

CHREV. 105

INTRODUCTION TO REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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1. INTRODUCTION

Analyses by liquid-liquid (LLC) or liquid-solid (LSC) chromatography are normally performed with a polar stationary phase (liquid or solid) and a non-polar mobile phase. With such systems, specific interactions occur between the solute and the stationary phase and the retention of compounds depends mainly on the type of functional groups present in the molecule.

The alternative technique of reversed-phase chromatography (RPC) was introduced in 1950 by Howard and Martin¹, and involves the use of non-polar stationary phases and polar eluents. Up to 1969, RPC could be carried out only in the LLC mode because of the lack of suitable adsorbents. The liquid stationary phase was a non-polar compound of high molecular weight, a polymer or the low-polarity phase of a ternary system. In his study of LLC, for example, Locke² used the system squalane-acetonitrile.

RPC has developed spectacularly since the introduction of chemically bonded stationary phases in 1969 by Halász and Sebastian³. New bonding techniques have been developed, and since 1974 have been applied to microparticles of silica, so that it is now possible to prepare very efficient columns packed with chemically bonded phases. It must be pointed out that it is possible to graft either non-polar or polar groups on the surface of silica particles and that bonded phases (BP) are not restricted to RPC but can also be used in "normal" chromatography. For this reason, Pryde⁴ stated that the development of the BP is the most important breakthrough in liquid chromatography. BP cannot solve all of the problems that arise, however. Recently, two new types of adsorbents have been introduced for RPC: various graphitized and non-graphitized modified carbon blacks and carbon-coated silica gels have proved to be very suitable non-polar adsorbents for liquid chromatographic applications⁵⁻⁸. Similarly good results have been obtained using styrene-divinylbenzene copolymers as a non-polar stationary phase⁹⁻¹².

There are many advantages to using bonded-phase chromatography (BPC) instead of LLC. Firstly, in LLC the mobile and stationary phases should be completely immiscible, which hardly ever occurs, so that it is necessary to use a saturation pre-column with a high loading of the stationary phase. Secondly, as the stationary phase is held on the solid support by only weak mechanical and interfacial forces, it is impossible to use a high solvent flow-velocity, for two reasons: on the one hand, a high velocity resulting in large shear forces would strip off the stationary phase, and on the other hand the heat generated locally by the forces would increase the miscibility of the phases. Thirdly, with a given system it is possible to work only in a narrow range of capacity factors and hence partition coefficients¹³, and it is almost impossible in LLC to increase the elution strength of the mobile phase except when working with ternary systems. Fourthly, it is impossible to use gradient elution and to work at high temperatures which might sometimes be useful with the viscous polar solvents generally used as the mobile phase in RPC. Finally, the column efficiency is often poor because of the thickness of the stationary liquid film, and it should also be noted that the eluent is always adversely affected by some concentration of dissolved stationary phase, which can prove troublesome when spectrometric investigations are required after the separation or in preparative LC.

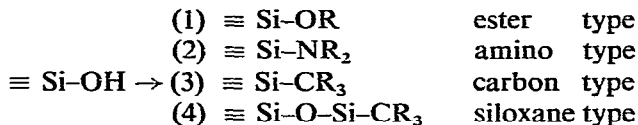
Important developments have been made in RPC in recent years. In a recent paper, Majors¹³ noted that 20% of the separations carried out in 1974 utilized RPC. It is probable that within 4 or 5 years at least 70% of the separations made by high-performance liquid chromatography (HPLC) will be performed using RPC. In view of this important prospect, it is interesting to compare critically the advantages and drawbacks of the various adsorbents used in RPC¹⁴.

This review describes the general properties of the BP used in RPC and the most popular methods of preparation and some applications are also surveyed. In

forthcoming papers a close comparison will be made between the most popular RP (octadecylsilane, ODS) and carbon adsorbents¹⁴.

2. DIFFERENT TYPES OF NON-POLAR BONDED PHASES AND THEIR PREPARATION

There are essentially four different bondings between an organic group and the silica surface which have been used to prepare bonded phases:



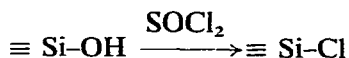
The organic groups (R) can be either polar or non-polar.

A. Ester type

Ester phases were first introduced by Halász and Sebestian³, and are obtained by direct reaction between silica gel and an alcohol (ROH). They are rarely used now because of their low stability: they are easily destroyed either by heating at moderate temperatures or by reaction with many solvents. Particularly because of hydrolysis of the esters, it is impossible to use them with aqueous solutions as the mobile phase, although such solutions are very useful in RPC applications.

B. Amino type

The amino phases are more stable than the ester phases towards solvolysis and can be used with aqueous solvents in the pH range 5–7 (refs. 4–13, 15). In order to prepare these phases, the silanols available on the silica surface are replaced with chlorine atoms using thionyl chloride:



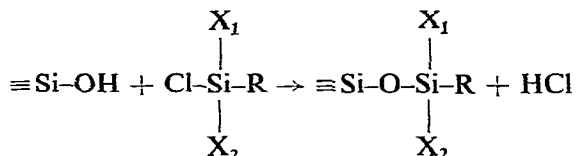
The ≡ Si-Cl groups then react with an amine (R₂NH or RNH₂) to give ≡ Si-NR₂. The structure is of the brush-type. Unwanted polymerization reactions are impossible (see later) and thus the resistance to mass transfer in the stationary phase is small⁸. However, it does not seem that there are many applications of these adsorbents, which are not commercially available to our knowledge.

C. Carbon type

The Si-CR₃ packings are prepared by reaction of a suitable Grignard derivative or an organolithium compound with ≡ Si-Cl groups obtained as described above. Locke *et al.*¹⁶ gave a complete description of the preparation techniques.

D. Siloxane type

The siloxane adsorbents are the most popular BPs. They are synthesized by reaction of an organohalogenosilane (generally a chlorosilane) with the silanol groups at the surface of the silica:



It is also possible to use an organoalkoxysilane, but Deelder *et al.*¹⁷ observed the formation of particle aggregates with this procedure.

The types of BPs summarized in sections A, C and D above can be obtained as monomeric adsorbents (reaction with one molecule, grafted to the surface) and as polymeric adsorbents when a multilayer and/or crosslinked coating is made, and these types are considered below in sections E and F, respectively.

E. Monomeric phases

Monomeric phases are the most widely used. As will be seen later, the efficiencies are much greater than those obtained with polymeric phases because of the faster diffusion rate in the stationary phase. Lynn and Filbert¹⁸ gave a list of the different moieties that can be grafted through silane reagents. The preparation reaction is carried out in an absolutely dry solvent (generally toluene) under prolonged reflux and the hydrogen chloride is removed continuously by bubbling pure nitrogen through the mixture.

When the reagent is a di- or a trichlorosilane, the absence of water is imperative in order to avoid crosslinking polymerization reactions.

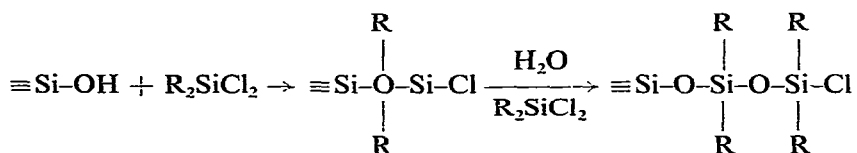
With monochlorosilane, it is obviously impossible for polymerization to occur. A variety of procedures are described in the literature (*e.g.* refs. 16, 17, 19–32). Unger *et al.*¹⁹ are probably the only workers who have described the preparation of silica gel modified in bulk. Majors and Hopper²⁰ gave interesting information about the bonding procedures. Kingston and Gerhart²¹ described in detail the modification of Porasil B with octadecyltrichlorosilane.

