Characterisation of RP Sorbents by Linear Solvation Energy Relationships (LSER)

Helmut Riering, Natalie Bilmann, MACHEREY-NAGEL GmbH & Co. KG

Fifteen HPLC sorbents based on the silicas NUCLEODUR® and NUCLEOSHELL® are characterised by the linear solvation energy relationship method. LSER coefficients are determined for these phases. They allow a discussion of the interactions between analytes and stationary phases, in particular the behaviour of surface modification under the chosen chromatographic conditions. A better understanding of these interactions may facilitate the selection of the stationary phase.

Introduction

The number of commercially available stationary phases for HPLC with an array of surface modifications and selectivities is increasing from year to year and allows the user to select the best phase for his separation problem. This requires a good characterisation of the sorbents. Numerous studies on the characterisation of the selectivity of RP phases have been published [1,2]. In general, the retention of certain compounds is investigated by comparison on HPLC phases and the results are assigned to certain phase properties such as hydrophobicity, steric selectivity or dipole interactions. Another method for phase characterisation is the linear solvation energy relationship approach [3]. According to this method, the logarithm of the retention factor k' can be described by the addition of a constant c and five types of interaction forces (equation 1).

(1) $\log k' = c + eE + sS + aA + bB + vV$

In this equation, the capital letters describe the properties of the respective analyte. E being the excess molar refraction, S the absolute dipolarity / polarisability, A and B the solute effective hydrogen bond acidity and basicity, and V the McGowan characteristic volume [4]. Values for many compounds are available in the literature [5]. The lowercase letters describe the corresponding values of the chromatographic system. If the chromatographic conditions like temperature or eluent composition are kept constant, these values depend only on the properties of the stationary phase. *Table 1* explains the meaning of the different coefficients for the characterisation of the stationary phase. The coefficients e - v of the stationary phases are determined by the measured retention factors and substance parameters E - V known from the literature using multiple linear regression. The LSER method compares always interactions between analyte / stationary phase and analyte / mobile phase. Positive values for the coefficients are obtained when the interaction of the analyte with the stationary phase is stronger; at negative values the interaction between solute and mobile phase is stronger. By using a larger number of test substances, the method is placed on a broader basis compared to other characterisation approaches. For known substance parameters it allows an estimation of the retention factors.

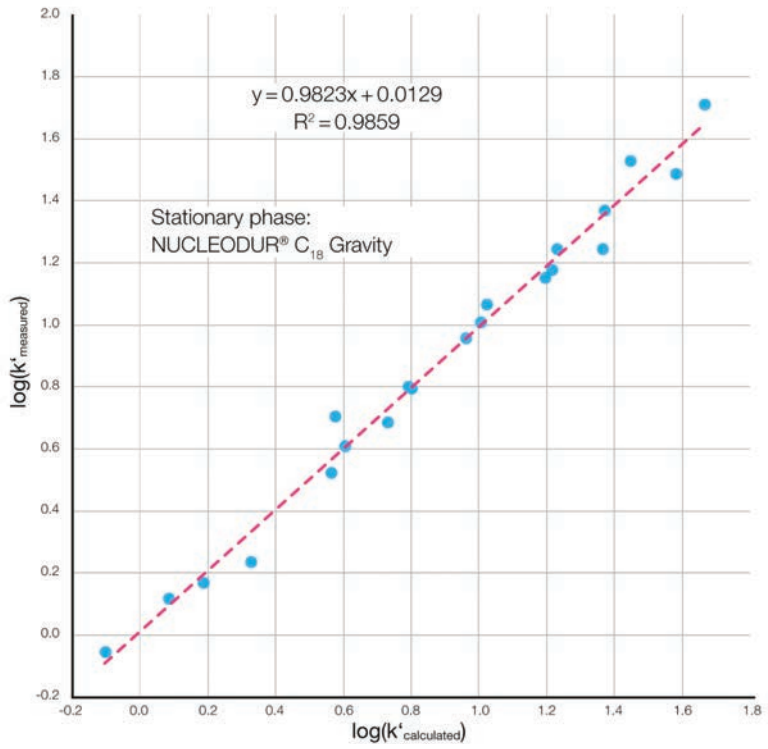

Figure 1: Plot of log(k'$_{measured}$) versus log(k'$_{calculated}$)

Experimental

The investigated stationary phases were prepared using the silicas NUCLEODUR® (pore diameter 110 Å, totally porous, surface area 340 m²/g, pore volume 0.9 mL/g) and NUCLEOSHELL® (pore diameter 90 Å, core shell silica, surface area 130 m²/g). Stainless steel columns with these sorbents (100 mm x 3 mm ID) are commercially available (MACHEREY-NAGEL, Germany). The structures of the surface modifications are shown in *Table 2*.

