Mechanisms: Normal Phase

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Introduction

In normal phase chromatography the dominant interactions between the solute and the stationary phase that cause retention and selectivity are polar in nature. If dispersive interactions dominate, then the separation system is called ‘reversed phase’ chromatography. To comprehend the retention mechanisms involved in normal phase chromatography, it is necessary to understand the nature of the different interactive forces that are involved between the solutes and the two phases and how they occur. The retention of a solute is directly proportional to the magnitude of its distribution coefficient (K) between the mobile phase and the stationary phase which, in turn, will depend on the relative affinity of the solute for the two phases, i.e. the interactive forces between the solute molecules and the two phases. Consequently, the stronger the forces between the solute molecule and the molecules of the stationary phase, the larger the distribution coefficient and the more the solute is retained. There are only three basic types of molecular force, all of which are electrical in nature. These three forces are called ‘dispersion forces’, ‘polar forces’ and ‘ionic forces’. Despite there being many different terms used to describe molecular interactions (e.g. hydrophobic forces, π–π interactions, hydrogen bonding, etc.) all interactions between molecules are the result of composites of these three basic molecular forces. Dispersion forces arise from charge fluctuations throughout a molecule resulting from random electron/nuclei vibrations. They are typical of those that occur between hydrocarbons and other substances that have either no permanent dipoles or can have no dipoles induced in them. In biotechnology and biochemistry, dispersive interactions are often referred to as ‘hydrophobic’ or ‘lyophobic’ interactions, apparently because dispersive substance such as the aliphatic hydrocarbons do not dissolve readily in water. Polar interactions arise from electrical forces between localized charges such as permanent or induced dipoles. Polar forces are always accompanied by dispersive interactions and may also be combined with ionic interactions. Polar interactions can be very strong and produce molecular associations that approach, in energy, to that of a weak chemical bond (e.g. ‘hydrogen bonding’). Ionic interactions arise from permanent negative or positive charges on the molecule and thus usually occur between ions. Ionic interactions are exploited in ion exchange chromatography where the counter-ions to the ions being separated are suited in the stationary phase. To achieve the necessary retention and selectivity between the solutes for complete resolution, it is necessary to select a phase system that will provide the optimum balance between dispersive, polar and ionic interactions between the solute molecules and the two phases.

To achieve retention, the forces between the solute molecules and the stationary phase must dominate. If the molecules are largely dispersive in character then dispersive interactions must dominate in the stationary phase and, by suitable choice of mobile phase, polar interactions are made to dominate in the mobile phase. Conversely, if the substances to be separated are largely polar or polarizable then polar interactions must dominate in the stationary phase and, by suitable choice of mobile phase, dispersive interactions must be made to dominate in the mobile phase. When polar interactions dominate in the stationary phase, historically, the separation system has been termed ‘normal’ chromatography. When dispersive interactions dominate in the stationary phase, historically, the separation system is said to be ‘reversed phase’ chromatography. Thus in normal chromatography polar interactions dominate in the stationary phase and dispersive interactions are made to dominate in the mobile phase.

The polar forces that retain solutes in normal chromatography vary in strength and somewhat in mechanism. The strongest polar forces arise from dipole–dipole interactions, where charge centres on the solute molecule interact with the opposite charge centres on the stationary phase. Compounds, such as those containing the aromatic nucleus and thus (π) electrons, are said to be ‘polarizable’. When such molecules are in close proximity to a molecule with a permanent dipole, the electric field from the dipole induces a counter dipole in the polarizable molecule. This induced dipole acts in the same manner as a permanent dipole and thus polar interactions occur between the molecules. Induced dipole interactions are, as with polar interactions, always accompanied by dispersive interactions. Aromatic hydrocarbons can be retained and separated purely by dispersive interactions when using a dispersive stationary phase (reversed phase chromatography) or they can be retained and separated by combined induced-polar and dispersive interactions by using a polar stationary phase such as silica gel (normal phase chromatography). The strongly polar hydroxyl groups inducing dipoles in the easily polarizable aromatic nucleus. A single molecule can possess different types of polarity; phenylethanol, for example, will possess both a permanent dipole as a result of the hydroxyl group and also be polarizable due to the aromatic ring. More complex molecules can have many different interactive groups.

