Mechanisms of retention in HPLC

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https://sites.google.com/site/fuschrom/
Research field of course teachers

The activity of the course teachers in HPLC started in 1992 in the field of micellar liquid chromatography by the hand of Alain Berthod (Université de Lyon, France), focused on the analysis of drugs in physiological fluids.

Eventually, they moved their interest to more fundamental studies and Chemometrics, aimed to extract the potential information contained in chromatographic signals and improve the separations, assisted by numerical methods. Particularly, they have worked in the development of new optimisation strategies, reliable peak models, purity assays, deconvolution methods, suppression of peak tailing for basic drugs, and quantitative-structure retention relationships.

Currently, they are involved in column characterisation, development of clean analytical methods, secondary equilibria in liquid chromatography, improvement of peak profile, fast chromatography and bidimensional separations.
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coffee break (30 min)

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Mechanisms of retention in HPLC

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https://sites.google.com/site/fuschrom/
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1.1. Elution strength and selectivity

In liquid-liquid chromatography (or simply, liquid chromatography), the separation is performed on a solid support inside a column, usually porous silica ($\text{SiO}_2$), covered by a thin layer of a liquid immiscible with another liquid, which percolates through the column, called the mobile phase. This thin layer acts as stationary phase.
The stationary phase liquid layer could simply wet the support. However, to avoid leakage, it is strongly bonded to the support.
The separation results from the **solubility equilibrium** between the two liquid phases: the liquid covering the solid support and the liquid that percolates through the support. However, other interactions in the stationary phase and mobile phase are also possible.

The **ability** of the mobile phase to sweep away the solutes retained on the stationary phase is called **elution strength**.

It depends on:

- stationary phase nature
- mobile phase composition
  - nature and concentration of solvents and additives
  - pH
- temperature
- solute molecular structure
Selectivity

Each solute experiences a particular elution strength !!!

1. Retention in reversed-phase, normal-phase and HILIC

- 15% ACN
- 0.1125 M SDS/17.5% ACN
- 0.1125 M SDS/25% ACN
- C18 column
- 10^{-3} M SDS/30% ACN
- 0.1125 M SDS/45% ACN
Elution strength

The elution strength should be carefully controlled !!!

- If the elution strength is too high:
  - retention times will be too short
  - resolution will be poor

- If the elution strength is too low:
  - retention times will be excessive
  - analysis time will be too long
  - signal-to-noise ratio at the peak maxima will be low

Ideally, the elution strength should be fixed to get retention factors in the ranges:

\[ 1 < k < 5 \quad \text{or at least} \quad 0.2 < k < 20 \]
In addition to water, many organic solvents can be used to prepare the mobile phase. Mixtures of solvents in different ratios, in the absence or presence of different reagents, are mainly used.

Solvent selection should be made according to the two chromatographic properties:

- elution strength (absolute retention)
- selectivity (relative retention among solutes)

This can make solvent selection for a given purpose a difficult task !!!
1.1. Elution strength and selectivity

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1.2. Columns and solvents in RPLC, NPLC and HILIC

Solutes are associated to the stationary phase and mobile phase based on their mutual affinities:

**like dissolves like**

**RPLC** (reversed-phase liquid chromatography)

**Stationary phase**: non-polar

The lower the solute polarity, the higher its retention: **Non-polar solutes are highly retained.**

- **Octadecyl-silica** (C18, ODS): most common choice
- **Octyl-** (C8) or **butyl-silica** (C4): less hydrophobic
decrease the high retention of some non-polar solutes
- **Triacontyl-silica** (C30): highly hydrophobic
increase the retention of scarcely retained solutes
- **Phenylpropyl-silica** or **cyanopropyl-silica**: offer different selectivity
A wide range of water-miscible organic solvents may be used as modifiers in RPLC, but only three are usual: acetonitrile (ACN), methanol (MeOH) and tetrahydrofuran (THF).

- Mobile phase: polar water to which a miscible organic solvent (the modifier) is added to decrease the polarity and increase the elution strength.

- As the mixture progressively resembles the non-polar stationary phase, ... competes better with the stationary phase for non-polar solutes.