HPLC grade solvents were used for the preparation of the eluents. Water was prepared by an ultra clear GP UV UF purifier (Evoqua, Germany). The various test compounds were of reagent grade or higher purity and were commercially obtained from various sources.

The HPLC equipment used in this work was a Nexera XR HPLC system (Shimadzu, Germany). The void volume has been determined by multiple injection of an uracil solution. All chromatographic investigations were carried out using acetonitrile / water (50:50 v/v) at a flow rate of 0.6 mL/min and a column temperature of 40°C. Detection was carried out with a diode array detector (220-300 nm). The LSER parameters of the analytes were taken from the literature [5]. The values of the test substances used here are listed in *table 3*.

The LSER coefficients of the sorbents are determined by multiple linear regression using Excel with the extension Realstatistics [6].

Results and discussion

Table 4 lists the retention factors of the test substances as well as the calculated LSER coefficients of the stationary phases determined by multiple linear regression. The values for R^2 as a quality measure of the regression are between 0.984 and 0.991. *Figure 1* shows the logarithmic plot of the measured retention factors against the values calculated with the coefficients of the sorbent NUCLEODUR® C_{18} Gravity. Ideally, this plot should lead to a straight line with slope 1 that intersects the origin. For the regression line, a slope of 0.98 and an intercept of 0.013 can be determined. The R^2 quality of the regression is 0,986. This proves that the LSER method satisfactorily describes the interactions between analytes and stationary phases. The small deviations are likely to be due to erroneous measurements of retention factors and literature values. For the other phases comparable plots have been obtained.

Figures 2, *3* and *4* show the LSER coefficients as a function of the stationary phase. The intercept c is mainly determined by the parent silica. Values of -0.1 are found for the totally porous NUCLEODUR®, while the core shell silica NUCLEOSHELL® yields values of -0.4. This general reduction of the interactions is due to the lower surface area of NUCLEOSHELL®.

The values of the coefficient e, which describes the stationary phase interactions with n- and π-electrons, show comparable orders of magnitude for the C18-phases a-e. The lower surface coverage of the phase b, caused by the bulky substituents, is responsible

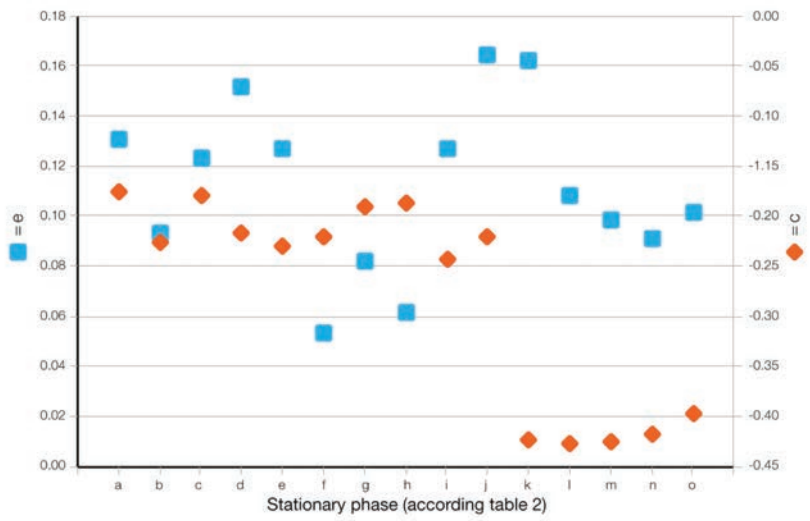

Figure 2: Determined values of coefficients c and e for the investigated stationary phases

