

# Effect of solvent strength and temperature on retention for a polar-endcapped, octadecylsiloxane-bonded silica stationary phase with methanol–water mobile phases

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## Abstract

Synergi™ Hydro-RP is a new type of polar-endcapped, octadecylsiloxane-bonded silica packing for reversed-phase liquid chromatography. Its retention properties as a function of solvent strength and temperature are evaluated from the change in retention factors over the composition range (0–70% v/v methanol) and temperature range (25–65 °C) using the solvation parameter model and response surface methodologies. The main factors that affect retention are solute size and hydrogen-bond basicity, with minor contributions from solute hydrogen-bond acidity, dipole-type and electron lone pair interactions. Within the easily accessible range for both temperature and solvent strength, the ability to change selectivity is much greater for solvent strength than temperature. Also, a significant portion of the effect of increasing temperature is to reduce retention without changing selectivity. Response surfaces for the system constants are smooth and non-linear, except for cavity formation and dispersion interactions (*v* system constant), which is linear. Modeling of the response surfaces suggests that solvent strength and temperature are not independent factors for the *b*, *s* and *e* system constants and for the model intercept (*c* term). © 2004 Elsevier B.V. All rights reserved.

**Keywords:** Solvent strength; Temperature effects; Stationary phases, LC; Mobile phase composition; Solvation parameter model

## 1. Introduction

Solvent type, solvent strength and temperature are the common factors used to control retention in isocratic reversed-phase liquid chromatography [1,2]. Solvent strength and temperature are explored in this report for a new type of stationary phase containing polar endcapping groups, introduced to facilitate separations with water as a mobile phase [3]. Our purpose is two-fold: to characterize the system properties for methanol–water as a mobile phase in a way that allows a direct comparison to other types of octadecylsiloxane-bonded stationary phases; and to elucidate the effect of solvent strength and temperature and their possible interactions on the retention mechanism. Two tools are used for this purpose. The solvation parameter model is used

to identify contributions from defined intermolecular interactions to the retention mechanism [1,4,5] and response surface methodology to determine the effect of solvent strength and temperature on the individual system constants of the solvation parameter model (i.e., the intermolecular interactions responsible for retention) [6–8].

The change in retention with solvent strength for a binary mobile phase in reversed-phase liquid chromatography is generally adequately described by:

$$\log k = \log k_w + a_1\phi + a_2\phi^2 \quad (1)$$

and if only a limited range of mobile phase compositions is considered by:

$$\log k = \log k_w + S_i\phi \quad (2)$$

where *k* is the retention factor, *k<sub>w</sub>* the retention factor for water as the mobile phase, *φ* the volume fraction of organic solvent, *S<sub>i</sub>* the slope of the experimental data after fitting to a linear

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regression model, sometimes taken as a general measure of the elution strength of the organic solvent, and  $a_1$  and  $a_2$  are regression constants for the second order model, which are not usually assigned any physical significance [1,5,9–11]. For a given mobile phase composition the effect of temperature on retention is usually described by the van't Hoff relationship:

$$\log k = A_i + \left( \frac{B_i}{T_K} \right) \quad (3)$$

or sometimes by empirical relationships of the general form

$$\log k = a_0 - a_1 T \quad (4)$$

if linear, or with the addition of further terms containing  $T$  (e.g.  $T^2$ ,  $\log T$ , etc.) if non-linear [1,12–14]. In the above relationships, the coefficients  $A_i$  and  $B_i$  have a thermodynamic meaning whereas  $a_0$ ,  $a_1$ , etc., are fitting constants, and are not usually assigned any physical significance.  $A_i$  is related to the standard partial molar enthalpy of transfer of solute  $i$  from the mobile phase to the stationary phase and  $B_i$  to the standard partial molar entropy of transfer of solute  $i$  from the mobile phase to the stationary phase and the phase ratio of the chromatographic system.  $T_K$  is the system temperature in Kelvin while  $T$  is often expressed in Celsius for convenience. Although Eq. (3) is formally correct, at least for a limited temperature range, exceptions are known. Examples include, for mixed retention mechanisms [13,15]; changes in solute (or stationary phase) conformations that affect binding [15–18]; retention mechanism influenced by secondary chemical equilibria (usually) in the mobile phase [19–21]; and for systems with a temperature-dependent phase ratio [14]. A more detailed account can be found in contemporary reviews of the effect of temperature on retention in reversed-phase liquid chromatography [1,13,22].