The contributions of different parameters to the quality of the BP and to the extent of the reaction (surface coverage) have been studied and are discussed later. The removal of the reaction by-products is sometimes tedious, especially for the $\equiv\text{Si}-\text{C}$ type where the preparation reaction involves the formation of a mineral salt²². The reactivity of the reagents also has to be considered. Karch *et al.*¹⁵ noted that in practice greater coverages of monomeric phases are obtained when using di- or trichlorosilanes under anhydrous conditions than with monochlorosilanes. It seems important to use a large excess of silanes and to remove the volatile products continuously^{19,23}.

Although Majors and Hopper²⁰ found very good reproducibility for the bonding reaction, Karch *et al.*¹⁵ noted that if it is not difficult to prepare a BP, "it is difficult to cook a good one".

F. Polymeric phases

Although most applications are carried out on monomeric packings, polymeric adsorbents (as well as multilayer ones) are sometimes useful. Deeler *et al.*¹⁷, for instance, using diatomaceous earths as supports found it necessary to carry out some polymerization of the bonding reagent in order to achieve the extensive coverages they were attempting to obtain. Polymerization is also advisable when coating porous layer beads (PLB), but the reaction is difficult to control¹³. Kirkland and DeStefano³³ used this technique to coat Zipax. The reagent is usually a di- or a trichlorosilane and trace amounts of water are added to the mixture. The water hydrolyses some $\equiv\text{Si-Cl}$ into $\equiv\text{Si-OH}$ groups, with the formation of a polymeric network. The preparation of multilayer adsorbents is carried out in several steps. The first is identical with that for a monomeric packing, then the unreacted chlorine atoms are hydrolysed and a second reaction is performed:



Because of the reactivity of dichlorodialkylsilanes, it is probable (see below) that the surface layer is, in fact, a mixture of monomeric and dimeric units. Commercially available bonded phases (either polar or non-polar) have been listed in different publications which deal with pre-packed columns and bulk packings. For more information, see refs. 4, 13, 20, 32 and 34.

G. *In situ* preparation

This method has been studied almost exclusively by Gilpin and co-workers^{24,25}, who described methods for the preparation of both monomeric and polymeric layers (multi-coating). The efficiencies of “*in situ*” columns are closely related to the volume of dry toluene used for the pre-treatment of the silica (there is an optimal volume of this solvent to be percolated through the column before the pumping of a 1:1 ODS-toluene mixture). The ratio H before to H after (where H is the height equivalent to a theoretical plate) is 0.55 in the best case. The advantages of this technique are time saving and the preparation of very reproducible columns.

H. Styrene-divinylbenzene copolymers

These adsorbents are used less often than BPs in liquid chromatography. Grieser and Pietrzyk⁹ made use of Amberlite XAD-2, a rigid material with a specific surface area of 300 m²/g and a pore diameter of 90 Å. The adsorbent can be used over a wide pH range (1–12) without any problems of stability. References to publications dealing with the properties of XAD-2 are given in ref .9.

The copolymer packing Hitachi Gel 3010 consists of spherical particles in the size range 20–30 μm. This crosslinked material has good chemical and physical

durability, can be used with acidic or basic mobile phases and has a high specific surface area of 800 m²/g (ref. 12).

A difficulty arises, however, from the fact that the particles swell in organic solvents and shrink in aqueous solvents, and it is necessary to use a specially designed injection system that can compensate for the variations in packing volume. A special sample injection port is described in Fig. 1 of ref. 12. Problems may arise when changing the solvent, resulting in poor efficiencies. Takahagi and Seno¹² packed the columns with the solvent mixture methanol–water (90:10) and used the following elution solvents: methanol, methanol–*n*-hexane (95:5), methanol–water (95:5) and methanol–water (90:10), which probably do not change the swelling–shrinking characteristics to a great extent. However, the situation may be different when preparing a gradient from, for instance, pure water to pure methanol.

More work must be carried out on this aspect as such phases could be very interesting for RPC applications. Nakae and Muto^{10,11} also used Hitachi Gel 3010 in theoretical and analytical studies, and this will be considered in later sections.

1. Extent of the reaction

The properties of the BP are closely related to the surface coverage, *i.e.*, the extent of the reaction. In dealing with silica adsorbents, the bonding reaction is controlled by the type, the activity and the steric hindrance of the reactive sites at the silica surface²⁶. Lynn and Filbert¹⁸, working on controlled-pore glass of specific surface area 70 m²/g, found that other parameters can also influence the concentration or the thickness of the bonded layer. They obtained constant coatings with concentrations of silane monomers greater than 4% and found that the pH has a negligible influence when it is less than 6, while at higher values the reaction yield is increased.

The coating density increases with increasing temperature at first and becomes constant above 75°. The reaction time is of minor importance when the reaction is carried out at high temperatures. According to Unger *et al.*¹⁹, however, the highest temperature and longest reaction times are desirable and Sorrell³⁵ also reported that increasing the temperature improves the surface coverage. He found that the logarithm of the surface coverage of silica gel by alcohols (ester-type phase) is proportional to the inverse of the temperature and suggested that the coating density is proportional to the equilibrium constant of the coating reaction. The reactivity of the bonded species must also be considered²². Diez-Cascon *et al.*²⁷ proposed the following order of increasing reactivity for derivatives of the alkyl type ($\equiv\text{Si}-\text{C}_n\text{H}_m$) and amino type ($\equiv\text{Si}-\text{NH}-\text{C}_n\text{H}_m$): Grignards < organolithium compounds < amines < silanes.

Another characteristic of the reagent is the length of the alkyl chain, and contradictory results have been reported in the literature. Karch *et al.*¹⁵ obtained the best coverages of LiChrosorb Si-100 when using dimethyldichlorosilane (8.7 $\mu\text{mole}/\text{m}^2$) and the lowest with octadecylmethylchlorosilane (2.6 $\mu\text{mole}/\text{m}^2$). Hemetsberger *et al.*²⁸, observed a slight decrease in the surface concentration between *n*-C₈H₁₇ and *n*-C₂₁H₄₃ bondings, the values being 3.74 and 3.35 $\mu\text{mole}/\text{m}^2$, respectively. They attributed this decrease to blocking of the micropores by the larger molecules. Unger *et al.*¹⁹ introduced the surface density, α_{exp} (mole/m²), to express the extent of coating: $\alpha_{\text{exp}} = W/(M \cdot S_{\text{BET}})$ where *W* is the weight of functional groups (gram per gram of adsorbent),

M the molar weight and S_{BET} the specific surface area of the starting support, corrected for the weight increase due to the grafting reaction (m^2/g). They measured α_{exp} for n -alkyl groups with n carbon atoms; α_{exp} remained constant as n increased. For $4 < n < 16$, the average value of α_{exp} was $3.5 \mu\text{mole}/\text{m}^2$ ($\pm 15\%$). On replacing the methyl group with a phenyl group a substantial decrease in α_{exp} occurred.

Kirkland²³ obtained a greater coverage density with octadecyltrichlorosilane than with trimethylchlorosilane or dimethylbenzylchlorosilane (3.1, 2.5 and $2.5 \mu\text{mole}/\text{m}^2$, respectively, using Spherosil XOA 400). He considered that the greater coverage obtained with octadecylsilane was probably due to the interaction of the excess of reagent with bonded groups that have active functions.

