Supporting Information

Role of electrostatic contributions in the separation of peptides with silica hydride stationary phases

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This supporting information contains an expanded description of experimental procedures as well as supplementary experimental results.

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Supporting Information Related To Experimental Procedures

Calculation of peptide charges. The total charge of a peptide (q_{tot}) as a function of pH can be defined, in the absence of any mobile phase effects due to the choice of a specific ACN content or buffer composition, as the sum of all positive charges (q^+) and all negative charges (q^-) of the individual ionizable functionalities in the peptide, *e.g.* $q_{tot} = \sum_{i=1}^{m} q_i^+ + \sum_{j=1}^{n} q_j^-$. The effective peptide charge (q_{eff}) at a specific mobile phase pH value can then be determined using the *Peptide Charge* software from the average values of q_{tot} and q^+ (or q^-) so obtained.

In contrast to the use of q_{eff}, the use of the total calculated charge, q_{tot}, is not suitable for the calculation of ΔG_{elec} since q_{tot} does not discriminate between peptides without any charge to those with the same number of positive and negative charges. Because peptides can conformationally orient themselves to promote retention,[1] two extreme cases were thus considered for their interactive behavior with the DH phase, namely attraction of positively charged peptides and repulsion of negatively charged peptides, *i.e.* when there is no contribution of negative charges to the retention (assuming no repulsion force occurs using q^+ for charge calculation) and when there is 100% contribution of those negative charges (using q_{tot} for charge calculation). The q_{eff} value was calculated from the average values of these two extreme cases.

Supporting Information Related To Additional Results

It can be noted that negative values of the correlation coefficients (*s*, *a* or *b*) mean that a decrease in retention is favored due to an increase in peptide-mobile phase interactions. In these circumstances, the magnitude of the mobile phase parameters related to dipolarity (π_m^*), hydrogen bonding acidity (α_m) and hydrogen bonding basicity (β_m) will also reflect the extent by which ACN in water is able to form solvent clusters.[2] Plots of the calculated and experimentally obtained log *k* values of the

peptides with mobile phases of different ACN content are shown in Figure S-2A-D, with R^2 being close to 1, as shown in Table S3. Consistent with the observation that peptides containing less polar amino acids tend to elute early in conventional aqueous normal phase chromatography,[3] peptide ***1*** with a less polar amino acid composition and with a lower q_{tot} eluted earlier than peptide ***2*** with ACN mobile phases containing 0.1% (v/v) FA (Figure 1). However, for peptides ***3***-***6*** in the presence of 0.1% (v/v) FA, the retention order was peptide ***6*** > ***4*** > ***3*** > ***5*** (Figure 1). Consequently, in these cases, the observed elution order did not follow the magnitude of the calculated total peptide charge, q_{tot}, as a function of pH (Figure S-1), confirming that the influence of the ACN content on the pK_a's of the individual ionizable functionalities of the peptides and thus the hydrogen bonding basicity parameter, *b*, need to be taken into account.

Table SI-1. Calculated pH of the mobile phases employed, approximated using the Peakmaster 5.2 software with pK_a correction.[4]

% (v/v) Acetonitrile	Calculated pH with additive			
	0.1% (v/v) FA	0.2% (v/v) AA	0.1% (v/v) AA	0.05% (v/v) AA
0	2.68	3.11	3.26	3.42
10	2.81	3.27	3.42	3.57
20	2.90	3.38	3.53	3.69
30	3.00	3.50	3.65	3.80
40	3.11	3.62	3.77	3.93
50	3.24	3.78	3.93	4.08
60	3.42	3.97	4.12	4.27

Table SI-2. Dependence of the values of the solvatochromic solvent descriptors on the ACN content for various aqueous/ACN mixtures.

