Dual retention mechanism on polar embedded stationary phases

Katarzyna Krzemieńska, Szymon Bocian, Boguslaw Buszewski

Nicolaus Copernicus University, Faculty of Chemistry, Chair of Environmental Chemistry & Bioanalytics, Gagarin 7 St., PL 87-100 Toruń
E-mail: katarzyna.krzeminska@poczta.pl

Introduction

For several years we are conducted intensive studies on the development of stationary phases which exhibit properties complementary to the classic alkyl stationary phases synthesized on a silica support, but they should provide alternative selective separation of substances by RP-LC. The progress that has been made in the preparation of packings enabled the introduction into alkyl chain polar functional group. There are many unexploited capabilities of the synthesis of chemically bonded stationary phases for liquid chromatography, which contain various functional groups incorporated into structure: Different functional groups allow to improve the resolution and selectivity. Additionally, they determine the hydrophilic/hydrophobic nature of the packing material. Incorporation into the alkyl chain polar functional group such as an amine, carboxyl, ether, or amide change the nature of the stationary phase. It allows to improve some chromatographic parameters such as selectivity, better stability in highly aqueous mobile phase and also improve shape and symmetry of the peaks for the substances with basic character in comparison to conventional phase used in the RPC. The polar functional group provides water adsorption and allow to use proper material in HILIC, while the hydrophobic chains have an influence on separation selectivity. The presence of amino groups provides preferential water adsorption. This specific effect is called "hydrophilic "pillow" [5] and it also provides a conformational freedom for the alkyl chains, thereby allowing their interaction with polar analytes. Polymers in RPC are shielded from the analytes. In this system, the retention of polar compounds is much lower than in HILIC. Responsible for this phenomenon are type and amount of the hydrophobic functional groups present in the stationary phase structure [6].

This approach to synthesis of packing materials has many advantages:

• stationary phase retains character assigned to the RPC phases;
• material has a different selectivity compared to the alkyl stationary phases especially in analysis of hydrophilic compounds;
• stationary phase is stable in highly aqueous conditions; there is no over-served problem of phase destabilization;
• eluted activity is limited due to effective presence of polar groups; it has been observed stabilizing effects from the presence of residual alkyl groups.

Selectivity in different conditions

Retention model

\[
\log k = a + m_{\text{UPLC}}(H_2O) = m_{\text{HILIC}} \log(1 + b \, \varphi(H_2O))
\]

where:

• \(k\) - log \(k\) in pure organic solvent \(\varphi(H_2O) = 0\); 
• \(a\) - the correction term for the HILIC retention in mobile phases with very low concentration of water 
• \(m_{\text{HILIC}}\) - measure of decreasing HILIC contribution to the retention in highly organic mobile phases [4]

Solvation effect

Conclusions

- Mixed mode surface of stationary phases with embedded polar groups allows to separate polar and hydrophobic compounds.
- In combination these approach provide hydrophilic and hydrophobic interactions between stationary phase, analyte and mobile phase.
- The importance role play secondary interactions, especially hydrogen bond formation.
- The water and acetone adsorption confirms these phenomena. It proves the preferential solution of hydrophilic and polar functional groups by organic solvent and water, respectively.
- Retention models are an important group of mathematical models used to select and optimize chromatographic conditions for the separation of chemical compounds.
- In liquid chromatography and related techniques it is possible to select the appropriate system parameters to achieve satisfactory selectivity.
- Application of embedded outer and phosphodextrin stationary phases allow to separate polar compounds using only pure water in mobile phase composition.

Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column type</td>
<td>Stationary</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>Pure water</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1.0 mL/min</td>
</tr>
<tr>
<td>Injection volume</td>
<td>1.0 µL</td>
</tr>
<tr>
<td>Column temperature</td>
<td>25 °C</td>
</tr>
<tr>
<td>Detection</td>
<td>Photodiode array detector</td>
</tr>
<tr>
<td>Chromatographic software</td>
<td>Wiley 6.21, MassLynx 4.21</td>
</tr>
</tbody>
</table>

Instrumentation & Chemicals

- High performance liquid chromatograph Shimadzu Nexera UHPLC (Shimadzu Corporation, Kyoto, Japan) equipped with binary pumps system (LC-20AD), double array detector (ESD SPD-M20A) autosampler (motor 3A5C) and the thermostat (CTO- \(N\)A).
- High performance liquid chromatograph Shimadzu Prominence system (Tokyo, Japan) equipped with binary pump (LC-20AD), refractive index detector RI (RI-20A), an autosampler (SIL-20A) and thermostat (CTO-20A).
- Data was collected using LabSolution software.
- Chromatographic columns non commercially available synthesized in Department of Environmental Chemistry & Bioanalytics, Faculty of Chemistry, Nicolaus Copernicus University, Toruń, Poland. Synthesis described [4]. Detailed informations Table 1.
- Dewaxing water from the Millipore system (Millipore, El Paso, Texas, USA).
- Organic solvent (methanol and acetonitrile) was high-purify "for HPLC" gradient grade from Sigma Aldrich (St. Louis, Mo, USA).
- Standards of pure HPLC-grade solvents (Sigma-Aldrich, St. Louis, Mo, USA).

Literature:


This study was supported by the National Science Centre, Poland Grant No. 2013/09/D/ST4/03897 for the period 2014-2017 and Grant No. 2016/23/N/ST4/00161 for the period 2017-2018.