The coverage density of the surface is obviously related to the number of reactive sites on the surface and to the functionality of the reagent. Some workers consider that there are 3.6 sites per 100 \AA^2 (ref. 35), 4 (refs. 22 and 29), 4.8 (ref. 19), 8 (refs. 15 and 35), or anything between 5 and 8 (ref. 26). Roughly, the maximum coverage density is about 6 molecules per 100 \AA^2 ($10 \mu\text{mole}/\text{m}^2$). With respect to the silane, it is generally accepted that for a di- or a trichlorosilane, a maximum of two chlorine atoms can react with the surface sites¹⁵. With a trichlorosilane, steric hindrance prevents the reaction of the third chlorine atom²⁸. The average area required by bonded hydrocarbon chains increases with the chain length and the molar weight; in fact, the governing factor is the crowding of groups close to the bonding sites rather than the molar weight. When working with a trichlorosilane, attention must be paid to the unreacted chlorine atoms as they are hydrolysed after the grafting reaction, providing a polar Si-OH group which can influence the chromatographic behaviour of the packing and its stability when working at $\text{pH} > 7$ ¹⁵. It is also important to avoid crosslinking, which is promoted by trace amounts of water. Finally, the activity of the bonding sites seems to have little effect on the coverage density under prolonged reaction conditions²⁸.

The coverage density can be measured in different ways. It seems pointless and misleading to use the percentage of carbon¹⁹ or of the stationary phase²², and a better parameter is the surface density of bonded species. As stated previously, it is possible to use Unger *et al.*'s α_{exp} value, the maximum values of which are $4.5 \mu\text{mole}/\text{m}^2$ for $(\text{CH}_3)_3\text{ClSi}$ and $1.5 \mu\text{mole}/\text{m}^2$ for $(\text{C}_6\text{H}_5)_3\text{ClSi}$ (a value of $4.5 \mu\text{mole}/\text{m}^2$ means that only 50% of the silanol have reacted). Locke²² proposed measuring the number of OH groups before and after the reaction. Infrared (IR) spectroscopy has also been used²⁰. For instance, comparison of the 1850 cm^{-1} band of silica with the 1620 cm^{-1} band of water gives an estimate of the surface silanols which have reacted with chlorosilane molecules. A complete method for IR analysis is given in ref. 20.

It is difficult to establish what the most suitable surface concentration is in order to obtain a "good" phase. It seems, however, that if at least half of the silanol groups have reacted the preparation is successful. Table I lists coverage ratios reported in different publications.

3. STRUCTURE OF THE BONDED PHASE

A. Structure of the silica gel before bonding

There are different kinds of sites on the surface of a silica particle. According

TABLE 1
COVERAGE DENSITIES OF BONDED PHASES

Concentration of free silanols ($\mu\text{mol}/\text{m}^2$)	Silica	Reagent	Coverage density of bonded groups ($\mu\text{mol}/\text{m}^2$)	Reference	
8	LiChrosorb	Chlorosilane- CH_3	8.7	15	
		- $n\text{-C}_4$	4.9		
		- $n\text{-C}_{10}$	3.8; 3.5		
		- $n\text{-C}_{18}$	2.4; 2.5; 2.6; 2.9		
8	Home-made	Chlorosilane- $(\text{CH}_3)_3$	4.5**; 4.1***	19	
		- $(\text{CH}_3)_2\text{Ph}^*$	2.6; -		
		- $(\text{Ph})_3$	1.5; -		
		- $(\text{CH}_3)_2\text{-}n\text{C}_4$	3.6; 3.7		
		- $(\text{pH})_2\text{-}n\text{C}_4$	1.8; 1.7		
		- $(\text{CH}_3)_2\text{-}n\text{C}_8$	3.8; 3.4		
		- $(\text{CH}_3)_2\text{-}n\text{C}_{16}$	3.4; 3.0		
		Spherosil XOA 400	Chlorosilane- $(\text{CH}_3)_3$	2.51	23
			- $(\text{CH}_3)_2\text{Ph}$	2.5	
		PSM 50	Trichlorosilane- $n\text{-C}_{18}$	3.13	
	Chlorosilane- $(\text{CH}_3)_3$		2.49		
		- $(\text{CH}_3)_2\text{Ph}$	2.36		
	PSM 500	Trichlorosilane- $n\text{-C}_{18}$	3.10		
		Chlorosilane- $(\text{CH}_3)_3$	3.25		
		- $(\text{CH}_3)_2\text{Ph}$	3.08		
		Trichlorosilane- $n\text{-C}_{18}$	4.00		
8.3; 10	Porasil A \rightarrow F	TMCS	3.85; 6.64 (depending on drying temp.)	26	
		DMCS	3.47; 3.58 (depending on specific surface area)		
	LiChrosorb SI-100	Trichlorosilane		28	
		-Ph	3.78		
		- $(\text{CH}_2)_2\text{Ph}$	3.70		
		- $(\text{CH}_2)_4\text{Ph}$	3.78		
		- $(\text{CH}_2)_6\text{Ph}$	4.13		
		- $n\text{-C}_8$	3.74		
		- $n\text{-C}_{11}$	3.52		
		- $n\text{-C}_{13}$	3.59		
		- $n\text{-C}_{15}$	3.47		
		- $n\text{-C}_{18}$	3.44		
		- $n\text{-C}_{21}$	3.35		

* Ph = phenyl.

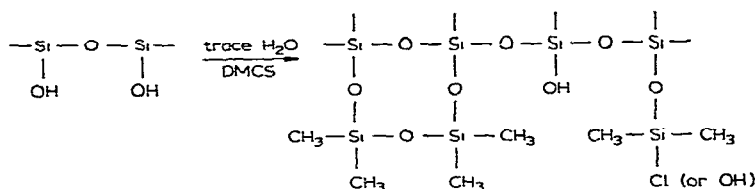
** Silica of $211 \text{ m}^2/\text{g}$.

*** Silica of $301 \text{ m}^2/\text{g}$.

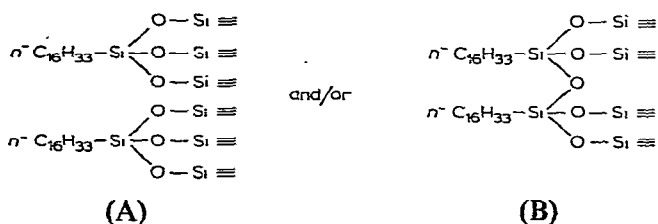
In their study on the effect of surface dehydration prior to reaction with dimethyl-dichlorosilane (DMCS) and trimethylchlorosilane (TMCS), it appeared that the percentage of bound carbon decreased continuously for DMCS when the temperature of dehydration increases from 150° to 350°, whereas in the same temperature range the effect for TMCS was less important, because DMCS can react with two silanols. They concluded that 150° is the optimal temperature (around 350° there is an important break in the graphs of percentage coverage *versus* dehydration temperature).