- Solutes elute according to decreasing polarities: most hydrophilic solutes prefer the polar mobile phase to elute the first; most hydrophobic solutes prefer the stationary phase to elute the last.
NPLC (normal-phase liquid chromatography)

**Stationary phase**: polar

- Cyanopropyl-silica: also used in RPLC
- Underivatised silica
- Diolpropyl-silica
- Aminopropyl-silica

The higher the solute polarity, the higher its retention: **Highly polar solutes are highly retained.**
1. Retention in reversed-phase, normal-phase and HILIC

HPLC’2013 (Amsterdam)

NPLC

**Mobile phase**: non-polar

- hydrocarbon mixed with a miscible polar solvent (the modifier)
  - to increase the polarity, and therefore, the elution strength

- As the mixture progressively resembles the polar stationary phase ...
  - ... competes better with the stationary phase for polar solutes

- **Solutes** elute according to increasing polarities:
  - elution order is reversed with regard to RPLC !!!

  - **most hydrophobic** solutes  ➔ elute the first
    - prefer the non-polar mobile phase
  
  - **most hydrophilic** solutes  ➔ elute the last
    - interact stronger with the stationary phase

**Hydrocarbons**: isoheptane, \( n \)-heptane and cyclohexane

**Modifiers**: chloroform, ethyl acetate, dichloromethane and isopropanol
Some polar solutes may be too weakly retained (if at all) in RPLC, but too strongly retained in NPLC !!!

**HILIC** (hydrophilic interaction liquid chromatography) : for highly polar solutes

**Stationary phase**: polar

- underivatised silica
- derivatised silica
  - amino, amide, cyano, diol, zwitterion, cyclodextrin, other polar functional groups
- ... but the real stationary phase is the rich-water layer adsorbed on the bonded chains.
HILIC

**Mobile phase**: non-polar

an organic solvent mixed with water (the modifier)

**Water miscible solvents:**
- acetonitrile (the most common), acetone, isopropanol, ethanol,
- 1,4-dioxane, dimethylformamide and methanol

<table>
<thead>
<tr>
<th>HILIC</th>
<th>RPLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACN &gt;&gt; H₂O</td>
<td>H₂O &gt;&gt; ACN</td>
</tr>
</tbody>
</table>

![Chemical structures and LogP values](image)
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1.3. Rationalisation of the elution strength

The strength of solute-solvent interactions is assumed to be represented by the interactions among solvent molecules. Hence, the elution strength in liquid chromatography can be explained by using a global measurement of the interactions that hold the solvent molecules altogether.

1.3.1. Global polarity measurements

- Hildebrand solubility parameter \((\delta)\)
- relative retention of solvents by adsorption on silica \((\varepsilon^0)\)
- adsorption on other solid surfaces such as alumina

quantitative polarity scales for solvents

There are discrepancies among the different scales due to the limitations inherent to the use of a single global polarity measurement and the experimental errors.
Hildebrand solubility parameter

\[ \delta = \left( -\frac{E}{v} \right)^{1/2} \]  
(1.1)

\(E\) : cohesive energy (exothermic, minus sign)

\(v\) : molar solvent volume

a common measurement
1. Retention in reversed-phase, normal-phase and HILIC

### NPLC solvents miscible with heptane

- Ethyl acetate < chloroform <
- *dichloromethane* < isopropanol

### RPLC solvents miscible with water

- MeOH < ACN < isopropanol << THF
Miscibility

- Mobile phases are usually composed by mixtures of solvents.

- Not all solvent mixtures are possible.

- As a rule, solvents are completely miscible if they are in the same third or half of the Hildebrand polarity scale (there are exceptions)
Particular case: dichloromethane/1,4-dioxane
- similar global polarity parameter
- but non-miscible

Dichloromethane: miscible with alkanes
- water is incapable of accepting protons from dichloromethane

1,4-Dioxane: miscible with water
- readily accepts protons from water

Big limitation of global polarity parameters: the contribution of each molecular interaction is not individually considered.
Polarity of solvent mixtures

A linear relationship

\[ \delta_M = \sum_j \delta_j \varphi_j \]  

(1.2)

\( \delta_j \): Hildebrand solubility parameter (other polarity scale can be used)

\( \varphi_j \): volumetric fraction of the \( j \) solvent in the mixture

For MeOH-water mixtures in RPLC (\( \delta_{\text{MeOH}} = 14.5, \delta_{\text{H}_2\text{O}} = 23.5 \))

\[ \delta_M = 14.5 \varphi_{\text{MeOH}} + 23.5 (1 - \varphi_{\text{MeOH}}) \]  