% (v/v) Acetonitrile	π^*_m	α_m	β_m
80	0.80	0.85	0.59
70	0.82	0.91	0.59
65	0.85	0.91	0.59
60	0.87	0.91	0.60
50	0.92	0.91	0.60
40	0.95	0.91	0.61
30	0.97	0.94	0.61
20	1.05	0.98	0.61
10	1.10	1.06	0.59

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69 **Table SI-3.** Multi-parametric relationships between log *k* of peptides ***1-6*** and the relevant
70 descriptors of the mobile phase contributions (π^*_m, α_m and β_m) with the different (log k)$_0$ values and
71 correlation coefficients *s*, *a* and *b* derived according to the corresponding LSER Eqn 1, using using
72 least chi square curve-fitting procedures.

Mobile phase	Analyte	Multiparametric relationships	R^2	Slope
0.10% (v/v) FA	peptide *1*	log k = 0.05 - 2.88π^*_m + 2.48α_m + 0.08β_m	0.967	1.033
	peptide *2*	log k = -0.08 - 2.98π^*_m + 2.65α_m + 0.24β_m	0.975	1.047
	peptide *3*	log k = 5.20 - 5.37π^*_m + 3.31α_m - 5.20β_m	0.955	1.163
	peptide *4*	log k = 5.56 - 5.68π^*_m + 3.54α_m - 5.65β_m	0.954	1.155
	peptide *5*	log k = 4.89 - 5.50π^*_m + 3.61α_m - 4.97β_m	0.949	1.186
	peptide *6*	log k = 6.63 - 5.44π^*_m + 3.32α_m - 6.93β_m	0.954	1.140
0.20% (v/v) AA	peptide *1*	log k = 6.97 - 0.97π^*_m - 1.72α_m - 7.51β_m	0.997	0.997
	peptide *2*	log k = 8.62 - 0.78π^*_m - 2.29α_m - 9.44β_m	0.989	1.000
	peptide *3*	log k = 16.40 - 1.89π^*_m - 4.10α_m - 16.95β_m	0.980	1.033
	peptide *4*	log k = 19.15 - 1.57π^*_m - 5.35α_m - 19.88β_m	0.978	1.028
	peptide *5*	log k = 18.08 - 1.76π^*_m - 4.92α_m - 18.55β_m	0.974	1.004
	peptide *6*	log k = 20.72 + 1.30π^*_m - 8.35α_m - 22.36β_m	0.996	1.004
0.10% (v/v) AA	peptide *1*	log k = 7.81 - 0.65π^*_m - 2.20α_m - 8.66β_m	0.948	0.978
	peptide *2*	log k = 9.36 - 0.88π^*_m - 2.48α_m - 10.13β_m	0.975	0.997
	peptide *3*	log k = 17.54 - 1.74π^*_m - 4.61α_m - 18.14β_m	0.971	1.000
	peptide *4*	log k = 20.28 - 1.75π^*_m - 5.55α_m - 20.97β_m	0.958	0.995
	peptide *5*	log k = 19.30 - 1.03π^*_m - 6.13α_m - 19.73β_m	0.959	1.000
	peptide *6*	log k = 23.36 + 1.73π^*_m - 9.54α_m - 25.37β_m	0.999	1.001
0.05% (v/v) AA	peptide *1*	log k = 8.84 - 0.39π^*_m - 2.71α_m - 9.88β_m	0.978	0.984
	peptide *2*	log k = 10.72 - 0.15π^*_m - 3.46α_m - 11.92β_m	0.972	0.904
	peptide *3*	log k = 18.74 + 1.52π^*_m - 7.87α_m - 20.14β_m	0.986	1.000
	peptide *4*	log k = 21.14 + 1.83π^*_m - 9.04α_m - 22.56β_m	0.989	1.000
	peptide *5*	log k = 20.28 + 1.53π^*_m - 8.60α_m - 21.47β_m	0.989	1.000
	peptide *6*	log k = 24.77 + 2.34π^*_m - 10.40α_m - 27.09β_m	0.988	0.998