B. Structure of silica gel surface after bonding

We have already mentioned that monochlorosilanes can yield only monomeric adsorbents. Using DMCS, Gilpin and Burke²⁶ found that one or the two chlorine atoms can react as follows, yielding a mixed surface structure:



Statistically, the ratio of dimeric to monomeric units is 1.25. Similarly, Grushka and Kikta³⁰ consider that both monomeric and dimeric zones are present when coating silica with a stationary phase such as 1-trimethoxysilyl-2-chloromethylphenylethane. From the reaction of hexadecyltrichlorosilane with Celite 545 Abel *et al.*⁴¹, proposed the following structures:



Form A is rather improbable because of steric considerations. In general, half of the silanols do not react; it is easy to check their concentration by IR spectroscopy²⁸ and another technique is based on the isotopic exchange with tritium-labelled water. It is thus possible to determine the OH concentration. Qualitatively, the methyl red adsorption is very suitable for rapid testing. Some workers have found that these silanols do not interfere with chromatographic properties, while other workers found the contrary. Kikta and Grushka²⁹ found that for C₁ groups, with a high coverage (70%), the underlying silica is shielded from any appreciable interaction with the solutes. However, for C₉ and C₁₈ (coverage 10.3 and 19%, respectively) the presence of unreacted silanols is of prime importance. The effect is smaller for C₁₈ than for C₉ because the higher density of the C₁₈ bonded phase and the bulkiness of the bonded species partially prevent the solutes from reaching the silica skeleton and interacting

with it. Hemetsberger *et al.*²⁸ also noted that the shorter the chain length, the more important is the influence of these silanols on chromatographic properties.

For extensive coverages, it is generally accepted that the unreacted silanols do not have a critical role. Theoretically, it is possible to change them into inactive species by reaction with DMCS or TMCS^{13,16,31}. In most instances it appears that no further increase in the carbon content is observed after reaction with DMCS or TMCS. Vivilecchia *et al.*³⁶ reported that when working with μ Bondapak C₁₈, the unreacted silanols are of no importance as the methyl red test is negative and no peak tailing is observed during the elution of polar solutes with dry *n*-heptane as solvent. Conversely, Karch *et al.*¹⁵ observed that when using the same solvent and polar solutes, the best shielding of the unreacted silanols is obtained with *n*-butyl chains. However, the role of these silanol groups is not negligible. In addition, Kirkland⁴² has defined the retention on "brush surfaces" as "adsorption chromatography at the monomolecular layer of organic modifier in conjunction with the activity of any residual silanol groups".

Very interesting studies of the influence of bonding on the structural properties of silica have been made by Unger *et al.*¹⁹ and Gilpin and Burke²⁶. Unger *et al.* reported that grafting can be associated with a modification of the pore structure and consequently of the specific surface area (S_{Sp}) and pore volume (V_p). When bonding an *n*-C₁₈ group, the relative decrease in S_{Sp} and V_p are 54% and 61%, respectively. The longer is the bonded alkyl chain, the more important are the variations. The column capacity factors on an *n*-C₁₈ BP are, however, larger than on an *n*-C₈ BP under the same conditions (see below). It appears from Unger *et al.*'s study that the best means of obtaining an extensive surface area is "bulk" modification. Other data regarding the decrease in S_{Sp} were tabulated by Sorrell and Rowan³⁵, and are in good agreement with those of Unger *et al.* When coating a silica gel of S_{Sp} 795 m²/g with TMCS (1.7 groups per 100 Å²), the S_{Sp} of the modified adsorbent is 374 m²/g (53% decrease). The actual area depends on the size of the organic groups and the density of the layer. It seems that the larger the pore size of the starting material, the smaller is the change in S_{Sp} .

Gilpin and Burke²⁶ studied the coating of Porasil with TMCS and DMCS. They found that the best agreement between the calculated area available for bonding (A_B) and surface area measurements using nitrogen adsorption (A_A) is obtained by assuming a maximum surface coverage of four TMCS groups per 100 Å² (for Porasil samples with a medium specific surface area of 200 m²/g). When plotting the surface area measured by silylation reactions for different Porasils versus A_A , a deviation appears for values greater than 50–100 m²/g. This phenomenon is probably due to the decrease in pore diameter with increasing surface area. They found that the number of molecules reacted per 100 Å² is $3.52 \pm 0.06\%$ for DMCS (based on the bonding surface area of TMCS). They concluded that "the number of groups available for reaction per unit surface area may be considered to be constant for Porasil series adsorbents". There is little difference between the results of Unger *et al.*¹⁹ and Gilpin and Burke²⁶ for the maximum coverage with TMCS (4.2 versus 5.9 μ mole/m² = 3.5 groups per 100 Å²). Unger *et al.* noted that 4.2 μ mole/m² is in excellent agreement with the theoretical value calculated from the molecular cross-sectional area of a trimethylsilyl group. On bonding an *n*-C₁₈ group on LiChrosorb SI-500 (35 m²/g), Karch *et al.*¹⁵ found a concentration of 5.4 molecules per 100 Å². This corresponds to

an area requirement of 18.5 \AA^2 per group, which appears to be a "good value" for a C_{18} phase (with a better estimation of S_{sp} of $92 \text{ m}^2/\text{g}$, they obtained a value of 48.6 \AA^2 for C_{18} ; this is in agreement with their data obtained on LiChrosorb SI-100 of $300\text{--}350 \text{ m}^2/\text{g}$). For a concentration of $2.6 \mu\text{mole}/\text{m}^2$ (1.56 groups per 100 \AA^2) no accessible silanol groups were detected by methyl red adsorption: the unreacted groups have no effect on the chromatographic properties. Some interesting observations have been made by Karch *et al.*¹⁵. Using LiChrosorb SI-100 and SI-500, the total column porosities are *ca.* 0.8, which is the usual value for silica columns. However, with LiChrosorb SI-60, the measured value is 0.6 and in this instance the adsorbent seems to behave more like porous layer beads, which could be very interesting in term of efficiency. Karch *et al.* noted that if this behaviour is not associated with too marked a decrease in the linear capacity of the column, then bonded $n\text{-}C_{18}$ on LiChrosorb SI-60 is among the best phases available for RPC.

In view of this discussion, it seems to us that the most important reason for the current controversy regarding the structure and properties of BPs and especially the retention mechanism arises from the great difficulties encountered in preparing batches of silica gels and modified silica gels which are reproducible or even repeatable. Most workers discuss the properties of different materials and comparisons between them are difficult.

4. CHARACTERISTICS OF REVERSED-PHASE LIQUID CHROMATOGRAPHY

A. Separation mechanism

The separation mechanism on chemically bonded non-polar phases is a sensitive topic. There are essentially three possibilities, according to the many works dealing with this problem. The first possible mechanism is partition between two liquid phases (the mobile and the stationary phases), RPC being then a kind of LLC. Another possible process is adsorption on the non-polar adsorbent. RPC would then be similar to classical LSC with, however, one fundamental difference: whereas in classical chromatography the main factor in determining the retention of solutes is the liquid–solid interactions controlled by specific, polar forces, in the reversed-phase technique the stationary phase–solute interactions are rather weak as they depend only on the non-specific dispersion forces of the Van der Waals type, and the behaviour in the mobile phase is therefore predominant. Finally, a third type of mechanism has been suggested by Knox and Pryde³¹, who used SAS silica (a reversed-phase packing). The organic modifier in the mobile phase, consisting of a mixture of water and an organic water-miscible solvent, is preferentially adsorbed and an adsorbed stationary phase layer is formed, the composition of which is different from that of the mobile phase. Partition of the solute molecules takes place between the mobile phase and the new "mixed" stationary phase. The role of the modified silica gel is simply to provide a surface on which the stationary phase is coated.