Although it is well established that temperature is a less powerful variable for reducing retention than solvent strength, changes in retention with temperature are only weakly correlated with solvent strength [22,23]. For neutral compounds a general rule of thumb is that a 4–5 °C change in temperature has about the same affect on retention as a 1% (v/v) change in mobile phase composition for binary mobile phases [15,22]. The introduction of simulation methods for the simultaneous optimization of solvent strength and temperature were important in establishing temperature as a useful and complementary variable for method development in reversed-phase liquid chromatography [22,24,25]. Four isocratic separations at two different values of solvent strength and temperatures are sufficient to allow the prediction of  $\log k$  as a function of  $\phi$  and  $T_K$  based on Eqs. (2) and (3). The main source of error in simulations is failure of these equations to accurately model the retention surface. These errors can be minimized in a reflection technique that requires an additional experiment at the predicted optimum to assess an offset value to re-estimate the true optimum conditions [25,26]. The combination of the increased recognition of temperature as a useful experimental variable for adjusting reten-

Table 1  
Solute descriptors used in the solvation parameter models

Solute	Descriptors				
	<i>V</i>	<i>E</i>	<i>S</i>	<i>A</i>	<i>B</i>
Acetanilide	1.113	0.870	1.40	0.50	0.67
Acetophenone	1.014	0.820	1.01	0	0.48
Aniline	0.816	0.955	0.96	0.26	0.50
Anisole	0.916	0.710	0.75	0	0.29
Benzamide	0.973	0.990	1.50	0.49	0.67
Benzene	0.716	0.610	0.52	0	0.14
Benzonitrile	0.871	0.740	1.11	0	0.33
Benzophenone	1.481	1.447	1.50	0	0.50
Benzyl alcohol	0.916	0.832	0.97	0.37	0.56
Benzyl benzoate	1.680	1.264	1.42	0	0.51
Biphenyl	1.324	1.360	0.99	0	0.26
1-Bromonaphthalene	1.260	1.598	1.13	0	0.13
3-Bromophenol	0.950	1.060	1.15	0.70	0.16
Butyrophenone	1.300	0.800	0.95	0	0.51
Caffeine	1.363	1.500	1.60	0	1.33
4-Chloroaniline	0.939	1.060	1.13	0.30	0.35
1-Chloronaphthalene	1.208	1.417	1.06	0	0.13
4-Chlorophenol	0.898	0.920	1.08	0.67	0.20
Cinnamyl alcohol	1.155	1.152	0.90	0.58	0.60
Coumarin	1.062	1.060	1.79	0	0.46
3,4-Dichloroaniline	1.061	1.158	1.24	0.35	0.25
Diethyl phthalate	1.711	0.729	1.40	0	0.88
2,6-Dimethylphenol	1.057	0.860	0.79	0.39	0.39
<i>N</i> -Ethylaniline	1.099	0.945	0.88	0.17	0.51
Ethylbenzene	0.998	0.613	0.51	0	0.15
Fluorene	1.357	1.588	1.06	0	0.25
Hexanophenone	1.580	0.720	0.95	0	0.50
4-Hydroxybenzyl alcohol	0.975	0.998	1.15	0.88	0.85
4-Hydroxybenzaldehyde	0.932	1.010	1.54	0.79	0.40
2-Methoxynaphthalene	1.285	1.390	1.13	0	0.35
2-Methylphenol	0.916	0.840	0.86	0.52	0.30
4-Methylphenol	0.916	0.820	0.87	0.57	0.31
Naphthalene	1.085	1.340	0.92	0	0.20
1-Naphthol	1.144	1.520	1.05	0.61	0.37
2-Naphthol	1.144	1.520	1.08	0.61	0.40
2-Nitroaniline	0.990	1.180	1.37	0.30	0.36
4-Nitroaniline	0.990	1.220	1.91	0.42	0.38
Nitrobenzene	0.891	0.871	1.11	0	0.28
4-Nitrobenzyl alcohol	1.090	1.064	1.39	0.44	0.62
4-Nitrotoluene	1.032	0.870	1.11	0	0.28
Octanophenone	1.859	0.720	0.95	0	0.50
Phenanthrene	1.454	2.055	1.29	0	0.26
Phenol	0.775	0.810	0.89	0.60	0.30
2-Phenylethanol	1.057	0.811	0.91	0.30	0.65
4-Phenylphenol	1.383	1.560	1.41	0.59	0.45
Progesterone	2.620	1.450	3.30	0	1.10
Propriophenone	1.160	0.800	0.95	0	0.51
Propylbenzene	1.139	0.604	0.50	0	0.15
Toluene	0.857	0.601	0.52	0	0.14
<i>m</i> -Toluidine	0.957	0.946	0.95	0.23	0.55
1,2,3-Trimethylbenzene	1.139	0.728	0.61	0	0.19
Valerophenone	1.440	0.800	0.95	0	0.50

