Dual retention mechanism on polar embedded stationary phases

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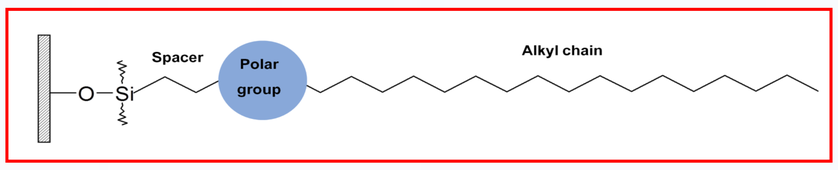
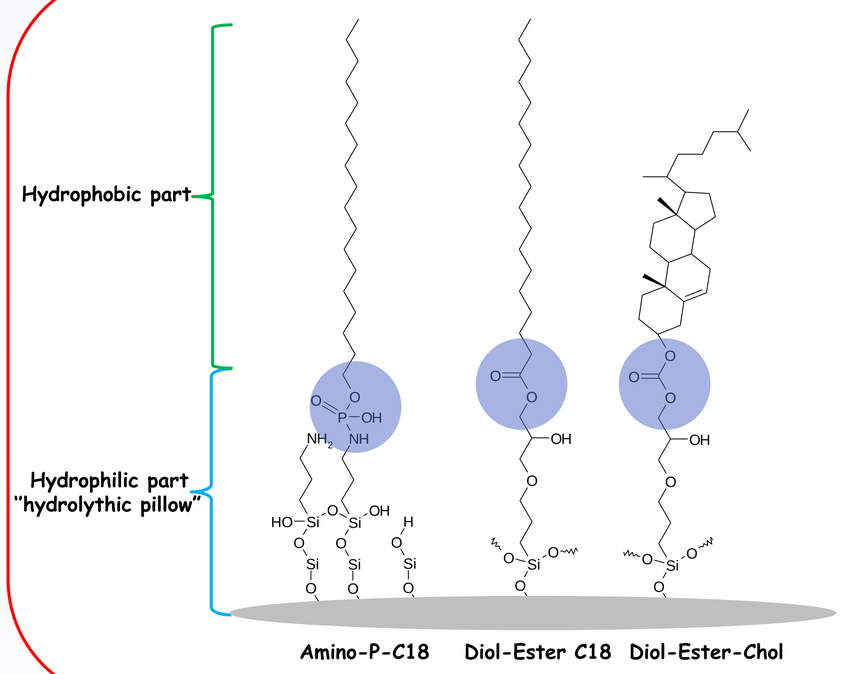
Introduction

For several years 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 RPLC. The progress that has been made in the preparation of packings enabled the introduction into alkyl chain polar functional group. There are many untapped capabilities of the synthesis of chemically bonded stationary phases for liquid chromatography, which contain varied 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 amide, carbamate, ether, or urea 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 RPLC. The polar functional group provides water adsorption and allow to use proposed 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 "hydrolytic pillow" [2]. It also provides a conformational freedom for the alkyl chains, thereby allowing their interaction with polar analytes. Polar groups in RPLC 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 [3].

This approach to synthesis of packing materials has many advantages:

- stationary phase retains character assigned to the RPLC 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 not observed a problem with phase dewetting
- silanol activity is limited due to the presence of polar groups; it has been observed shielding effects from the presence of residual silanol groups.





Instrumentation & Chemicals

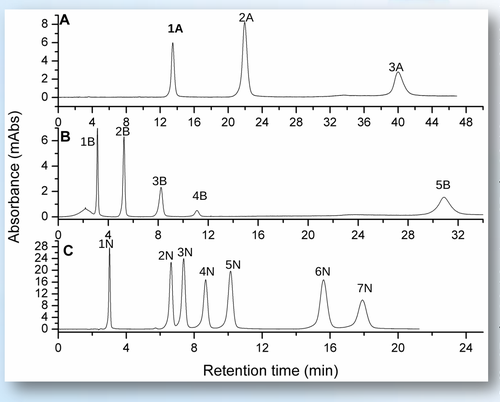
- Ultra high performance liquid chromatograph Shimadzu Nexera UHPLC (Shimadzu Corporation, Kyoto, Japan) equipped with binary pumps system (LC-30AD), diode array detector (DAD SPD-M20A) autosampler (motor 30AC) and the thermostat (CTO-30-A)
- High performance liquid chromatograph Shimadzu Prominence system (Tokyo, Japan) equipped with a pump (LC-20AD), refractive index detector RI (RI-20A), an autosampler (SIL-20A) and thermostat (CTO-10AS VP)
  Data were 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 [5,6]. Detailed informations Table 1.
- Deionized water from the Milli-Q system (Millipore, El Passo, Texas, USA)
- Organic solvent (methanol and acetonitrile) was high-purity "for HPLC" gradient grade from Sigma Aldrich (St. Louis, MO, USA).
- Standards of purine alkaloids, 4-aminoacetophenone (Sigma-Aldrich, St. Louis, MO, USA), nucleobases and nucleosides (AppliChem GmbH, Darmstadt, Germany)