The most common, and in fact traditional, normal phase system used in liquid chromatography consists of silica gel as the stationary phase and a mobile phase that is predominantly an alkane or a mixture containing a high proportion of an alkane. This system will be used to examine the type of interactive mechanisms that can take place in normal phase chromatography. The second solvent(s) can be more dispersive such as methylene chloride or a more polar such as ethyl acetate, *n*-propanol or even ethanol. To reduce retention, either the interactive character of the stationary phase must be reduced or the interactive character of the mobile phase increased or both. Both effects can be achieved by modifying the mobile phase. However, in normal phase chromatography, when employing mixed mobile phases, the interactions on the silica surface can become quite complex and the mechanism of retention needs some discussion. The mechanisms involved in mobile phase interactions with the solute are quite different to those involved with the stationary phase and thus, they will be considered separately.

The Nature of the Surface of Silica Gel

When the silica surface is in contact with a solvent, the surface is covered with a layer of the solvent molecules. If the mobile phase consists of a mixture of solvents, the solvents compete for the surface which is partly covered by one solvent and partly by the other. Thus, any solute interacting with the stationary phase may well be presented with two, quite different types of surface with which to interact. The probability that a solute molecule will interact with one particular type of surface will be statistically controlled by the proportion of the total surface area that is covered by that particular solvent.

Mono-Layer Adsorption

A solvent can be adsorbed from a solvent mixture on the surface of silica gel according to the Langmuir equation for monolayer adsorption. Examples of mono-layer adsorption isotherms obtained for benzene, chloroform and butyl chloride are shown in **Figure 1**. It is seen that the surface becomes completely covered, and its interactive characteristics severely modified at relatively low concentrations of the second solvent in the mobile phase ($> 10\%$ v/v). If the second solvent is more polar, a quite different type of adsorption isotherm applies. In this case, bi-layer adsorption can take place as shown by the isotherms in **Figure 2**. Bi-layer adsorption is not uncommon and the bi-layer adsorption isotherm equation can be derived by a simple extension of the procedure used to derive the Langmuir adsorption isotherm. It should be noted that, due to the strong polarity of the hydroxyl groups on the silica, the initial adsorption of the ethyl acetate on the silica surface is extremely rapid.

The individual isotherms for the two adsorbed layers of ethyl acetate are included in Figure 2. The

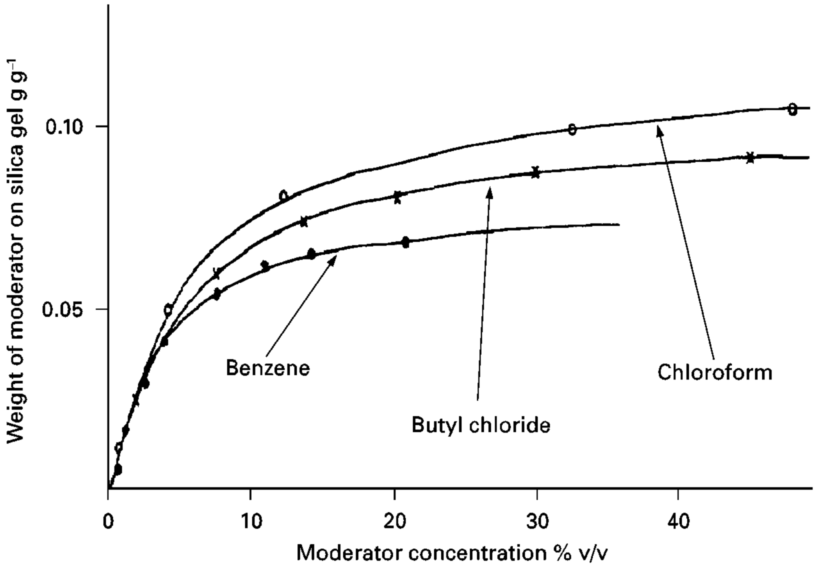

Figure 1 Langmuir adsorption isotherms for benzene, butyl chloride and chloroform.