tion in reversed-phase liquid chromatography and the availability of stationary phases with improved thermal stability has resulted in increased interest in high temperature liquid chromatography [13], pressurized hot water chromatography [27–29], thermally tuned series coupled columns [30] and temperature-programmed separations using small diam-

eter columns [31]. These latter developments are beyond the scope of this work and are mentioned here for completeness and to illustrate the heightened interest in temperature as a separation variable in reversed-phase liquid chromatography.

The solvation parameter model in a form suitable for describing retention in reversed-phase liquid chromatography is set out below [1,4,32]:

$$\log k = c + eE + sS + aA + bB + vV \quad (5)$$

The model equation is made up of product terms representing solute properties (descriptors), indicated by capital letters, and the complementary properties characteristic of the separation system, indicated by the lower case letters in italics. Each product term defines the relative contribution of a specified intermolecular interaction to the correlated property, in this case  $\log k$ . The contribution from electron lone pair interactions is defined by  $eE$ , interactions of a dipole-type by  $sS$ , hydrogen-bond interactions by  $aA$  and  $bB$ , and differences in cavity formation and dispersion interactions in the mobile and stationary phases by  $vV$ . The solute descriptors are formally defined as the excess molar refraction,  $E$ , dipolarity/polarizability,  $S$ , effective hydrogen-bond acidity,  $A$ , effective hydrogen-bond basicity,  $B$ , and McGowan's characteristic volume,  $V$ . Descriptors are available for about 4000 compounds with others accessible through calculation and estimation methods [4,32].

The system constants characterize the retention properties of the separation system. System constants with a positive sign indicate a more favorable interaction with the solvated stationary phase than the mobile phase and an increase in retention. The opposite is true for system constants with a negative sign. The system constants are defined as the difference in contributions from electron lone pair interactions,  $e$ , dipole-type interactions,  $s$ , hydrogen-bond basicity,  $a$ , hydrogen-bond acidity,  $b$ , and cohesion and dispersion interactions,  $v$ , for the mobile phase and the solvated stationary phase. The system constants are obtained by multiple linear regression analysis for a varied group of solutes selected to satisfy the statistical and chemical requirements of the model [1,4,32,33].

Table 2  
Coded variables used in the calculation of response surfaces

Methanol % (v/v)	Coded variable	Temperature (°C)	Coded variable
0	-1.0	25	-1.0
10	-0.714	35	-0.5
20	-0.571	45	0
30	-0.143	55	0.5
40	0.143	65	1.0
50	0.571		
60	0.714		
70	1.0		

## 2. Experimental

Common chemicals were reagent grade or better and obtained from several sources. Water was prepared using a Milli-Q system (Millipore, Bedford, MA, USA). The water had a pH 5.3–5.4 and a resistance of 18.2 mΩ/cm. The 150 mm × 4.6 mm i.d. Synergi<sup>TM</sup> Hydro-RP column, 4 μm particles, 8 nm pore size, was obtained from Phenomenex (Torrance, CA, USA). The column packing is based on a high purity spherical silica substrate reacted with a monomeric octadecylsilane reagent and proprietary polar endcapping reagent. The column packing has a typical surface area of 475 m<sup>2</sup>/g and a bonded phase concentration of 2.45 μmol/m<sup>2</sup> (C load 19%).