Using polymeric phases, it is generally accepted that the mechanism is partition (*e.g.*, refs. 33 and 34). Kirkland and DeStefano³³ and Locke²² consider that the polymeric phase is an inert sponge. The thermodynamic activity of the eluent inside the gel is different from that of the bulk liquid in the interstitial volume. Partition-

ing of the solutes occurs between the two liquid phases in which the solute solubility is different. It is also possible that adsorption takes place on sites of the polymer⁴³. The selectivity of the stationary "liquid" changes considerably with the solvent used at high loadings, while surface adsorption is the major or the only mechanism at low loadings²². According to Kirkland and DeStefano, the major contribution to the retention mechanism is partition, because the capacity factor (k') increases with increasing amount of bonded material (they used an ether phase but the phenomenon must be the same with an apolar phase) while the specific surface area remains constant. We have observed the same phenomenon, however, when using silica gel modified with a layer of pyrocarbon, on which retention can occur only by adsorption⁴⁴. Rehak and Smolkova³² consider that both partition and adsorption are responsible for retention, partition being more important for a polymeric phase, although the structure of the bonded layer is more similar to that of a solid than a liquid. Schmitt *et al.*⁴⁵ explained retention on Zipax coated with high-molecular-weight octadecyl polymers by a combination of different effects: there is not only partition in the polymeric phase, which seems to behave as a partitioning liquid, but also preferential adsorption of the eluent molecules in the polymeric network which provides a stationary phase different from the pure non-bonded polymer.

It might seem natural at first to think that the chromatographic behaviours of a mechanically held liquid and the same liquid chemically bonded on the same adsorbent would be identical, and that BPC would be equivalent to LLC. It is not surprising that many workers have considered BPC as a form of LLC, for instance Twitchett and Moffat⁴⁶ using μ Bondapak C₁₈, Hatwick and Brown⁴⁷ using Partisil 10 ODS and Schmitt *et al.*⁴⁵ using ODS Permaphase. From studies with Hitachi gel, Nakae and Muto¹⁰ concluded that the retention behaviour is better accounted for in terms of partition than of adsorption chromatography. As discussed in a forthcoming publication¹⁴, their work is not very convincing because what they consider as characteristic LLC behaviour can also be observed in pure LSC. Finally, we wish to point out two major differences between a mechanically held liquid and the same compound all the molecules of which are bound to the same adsorbent: in the former instance, liquid chromatography is impossible as the stationary liquid quickly dissolves in the eluent, so that the eluent molecules penetrate the bonded layer and swell it as much as is permitted by the fixing of all of the ends of the molecules. The second fact is still more striking: all stationary liquid molecules are permanently bonded to the substrate and cannot move. Hence the entropy of this liquid is markedly lower than that of the bulk and its properties are different. They can be likened to some extent to those of a liquid crystal, although in the latter instance the molecules can move long distances from each other, which they cannot do in BPs. It can be expected, for example, that the average number of closest neighbours of a solute molecule will be lower with BPs than within the normal bulk liquid.

The difference with the adsorption mechanism is also marked. Adsorption on non-polar surfaces is controlled mainly by geometrical considerations: the larger the number of heavy atoms (C, O, N, etc.) which can come in close contact with the surface, the greater the retention. There is little, if any, lateral contact between the sorbed molecule and the adsorbent material, as the surface is most often planar locally. This is not so with BPs, at least when the bonded group is long enough, and the molecule interacting with the stationary phase can be in contact with several such

groups or with different parts of a given group in addition to possible interactions with the basic substrate.

In summary, we have a pseudo-liquid layer oriented by the underlying surface and deprived of its translational degrees of freedom but which can contact the interacting molecules in several different parts, directly or through interaction with molecules of the mobile phase. Depending on the layer density and the length of the bonded molecule, we can expect to observe very different properties and unusual behaviour.

It is not surprising that for some workers the situation appears to be unclear¹³ and that contradictory conclusions have been drawn by various workers using widely different materials. Hemetsberger *et al.*²⁸ favoured an adsorption to a partition mechanism because the average distance between the bristles is too large. However, the interactions between the solute and the stationary phase are non-selective, weak⁴⁸, *i.e.*, weaker than in adsorption, and should be similar to the intermolecular interactions in a liquid. Rehak and Smolkova³² considered the mechanism to be very complex and probably a mixture of adsorption and partition, their relative contributions depending on the nature and the chemical composition of the stationary phase, the mobile phase and the solute. However, it is not possible to consider a monomolecular layer as a liquid and the most important effect is probably adsorption. In their work on $\equiv\text{Si}-\text{C}$ phases, Locke *et al.*¹⁶ noted that these packings can be considered neither as partitioning liquids nor as classical adsorbents. In another publication, Locke²² noted that on the brush-type material (monomeric packing) the retention mechanism cannot be accounted for in terms of solution-like interactions for brush lengths shorter than several thousands of Ångströms (which is the situation for all classical BPs of the brush type). Hanai and Fujimura⁴⁹ and Leitch and DeStefano³⁴ supported an adsorption rather than a partition mechanism. Karch *et al.*⁵⁰ considered that a possible mechanism is the existence of dispersive (London) forces between the solute and the non-polar stationary phase. An interesting comparison was made by Telepchak⁵¹ between a column packed with Sil-X II RP (ODS packing) and a column made of natural diamond particles. He presented arguments in favour of the partition and the adsorption mechanism and concluded that the retention process on bonded phases should be one of reversed-phase adsorption. This was also the opinion of Kirkland⁴².

A complete and pertinent study was published recently by Horváth *et al.*⁵². They consider retention as an adsorption mechanism, solely due to the formation of reversible complexes by solvophobic interactions between solute molecules and chemically bonded moieties at the surface of silica. An expression for the capacity factor was derived which accounts for the role of all of the parameters of the chromatographic system, and the very important role of the mobile phase was pointed out. This topic has been also studied by other workers. Karger *et al.*⁵³, for instance, introduced a new topological index, the molecular connectivity, which is related to the area of the cavity created inside the solvent (see also ref. 52).

If the absolute retentions are determined by both the stationary and the mobile phase, the relative retentions, however, depend mainly on the solvent used in RPC; more will be said about that elsewhere¹⁴. Takahagi and Seno¹² and Grieser and Pietrzyk⁹, using Hitachi gel, drew opposite conclusions to Nakae and Muto¹⁰, *i.e.*, that retention is due to a kind of hydrophobic adsorption by means of Van der Waals-type forces. The hydrophobic part of the solute is preferentially adsorbed on the hydro-

phobic surface of the polymer (it is just lipophilic affinity), and the hydrophilic part of the solute is oriented towards the polar mobile phase. The adsorption process is modified when the hydrophobic-hydrophilic balance effects of the solute and/or solvent are changed. It is suggested that adsorption occurs essentially at the polystyrene surface and that the solute molecules do not penetrate deeply the porous interstices of the solid.

Pryde⁴ found a compromise by calling this technique liquid-solid-partition chromatography and noted "that it seems a little irrelevant to argue whether the mechanism is by partition or by adsorption for neither term is strictly applicable".

In the following sections we deal mainly with monomeric packings.

B. Dependence on solvent composition

In most BRPC applications, the eluent is a mixture of water and an organic water-soluble solvent, usually methanol, ethanol, acetonitrile or dioxane. Water is the weakest solvent, while methanol (the most commonly used organic modifier) is generally strong enough to elute all of the solutes from the column. In most instances, a gradient of pure water to pure methanol permits the elution of all of the components of the mixtures being analysed. The return to the initial conditions is very rapid and virtually no equilibration time is necessary before starting a new analysis.