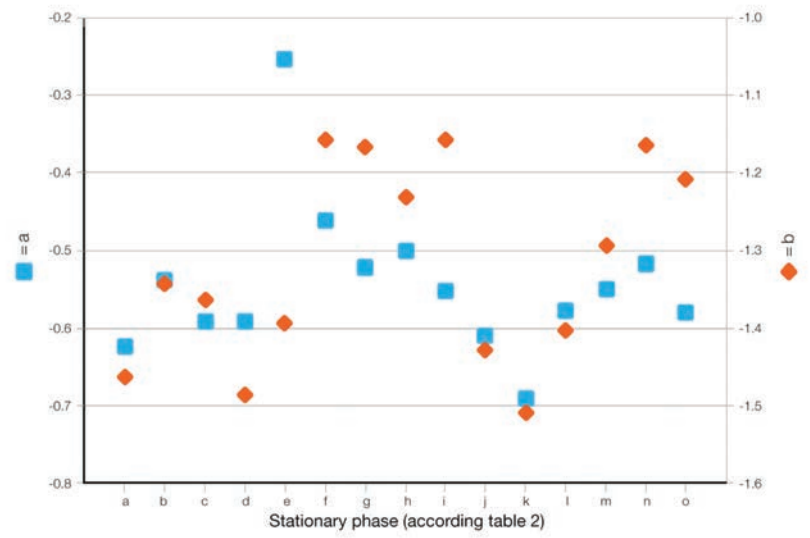

Figure 3: Determined values of coefficients a and b for the investigated stationary phases

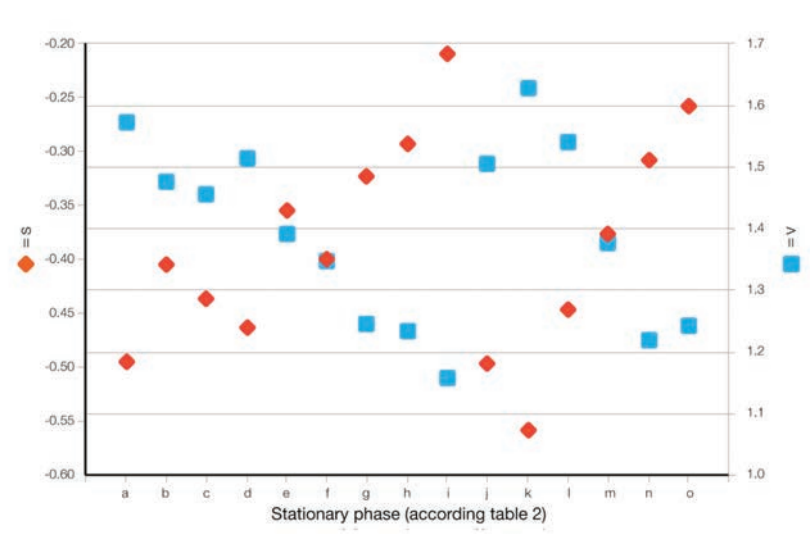

Figure 4: Determined values of coefficients s and v for the investigated stationary phases

for its low value, while the polymeric modified phase d the coefficient e has a high value. Despite the presence of double bonds and lone pairs of electrons, the polar embedded phase NUCLEODUR® PolarTec (e) shows an inconspicuous behaviour here. The change from octadecyl to octyl ligands leads to a significant decline of this value for NUCLEODUR® C_8 Gravity (f). Interestingly, phases g and h also show low values for coefficient e, although they carry aromatic ligands. However, for biphenyl or pentafluorophenyl ligands, the influence of this interaction increases significantly. A similar, albeit less pronounced, behaviour is shown by the NUCLEOSHELL® based phases k - n.

The hydrogen bonding acceptor properties (coefficient a) of all phases are of similar magnitude (*Figure 3*). Only NUCLEODUR® PolarTec (e) shows a high value for this coefficient, which should be due to the polar group embedded in ligand. In contrast to the RP18 phases, the C_8 phase f and the aryl phases g, h, i, m, n, and o show a larger proportion of donor properties (coefficient b). In contrast, the pentafluorophenyl modified NUCLEODUR® PFP (j) behaves like an RP18 phase.