The liquid chromatograph consisted of a Waters (Milford, MA, USA) 600E multisolvent delivery system, a Waters 717 plus autosampler, an Eppendorf TC50 (Westbury, NY, USA) column heater, a Waters 486 variable wavelength absorbance detector, and a Vectra VL pentium II computer (Agilent Technologies, Wilmington, DE, USA) running Waters Millennium 32 software for instrument control and data acquisition. The mobile phase reservoir was continuously purged with helium at 1.5 ml/min. All separations were performed with a flow rate of 1.5 ml/min. The column hold-up time was determined by injection of an aqueous solution of sodium nitrate (26 mg/ml).

Multiple linear regression analysis and statistical calculations were performed on a Gateway E-4200 computer (North Sioux City, SD, USA) using the program SPSS v10.1 (SPSS, Chicago, IL, USA). The solute descriptors used to determine system properties were taken from an in-house data base and are summarized in Table 1. Response surfaces were calculated using Design-Expert v. 5.0.8 (Stat-Ease Corporation, Minneapolis, MN, USA). The experimental variables were coded with the highest value of temperature or solvent strength assigned a value of 1.0 and the lowest value -1.0 as indicated in Table 2. This simplifies calculations and interpretation of the response surfaces.

## 3. Results and discussion

Retention factors were determined for a varied group of compounds at methanol–water compositions from 0 to 70% (v/v) methanol in 10% (v/v) methanol increments and at temperatures from 25 to 65 °C in 10 °C intervals. This created a matrix of retention factors for 40 combinations of temperature and solvent strength for compounds with  $\log k$  values constrained to the range  $-0.75 < \log k < 2.6$  approximately for experimental convenience. The solutes were selected to provide a wide range of descriptor properties ( $V = 0.7$ – $2.6$ ,  $E = 0.6$ – $2.1$ ,  $S = 0.5$ – $3.3$ ,  $A = 0$ – $0.9$  and  $B = 0.1$ – $1.4$ ) with minimal cross-correlation, Table 3. The largest correlation is between the  $V$  and  $A$  solute descriptors ( $r = 0.54$ ). This is well below the range of values where cross-correlation is a likely cause of uncertainty in the calculation of the number of sys-

Table 3  
Cross-correlation matrix for solute descriptors (*r* values)

	<i>V</i>	<i>E</i>	<i>S</i>	<i>A</i>	<i>B</i>
<i>V</i>	1.00	−0.232	−0.369	0.540	−0.340
<i>E</i>	−0.232	1.00	−0.379	−0.164	0.296
<i>S</i>	−0.369	−0.379	1.00	−0.201	−0.412
<i>A</i>	0.540	−0.164	−0.201	1.00	−0.210
<i>B</i>	−0.340	0.296	−0.412	−0.210	1.00

tem constants. At each combination of solvent strength and temperature the solvation parameter model was used for data reduction and interpretation. This allowed the retention data to be expressed by a smaller number of factors corresponding to defined intermolecular interactions with the results summarized in Table 4. The statistics for the fit of each solvation parameter models are acceptable with multiple correlation coefficients from 0.978 to 0.994, standard errors of the estimate 0.07 to 0.15 and Fischer *F*-statistics 150–725.