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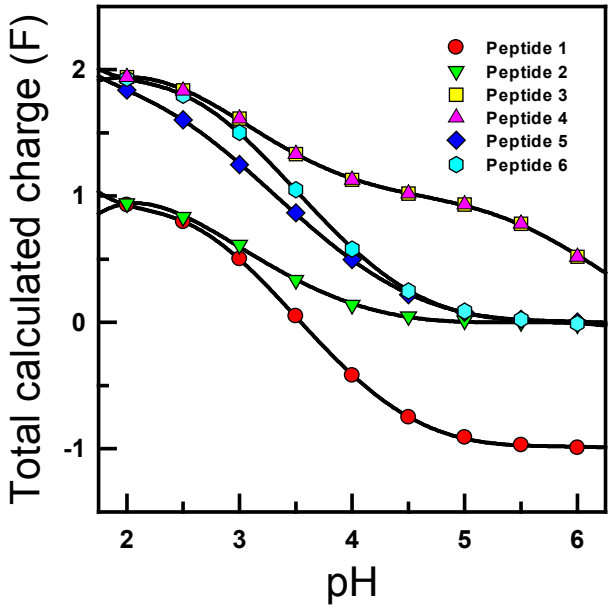
Table SI-4. Modified multi-parametric relationships between log *k* and including the correction of the electrostatic contribution (log k_{corr}) of peptide ***1-6*** and the descriptors of the mobile phases contributions (π_m^*, α_m and β_m) with different $(\log k^*)_0$ values and correlation coefficients *s*, *a* and *b* derived according to the corresponding LSER Eqn 7 using least chi square curve-fitting procedures.

Mobile phase	Analyte	Modified multiparametric relationships	R^2	Slope
0.10% (v/v) FA	peptide *1*	$\log k_{corr} = -4.35 - 1.96\pi_m^* + 2.37\alpha_m + 5.94\beta_m$	0.947	1.016
	peptide *2*	$\log k_{corr} = -3.42 - 1.27\pi_m^* + 1.50\alpha_m + 4.66\beta_m$	0.971	1.003
	peptide *3*	$\log k_{corr} = -7.12 - 4.93\pi_m^* + 5.44\alpha_m + 10.88\beta_m$	0.957	0.960
	peptide *4*	$\log k_{corr} = -6.68 - 5.22\pi_m^* + 5.63\alpha_m + 10.32\beta_m$	0.954	0.959
	peptide *5*	$\log k_{corr} = -3.00 - 3.67\pi_m^* + 3.38\alpha_m + 5.24\beta_m$	0.958	0.955
	peptide *6*	$\log k_{corr} = -1.44 - 2.28\pi_m^* + 1.57\alpha_m + 3.27\beta_m$	0.973	1.064
0.20% (v/v) AA	peptide *1*	$\log k_{corr} = 6.47 - 0.08\pi_m^* - 2.43\alpha_m - 7.20\beta_m$	0.984	0.997
	peptide *2*	$\log k_{corr} = 5.82 + 0.19\pi_m^* - 2.54\alpha_m - 6.25\beta_m$	0.986	1.003
	peptide *3*	$\log k_{corr} = 5.64 - 1.80\pi_m^* + 1.07\alpha_m - 7.93\beta_m$	0.974	1.025
	peptide *4*	$\log k_{corr} = 6.80 - 1.66\pi_m^* + 0.02\alpha_m - 8.26\beta_m$	0.970	1.013
	peptide *5*	$\log k_{corr} = 8.89 - 0.43\pi_m^* - 3.57\alpha_m - 8.17\beta_m$	0.981	1.016
	peptide *6*	$\log k_{corr} = 9.45 + 0.46\pi_m^* - 4.75\alpha_m - 8.54\beta_m$	0.984	0.987
0.10% (v/v) AA	peptide *1*	$\log k_{corr} = 5.69 - 1.29\pi_m^* - 1.18\alpha_m - 5.88\beta_m$	0.951	0.997
	peptide *2*	$\log k_{corr} = 4.49 - 1.69\pi_m^* - 0.60\alpha_m - 3.99\beta_m$	0.985	0.999
	peptide *3*	$\log k_{corr} = 5.17 - 1.40\pi_m^* - 1.42\alpha_m - 3.90\beta_m$	0.973	1.010
	peptide *4*	$\log k_{corr} = 7.14 - 1.00\pi_m^* - 2.60\alpha_m - 5.75\beta_m$	0.967	0.990
	peptide *5*	$\log k_{corr} = 6.64 - 1.70\pi_m^* - 2.21\alpha_m - 4.43\beta_m$	0.977	1.000
	peptide *6*	$\log k_{corr} = 9.63 - 1.33\pi_m^* - 3.44\alpha_m - 7.87\beta_m$	1.000	1.000
0.05% (v/v) AA	peptide *1*	$\log k_{corr} = 8.69 - 0.92\pi_m^* - 2.40\alpha_m - 9.45\beta_m$	0.990	1.004
	peptide *2*	$\log k_{corr} = 7.31 - 0.67\pi_m^* - 2.32\alpha_m - 7.55\beta_m$	0.997	0.999
	peptide *3*	$\log k_{corr} = 4.67 - 0.04\pi_m^* - 2.53\alpha_m - 3.44\beta_m$	0.985	1.001
	peptide *4*	$\log k_{corr} = 5.64 - 0.13\pi_m^* - 3.02\alpha_m - 3.91\beta_m$	0.989	1.001
	peptide *5*	$\log k_{corr} = 9.33 - 0.75\pi_m^* - 3.62\alpha_m - 8.08\beta_m$	0.992	1.000
	peptide *6*	$\log k_{corr} = 10.99 - 2.53\pi_m^* - 2.67\alpha_m - 9.17\beta_m$	0.999	0.998