The coefficient s as a measure of dipolarity and polarisability is naturally low for the RP18 phases (*Figure 4*). The lower surface coverage of phase b, the hydrophilic endcapping of phase c and the polar group of phase e increase their values of s. All aryl phases also show increased values for s. For the biphenyl phases i and o the highest values can be determined within this series of measurements. NUCLEODUR® PFP (j) also shows a contrary behaviour here.

The coefficient v as a measure of the hydrophobicity is the most pronounced in the RP18 phases. It is reduced by the coverage density (phase b), hydrophilic endcapping (phase c), polar groups (phase e) and shorter octyl chains (phase f). The aryl phases, with the exception of NUCLEODUR® PFP (j), also show only small values for coefficient v. Despite the large number of carbon atoms, NUCLEODUR® π^2 (i) has the lowest hydrophobicity using the LSER model under the chosen conditions.

Table 1: Description of the different LSER coefficients

LSER coefficients	Interaction
c	Intercept
e	Interactions of the phase with n and π electrons of the analyte
s	Dipolarity / polarisability of the phase
a	Hydrogen bonding acceptor properties of the phase
b	Hydrogen bonding donor properties of the phase
v	Strength of hydrophobic interactions

Table 2: Description of the investigated stationary phases

	Stationary phase	Surface modification	Structures of surface modification
a	NUCLEODUR® C_{18} Gravity	Brush type, dense C_{18} layer, cross-linked	
b	NUCLEODUR® C_{18} Gravity-SB	C_{18} modification with bulky substituents	
c	NUCLEODUR® C_{18} Pyramid	Brush type, dense C_{18}-layer, hydrophilic endcapping	
d	NUCLEODUR® C_{18} Isis	Polymeric C_{18} layer	
e	NUCLEODUR® PolarTec	C_{18} modification with polar embedded groups	
f	NUCLEODUR® C_8 Gravity	Brush type, dense C_8 layer, cross-linked	
g	NUCLEODUR® Sphinx RP	Brush type, dense mixed phase with C_{18} und C_6H_5 ligands	
h	NUCLEODUR® Phenyl-Hexyl	Brush type, phenylhexyl modification	
i	NUCLEODUR® π^2	Brush type, biphenyl modification	
j	NUCLEODUR® PFP	Brush type, pentafluorophenyl phase	
k	NUCLEOSHELL® RP 18	Brush type, dense C_{18} layer, crosslinked	
l	NUCLEOSHELL® RP 18plus	C_{18} modification with bulky substituents at the silicium atom	
m	NUCLEOSHELL® Bluebird	Brush type, dense C_{18}-layer, hydrophilic endcapping	
n	NUCLEOSHELL® Phenyl-Hexyl	Brush type, phenylhexyl modification	
o	NUCLEOSHELL® Biphenyl	Biphenylpropyl modification with bulky substituents	

Table 3: LSER coefficients of used analytes [5]

Analyte	E	S	A	B	V
Acenaphthylene	1.75	1.14	0	0.26	1.2156
Pentylbenzene	0.594	0.51	0	0.15	1.4209
Anisol	0.708	0.75	0	0.29	0.916
Fluorene	1.588	1.03	0	0.2	1.357
Chlorobenzene	0.718	0.65	0	0.07	0.8288
Fluoranthene	2.377	1.53	0	0.2	1.585
Phenol	0.805	0.89	0.6	0.3	0.7751
Naphthaline	1.34	0.92	0	0.2	1.0854
Anthracene	2.29	1.34	0	0.26	1.454
Toluene	0.601	0.52	0	0.14	0.8573
Propylbenzene	0.604	0.5	0	0.15	1.1391
Butylbenzene	0.6	0.51	0	0.15	1.28
Acetophenone	0.818	1.01	0	0.48	1.0139
Butyl benzoate	0.668	0.8	0	0.46	1.4953
Biphenyl	1.36	0.99	0	0.26	1.3242
4-Nitrotoluene	0.87	1.11	0	0.28	1.032
Ethyl benzoate	0.689	0.85	0	0.46	1.214
Ethylbenzene	0.613	0.51	0	0.15	0.9982
N,N-Dimethylaniline	0.957	0.84	0	0.47	1.098
m-Cresol	0.822	0.88	0.57	0.34	0.916
o-Cresol	0.84	0.86	0.52	0.3	0.916
p-Cresol	0.82	0.87	0.57	0.31	0.916
2,6-Dimethylphenol	0.86	0.79	0.39	0.39	1.057
2,6-Dimethylaniline	0.972	0.89	0.2	0.46	1.098
Nitrobenzene	0.871	1.11	0	0.28	0.8906
Dimethyl phthalate	0.78	1.41	0	0.88	1.429
Diethyl phthalate	0.729	1.4	0	0.88	1.711
1-Phenylethanol	0.784	0.83	0.3	0.66	1.057
Pyrene	2.808	1.71	0	0.29	1.585
Chrysene	3.027	1.73	0	0.36	1.823