(1.3)

**RPLC**: Linearity of the global polarity (elution strength) is only approximately valid below 30% (v/v) organic solvent

**NPLC**: The effect of minute amounts of a polar solvent in heptane can be much larger than the effect of adding larger amounts

**HILIC**: a U shape relationship is obtained

In spite of these limitations, Eq. (1.2) is useful to estimate the polarity of solvent mixtures in RPLC.
1.3.3. Schoenmakers’ rule: estimation of solute polarity ranges

Two conditions should be fulfilled to obtain retention factors within an appropriate range:

- Solute polarity not far from the mean polarity of mobile phase and stationary phase:
  \[
  \delta_i \approx \frac{\delta_M + \delta_S}{2} \quad (1.4)
  \]
  Otherwise, solutes will show an excessive preference for one of the phases.

- For a mixture of solutes in a wide polarity range, the polarities of both phases should differ significantly.

  If \( \delta_M \approx \delta_S \) : \( \delta_i \) for most solutes will not be in between.

Schoenmakers’ rule: The retention factors will be within the optimal target region when

\[
(\delta_M + \delta_S - 2\delta_i)(\delta_M - \delta_S) \approx 0 \quad (1.5)
\]

this should be \( \approx 0 \) as large as possible.
A graphical representation of the Schoenmakers’ rule

- Rather polar solutes ($\delta_i \approx 15.5$) are properly eluted with water ($\delta_M = 23.5$) on a C18 stationary phase ($\delta_S \approx 6.5$-$7.0$).
- With 100% ACN, rather low polar solutes with $\delta_i \approx 10$ are properly eluted.

Within the limits of predictions based on the Hildebrand solubility parameter, solutes in the range $10 > \delta_i > 15.5$ are properly eluted using 0 to 100% ACN. Less polar solutes, going down to $\delta_i \approx 8.5$, will be eluted with THF. With MeOH, the range is smaller.
1. Retention in reversed-phase, normal-phase and HILIC

**Schoenmakers’ rule**

Silica column / heptane-isopropanol mixtures:

\[11.5 < \delta_i < 13.5\]

Solute polarity range narrower with regard to RPLC

Water layer (\(\delta_S \approx 23.5\)) / water-ACN mixtures:

\[18 < \delta_i < 21\]

highly polar solutes / water content 5 – 50%
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1.4. Snyder’s solvent selectivity triangle (1974):
A simple approach for solvent characterisation

Six types of interactions contribute to the Hildebrand solubility parameter:

- between permanent dipoles
- between induced dipoles
- between permanent and induced dipoles
- hydrogen ion donation (acidity)
- hydrogen ion acceptance (basicity)
- electrostatic interactions

Selectivity

Owing to the different contributions, solutes with exactly the same global polarity, but structural differences will yield similar but not necessarily identical retention.
Snyder’s solvent selectivity triangle

Solvents were characterised according to their capacity to interact with three probes: ethanol, 1,4-dioxane and nitromethane.

Properties:

- electrostatic interactions were neglected
- permanent and induced dipole interactions were gathered in a single property: dipolarity: polarity + polarizability
- only three solvent properties: acidity, basicity and dipolarity:
- a triangle was drawn where each vertex represents each property
Probes (Rohrschneider): do not represent the pure properties!!!

- **ethanol (e)**: hydrogen ion donor (acidic, weak acceptor / moderately dipolar)
- **1,4-dioxane (d)**: hydrogen ion acceptor (basic, weakly dipolar)
- **nitromethane (n)**: dipolar (weakly acidic and weakly basic)

A global measurement, called the **Snyder's global polarity**, was defined as the sum of the contributions of the three properties for ethanol, 1,4-dioxane and nitromethane:

\[
P' = \log k'_e + \log k'_d + \log k'_n
\]  

(1.6)

**gas-liquid partition coefficients** for the probes, determined from the equilibrium concentrations in a sealed vial containing a fixed volume of the solvent being characterised.
### Snyder’s global polarity