Table 1
Parameters of stationary phases using in this study

Stationary phase	Column dimension [mm]	Pore diameter [Å]	Particle size [µm]	Carbon content [%]	Coverage density [µmol/m²]	Silanol activity*	Hydrophobicity*
Amino-P-C18	125x4.6	100	5	9.33	1.04	2.24	0.80
Diol-Ester C18	125x4.6	100	5	7.65	0.90	4.57	0.74
Diol-Ester-Chol	125x4.6	100	5	10.73	0.93	-2.92	1.48

*measured according to Galushko test

Pure water | RP-LC | Mixed mode | HILIC



Elution order:
1A) Theobromine
2A) Theophiline
3A) Caffeine

1B) Uracil
2B) Thymine
3B) Guanine
4B) Cytosine
5B) Adenine

1N) Uridine
2N) Thymidine
3N) 1-methylinosine
4N) Guanosine
5N) 1-methylguanosine
6N) Adenosine
7N) N^2-methylguanosine

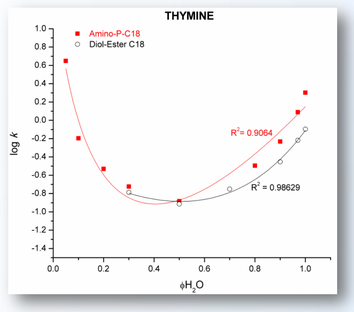
Column: Amino-P-C18, 5µm, 125x4.6 mm, mobile phase: 100% *(v/v)* H_2O; temperature 30°C, detector DAD UV-Vis λ=254 nm, flow rate 1mL/min.

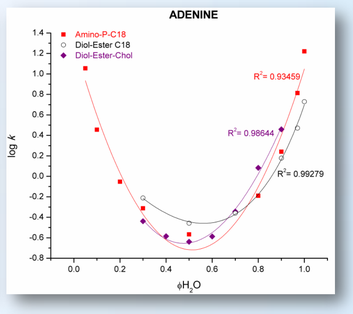
A – purine alkaloids **B** – nucleobases **C** – nucleosides

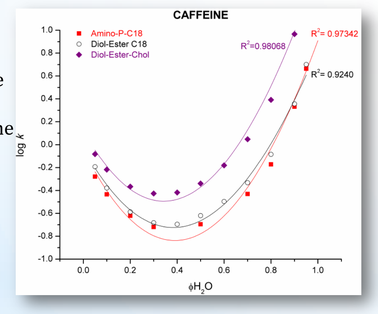
Retention model

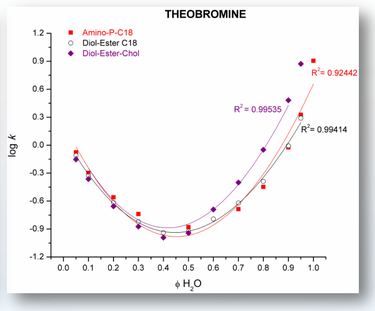
$$\log k = a + m_{RP}\varphi(H_2O) - m_{HILIC}\log[1+b\,\varphi(H_2O)]$$

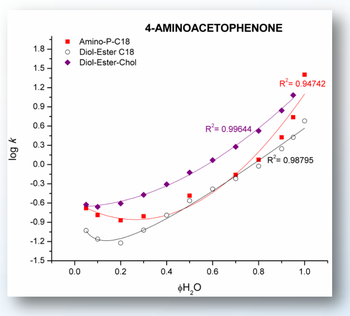
a – $\log k$ in pure organic solvent $\varphi(H_2O)$ =0
b – (b>1) the correction term for the HILIC retention in mobile phases with very low concentration of water
m_{RP} – the effect of increasing concentration of water in the mobile phase on enhancing the contribution of the RP mechanism to the retention
m_{HILIC} – measure of decreasing HILIC contribution to the retention in highly organic mobile phases [4]