Table 4  
Variation of the system constants with methanol-water composition and temperature for the Synergi<sup>®</sup> Hydro-RP column<sup>a</sup>

Methanol % (v/v)	Temperature (°C)	System constants						Statistics <sup>b</sup>			
		<i>v</i>	<i>e</i>	<i>s</i>	<i>a</i>	<i>b</i>	<i>c</i>	$\rho$	SE	<i>F</i>	<i>n</i>
10	25	3.64 (0.20)	0.29 (0.11)	−0.58 (0.06)	−0.59 (0.07)	−1.99 (0.09)	−0.46 (0.14)	0.986	0.093	191	34
20	25	3.18 (0.18)	0.34 (0.095)	−0.67 (0.06)	−0.58 (0.06)	−2.13 (0.08)	−0.29 (0.13)	0.990	0.086	311	36
30	25	2.81 (0.085)	0.36 (0.06)	−0.71 (0.05)	−0.58 (0.05)	−2.05 (0.07)	−0.24 (0.08)	0.993	0.080	560	43
40	25	2.50 (0.07)	0.39 (0.05)	−0.71 (0.05)	−0.56 (0.05)	−1.95 (0.07)	−0.28 (0.07)	0.994	0.085	722	49
50	25	2.14 (0.06)	0.36 (0.05)	−0.61 (0.05)	−0.58 (0.06)	−1.79 (0.07)	−0.37 (0.06)	0.993	0.091	674	54
60	25	1.69 (0.05)	0.37 (0.04)	−0.61 (0.05)	−0.52 (0.05)	−1.49 (0.06)	−0.36 (0.06)	0.993	0.075	612	52
70	25	1.37 (0.05)	0.31 (0.04)	−0.56 (0.05)	−0.50 (0.05)	−1.24 (0.06)	−0.44 (0.06)	0.991	0.075	498	52
10	35	3.60 (0.19)	0.29 (0.11)	−0.59 (0.06)	−0.60 (0.07)	−1.97 (0.09)	−0.46 (0.14)	0.986	0.090	199	34
20	35	3.15 (0.17)	0.33 (0.09)	−0.62 (0.05)	−0.58 (0.06)	−2.07 (0.07)	−0.42 (0.13)	0.991	0.078	313	34
30	35	2.80 (0.08)	0.30 (0.08)	−0.66 (0.05)	−0.56 (0.07)	−2.07 (0.08)	−0.23 (0.08)	0.993	0.085	356	44
40	35	2.48 (0.08)	0.39 (0.06)	−0.71 (0.06)	−0.55 (0.06)	−1.97 (0.07)	−0.30 (0.09)	0.994	0.088	620	46
50	35	2.15 (0.05)	0.33 (0.05)	−0.59 (0.04)	−0.56 (0.06)	−1.81 (0.07)	−0.43 (0.06)	0.993	0.090	642	50
60	35	1.73 (0.06)	0.30 (0.05)	−0.52 (0.04)	−0.54 (0.05)	−1.52 (0.07)	−0.48 (0.06)	0.991	0.087	485	51
70	35	1.31 (0.04)	0.32 (0.04)	−0.54 (0.04)	−0.51 (0.04)	−1.19 (0.05)	−0.48 (0.06)	0.991	0.070	499	49
10	45	3.50 (0.21)	0.26 (0.11)	−0.61 (0.06)	−0.58 (0.07)	−1.91 (0.09)	−0.44 (0.16)	0.987	0.085	192	32
20	45	3.17 (0.18)	0.32 (0.09)	−0.67 (0.06)	−0.57 (0.06)	−2.08 (0.08)	−0.38 (0.14)	0.991	0.081	298	34
