such systems are well known. As shall be seen, the selectivity phenomena in RP systems can be more complex than normal-phase systems, from a theoretical standpoint. But in practice the employment of selectivity effects to improve separations is just as simple.

Reversed-phase retention mechanisms

Although it has been universally accepted for some time that in RP systems the most polar solutes are the least retained and the most polar solvents have the weakest eluting strength, consensus has yet to be reached on the precise mechanism of retention. Karch *et al.* have suggested it is due to solute interaction with the non-polar stationary phase by dispersion forces[7]. They expect the effect of solute structure on retention to be similar to that in gas chromatography (GC) with graphitized carbon black stationary phase, retention increasing with increasing apolar chain length. Their experimental data support the predicted elution order; for example, pentanols are eluted after butanols, and within a butanol homologous series the elution order is tertiary, secondary and normal.

In contrast, Locke considers the solute interactions with the non-polar stationary phase to be weak and non-selective[35]. Retention is considered primarily a function of solution phenomena in the mobile phase. Taking note of this, Karger *et al.* have emphasized the importance of understanding water solubility[36]. Starting

with water solubility theories which use the idea of cavity formation[37,38], they discussed hydrophobic effects to explain their experimental observations that RP systems exhibit a marked selectivity for the hydrocarbon structure of solutes. Retention could be predicted by using a topological index termed "molecular connectivity", in which solute surface area is estimated by looking only at the hydrocarbon skeleton of a solute.

Horváth *et al.* also affirm the paramount role of the mobile phase[5]. They have adopted solvophobic theory[39,40], and point out that the most pronounced solvophobic effect is the hydrophobic effect, which results from the very high cohesive density of water. Mobile phase surface tension is shown to play a key role. They further point out that, whereas in stationary phases having ionic or hydrogen bonding moieties (normal-phase systems) the driving force of retention is predominantly the attraction between the solute and stationary phase, in stationary phases having a hydrocarbon character (RP systems) the driving force of retention is the concomitant decrease in the non-polar surface area exposed to the solvent. That is, the hydrophobic "bond" between solute and stationary phase results primarily from the aqueous solvent forcing the molecules to associate, rather than from any real attraction.

Snyder has provided a clear description of this effect within a general discussion of polarity and the four bond-breaking or bond-making steps involved in the transfer of a solute from mobile to stationary phase[41]. The energetically favorable transfer of non-polar solutes from the aqueous mobile phase to the hydrophobic stationary phase is related to the heat required to form a cavity within the water structure into which the solute is placed. The solute is repelled or "squeezed out" of the water because its interactions with water are weaker than the interactions of water with itself[42].

Reversed-phase selectivity mechanisms

As previously mentioned, in normal-phase chromatography the role of the mobile phase in controlling selectivity has been well documented[9,10,29–33], although little use has been made of these effects in practical HPLC. Thin-layer chromatography solvent systems are not infrequently ternary mixtures of solvents chosen for selectivity enhancement.

In RP HPLC the selectivity effects are only recently being explored. Karger *et al.* found that regardless of the class of solute, a methylene group increment caused a k' increment of 4 in pure water[36]. The k' increment decreased as organic modifier was added, the extent of decrease being a function of the type of modifier. However, when both modifier type and percentage composition were simultaneously varied so as to maintain k' constant for a given solute, *i.e.*, time-normalized conditions, the methylene group increment caused a roughly constant k' increment of 2 to 3. Thus although RP systems show a strong ability to distinguish different hydrocarbon moieties, different solvents do not show different selectivities for these moieties.

In the same paper, the authors suggested that solvent selectivity for different functional groups in RP HPLC would exist to some extent. An experiment with five-carbon ketones, esters and alcohols showed differences in relative retention between mobile phases containing methanol, propanol and acetonitrile. Others have done limited work in this area[6,7,11].

EXPERIMENTAL

Columns, solvents and samples

The majority of the work was done using 250 × 4.6 mm I.D. stainless-steel columns packed with 10-μm Lichrosorb RP-8, a totally porous granular material with covalently bonded octyl functionality. The gradient work used 250 × 3.1 mm I.D. stainless-steel columns packed with 10-μm Spherisorb ODS, a totally porous spherical material with covalently bonded octadecyl functionality. Both are available from Spectra-Physics, Santa Clara, Calif., U.S.A.