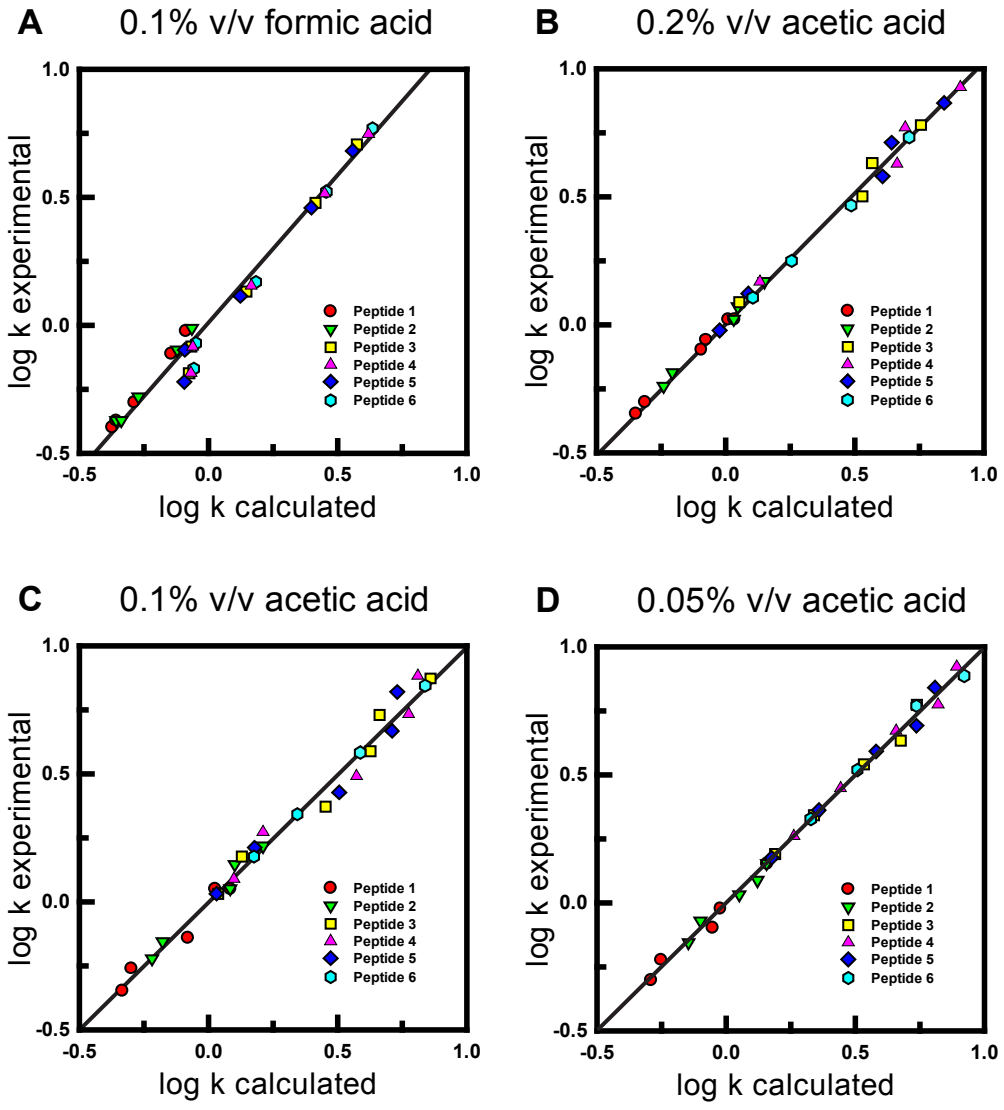
Supporting Information Related to the Total Calculated Charges (Q_{tot}) of Different Peptides as a Function of pH