Different relationships have been derived experimentally between k' and the eluent composition. In their work on the separation of nucleosides, Hartwick and Brown⁴⁷ obtained a linear relationship when plotting $1/V_R$ (where V_R is the absolute retention volume) versus the methanol concentration (mole/l). It is frequently observed that $\log k'$ varies linearly with the volume percentage of methanol¹³. Karch *et al.*⁵⁰ found that this linear relationship holds if the volume percentage of methanol is greater than 50 and also that the slope of the plots seems to be independent of the brush length and of the solute. However, Majors⁵⁴, using Micropack CH, noted that different solutes show different concentration dependences, possibly owing to different degrees of solvation. Karger *et al.*⁵³ studied mixtures of water with methanol, acetonitrile and acetone, and observed good linearity between $\log k'$ and the volume percentage of organic modifier for the system water-methanol. This linearity does not hold for the two other systems when the mixtures contain more than 40% of the organic solvent. On the other hand, Horváth *et al.*⁵² predicted and obtained good linearity

TABLE 2
ELUOTROPIC SERIES (ORDER OF INCREASING SOLVENT STRENGTH)

<i>Ref. 45</i>	<i>Ref. 50</i>
Ethylene glycol	Methanol
Methanol	Acetic acid
Dimethyl sulphoxide	Ethanol
Ethanol	Acetonitrile
Acetonitrile	2-Propanol
Dioxane	Dimethylformamide
Isopropanol	Acetone
	<i>n</i> -Propanol
	Dioxane

between $\log k'$ and volume percentage with the system water-acetonitrile (except near the regions of pure water and pure acetonitrile) and a less regular plot with water-methanol, the solute being *o*-toluic acid. The eluotropic powers of different organic modifiers have been classified and are listed in Table 2.

However, k' does not vary as simply as predicted by the solvent strength. Indeed, there are sometimes specific interactions in the RP systems which can give significant deviations from the values predicted by Table 2⁵⁰. In general, a 10% increase in the organic solvent concentration produces roughly a 2-3-fold decrease in k' (ref. 13). Kirkland²³ advised the use of aqueous solvent mixtures containing more than 10% of organic modifier if the coating is important (to improve the wetting of the stationary phase).

In their thermodynamic treatment of the solvophobic effect, Horváth *et al.*⁵² predicted and verified experimentally that $\log k'$ increases linearly with the salt concentration (mole/l). Know and Pryde³¹ noted that the addition of small concentrations of sodium lauryl sulphate considerably affects the capacity factor of catecholamines on ODS/TMS silica. In this instance the retention mechanism probably becomes a kind of ion-pair partition.

C. Dependence on temperature

It is well known that retentions in LC are far less dependent on temperature than in GC. It is difficult, if not impossible, to study the actual effect of temperature on the retention on silica gel; a careful adjustment of the water concentration of the eluent is needed at each temperature, otherwise the adsorbent activity changes because of the modification of the water concentration at the surface of the gel.

Using BP and particularly RBP, it is possible to use temperature as a chromatographic variable as in GC; the column capacity factors decrease with increasing temperature. As expected, plots of logarithm of k' versus the inverse of the temperature yield almost straight lines as the change in adsorption (or partition) enthalpies (ΔH) is fairly constant in the range of temperatures used in LC applications (15-80°). A 10° increase in temperature reduces the retention by a factor of 2-3^{13,45}. If the solvent can be used at a temperature of 100°, the capacity factor can be reduced roughly 7-fold. Such linear relationships have been obtained by many workers (see, for example, refs. 13, 45, 52 and 54). The slopes of the plots obtained depend on the solute, providing a selectivity which varies with the temperature¹³. These plots have been used to obtain ΔH and the corresponding entropy term, ΔS (e.g., refs. 30 and 55).

Increasing the column temperature also increases its efficiency and decreases the pressure drop necessary to achieve a given flow-rate¹³, because the solvent viscosity decreases and the diffusion coefficient increases with increasing temperature. This effect is useful as the solvents used in RPC are generally viscous; for example, water-methanol mixtures exhibit a viscosity maximum of 1.4 cP for a 70% (v/v) water content. Increasing the temperature decreases this viscosity markedly and consequently increases the diffusion coefficients (D_m). Knox⁵⁵ established that the improvement in HETP is due solely to the change in D_m as, at constant reduced velocity (v), the reduced HETP (h) is independent of temperature.

The column capacity or maximum sample size also increases with increasing temperature. For instance, Schmitt *et al.*⁴⁵ showed that the capacity of ODS Perma-

phase (a polymeric packing) increases from 1–40 $\mu\text{g/g}$ at 25° to more than 100 $\mu\text{g/g}$ at 80°. Majors¹³ reported that the capacity increases because of a better solubility in the mobile phase. This is particularly interesting for preparative applications.

Finally, another advantage of high temperatures is the improvement in peak symmetry³⁰. According to Knox and Vasvari⁵⁵, who found that peak asymmetry increases with decreasing velocity, this is due to a linearization of the partition isotherm, the very reason why the column capacity is increased.

D. Type of packing loading

In adsorption chromatography, the capacity factors are proportional to the total surface area in the column (S_{BET}) and to the concentration of the functional groups attached to the surface. The role of the bonded n -alkyl groups in RPC is of importance¹⁹. The quantity $\log(k'/S_{\text{BET}})$ is independent of the specific surface area (for a given bonded group) and yields almost straight lines when plotted against the number of carbon atoms in the n -alkyl chains. This result suggests that the total area of the bonded group is accessible for the solvophobic interactions. Moreover, the selectivity is independent of the chain length. Hemetsberger *et al.*²⁸ obtained very good linearity between the capacity factor and the percentage of carbon when using LiChrosorb SI-100 coated with n -C₈ to n -C₂₁ alkyl groups, indicating that the type of interaction is the same for n -alkyl groups of different lengths. The selectivity again appears to be independent of the number of carbon atoms in the bonded material (in fact it increases slightly up to n -C₁₃ and then remains constant). Majors and Hopper²⁰ obtained similar data although it seems that the selectivity is markedly improved up to n -C₁₂ (and then remains constant). From the data of Karch *et al.*⁵⁰, it is possible to plot $\log k'$ versus percentage of carbon, and straight lines are obtained similar to those of Hemetsberger *et al.*²⁸. The selectivity appears to be slightly affected by the chain length and to increase slowly with the brush length. However, as pertinently pointed out by Karch *et al.*⁵⁰, a weaker retention on a smaller brush can easily be overcome by changing the solvent composition (higher water content).

A very characteristic feature of RPC is the linear dependence between $\log k'$ and the number of carbon atoms for solutes belonging to homologous series, and many workers have observed this linearity (*e.g.*, refs. 10 and 56). For a constant carbon number, linear molecules are more retained than branched chain molecules^{10,15}. The slope of the plots increases with the chain length of the grafted group and with the degree of coverage of the support surface. If the solvent polarity increases (more water), the difference in selectivity as a characteristic of the BP decreases although the selectivity for a given packing increases¹⁵. More will be said about the solute in a later section.

Little has been written about the effect of the nature of the grafted group on the loadability in RPC. Karch *et al.*¹⁵, however, measured the column capacity (θ) by plotting the variations of h and k' with the sample size at constant velocity. They found that θ increases with the brush length (2-fold from C₄ to C₁₈) and appears to be larger than with bare silica (2 mg/g for n -C₁₈).

5. ADVANTAGES AND USE OF REVERSED-PHASE LIQUID CHROMATOGRAPHY

There are numerous publications on RPC, which give many good reasons for using this technique and also discuss the advantages of one type of BP over the others that are available. We shall restrict ourselves to a survey of the most important problems.

A. Stability of phases

The crosslinked silicone type have exceptional stability with regard to the solvents used in RPC²². However, the main drawback of polymeric phases is the slow mass transfer within the polymeric network with, as a consequence, poor column efficiency^{13,20,31,54}, poor wettability and long equilibration time in gradient elution. Of the monomeric phases the most stable are the $\equiv\text{Si-C}$ and the $\equiv\text{Si-O-Si-C}$ types³¹. Majors¹³ noted that the $\equiv\text{Si-C}$ are potentially more stable than the $\equiv\text{Si-O-Si-C}$ phases, but in fact the silica skeleton is destroyed by acidic or basic solvents before the skeleton-brush bondings.