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Normal boiling point (°C)</th>
<th>Cutoff wavelength (nm)</th>
<th>Viscosity at 20°C (mPa·s)</th>
<th>Solubility parameter, δ</th>
<th>Snyder global polarity, P'</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isooctane</td>
<td>99.2</td>
<td>200–210</td>
<td>0.50</td>
<td>7.0</td>
<td>-0.4</td>
</tr>
<tr>
<td>Diisopropyl ether</td>
<td>68.0</td>
<td>380</td>
<td>0.33</td>
<td>7.1</td>
<td>1.8</td>
</tr>
<tr>
<td>n-Heptane</td>
<td>98.4</td>
<td>200</td>
<td>0.42</td>
<td>7.5</td>
<td>0.0</td>
</tr>
<tr>
<td>n-Hexane</td>
<td>68.7</td>
<td>200</td>
<td>0.31</td>
<td>7.5</td>
<td>0.0</td>
</tr>
<tr>
<td>Triethylamine</td>
<td>89.5</td>
<td>235</td>
<td>0.38</td>
<td>7.5</td>
<td>1.8</td>
</tr>
<tr>
<td>Cyclohexane</td>
<td>80.7</td>
<td>200</td>
<td>0.98</td>
<td>8.2</td>
<td>0.0</td>
</tr>
<tr>
<td>Carbon tetrachloride</td>
<td>76.8</td>
<td>263</td>
<td>0.97</td>
<td>8.6</td>
<td>1.7</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>77.1</td>
<td>256</td>
<td>0.46</td>
<td>8.9</td>
<td>4.3</td>
</tr>
<tr>
<td>Toluene</td>
<td>110.6</td>
<td>284</td>
<td>0.59</td>
<td>8.9</td>
<td>2.3</td>
</tr>
<tr>
<td>Tetrahydrofuran</td>
<td>66.0</td>
<td>212</td>
<td>0.55</td>
<td>9.1</td>
<td>4.2</td>
</tr>
<tr>
<td>Chloroform</td>
<td>61.2</td>
<td>245</td>
<td>0.58</td>
<td>9.2</td>
<td>4.4</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>40.0</td>
<td>232</td>
<td>0.44</td>
<td>9.6</td>
<td>4.3</td>
</tr>
<tr>
<td>Methyl ethyl ketone</td>
<td>79.6</td>
<td>329</td>
<td>0.42 (15°C)</td>
<td>9.5</td>
<td>4.5</td>
</tr>
<tr>
<td>Acetone</td>
<td>56.3</td>
<td>330</td>
<td>0.30 (25°C)</td>
<td>9.6</td>
<td>5.4</td>
</tr>
<tr>
<td>Carbon disulfide</td>
<td>46.0</td>
<td>220</td>
<td>0.36</td>
<td>10.0</td>
<td>1.1</td>
</tr>
<tr>
<td>1,4-Dioxane</td>
<td>101.3</td>
<td>215</td>
<td>1.44 (15°C)</td>
<td>10.1</td>
<td>4.8</td>
</tr>
<tr>
<td>Pyridine</td>
<td>115.3</td>
<td>330</td>
<td>0.95</td>
<td>10.6</td>
<td>5.3</td>
</tr>
<tr>
<td>Isopropanol</td>
<td>82.3</td>
<td>205</td>
<td>2.86 (15°C)</td>
<td>11.4</td>
<td>4.3</td>
</tr>
<tr>
<td>1-Butanol</td>
<td>117.7</td>
<td>215</td>
<td>2.95</td>
<td>11.6</td>
<td>3.9</td>
</tr>
<tr>
<td>2-Methoxy-ethanol</td>
<td>124.6</td>
<td>210</td>
<td>1.72</td>
<td>11.7</td>
<td>5.7</td>
</tr>
<tr>
<td>Dimethylformamide</td>
<td>155.0</td>
<td>268</td>
<td>0.92</td>
<td>11.8</td>
<td>6.4</td>
</tr>
<tr>
<td>Ethanol</td>
<td>78.3</td>
<td>205–210</td>
<td>1.2</td>
<td>12.0</td>
<td>5.2</td>
</tr>
<tr>
<td>Dimethyl sulfoxide</td>
<td>189.0</td>
<td>286</td>
<td>2.20</td>
<td>12.0</td>
<td>6.5</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>81.6</td>
<td>190</td>
<td>0.34</td>
<td>12.1</td>
<td>6.2</td>
</tr>
<tr>
<td>1-Propanol</td>
<td>97.2</td>
<td>210</td>
<td>2.26</td>
<td>12.2</td>
<td>3.9</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>117.9</td>
<td>210</td>
<td>1.31 (15°C)</td>
<td>13.0</td>
<td>6.2</td>
</tr>
<tr>
<td>Methanol</td>
<td>64.7</td>
<td>205</td>
<td>0.55</td>
<td>14.5</td>
<td>6.6</td>
</tr>
<tr>
<td>Formamide</td>
<td>210.5</td>
<td>210</td>
<td>3.5</td>
<td>19.2</td>
<td>7.3</td>
</tr>
<tr>
<td>Water</td>
<td>100.0</td>
<td>&lt;190</td>
<td>1.00</td>
<td>23.5</td>
<td>9.0</td>
</tr>
</tbody>
</table>
To eliminate the differences among the solvent global polarities, the partition coefficients were normalised.