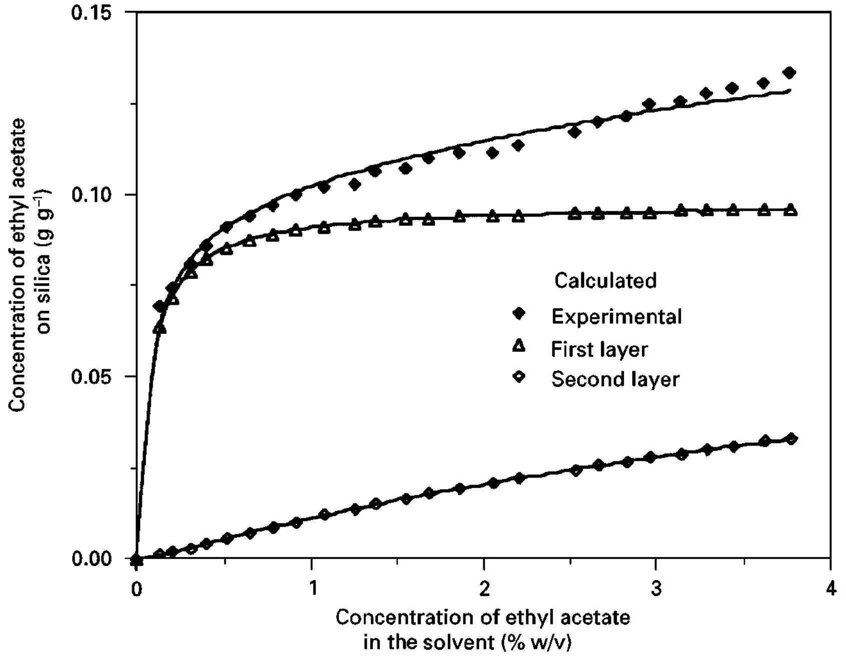

Figure 2 The individual and combined adsorption isotherms for ethyl acetate on silica gel.

two curves, although similar in form, are quite different in magnitude. The first layer which is very strongly held is complete when the concentration of ethyl acetate is only about 1% w/w. At concentrations in excess of 1% w/w the second layer has only just begun to be formed. The formation of the second layer is much slower and the interactions between the solvent molecules with those already adsorbed on the surface are much weaker. Assuming that the total area covered by the first layer will be the same as the area covered by the second layer, then only about one third of the layer is complete at a concentration of about 4% w/v. This is in striking contrast to the formation of the first layer which is virtually complete at an ethyl acetate concentration of 1% w/v.

From the point of view of solute interaction with the surface, it is seen that it can now be very complex indeed. In contrast to the less polar or dispersive solvents, the character of the interactive surface will be modified dramatically as the concentration of the polar solvent ranges from 0 to 1% w/v. However, above 1% w/v, the surface will be modified more subtly, allowing a more controlled adjustment of the interactive nature of the surface.

Multi-layer adsorption is also feasible, for example the second layer of ethyl acetate might have an absorbed layer of the dispersive solvent *n*-heptane on it. However, any subsequent solvent layers that may be generated will be situated further, and further, from the silica surface and are likely to be very weakly held and sparse in nature. Under such circumstances their presence, if, in fact, real, may have little impact on solute retention.

Interactive Mechanisms on the Surface of Silica Gel in Normal-Phase Liquid Chromatography

It is clear that the interaction of the solute molecules with the stationary phase surface can be quite complex and also change with the composition of the mobile phase. There are two ways a solute can interact with a stationary phase surface. The solute molecule can interact with the adsorbed solvent layer and rest on the top of it. This is called 'sorption interaction' and occurs when the molecular forces between the solute and the stationary phase are relatively weak compared with the forces between the solvent molecules and the stationary phase. The second type is where the solute molecules displace the solvent molecules from the surface and interact directly with the stationary phase itself. This is called 'displacement interaction' and occurs when the interactive forces between the solute molecules and the stationary phase surface are much stronger than those between the solvent molecules and the stationary phase surface. An example of sorption interaction is shown in **Figure** 3(A). Its linear relationship is clearly demonstrated and it is seen that the distribution coefficient (which controls retention) can be adjusted to any selected value by choosing the appropriate mixture of the two solvents.