The siloxane phases are more stable than the ester or amino phases¹⁹. The range of pH over which they can be used is 2–9 (ref. 13) or 1–8.5 (ref. 15). Unger *et al.*¹⁹ gave limiting values of pH 0 and 8.5 for ODS packings and noted that the stability in aqueous solutions at pH > 7 is influenced by the presence of organohydroxylsilyl groups resulting from the hydrolysis of Si-Cl groups when the original reagent is of the type SiR_2Cl_2 or SiRCl_3 . In salt solutions at pH > 7, the stability of the packing is very sensitive to the presence of silanol groups which act as weakly acidic ion-exchange groups. It is therefore very important to achieve a dense coverage by the organic groups. Infrared spectrometric studies¹⁸ showed no variation in the 2850 cm^{-1} and 2925 cm^{-1} bonds (CH stretching) up to 350° under vacuum, indicating good thermal stability. Moreover, similar results were obtained on samples of BP treated by boiling them under reflux in a large number of different solvents. Gilpin and Burke²⁶ studied the stability of particles bonded with TMCS and DMCS in water and methanol. It appears that when TMCS-coated silica is heated in a solution of methanol and toluene with hydrochloric acid under reflux, all of the trimethylsilyl groups are replaced with methoxy groups (the same phenomenon is observed with water and the groups are replaced with OH groups). With DMCS-coated supports to which both monomeric and dimeric units are bonded, it is possible to remove only the monomeric units. Consequently, one must avoid the presence of any acid in the solvents used to regenerate packings, in preparative applications for instance.

B. Reproducibility of retention data

As the polymerization reactions are often difficult to control, the most reproducible phases are made of monolayers¹⁹. With reference to the chromatographic properties, it is generally accepted that the RP packings give very reproducible data, owing chiefly to the weak influence of the adsorbent activity which cannot be changed substantially by the adsorption of impurities from the solvents or samples. There are no specific interactions in BRPC and consequently the retention cannot be influenced by "impurities" adsorbed on the packing. On the other hand, it is not necessary to

make a very close adjustment of the solvent composition and consequently gradients of solvents are more reproducible.

Moreover, it is not a serious problem to maintain constant the retention properties of a reversed-phase column; the water content of the adsorbent has no influence (the opposite of the effect with silica gel columns) and all impurities are easily washed from the column by percolating methanol or ethanol through it. Majors¹³ noted that the equilibration time in gradient elution is very short (longer for polymeric than for monomeric adsorbents). The change of solvent can be much faster than in classical adsorption^{13,15,28}. Majors⁵⁴ used step-gradient elution with the system methanol-water and found that the capacity factors of the compounds of the test mixture (2, 6 and 20 respectively) achieved their constant equilibrium values within 6 min for high methanol concentrations and 15 min for low concentrations (the mobile phase velocity was 1 cm/sec and the concentration change was 10%).

In studies of the separation of nucleosides using μ Bondapak C₁₈, Hartwick and Brown⁴⁷ found that the reproducibility of k' was better than 1.5% during 1 month of gradient elution analysis [the gradient solvents were 0.01 M KH₂PO₄ at pH 5.5 and water-methanol (1:4)] and, under the same conditions, the peak area reproducibility was better than 2%.

Kirkland⁴² pointed out that with polymeric packings the partition coefficients increase with decreasing velocity and the higher the polymer concentration, the more important this change is. For concentrations lower than 1%, the partition coefficients are constant (the packing was an ether-bonded phase). Kikta and Grushka^{29,30} reported the same observations. The effect becomes more important at high temperatures. Conversely, they found³⁰ that k' first increases with velocity and then remains stationary. The higher the temperature, the more important is the initial increase.

C. Efficiency

We have already mentioned that the efficiency is poor on polymeric packings. Horgan and Little⁵⁷, however, obtained better results than with mechanically held liquids. Polymeric species bonded on totally porous packings have given very poor results⁴⁷. Porous layer beads coated with a polymeric liquid are less efficient than porous supports that have comparable liquid loadings of mechanically held organic phases^{33,42}. According to Williams⁵⁸, columns packed with chemically bonded phases are less efficient than those made with the same support coated with the corresponding liquid, but are more efficient than those made with the corresponding polymer coated on the same support. Moreover, the efficiency sometimes varies considerably with the capacity factor (*cf.*, ref. 30 for instance). Grushka and Kikta³⁰ explained this effect in terms of the presence of some stagnant mobile phase in the polymer, concluding that the polymeric zones are important in governing the overall efficiency of the column.

The monomeric packings give much better results. Majors and Hopper²⁰ consider that the best results are obtained when 10- μ m silica particles are reacted directly with an organosilane. Some workers consider that the efficiencies obtained are lower with RPC than on the corresponding silica gel columns. The argument is based on the poor wettability of the non-polar surface adsorbent by the polar eluent²⁹. It should be noted that the same can be said of normal chromatography (polar surface, non-polar solvent). Kikta and Grushka observed better efficiencies with *n*-C₁₈ than with

n -C₉ derivatives but could not give a satisfactory explanation. Others found that the efficiency is not influenced by the length of the organic bristle¹⁵. Karch *et al.*¹⁵ mentioned that it is more difficult to pack an RP column than a column made of silica. The packing density seems to depend on the packing procedure. In a very simple way it can be said that the efficiency of columns increases with decreasing packing density. The lifetime of low-density packed columns is, however, not very long. Longer lifetimes are obtained with densely packed columns and column efficiencies always have satisfactory reproducibilities when the initial experimental conditions (on LiChrosorb SI-100/C₁₈) are restored.

Knox and Pryde³¹ observed that grafting can improve the efficiency of columns. They packed very efficient columns with several different RP packings and a reduced HETP of 2.5 was measured under optimal conditions: sometimes h was less than 8 for $\nu > 100$. They derived Knox's "C" parameters from the (h, ν) plots ($h = B/\nu + A\nu^{0.33} + C\nu$). The C values suggest that the mass transfer is better in the bonded phase than in the starting silica gel ($C = 4 \cdot 10^{-2}$ for ODS/TMS silica and $2 \cdot 10^{-2}$ – $4 \cdot 10^{-2}$ for SAS silica; $A = 0.7$ – 0.9 ; SAS silica is an RP packing).

D. Conditions of use

As mentioned previously, a siloxane packing can be used in the pH range 1–9. It can be used in all solvents (pH < 9), including buffers³¹, and elution at temperatures above 80° are also possible. All solutes can be chromatographed without any problem of irreversible "adsorption". *A priori* there are no restrictive conditions to the use of siloxane phases.

E. Analytical properties of bonded reversed-phase chromatography

It is well established that the elution order in RPC is the opposite of that in "normal" chromatography¹³; the more polar the solute, the shorter its retention time⁵⁰. It is also accepted that the solutes are eluted according to their solubilities in the mobile phase¹³. In fact, these opinions must be considered only as general trends. It is obvious that the chromatographic behaviour of solutes is closely related to the solubility in the eluent^{53,59}. For solutes with similar geometries and structures, we agree with Locke⁵⁹: the differential solute–adsorbent interactions are small and the selectivity is mostly determined by the eluent. We have shown, however, that for the substituted methylphenols, for instance, the elution order does not correspond to the solubility in the mobile phase¹⁴. Hence it is difficult to make predictions regarding the elution order; as retention depends on solute–adsorbent, solute–solvent and solvent–adsorbent interactions, it is very difficult to formulate precise rules^{7,60}.