30	45	2.78 (0.09)	0.33 (0.07)	−0.70 (0.05)	−0.57 (0.06)	−2.01 (0.07)	−0.26 (0.07)	0.983	0.130	275	35
40	45	2.46 (0.08)	0.37 (0.06)	−0.70 (0.05)	−0.59 (0.06)	−1.92 (0.07)	−0.33 (0.08)	0.994	0.084	701	45
50	45	2.07 (0.06)	0.31 (0.05)	−0.60 (0.04)	−0.56 (0.06)	−1.69 (0.07)	−0.43 (0.06)	0.994	0.087	642	48
60	45	1.70 (0.05)	0.27 (0.04)	−0.52 (0.04)	−0.55 (0.05)	−1.45 (0.07)	−0.50 (0.06)	0.992	0.083	509	48
70	45	1.36 (0.05)	0.28 (0.04)	−0.45 (0.04)	−0.51 (0.05)	−1.15 (0.06)	−0.65 (0.05)	0.990	0.076	400	48
10	55	3.38 (0.14)	0.33 (0.15)	−0.60 (0.11)	−0.61 (0.12)	−1.87 (0.15)	−0.50 (0.18)	0.986	0.150	156	32
20	55	3.06 (0.16)	0.34 (0.09)	−0.70 (0.05)	−0.61 (0.06)	−2.04 (0.08)	−0.32 (0.12)	0.992	0.079	352	34
30	55	2.72 (0.14)	0.35 (0.08)	−0.68 (0.05)	−0.55 (0.05)	−1.96 (0.06)	−0.32 (0.10)	0.994	0.072	517	35
40	55	2.32 (0.10)	0.36 (0.06)	−0.69 (0.05)	−0.61 (0.06)	−1.85 (0.07)	−0.35 (0.09)	0.994	0.082	627	42
50	55	2.05 (0.06)	0.31 (0.05)	−0.58 (0.04)	−0.53 (0.05)	−1.65 (0.07)	−0.50 (0.06)	0.993	0.085	602	47
60	55	1.68 (0.06)	0.28 (0.04)	−0.51 (0.04)	−0.53 (0.05)	−1.40 (0.07)	−0.57 (0.07)	0.992	0.081	481	47
70	55	1.35 (0.05)	0.27 (0.04)	−0.44 (0.04)	−0.52 (0.05)	−1.15 (0.06)	−0.67 (0.05)	0.990	0.074	405	47
10	65	3.45 (0.17)	0.29 (0.11)	−0.63 (0.09)	−0.49 (0.10)	−1.93 (0.13)	−0.53 (0.15)	0.978	0.097	194	37
20	65	3.15 (0.16)	0.25 (0.10)	−0.67 (0.06)	−0.51 (0.06)	−2.05 (0.08)	−0.42 (0.11)	0.989	0.086	267	37
30	65	2.68 (0.08)	0.35 (0.06)	−0.75 (0.05)	−0.55 (0.06)	−1.98 (0.07)	−0.29 (0.07)	0.993	0.091	511	40
40	65	2.36 (0.08)	0.35 (0.06)	−0.69 (0.06)	−0.56 (0.06)	−1.87 (0.08)	−0.39 (0.08)	0.993	0.090	553	46
50	65	2.04 (0.07)	0.26 (0.05)	−0.58 (0.05)	−0.49 (0.06)	−1.63 (0.07)	−0.52 (0.06)	0.992	0.088	491	47
60	65	1.67 (0.06)	0.24 (0.04)	−0.50 (0.04)	−0.50 (0.05)	−1.42 (0.06)	−0.57 (0.05)	0.991	0.078	469	47
70	65	1.36 (0.06)	0.22 (0.04)	−0.45 (0.04)	−0.46 (0.05)	−1.15 (0.06)	−0.67 (0.05)	0.988	0.077	335	47