Table 4: Chromatographic results und calculated LSER coefficients of the stationary phases

LSER coefficient / retention factor (Analyte)	NUCLEODUR® C_{18} Gravity	NUCLEODUR® C_{18} Gravity-SB	NUCLEODUR® C_{18} Pyramid	NUCLEODUR® C_{18} Isis	NUCLEODUR® PolarTec	NUCLEODUR® C_8 Gravity	NUCLEODUR® Sphinx RP	NUCLEODUR® Phenyl-Hexyl	NUCLEODUR® π^2	NUCLEODUR® PFP	NUCLEOSHELL® RP 18	NUCLEOSHELL® RP C18plus	NUCLEOSHELL® Bluebird	NUCLEOSHELL® Phenyl-Hexyl	NUCLEOSHELL® Biphenyl
c	-0.175	-0.226	-0.178	-0.217	-0.229	-0.219	-0.189	-0.186	-0.242	-0.221	-0.422	-0.426	-0.425	-0.417	-0.396
e	0.131	0.094	0.123	0.152	0.127	0.054	0.082	0.062	0.127	0.165	0.163	0.109	0.099	0.091	0.102
s	-0.495	-0.404	-0.436	-0.462	-0.354	-0.400	-0.324	-0.294	-0.209	-0.496	-0.558	-0.447	-0.376	-0.307	-0.258
a	-0.624	-0.539	-0.592	-0.591	-0.253	-0.462	-0.521	-0.501	-0.551	-0.611	-0.693	-0.578	-0.550	-0.517	-0.580
b	-1.463	-1.342	-1.363	-1.487	-1.393	-1.158	-1.166	-1.233	-1.158	-1.429	-1.511	-1.403	-1.294	-1.164	-1.209
v	1.573	1.475	1.455	1.515	1.392	1.346	1.246	1.234	1.158	1.506	1.627	1.541	1.377	1.217	1.241
R^2	0.988	0.989	0.990	0.989	0.986	0.984	0.989	0.990	0.991	0.990	0.989	0.989	0.989	0.990	0.991
k'(acenaphthylene)	11.65	9.19	9.93	10.67	9.67	6.47	7.08	7.07	7.61	9.88	7.16	6.44	5.00	4.14	5.34
k'(pentylbenzene)	51.46	37.57	38.89	43.96	33.36	24.72	21.67	21.03	16.92	40.19	34.16	28.11	18.18	11.92	14.32
k'(anisol)	3.33	2.88	3.05	3.03	3.01	2.41	2.51	2.48	2.50	2.86	1.93	1.91	1.64	1.47	1.73
k'(fluorene)	17.62	13.70	14.56	16.39	12.80	8.89	9.63	9.76	10.73	14.94	11.13	9.75	7.13	5.76	7.52
k'(chlorobenzene)	6.25	4.95	5.44	6.00	4.77	3.08	3.98	3.86	3.76	5.44	3.81	3.39	2.79	2.31	2.69
k'(fluoranthene)	30.72	22.89	24.93	30.31	23.52	12.79	14.92	14.96	18.55	26.72	19.62	16.54	11.88	8.89	12.41
k'(phenol)	0.88	0.88	0.87	0.91	1.25	0.81	0.84	0.86	0.83	0.78	0.45	0.54	0.51	0.50	0.54
k'(naphthaline)	9.07	7.22	7.77	7.82	6.86	5.40	5.53	5.42	5.74	7.79	5.58	4.99	3.94	3.27	4.07
k'(anthracene)	23.45	17.72	19.17	20.81	17.63	10.72	12.00	11.81	14.46	20.73	14.99	12.69	9.23	7.15	9.77
k'(toluene)	6.32	5.01	5.44	5.48	4.59	4.14	3.92	3.71	3.55	5.44	3.91	3.44	2.77	2.25	2.59
k'(propylbenzene)	17.61	13.48	14.21	14.51	11.34	10.10	9.08	8.52	7.72	14.43	11.32	9.62	6.91	5.14	6.06
k'(butylbenzene)	33.83	22.56	23.52	24.18	18.30	15.97	13.95	12.95	11.41	23.99	19.71	16.45	11.17	7.83	9.35