A phenyl phase can be used successfully either in "normal" chromatography or in RPC¹³. There are, in fact, only small differences in selectivity between the non-polar adsorbents. From Kirkland's work²³, it appears that a dimethylbenzyl bonded phase has a selectivity similar to a C₁₈ phase for non-polar solutes, with some useful exceptions. With a given carbon number, the alkylaryl phases give smaller retentions than alkyl phases. The elution order is not modified but some changes in the selectivity can occur²⁸. Williams⁵⁸ and Majors¹³ observed that polar solutes are better

separated on a phenyl packing than on an ODS and better selectivity and peak shape are obtained on the phenyl phase; the reverse occurs for substituted naphthalenes. However, very good results have been obtained for solutes of high polarity that contain a large number of functional groups (which give very asymmetrical peaks in "normal" chromatography on SAS silica³¹).

In general, RPC is said to be excellent for separations of non-polar or slightly polar solutes^{13,43} and of very polar solutes^{23,31}. Solute that have weak retentions in normal LLC, LSC and BP chromatography are better separated in RPC⁵⁴. Good results are obtained in RPC when the solutes are weakly soluble in water and fairly soluble in alcohols and alkanes. The technique is complementary to conventional LLC or LSC and is very useful for the analysis of compounds that are too soluble in alkanes to be separated successfully by LLC (or LSC) using polar stationary phases⁴⁵. An interesting comparison has been made between ODS and other adsorbents (ETH Permaphase, phenyl-bonded Zipax and nitrophenyl-bonded Zipax)⁵⁴. It appears that, in general, the best results are obtained with the ODS packing. O'Hare *et al.*⁶¹ used RPC because it permits the separation of compounds that have a wide range of polarity with a high selectivity. We report below some applications of RPC that appear to us to be particularly interesting. It should be emphasized that it is not a systematic survey of the analytical possibilities of RPC and that there are many other interesting separations.

For aromatic polynuclear compounds, the selectivity is better on a C₁₈ BP than on a phenyl phase and, in general, is better with an RP system than with classical silica gel. Very good separations of such compounds have been carried out on ODS Permaphase using a water-methanol gradient at 50°C⁴⁵ or under isocratic conditions with methanol-water (87.5:12.5) on PSM 50/48 (ref. 23). Sleight⁵⁶ also studied the behaviour of aromatic hydrocarbons with and without alkyl substituents.

Majors⁵⁴ showed that good separations of alkyl bromides can be achieved with gradient elution using a column of Micropack CH. This packing is also very suitable for the resolution of hindered phenolic antioxidants and phthalate plasticizers. Pesticides can be analyzed on Vidac RP with the solvent mixtures water-methanol and water-ethanol⁴³. Good correlations are obtained between the capacity factors relative to methoxychlor and the partition coefficients determined in the system *n*-heptane/ethanol-water (9:1).

Very good separations of drugs have been achieved on μ Bondapak C₁₈ (ref. 46): 30 different compounds covering a wide range of p*K* values, solubilities in the mobile phase and molecular weights were analyzed. For acidic solutes, the capacity factors increase with increasing pH and with decreasing methanol content, except for nicotine, the *k'* value of which is minimal for 55% methanol. The elution of neutral compounds is not influenced by the pH. Good correlations are found for each series of drugs between retention volume and solubility as measured by the partition coefficient in the system water-*n*-octanol. The efficiency is very good for acidic and neutral solutes but very poor for basic solutes. Using Partisil 10 ODS and μ Bondapak C₁₈, Hartwick and Brown⁴⁷ separated nucleosides and the corresponding bases. They studied the effect of pH and ionic strength on retention and drew correlations between pH and p*K*_a. It was found that for the compounds studied, RPC is more powerful than ion exchange and gives more reproducible results (see above). The analysis of steroids on Zorbax ODS was undertaken by O'Hare *et al.*⁶¹. Three gradient systems

were used, consisting of water with either methanol, acetonitrile or dioxane for the elution of adrenal steroids, testis steroids and polar steroids.

Separations of several complex mixtures were presented by Karch *et al.*⁵⁰. The adsorbents used were LiChrosorb SI-100/C₄ and SI-100/C₁₈. The chromatograms include pesticides, fatty acids (without derivatization), quinones, androgenic hormones, corticosteroids, progesterones and heart glycosides. The analysis of catecholamines and their metabolites was performed on ODS/TMS and ODS silica; for tetracyclines the packing material was SAS silica³¹. Williams *et al.*⁶² described the quantitative separation of fat-soluble vitamins and their esters. The phases used were Permaphase ODS and Zipax HCP (hydrocarbon polymer, mechanically held) and the reproducibility of the retention times was better than 1% and that of the relative responses was better than 2% under isocratic conditions. The corresponding figures for gradient elution at 50° were essentially identical. Recently, a separation of naturally occurring phenolic compounds has been reported⁶³. Reversed HPLC on Bondapak C₁₈ provides much shorter analysis times compared with other methods. Finally, a preparative application was described by Carnes *et al.*⁶⁴, relating to the purification and fragmentation processes of a cytokinin complex from tomato-root pressure exudate on Bondapak C₁₈/Porasil B.

6. CONCLUSION

Reversed-phase chromatography can now be performed under optimal conditions as a result of the introduction of 10- μ m diameter particles coated with chemically bonded moieties. With such chemically bonded phases, all of the major difficulties associated with liquid-liquid chromatography are avoided. The small particles permit the production of very efficient columns, *e.g.*, with 10,000 theoretical plates for a 2-ft. long column. They can be chemically coated easily with both polar and non-polar groups.

The advantages of the reversed-phase techniques are numerous, the most outstanding being the extremely simple operating conditions. Indeed, there is no need for an equilibration time with the solvent (in particular the water content has no effect), gradients are particularly easy to perform and to reproduce and the columns give very reproducible results over long periods with no need for special care or storage. Increasing numbers of separations are now being performed using the reversed-phase mode and it seems that within a few years this mode of chromatography could become the most popular of the liquid chromatographic techniques.

7. SUMMARY

Reversed-phase liquid chromatography (RPC) is being increasingly used. The different types of bonded phases (BP) for RPC applications and their preparation are reviewed. The extent of the bonding reaction, which is an important parameter affecting the quality of the BP, is discussed. The surface coverage is closely related to the structure of the starting silica. Activation conditions are reviewed and the structure of the final adsorbent is discussed, particularly the role of unshielded silanols. The main characteristics of bonded reversed-phase chromatography (BRPC) are described and some of the advantages of this technique and a few typical applications are reported.

EDITOR'S NOTE

Some readers will find the discussion of the mechanism of reversed-phase chromatography somewhat confusing, the reason being that most HPLC workers found their way to this technique via gas chromatography and usually do not have a detailed knowledge of the extensive literature on reversed-phase paper, thin-layer and column chromatography that is already available.

Bonded phases are already well known. It suffices to mention acetylated cellulose papers and thin layers, grafted papers, Sephadex LH-20, salting-out and salting-in chromatography on ion-exchange resins and resin papers and some of the work on polyamide chromatography. Results that could solve most of the problems discussed here concerning the mechanism of retention are already available for such supports. A study of this literature would be rewarding for chromatographers using HPLC.

AUTHORS' NOTE

We fully agree with the Editor that there is a large amount of useful TLC data which are ignored by chromatographers working with HPLC and which they should read. We are of the opinion, however, that the structure of chemically bonded phases is different from that of porous polymeric material and that the retention mechanism is consequently slightly but significantly different, although the basic interactions are of course the same.

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