<sup>a</sup> Chromatographic conditions: Synergi<sup>™</sup> Hydro-RP column 150 mm × 4.6 mm;  $d_p = 4 \mu\text{m}$ ; 10  $\mu\text{l}$  injection; and UV detection at the maximum absorption wavelength for each compound.

<sup>b</sup>  $\rho$  is the multiple correlation coefficient, SE the standard error in the estimate, *F* the Fischer statistic and *n* the number of solutes. The numbers in parentheses are the standard deviations for the system constants.

### 3.1. General interpretation of system constants

A general description of the relative affect of solvent strength and temperature on the system constants of the solvation parameter model is useful to enable interpretation of the system surfaces discussed subsequently. The change in system constants near the center point of the data matrix is described first to illustrate typical trends for solvent strength and temperature and then the ability of these factors to affect the system constants over the whole factor space is discussed.

Fig. 1 illustrates the change in system constants and model constant [*c* term in Eq. (5)] at a temperature of 45° for the mobile phase composition of 0–70% (v/v) methanol. System constants with a positive sign contribute to higher retention. In this case, the *v* and *e* system constants, indicating that an increase in solute size and/or capacity for electron lone pair

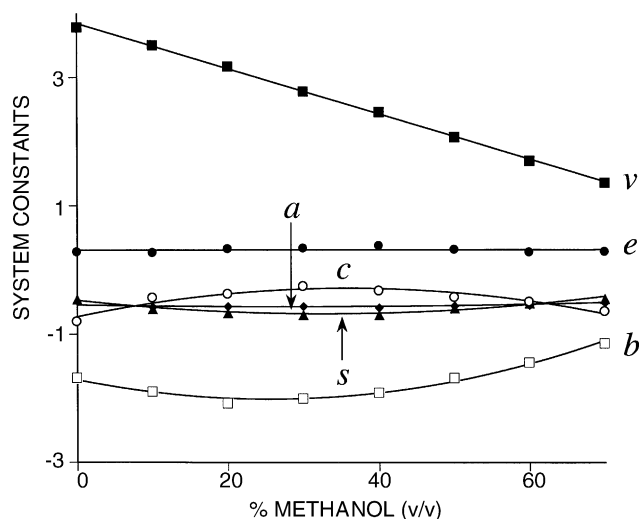


Fig. 1. Variation of the system constants and model constant with methanol composition (v/v) at a constant temperature of 45 °C. Chromatographic conditions: Synergi™ Hydro-RP column 150 mm × 4.6 mm;  $d_p = 4 \mu\text{m}$ ; 10  $\mu\text{l}$  injection; and UV detection at the maximum absorption wavelength for each compound.

interactions will increase retention at any methanol composition. System constants with a negative sign ( $s$ ,  $a$  and  $b$ ) reduce retention. That is, solutes with greater ability to enter into dipole-type and hydrogen-bonding interactions are less well retained at any methanol composition. The  $v$  system constant changes approximately linearly with methanol composition while the  $e$  system constant is only weakly affected by the composition of the mobile phase. The  $s$ ,  $a$  and  $b$  system constants change non-linearly with methanol composition with changes in the  $b$  system constant significantly larger than for the  $s$  and  $a$  system constants. In terms of relative magnitude, the  $e$ ,  $s$  and  $a$  system constants cannot be ignored for modeling retention, but over the composition range studied, retention is dominated by changes in the  $v$  and  $b$  system constants, particularly for water-rich compositions. Since water is more cohesive and hydrogen-bond acidic than methanol, this is an illustration of the dominant role of water in the reversed-phase retention mechanism. The model constant ( $c$  term) also changes smoothly and non-linearly with mobile phase composition. The main physical contribution to the model constant is the phase ratio for the separation system when the retention factor is the dependent variable for the model. However, the model constant is also affected by all sources of error, lack-of-fit and descriptor scaling, and in physical terms, its interpretation is ambiguous.

Fig. 2 illustrates the change in system constants and model constant for the 40% (v/v) methanol–water mobile phase for the temperature range 25–65 °C. Changes in the system constants and model constant are approximately linear with temperature. The small slopes are an indication that the effect of temperature on the retention mechanism is not as great as for solvent strength. Given the uncertainty in the system constants the slopes for the  $e$ ,  $s$  and  $a$  system constants are not

statistically different from zero at the 95% confidence level. The predominant affect of higher temperature, therefore, is to decrease retention by a reduction in the difference in cohesive energy between the mobile and stationary phases and to decrease the hydrogen-bond acidity of the mobile phase relative to the solvated stationary phase. These conclusions are similar to those observed for the effect of temperature on retention for a porous polymer stationary phase under reversed-phase conditions [6]. The significant change in the model constant with temperature is interesting, since this suggests that a significant portion of the effect of temperature on retention is explained by a fixed effect factor rather than selectivity changes. This was previously shown to be the case for water as a mobile phase and qualitatively explained by changes in the phase ratio of the separation system with temperature [3,28].

The above results are not too surprising. Since there is no practical means to adjust the temperature of one phase with respect to the other by varying the system temperature, the simultaneous changes in intermolecular interactions in both the mobile and stationary phases cancel each other to a significant extent. While variation in the composition of the mobile phase are not independent of changes in the solvated stationary phase (composition of active volume of the stationary phase), these changes are probably no more than proportional, and consequently, less likely to offset each other. Thus, typical changes in solvent strength are more likely to affect selectivity than typical changes in temperature. In addition, it is indicated that changes in system properties, such as the phase ratio, are important in explaining changes in absolute retention with variation in solvent strength and temperature.