Mobile phases were prepared from distilled-in-glass solvents (Burdick & Jackson Labs., Muskegon, Mich., U.S.A.). Distilled water was used. No attempt was made to control pH other than that all solvents were continuously helium degassed[34].

Samples were from Chem Service (West Chester, Pa., U.S.A.) and Aldrich (Milwaukee, Wis., U.S.A.). They were dissolved in methanol.

Control of flow, composition and temperature

A Spectra-Physics Model SP 8000 research liquid chromatograph was used. It employs a single pump, attached to a low pressure composition forming module (ternary proportioning valve). The hardware functions of flow and composition control are thus totally separated[34]. Up to three different solvents can be separately stored and mixed in the valve according to the operator instructions in both isocratic and gradient elution. The column temperature was controlled by an air oven.

Detection and retention time measurement

The detector was a Spectra-Physics Model SP 8200 multiple-wavelength UV photometer operated at 254 nm. Peak retention times were measured by a computing integrator inboard the SP 8000 chromatograph.

RESULTS

Hydrocarbon structure and functional groups

The general scheme of the experimental work involved studying how different solvents affected the retention of different functional groups. To gain insight into the mechanisms and allow generalizations, a variety of hydrocarbon structures were examined. Table I shows the matrix of structure and functionality, and lists the code symbols (abbreviations) we chose for the various compounds. Table II lists the names of these compounds. Results for some of these compounds are not reported here, but will be incorporated into future publications.

Three different methods were used to study solvent selectivity:

(1) Binary mixtures of water–methanol, water–acetonitrile and water–tetrahydrofuran (THF) were employed. Using water–methanol (1:1, v/v) as a reference, the acetonitrile and THF were made up in a concentration sufficient to provide the same retention time for benzene as was obtained with the water–methanol (1:1) solvent. That is, all data is referred to the retention of benzene in 50% methanol solutions. These were all isocratic runs.

(2) Ternary mixtures of water (40%), methanol (50%) and a third solvent (10%) were employed. The third solvent was varied among acetonitrile, tetrahydro-

TABLE I

HYDROCARBON STRUCTURE, FUNCTIONALITY AND CODE SYMBOLS

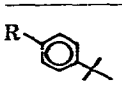
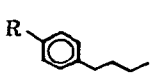
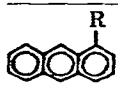
Functionality		*Hydrocarbon structure*				
Name	*R*	C_6	C_{10}			C_{14}
						
	no group	Ø	Ø^t	Ø^n	Ø^2	Ø^3
Chloro	–Cl	Cl			Cl^2	Cl^3
Methoxy	–O–CH_3	OM	OM^t		OM^2	
Ketone	–C(=O)–CH_3	OK	OK^t		OK^2	
Aldehyde	–CH	OA			OA^2	
Phenol	–OH	OH			OH^2	
Carboxyl	–C(=O)–OH	CO_2H	CO_2H^t		CO_2H^2	CO_2H^3
Methyl ester	–C(=O)–O–CH_3	CO_2M				
Amide	–C(=O)–NH_2	CON				
Amine	–NH_2	NH_2		NH_2^n	NH_2^2	NH_2^3
Nitro	–NO_2	NO_2			NO_2^2	NO_2^3
Nitrile	–C≡N	CN			CN^2	

TABLE II

CODE SYMBOLS AND TEST SOLUTE NAMES

Code symbol	*IUPAC name*	*Code symbol*	*IUPAC name*
Ø	Benzene	CO_2H	Benzoic acid
Ø^t	*tert.*-Butylbenzene	CO_2H^t	*p-tert.*-Butylbenzoic acid
Ø^n	*n*-Butylbenzene	CO_2H^2	1-Naphthoic acid
Ø^2	Naphthalene	CO_2H^3	Anthracene-9-carboxylic acid
Ø^3	Anthracene	CO_2M	Methyl benzoate
Cl	Chlorobenzene	CON	Benzamide
Cl^2	1-Chloronaphthalene	NH_2	Aniline
Cl^3	Chloroanthracene	NH_2^n	*p-n*-Butylaniline
OM	Anisole (methoxybenzene)	NH_2^2	1-Aminonaphthalene
OM^t	*p-tert.*-Butylanisole	NH_2^3	1-Aminoanthracene
OM^2	1-Methoxynaphthalene	NO_2	Nitrobenzene
OK	Acetophenone	NO_2^2	1-Nitrophthalene
OK^t	*p-tert.*-Butylacetophenone	NO_2^3	9-Nitroanthracene
OK^2	1-Acetonaphthone	CN	Cyanobenzene
OA	Benzaldehyde	CN^2	1-Cyanonaphthalene
OA^2	1-Naphthaldehyde		
OH	Phenol		
OH^2	1-Naphthol		