Reiterating the equation proposed by Purnell, for two solvents (A) and (B) in GC (see **Figure 4**):

$$V'_{AB} = \alpha(V'_A - V'_B) + V'_B \qquad [1]$$

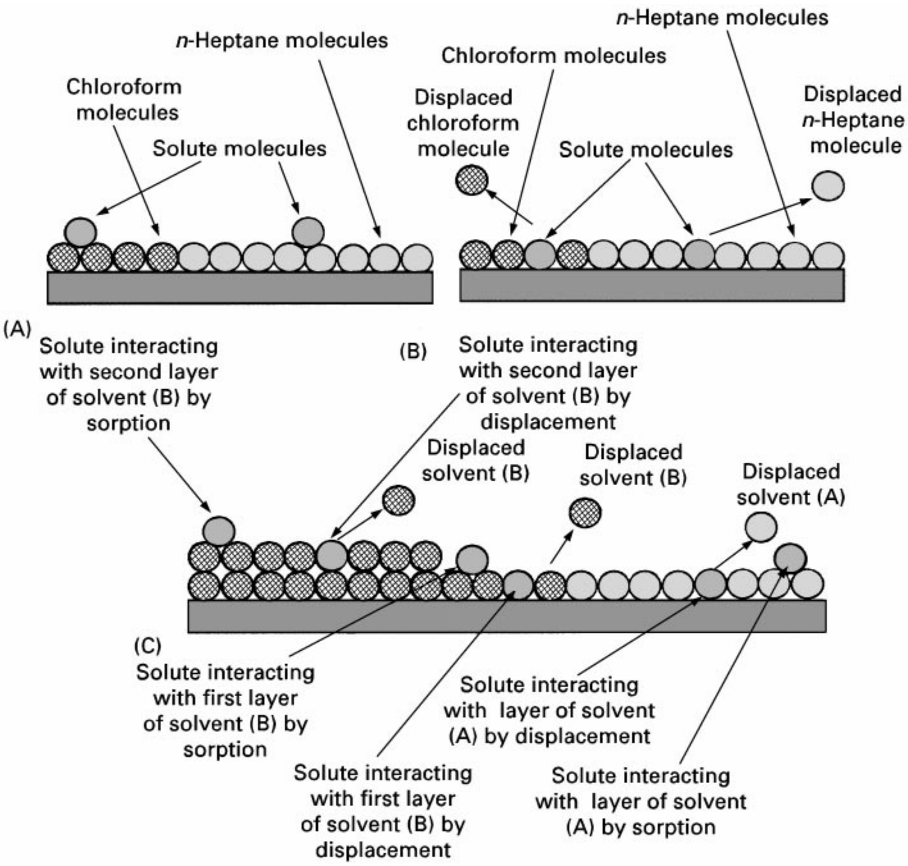

Figure 3 Different forms of molecular interaction with the silica gel surface.

The results of Katz *et al.* can be algebraically expressed in a similar form for LC:

$$K_{AB} = \alpha(K_A - K_B) + K_B \qquad [2]$$

For chromatography purposes the product of the distribution coefficient and the volume of stationary phase, or stationary phase surface area, gives the corrected retention volume, i.e.:

$$V'_{AB} = K_{AB}\phi, \quad V'_A = K_A\phi \quad \text{and} \quad V'_B = K_B\phi$$

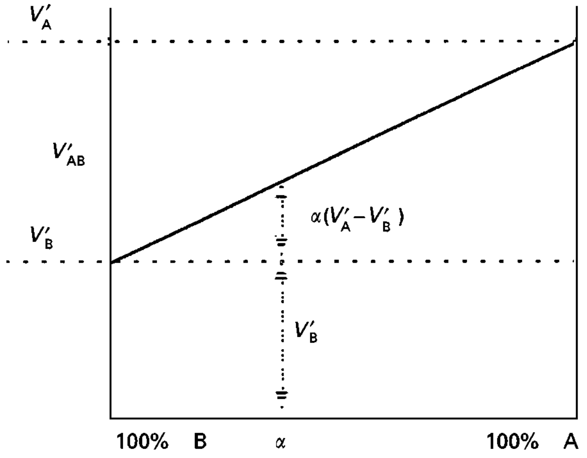

Figure 4 Graph of corrected retention volume against volume fraction of stationary phase.