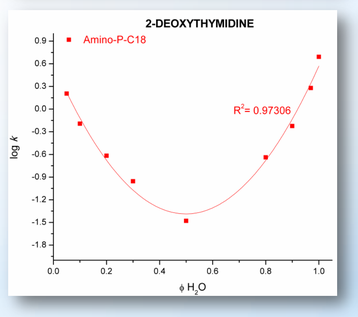


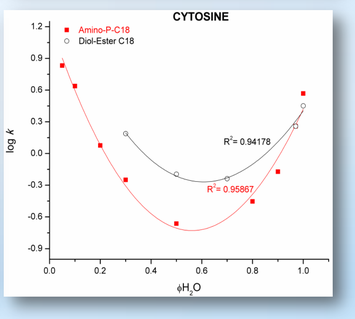












Selectivity in different conditions

Column: Diol – Ester C18, 5µm, 125x4.6 mm, temperature 30°C, detector DAD UV-Vis λ=254 nm, flow rate1 mL/min, inject 0.1 – 0.2 µL

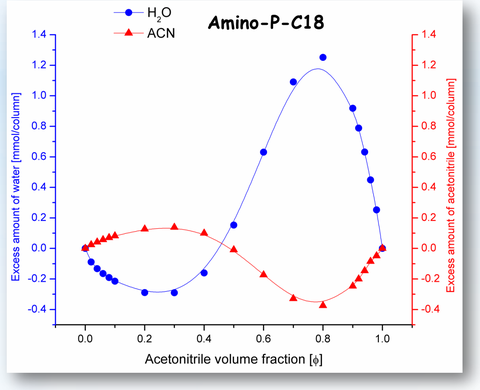
T – theobromine **4a** – 4-aminoacetophenone **C** – caffeine

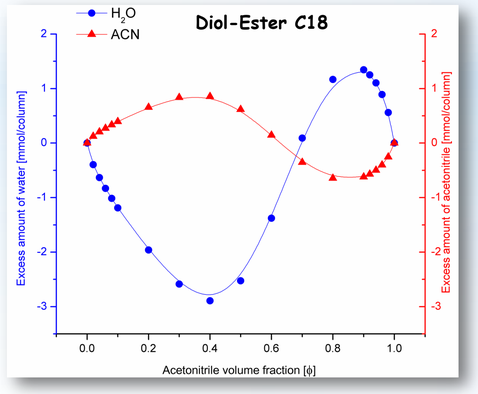
A) Mobile phase: 3/97% *(v/v)* ACN/H_2O $\alpha_{4a/T}$ = 1.155
$\alpha_{K/4a}$ = 2.339
$\alpha_{K/T}$ = 2.702

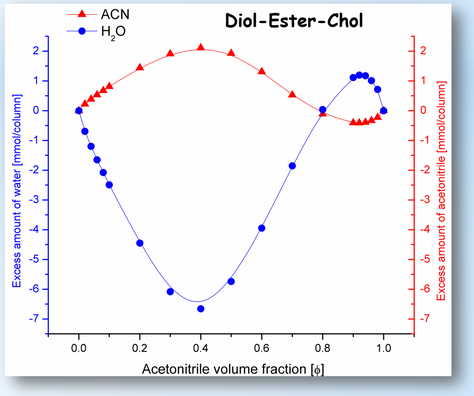
B) Mobile phase: 40/60% *(v/v)* ACN/H_2O $\alpha_{4a/T}$ = 2.584
$\alpha_{4a/K}$ = 1.304
$\alpha_{K/T}$ = 1.981

C) Mobile phase: 97/3% *(v/v)* ACN/H_2O $\alpha_{T/4a}$ = 5.960
$\alpha_{K/4a}$ = 4.720
$\alpha_{T/K}$ = 1.263

Solvation effect







Conclusions

- Mixed mode surface of stationary phases with embedded polar groups allows to separate polar and hydrophobic compounds.
- In retention take part both hydrophobic and hydrophilic interactions between stationary phase, analyte and mobile phase. The important role play secondary interactions also.
- The water and acetonitrile adsorption comparision confirms the heterogeneity of chemically bonded phases. It proves the preferential solvation of hydrophobic 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 ester and phosphodiester stationary phases allow to separate polar compounds using only pure water in mobile phase composition.

Literature:

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