furan, diethyl ether, methylene chloride, dimethylformamide, dimethyl sulfoxide, and water 50% saturated with hexane. These were all isocratic runs.

(3) Binary and ternary gradient mixtures were employed. This work included some runs where the third solvent is programmed independently from the second. That is, the water concentration changes in some manner (typically a linear reduction in concentration during the run), and the combined second and third solvent changes in a corresponding opposite manner. But the ratio of the second solvent to the third is a program variable independent of the water program. This provides the ability to program solvent strength (polarity) independently from selectivity. There is no analogous operation in GC, nor in currently practiced HPLC.

Selectivity in isocratic binary mixtures

Table III shows the retention (k') of the eleven functional groups on both benzene and naphthalene in the three binary systems. They are listed in the order of elution with the 50% methanol solvent. Note that the percentage composition of the acetonitrile and THF solvent mixtures have been adjusted to produce a retention of benzene identical to that obtained for 50% methanol ($k' = 4.7$).

TABLE III

RETENTION (k') OF TEST SOLUTES IN THREE BINARY SOLVENTS

See Tables I and II for structures and names of test solutes. NaØS is sodium benzene sulfonate used to mark the unretained time for k' calculations. PM, PE, PP, and PB are methyl-, ethyl-, propyl- and butyl-parabens (*p*-hydroxybenzoates), respectively.

Test solute	*50% Methanol*	*40% Acetonitrile*	*37% THF*
NaØS	0	0	0
CO_2H	0.5	0.3	0.4
CO_2H^2	0.7	0.4	0.8
CON	0.9	0.7	0.7
N_2	1.3	1.5	1.7
OH	1.6	1.4	2.3
OA	2.2	2.2	1.9
PM	2.3	1.5	2.0
CN	2.3	2.7	2.3
OK	2.7	2.0	1.9
NO_2	3.4	3.6	3.4
N_2^2	3.7	3.5	3.5
PE	3.8	2.2	2.8
OM	4.5	4.3	3.9
Ø (reference)	4.7	4.7	4.7
CO_2M	5.0	3.9	2.9
OH^2	5.4	3.9	4.9
PP	7.0	3.6	4.2
OK^2	8.2	6.1	3.6
OM^2	8.2	6.0	[illegible]
OA^2	8.7	6.1	3.7
Cl	9.2	7.7	6.5
CN^2	9.8	7.3	4.2
NO_2^2	12.0	9.0	5.7
PB	13.4	5.9	6.2
$Ø^2$	15.1	11.0	7.3
Cl^2	35.8	17.3	10.0