In the experiments of Katz *et al.* (see **Figure 5**), that validated the relationship given in eqn [4], the distribution coefficients (K) were referred to the solvent phase (mobile phase) whereas in LC it is the mobile phase composition that is changed and the distribution coefficients (K″) are referred to the stationary phase. Thus:

$$K''_{AB} = \frac{1}{K_{AB}}, \quad K''_A = \frac{1}{K_A} \quad \text{and} \quad K''_B = \frac{1}{K_B}$$

or:

$$V''_{AB} = \frac{1}{V'_{AB}}, \quad V''_A = \frac{1}{V'_A} \quad \text{and} \quad V''_B = \frac{1}{V'_B} \qquad [3]$$

Substituting for the corrected retention volumes in eqn [3] for inverse phase system:

$$V''_{AB} = \frac{1}{\alpha\left(\frac{1}{V''_A} - \frac{1}{V''_B}\right) + \frac{1}{V''_B}} \qquad [4]$$

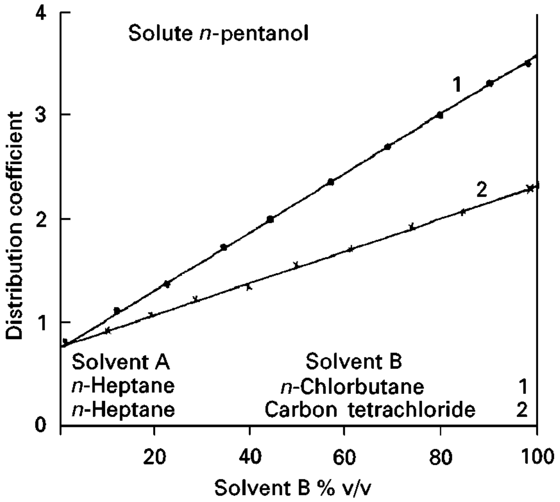

Figure 5 Graphs relating distribution coefficient to solute retention for *n*-pentanol.

Simplifying:

$$V''_{AB} = \frac{V''_A V''_B}{\alpha(V''_B - V''_A) + V''_A} \qquad [5]$$

Thus, when $\alpha = 0$, $V''_{AB} = V''_B$ and when $\alpha = 1$, $V''_{AB} = V''_A$.

In practice a more convenient way of expressing solute retention in terms of solvent concentration for a binary solvent mixture as the mobile phase is to use the inverse of eqn [5], i.e.:

$$\frac{1}{V''_{AB}} = \alpha\left(\frac{1}{V''_A} - \frac{1}{V''_B}\right) + \frac{1}{V''_B} \qquad [6]$$

If the corrected retention volume in the pure strongly eluting solute is very small compared with the retention volume of the solute in the other pure solvent, i.e. $V''_A \ll V''_B$ which is very often the case in practical LC then eqn [6] simplifies to the simple reciprocal relationship:

$$V''_{AB} = \frac{V''_A}{\alpha} \qquad [7]$$

Under these conditions the reciprocal relationship fits experimental data extremely well, particularly at volume fractions below 0.5 of the strongly eluting solute.

In addition, under these conditions the inverse will also apply, i.e.

$$\frac{1}{V''_{AB}} = \frac{\alpha}{V''_A} \qquad [8]$$

Equation [8] has been validated by the results from a number of workers in the field and an example of the expected correlation is given by the curves in Figure 6 for the two enantiomers, (S) and (R) 4-benzyl-2-oxazolidinone. The column chosen was 25 cm long 4.6 mm i.d. packed with 5 μm silica particles bonded with the stationary phase Vancomycin (Chirobiotic V).

This stationary phase is a macrocyclic glycopeptide Vancomycin that has a molecular weight of 1449.22, and an elemental composition of 54.69% carbon, 5.22% hydrogen, 4.89% chlorine, 8.70% nitrogen and 26.50% oxygen. Vancomycin is a strongly polar stationary phase which contains 18 chiral centres surrounding three 'pockets' or 'cavities' which are bridged by five aromatic rings and thus can readily offer unique enantiomeric selectivity to a wide range of chiral substances. The column was operated in the normal phase mode using mixtures of n-hexane and ethanol as the mobile phase. Equation [7] is unambiguously validated by the curves relating the corrected retention volume to the reciprocal of the volume fraction of ethanol shown in Figure 6. It is also seen that an excellent linear relationship is obtained with an index of determination very close to unity.