Figure SI-1. Plots of the total calculated charges (q_{tot}) of different peptides (red ●, green ▼, yellow ■, pink □, dark blue ◆ and light blue ● for peptide ***1-6***, respectively) as a function of pH, covering the range of mobile phase pH values used in this study (Table SI-1) and including the pK_a values of the peptide functionalities. F = faraday (unit of charge). Since peptides ***3*** and ***4*** are isomers, they shared the same calculated q_{tot} charge value.



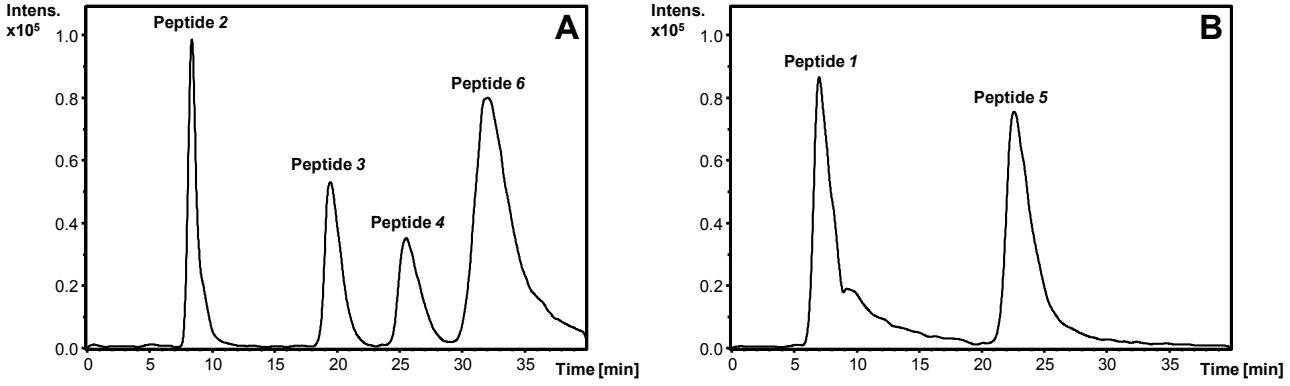
Supporting Information Related to the Correlations between the Calculated Log *K* Values and the Corresponding Experimentally Obtained Log *k* Values for the Peptide *1-6*