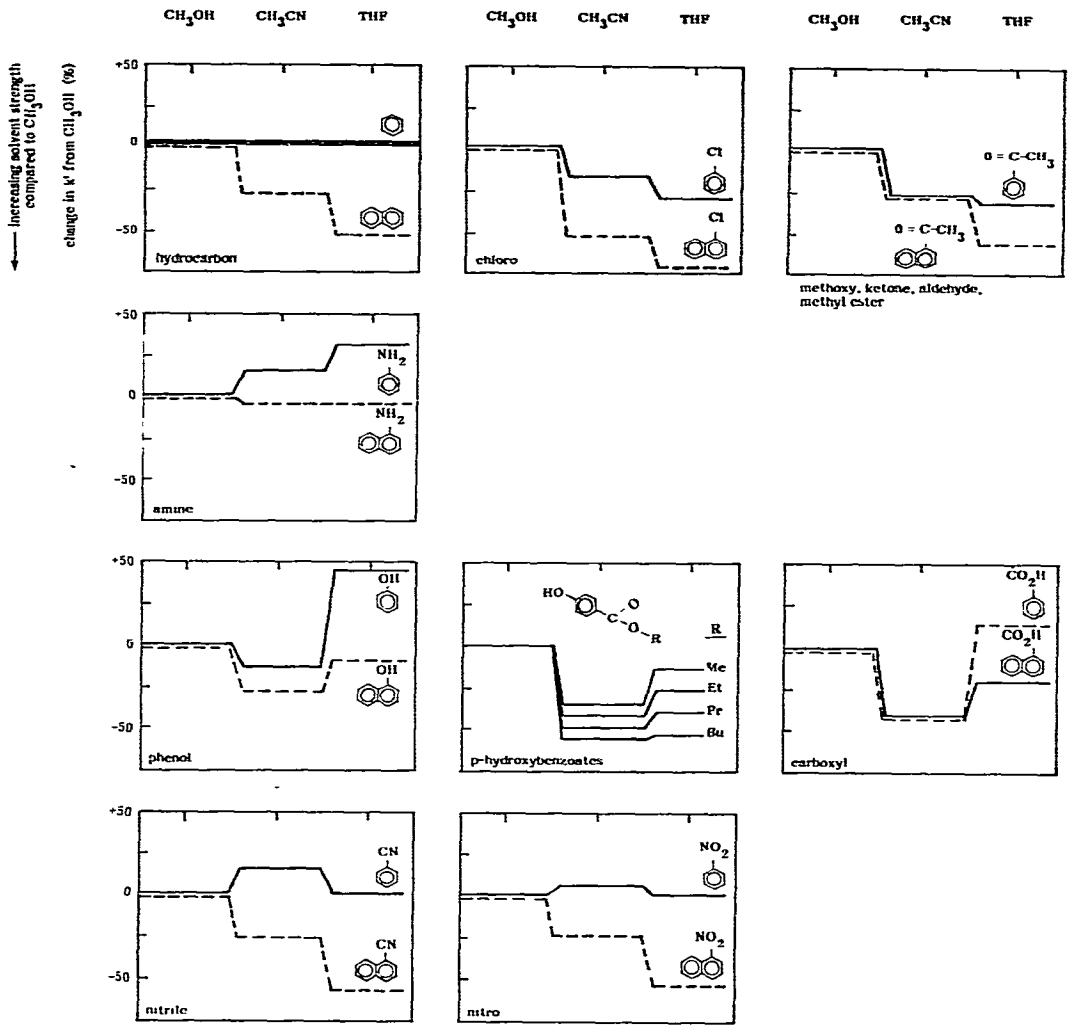


Fig. 1. Solvent selectivity *vs.* functionality. Column, 250 × 4.6 mm I.D.; packing, 10-μm Lichrosorb RP-8; solvent, 50% methanol, 40% acetonitrile, 37% tetrahydrofuran in water; flow-rate, 5 ml/min; pressure, 2000–4000 p.s.i.; temperature, 35°; samples, see Tables I and II for names and structures of compounds, using code symbols.

Fig. 1 presents these results in a way which makes the selectivity of the three solvent systems toward different functional groups immediately apparent. Each graph plots the percentage change in k' for the solute in going to the acetonitrile and THF solvents. Note again the reference to benzene.

Selectivity in isocratic ternary mixtures

Table IV shows the retention (minutes) of several compounds in eight different ternary solvent systems. The unretained peak has a retention time of 1.8 min, so a retention time of 10 represents a k' of about 4.6.