Although silica gel was taken as an extreme example of a normal phase system, any stationary phase that is polar in nature (bonded silica or polymer based), when used with a predominantly dispersive solvent as the mobile phase, will constitute normal phase chromatography. Expressed in a slightly different way, although the interactive mechanism can be extremely complex and difficult to quantitatively predict, if the prevailing forces in the stationary phase are polar and the eluting forces in the mobile phase

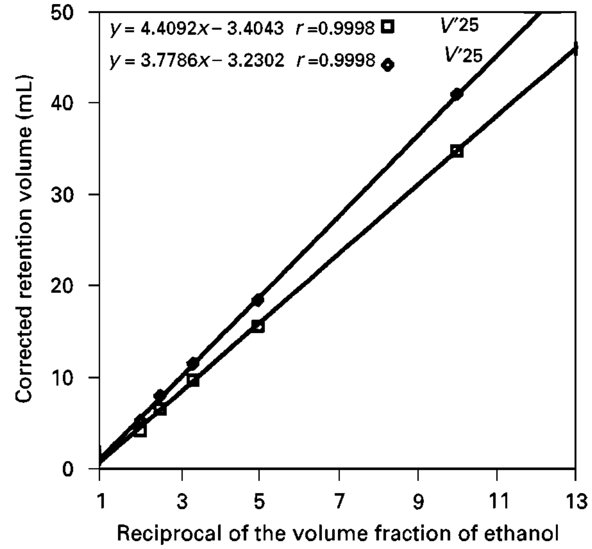

Figure 6 Graph of corrected retention volume of the enantiomers of 4-benzyl-2-oxazolidinone against the reciprocal of the volume fraction of ethanol.

are predominantly dispersive then it is a normal phase system. In practice, normal phase systems are used to separate mixtures of polar substances; they are largely ineffective in separating substances that are largely or exclusively dispersive. For reversed phase chromatography, the converse applies.

See also: **II/Chromatography: Liquid:** Column Technology; Theory of Liquid Chromatography.

Further Reading

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Mechanisms: Reversed Phases

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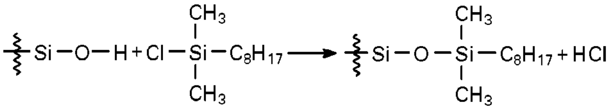
Introduction

In the early days of liquid chromatography (LC), the most commonly used stationary phase was silica gel, which was usually loaded with water and employed with a hydrocarbon mixture such as petroleum ether as the mobile phase. To change the selectivity of the distribution system, the hydrocarbon was sometimes adsorbed on the silica, and water or an aqueous alcohol mixture was used as the mobile phase. For obvious reasons, the latter was termed a reversed-phase system. Since that time, the reversed phase has been defined in a number of different ways: in this article, it is regarded as consisting of hydrocarbon moieties chemically bonded to a silica matrix. Reversed phases are considered to exhibit predominantly dispersive interactions with any solute or solvent with which they are in contact.

The first reversed phase was synthesized by Hálasz and Sebastian in 1969 by refluxing silica with a suitable alcohol (e.g. *n*-octanol) to form the silyl ester, the hydrocarbon chain being linked to the silica by carbon–oxygen–silicon bonds. This bonding proved to be labile, as the hydrocarbon moiety was easily removed by hydrolysis, but was sufficiently stable to allow the potential of such phases to be established. The next year Kirkland and DeStefano used chlorosilane reagents to attach the hydrocarbon chain to the silica by a silicon–oxygen–silicon bond which proved to be far more stable, at least between pH 4 and pH 8.



$$\text{Si}-\text{O}-\text{H}+\text{Cl}-\overset{CH_3}{\underset{CH_3}{\text{Si}}}-C_8H_{17} \longrightarrow \text{Si}-\text{O}-\overset{CH_3}{\underset{CH_3}{\text{Si}}}-C_8H_{17}+\text{HCl}$$

Subsequently, other silyl reagents, such as the silyl esters, were also shown to react with silica. Some of these reagents are now commonly used in the production of reversed phases.

Brush Phases

There are three basic types of bonded phases which are produced by the use of the mono-, di- and trisubstituted silanes: brush phases, oligomeric phases and bulk phases. For example, the monochlorsilanes (e.g. octyldimethylchlorsilane) react with the hydroxyl groups on the silica surface to produce dimethyloctylsilyl chains attached to the silica. The alkyl chains are thought to stand out from the surface like the bristles of a brush, hence the term brush phase. After reaction, the material is usually treated with trimethylchlorsilane or hexamethyldisilazane to eliminate any remaining unreacted hydroxyl groups. This procedure is called capping the bonded phase.

The extent to which the silyl groups are reacted is still a subject of some debate. It is thought that the two methyl groups next to the silicon atom of the silyl reagent hinder reaction with adjacent hydroxyl groups on the silica gel surface. Consequently, a considerable amount of unreacted hydroxyl groups will