\[ P' = \log k_e' + \log k_d' + \log k_n' \]  \hspace{1cm} (1.6)

\[ 1 = \frac{\log k_e'}{P'} + \frac{\log k_m'}{P'} + \frac{\log k_n'}{P'} = x_e + x_d + x_n \]  \hspace{1cm} (1.7)

- The Snyder’s approach assumes that:
  - a solvent that strongly retains ethanol should have a predominantly basic character
  - a solvent that strongly retains 1,4-dioxane should have a predominantly acidic character
  - a solvent that strongly retains nitromethane has a strong dipolar character

**Solvent properties:**

- \( x_e \): solvent basic character
- \( x_d \): solvent acidic character
- \( x_n \): solvent dipolar character
The $x_e$, $x_d$ and $x_n$ data for a large number of solvents were plotted.
Snyder’s solvent selectivity triangle

Plotted properties were obtained from solutes with a mixed character, and therefore the vertices do not represent pure properties.
If the triangle scales would correspond to pure properties (each vertex representing 100% acidity, 100% basicity and 100% dipolarity), mixtures of three hypothetical solvents, each one located at each vertex, would provide a whole universe of possibilities.

However, such solvents do not exist !!!
1. Retention in reversed-phase, normal-phase and HILIC

Families of solvents

- BASIC
  - triethylamine
  - MeOH
- ISO
  - isoPrOH
  - diisopropyl ether
- ACIDIC
  - glycols, acetic acid
  - H$_2$O, HAcO
  - phenols, water
  - aromatic hydrocarbons, nitrocompounds
  - aliphatic alcohol
  - aliphatic ethers, amines
  - pyridine, THF
  - aliphatic ketones, esters, 1,4-dioxane, and nitriles
  - dichloromethane, dichloroethane

DIPOLAR
The scales should be read counterclockwise.
The most common solvents in RPLC provide different selectivity, since they have different positions with rather different profiles of the three properties defined in the triangle.
1. Retention in reversed-phase, normal-phase and HILIC

Common solvents in RPLC

- **Water**: strong hydrogen ion donor and acceptor, but weakly polarizable
- **ACN**: less acidic than water, but appreciably more dipolar
- **MeOH**: appreciably more basic and more dipolar than water, and less dipolar than ACN
- **THF**: both acidic and basic, and more dipolar than water

**Common solvents in RPLC**

- **Water**: strong hydrogen ion donor and acceptor, but weakly polarizable
- **ACN**: less acidic than water, but appreciably more dipolar
- **MeOH**: appreciably more basic and more dipolar than water, and less dipolar than ACN
- **THF**: both acidic and basic, and more dipolar than water
The positions of ACN, MeOH and THF are intermediate in the triangle, being excellent choices to achieve a wide range of properties in RPLC.

Not surprisingly, these solvents were already popular by the time the triangle was developed.
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Chromatographic properties:

- **Elution strength**
  
  depends on the **global polarity of**
  
  stationary phase and mobile phase
  
  is tuned by changing the **modifier concentration**

- **Selectivity**

  depends on the **specific interactions of solutes with stationary phase and mobile phase**
  (profile of the contributions to the global polarity of solutes and phases)
  
  is tuned by changing the **nature of the solvent mixture** (for ionisable solutes also **pH**)

A **key question** in selectivity optimisation is how to modify the nature of the solvent mixture (the selectivity), without altering the elution strength.
Isoeluotropic mixtures: mixtures with the same elution strength but prepared with different modifiers (different interactions, different selectivity).