TABLE IV

RETENTION TIMES OF VARIOUS SOLUTES WITH DIFFERENT MODIFIER

Solute	*Symbol*	*"C" Solvent in ternary mixture (40% water, 50% methanol, 10% "C")*							
		Methanol	*Aceto-nitrile*	*THF*	*Diethyl ether*	*Dichloro-methane*	*DMF*	*DMSO*	*50% sat. C_7*
Acetanilide	—	2.9	2.8	2.4	2.3	2.8	2.5	3.3	2.4
Aniline	NH_2	2.9	2.9	2.4	—	2.8	2.5	3.3	2.5
Benzyl alcohol	—	3.1	2.9	2.6	2.4	3.0	2.6	3.3	2.6
Phenol	OH	3.1	2.9	2.7	2.8	2.9	2.7	3.3	2.6
Acetophenone	OK	4.0	3.5	2.8	2.7	4.6	3.1	4.1	3.5
N-Ethylaniline	—	5.2	4.6	4.1	—	—	4.4	6.9	—
Benzene	Ø	5.5	4.7	4.3	4.5	6.6	4.4	5.6	5.2
Anisol	OM	5.5	4.7	3.9	4.1	6.4	4.3	5.6	5.0
N,N-Dimethyl aniline	—	6.8	5.7	4.9	—	9.9	5.4	8.8	6.7
Toluene	—	8.3	6.6	5.9	6.4	10.0	6.4	8.6	8.7
Chlorobenzene	Cl	8.5	6.8	5.9	6.5	9.3	6.5	8.7	8.9
Bromobenzene	—	9.5	7.4	6.7	7.1	10.6	7.2	9.9	10.0
1,2-Dichloro-benzene	—	13.0	9.7	8.4	9.0	13.1	9.7	13.7	14.0

Selectivity in gradient binary and ternary mixtures

Fig. 2 shows the chromatograms resulting from four different solvent programs. The ternary program has all peaks resolved, and numbered accordingly. The marked change in selectivity (relative retention) as one goes from system to system is readily apparent.

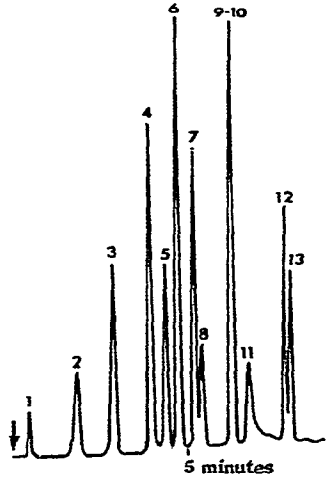
DISCUSSION

It is clear that there are abundant selectivity effects in RP systems. Fig. 1 shows that a solvent different from methanol may decrease retention for one functional group, but that the same solvent may increase retention for a different functional group. As an example consider aniline (NH_2) *vs.* phenol (OH). Compared to the methanol solvent, acetonitrile causes aniline retention to increase, but phenol retention to decrease.

The larger the hydrocarbon skeleton (naphthalene *vs.* benzene) the stronger the acetonitrile and THF appear relative to methanol. Yet the selectivity pattern is the same, when normalized against naphthalene. The case of the parabens (*p*-hydroxybenzoates) illustrates this also.

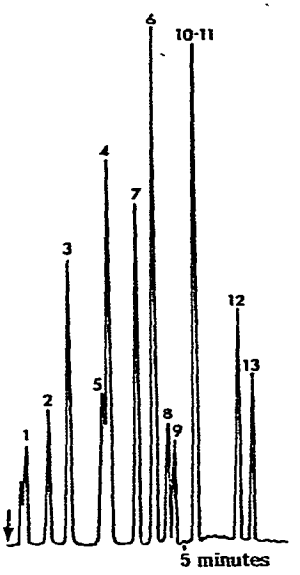
A study of Table IV and Fig. 2 illustrates the same point; there is a marked solvent selectivity in RP HPLC. A detailed theoretical discussion to account for these effects is beyond the scope of the present paper. However, we can point out some preliminary considerations.

Fig. 3 diagrams the solvent–solute interactions which take place. What happens when acetonitrile is substituted for THF in the aqueous mixture? Acetonitrile is more polar than THF and the net polarity of the solvent is increased. This tends to reduce solvent strength. But to the extent that there are specific intermolecular