Figure SI-2. Correlations between the calculated log *k* values derived from the multi-parametric relationships (as defined in Equation 1 and shown in Table S-3) and the corresponding experimentally obtained log *k* values for the peptide ***1-6*** (red ●, green ▼, yellow ■, pink □, dark blue ♦ and light blue ● for peptide ***1-6***, respectively) using aqueous/ACN mobile phases with different ACN contents and acid additives, A: 0.1% (v/v) formic acid (with 10, 20, 30, 40, 50 and 60% (v/v) of ACN), B: 0.2% (v/v) acetic acid, C: 0.1% (v/v) acetic acid and D: 0.05% (v/v) acetic acid (all with 10, 20, 30, 40, 45 and 50% (v/v) of ACN).



Supporting Information Related to the chromatogram and peak symmetry of the Peptide *1-6*

Figure SI-3. Isocratic separation of peptides ***2***, ***3***, ***4*** and ***6*** (A) and peptide ***1*** and ***5*** by using the Diamond Hydride stationary phase with the mobile phase containing 40% (v/v) ACN and 0.1% (v/v) AA.



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Abbreviations: **AA**, acetic acid; **ACN**, acetonitrile; **AF**, ammonium formate; **ANP**, aqueous normal-phase; **DH**, Diamond Hydride™; **EOF**, electro-osmotic flow; **ESI**, electrospray ionization; **FA**, formic acid; **HILIC,** hydrophilic interaction liquid chromatography; **HR/MAS NMR**, high-resolution/magic-angle-spinning nuclear magnetic resonance; **LC**, liquid chromatography; **LSER**, linear solvation energy relationships; **RP**, reversed-phase.

Symbols:

a	Correlation coefficient of analyte contribution to solute-mobile phase interactions related to hydrogen bonding acidity
$a_{\text{(particle)}}$	Radius of the particles
α_m	Parameter describing the mobile phase contribution to solute-mobile phase interactions related to hydrogen bonding acidity
α_s	Parameter describing the stationary phase contribution to solute-stationary

		phase interactions related to hydrogen bonding acidity
b		Correlation coefficient of analyte contribution to solute-mobile phase interactions related to hydrogen bonding basicity
β_m		Parameter describing the mobile phase contribution to solute-mobile phase interactions related to hydrogen bonding basicity
β_s		Parameter describing the stationary phase contribution to solute-stationary phase interactions related to hydrogen bonding basicity
ΔG_{tot}		Total free energy change associated with retention
ΔG_{corr}		Free energy change associated with retention contributed from non-electrostatic interactions
ΔG_{elec}		Free energy changes associated with retention contributed from electrostatic interactions
$\%\Delta G_{elec}$		Percent contribution towards spontaneous retention due to electrostatic interactions
E		Electrostatic energy
ε		Dielectric constant
F		Faraday constant
$f(\kappa a)$		Henry's function
k		Retention factor
k_{corr}		Retention factor arising from all non-electrostatic interactions
k_{elec}		Retention factor contributions arising from all electrostatic interactions
κ		Inverse Debye length
$(\log k)_0$		Intercept of the regression in the multi-parametric analysis as defined in Equation 1

$(\log k^*)_0$	Intercept of the regression in the multi-parametric analysis as defined in Equation 7
η	Viscosity
π_m^*	Parameter describing the mobile phase contribution to solute-mobile phase interactions related to dipolarity/polarizability
π_s^*	Parameter describing the stationary phase contribution to solute-stationary phase interactions related to dipolarity/polarizability
q^+	Sum of all positive charges on a peptide
q_{eff}	Effective peptide charge
q_{tot}	Total charge of a peptide
q^-	Sum of all negative charges on a peptide
R	Universal gas constant
s	Correlation coefficient derived from the multi-parametric analysis due to solute-mobile phase interactions related to dipolarity/polarizability
T	Absolute temperature
u_e	Mobility of particles
φ	Phase ratio
ζ	Zeta potential