RPLC

\[ \delta_M = \sum_j \delta_j \phi_j \]  \hspace{1cm} (1.2)

For binary mixtures of MeOH, ACN or THF / water, the elution strength will be the same for:

\[ \delta_M = \delta_{\text{MeOH}} \phi_{\text{MeOH}} + \delta_{\text{H2O}} (1 - \phi_{\text{MeOH}}) = \delta_{\text{ACN}} \phi_{\text{ACN}} + \delta_{\text{H2O}} (1 - \phi_{\text{ACN}}) = \delta_{\text{THF}} \phi_{\text{THF}} + \delta_{\text{H2O}} (1 - \phi_{\text{THF}}) \]  \hspace{1cm} (1.8)

\[ \delta_{\text{H2O}} = 23.5, \ \delta_{\text{MeOH}} = 14.5, \ \delta_{\text{ACN}} = 12.1, \ \delta_{\text{THF}} = 9.1 \]

\[ \phi_{\text{MeOH}} = 1.27 \ \phi_{\text{ACN}} = 1.60 \ \phi_{\text{THF}} \]  \hspace{1cm} (1.9)

THF is the most hydrophobic solvent

the same elution strength is achieved with a smaller percentage

Example: The same elution strength as 30% MeOH is approximately obtained for

23.6% ACN or 18.8% THF
The predictions of elution strength depart from linearity at large modifier concentrations.

Estimation of isoeluotropic binary mixtures ACN, MeOH or THF / water:
- non-linear relationships
- nomograms

Due to the limitations inherent to the global polarity parameters, predictions are only approximate, and depend largely on the solute type.
1.1. Elution strength and selectivity

1.2. Columns and solvents in RPLC, NPLC and HILIC

1.3. Rationalisation of the elution strength
   
   1.3.1. Global polarity measurements
   
   1.3.2. Global polarity for solvent mixtures
   
   1.3.3. Schoenmakers’s rule

1.4. Isoeluotropic mixtures

1.5. Snyder’s solvent-selectivity triangle

1.6. Column characterisation

1.7. Recommended literature
1.6. Column characterisation

Stationary phase properties

**Hydrophobicity:** refers to the dispersion interactions that the solutes can establish with the bonded alkyl chains (which dominate the RPLC retention process).

**Depends on:** length of the bonded chain
bonding density
specific surface area of the silica support
Shape selectivity: capacity of a stationary phase to discriminate compounds of identical elemental composition but different three-dimensional structure (as isomers).

The penetration capability of solutes in the stationary phase liquid layer depends on the length-to-breadth ratio and molecule planarity.

Depends on: length of the bonded chain
bonding density
monomeric or polymeric nature of the stationary phase
Steric selectivity: related to the difficulty of a compound to penetrate in the stationary phase depending on its size (size exclusion).

The penetration of large solutes is limited and thus diminishes their retention.

Depends on: pore size
bonding density
Silanol activity: Underivatised silanols can interact with neutral solutes by hydrogen bonding, and with positively charged basic compounds by electrostatic attraction. This increases their retention and deteriorates the peak profile.

Depends on: amount of available silanol groups
relative acidic character
type (isolated, geminal or vicinal)
presence of metal impurities
Two main types of tests are performed to characterise columns: based on probes having well defined properties (such as the Tanaka, Engelhardt, Eyman, Walters, Daldrup tests), or based on retention models obtained with the data for a large number of varied compounds (solvation parameter model and hydrophobic subtraction model).

**Tanaka test: probes**

![Chemical structures](image)
Tanaka test: Parameters

Retention capacity or absolute hydrophobicity, which depends on the carbon load and the specific stationary phase surface area: \( k \) (pentylbenzene)

Hydrophobic selectivity or methylene group selectivity:
\[
\frac{k \text{ (pentylbenzene)}}{k \text{ (butylbenzene)}}
\]

Shape selectivity: \( \frac{k \text{ (triphenylene)}}{k \text{ (o-terphenyl)}} \)

Hydrogen bonding: \( \frac{k \text{ (caffeine)}}{k \text{ (phenol)}} \)

Electrostatic interactions: \( \frac{k \text{ (benzylamine)}}{k \text{ (phenol)}} \) at two pH values:
- pH 2.7: protonated silanols (neutral)
- pH 7.6: anionic silanols
- benzylamine: protonated with a positive charge at both pH values
Tanaka test: industrial standard

- Retention capacity
- Hydrophobicity
- Ion exchange capacity
- Steric selectivity
- Silanol capacity