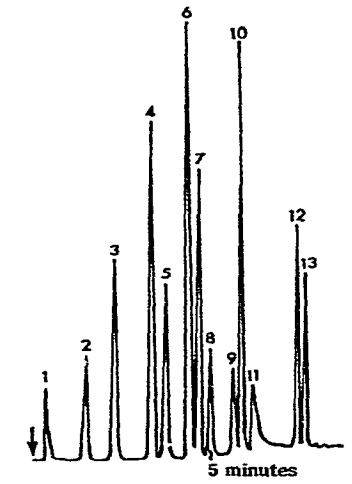
time	H_2O	$\%CH_3OH$
0	85	15
10	15	85

CH_3OH binary



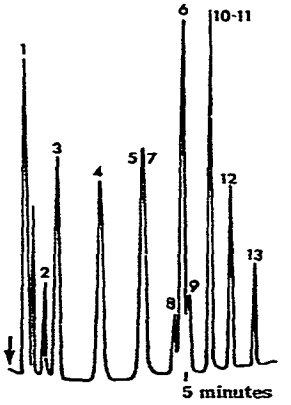
time	$\%H_2O$	CH_3CN
0	85	15
10	15	85

CH_3CN binary



time	$\%H_2O$	$\%CH_3OH$	$\%CH_3CN$
0	85	10	5
10	15	80	5

CH_3OH/CH_3CN ternary
(all 13 peaks resolved)



time	$\%H_2O$	THF
0	85	15
10	15	85

THF binary

Fig. 2. Gradient solvent selectivity. Column, 250 × 3.1 mm I.D.; packing, 10-μm Spherisorb ODS; solvent as noted; flow-rate, 2.0 ml/min; pressure 1000–2000 p.s.i.; temperature, 50°; detector, Model 8200 at 254 nm; sample size, 10 μl. Peaks: 1 = uridine; 2 = sulfamerazine; 3 = acetanilide; 4 = benzaldehyde; 5 = methyl *p*-hydroxybenzoate; 6 = nitrobenzene; 7 = cinnamyl alcohol; 8 = anisole; 9 = methyl benzoate; 10 = *o*-nitrotoluene; 11 = methyl salicylate; 12 = 1-nitronaphthalene; 13 = diphenylamine.

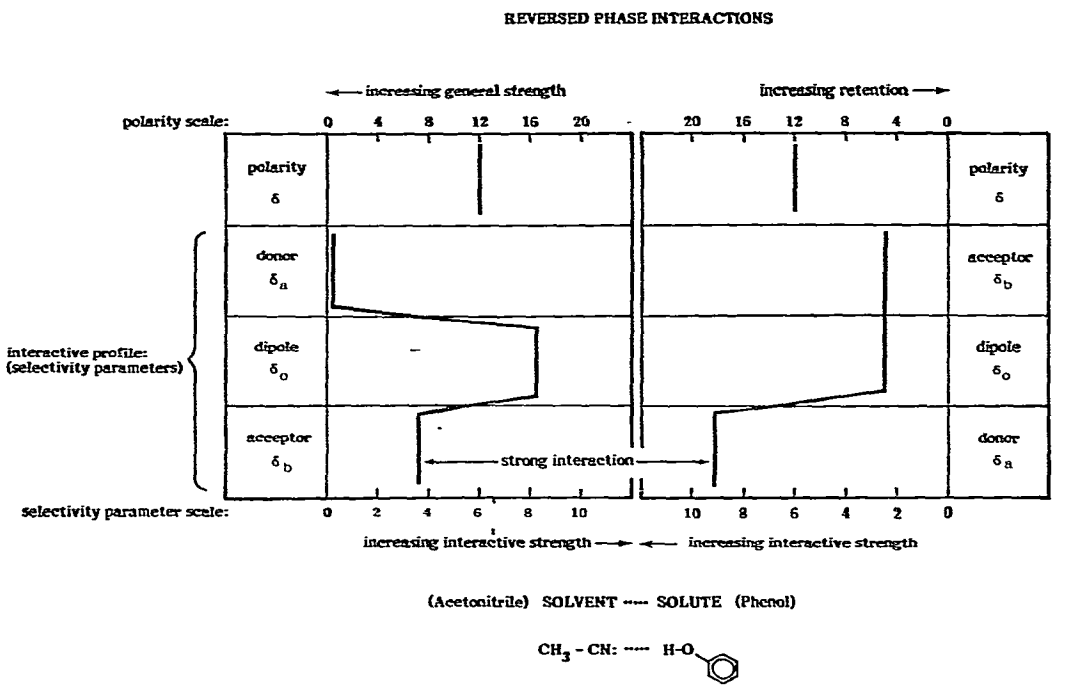


Fig. 3. Reversed-phase interactions. The polarity scale is the polarity index of Karger *et al.*[22] (see Table V). The selectivity parameter scale refers to the selectivity parameters (Table V). The values graphed are for acetonitrile (solvent) and phenol (solute). The molecular diagram indicates a specific interaction between the solvent and solute, for example dipole and/or hydrogen bonding. The interactive profiles of the solvent and solute are seen to be such as to cause fairly high solubility and thus acetonitrile is a strong solvent for phenol.

interactions between acetonitrile and the solute which are stronger than the specific THF–solute interactions, the solvent strength will be increased. The picture is therefore somewhat more complex than normal-phase chromatography.