k'(acetophenone)	1.72	1.64	1.65	1.47	1.54	1.40	1.48	1.43	1.41	1.46	0.94	1.03	0.93	0.86	1.05
k'(butyl benzoate)	14.21	11.86	11.90	11.35	9.67	8.72	8.06	7.50	7.02	11.33	8.63	8.24	5.95	4.51	5.44
k'(biphenyl)	15.06	12.01	12.60	12.49	10.69	8.59	8.76	8.29	9.24	12.45	9.35	8.43	6.29	5.16	6.67
k'(4-nitrotoluene)	4.07	3.74	3.74	3.46	3.47	2.96	3.14	3.06	3.55	3.39	2.28	2.43	2.05	1.86	2.40
k'(ethylbenzoate)	4.86	4.26	4.37	4.05	3.77	3.45	3.39	3.14	3.20	3.97	2.82	2.83	2.32	1.93	2.29
k'(ethylbenzene)	10.23	8.03	8.56	8.59	7.02	6.42	5.90	5.32	5.23	8.40	6.29	5.61	4.28	3.37	3.95
k'(N.N-dimethylaniline)	5.08	4.27	4.50	4.31	3.86	3.48	3.62	3.20	3.53	4.24	3.04	2.90	2.34	2.07	2.49
k'(m-cresol)	1.31	1.30	1.26	1.16	1.79	1.20	1.17	1.14	1.13	1.14	0.69	0.80	0.72	0.68	0.76
k'(o-cresol)	1.48	1.45	1.43	1.31	2.00	1.34	1.30	1.26	1.26	1.29	0.79	0.91	0.81	0.77	0.85
k'(p-cresol)	1.31	1.28	1.26	1.16	1.79	1.19	1.16	1.11	1.13	1.14	0.69	0.80	0.72	0.68	0.75
k'(2.6-dimethylphenol)	2.44	2.29	2.28	2.11	2.73	2.02	1.96	1.85	1.88	2.08	1.33	1.48	1.26	1.16	1.31
k'(2.6-dimethylaniline)	2.26	2.08	2.11	1.94	1.99	1.81	1.86	1.72	1.89	1.91	1.25	1.35	1.17	1.11	1.28
k'(nitrobenzene)	2.54	2.37	2.42	2.18	2.28	2.05	2.15	1.98	2.36	2.14	1.38	1.51	1.36	1.27	1.58
k'(dimethyl phthalate)	1.79	1.79	1.73	1.49	1.55	1.58	1.64	1.50	1.74	1.46	0.96	1.12	1.01	0.95	1.17
k'(diethyl phthalate)	4.26	4.08	3.88	3.46	3.24	3.48	3.37	2.96	3.28	3.39	2.38	2.66	2.15	1.91	2.36
k'(1-phenylethanol)	0.99	1.00	0.97	0.87	1.06	0.95	0.91	0.85	0.89	0.86	0.53	0.61	0.56	0.53	0.60
k'(pyrene)	33.46	24.29	26.96	29.86	25.77	13.77	15.49	12.75	19.41	29.18	21.73	17.67	12.64	9.24	13.00
k'(chrysene)	51.59	37.32	40.63	47.78	39.65	18.68	22.60	18.30	31.11	46.58	33.88	27.32	18.82	13.36	19.79

Conclusion

The LSER method allows the characterisation of HPLC phases. The retention factor is attributed to multiple interactions between analyte and sorbent under the selected chromatographic conditions. This improves the understanding of the interactions between analyte and sorbent. If the LSER parameters (E, S, A, B, V) of the analytes are known from literature, their retention factors can be at least estimated. But even without knowledge of these parameter, the understanding of the interactions may facilitate the choice of a stationary phase.

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