Table V compares the interactive profiles of methanol, acetonitrile and THF as conceived by various solubility parameter theories. Included is a new and somewhat simplistic empirical characterization based on k' for three test solutes: nitrobenzene, anisol and phenol. These solutes are analogous to those used by Snyder[24] in his empirical solvent classification scheme.

These comparisons are presented without further comment, pending the collection of additional chromatographic data for a wider range of solvents and solutes.

CONCLUSIONS

There is general agreement that in GC there are too many liquid phases[44]. However, there will always be a need for several, because many different phases do in fact show marked selectivity amongst different compounds. In GC only the stationary phase can be conveniently changed, the mobile phase being essentially inactive.

In RP HPLC the situation is somewhat the mirror image of this. The mobile phase is quite an active partner in the separation process, and the hydrocarbon

TABLE V

COMPARISON OF INTERACTIVE PROFILES

References	*Formulae*	*Interaction*	*Symbol*	*Methanol*	*Acetonitrile*	*THP*
15–18		Cohesive energy density	δ	14.5	12.1	9.1
21, 22 (extension of 20)	$\delta_T^2 = \delta_d^2 + 2\,\delta_{ln}\delta_d + \delta_o^2 + 2\,\delta_a\delta_b$	Total solubility parameter	δ_T	14.5	12.1	9.1
		Orientation (dipole)	δ_o	4.9	8.2	3.5
		Acid (proton donor)	δ_a	8.3	—	—
		Base (proton acceptor)	δ_b	8.3	3.8	3.7
23	$\delta_T^2 = \delta_d^2 + \delta_o^2 + 2\,\delta_a\delta_b$	Total solubility parameter	δ_T	15.9	13.2	9.9
		Orientation (dipole)	δ_o	6.7	10.3	3.0
		Acid (proton donor)	δ_a	7.2	0.4	0.8
		Base (proton acceptor)	δ_b	12.9	17.6	16.3
24 (extension of 43)	$P' = \log K_{\text{nitromethane}} +$ $+ \log K_{\text{dioxane}} + \log K_{\text{ethanol}}$	Total polarity index	P'	6.6	6.2	4.2
	$\chi_n = \log K_{\text{nitromethane}}/P'$	Solubility for nitromethane ("dipole")	χ_n	0.3	0.4	0.4
	$\chi_d = \log K_{\text{dioxane}}/P'$	Solubility for dioxane ("proton donor")	χ_d	0.2	0.3	0.2
	$\chi_e = \log K_{\text{ethanol}}/P'$	Solubility for ethanol ("proton acceptor")	χ_e	0.5	0.3	0.4
	$S' = 1/\log k'_{\text{nitrobenzene}} +$ $+ 1/\log k'_{\text{anisol}} + 1/\log k'_{\text{phenol}}$	Total solvent strength	S'	7.7	10.0	6.4
	$S_n = \dfrac{1/\log k'_{\text{nitrobenzene}}}{S'}$	Strength for nitrobenzene	S_n	0.15	0.18	0.30
	$S_a = \dfrac{1/\log k'_{\text{anisole}}}{S'}$	Strength for anisole	S_a	0.21	0.16	0.30
	$S_p = \dfrac{1/\log k'_{\text{phenol}}}{S'}$	Strength for phenol	S_p	0.64	0.66	0.44

stationary phase is relatively passive. We have, then, the situation in HPLC where selectivity can be manipulated by mobile phase changes as well as stationary phase changes. It may well be that the standard column becomes a hydrocarbon bonded phase (analogous to the nitrogen gas mobile phase of gas chromatography), and that selectivity is adjusted by changing the mobile phase only (analogous to the column in GC).

The increasing ease with which HPLC methods can be developed, and in particular the availability of instrumentation with the capability of programmed automatic operation as well as three-solvent (ternary) operation[34], should encourage the chemist to take advantage of selectivity effects.

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