### 3.2. Comparison of Synergi™ Hydro-RP to other octadecylsiloxane-bonded silica stationary phases

Although system constants for many stationary phases are now available [4,5], in most cases different mobile

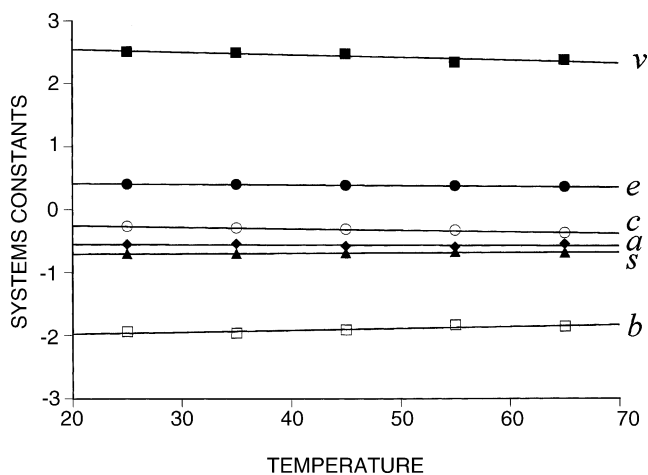


Fig. 2. Variation of the system constants and model constant with temperature (25–65 °C) at a constant mobile phase composition of 40% (v/v) methanol–water. Chromatographic conditions as for Fig. 1.



Table 5  
System constants for different octadecylsiloxane-bonded silica stationary phases for 50% (v/v) methanol–water as the mobile phase

Stationary phase	System constants					Temperature <sup>a</sup> (°C)	Reference
	<i>v</i>	<i>e</i>	<i>s</i>	<i>a</i>	<i>b</i>		
Nucleosil C18	1.78	0.20	−0.52	−0.45	−1.62	r.t.	[5]
J.T. Baker ODS	2.03 (0.12)	0.16 (0.08)	−0.40 (0.08)	−0.34 (0.06)	−1.51 (0.11)	r.t.	[35]
Chromolith RP-18e	2.10 (0.06)	0.30 (0.06)	−0.67 (0.06)	−0.41 (0.05)	−1.77 (0.07)	r.t.	[11]
Synergi <sup>TM</sup> Hydro-RP	2.14 (0.06)	0.36 (0.05)	−0.61 (0.05)	−0.58 (0.06)	−1.79 (0.07)	25	
Spherisorb ODS-2	2.14	0.36	−0.68	−0.47	−1.84	30	[5]
Supelcosil LC-ABZ	2.22 (0.09)	0.47 (0.07)	−0.59 (0.08)	0	−2.53 (0.15)	r.t.	[38]
Capcell Pak C18	2.23	0.18	−0.47	−0.76	−2.03	r.t.	[34]
Partisil ODS	2.28 (0.08)	0.44 (0.06)	−1.07 (0.09)	−0.48 (0.06)	−2.07 (0.10)	30	[37]
Nucleosil C18 (HD)	2.37	0.20	−0.38	−0.20	−2.01	r.t.	[36]
Hypersil ODS	2.46	0.17	−0.66	−0.20	−1.84	r.t.	[5]
Zorbax ODS	2.68	0.38	−0.83	−0.29	−2.17	r.t.	[5]

Values in parentheses are the standard deviations for the system constants were available.

<sup>a</sup> r.t. = room temperature (exact temperature unspecified).

phase compositions were used for their determination, and a comparison of their sorption properties is difficult. Most data are available for 50% (v/v) methanol–water and 30% (v/v) acetonitrile–water at room temperature. To facilitate a comparison of the sorption properties of the Synergi<sup>TM</sup> Hydro-RP stationary phase with other octadecylsiloxane-bonded silica stationary phases their system constants for 50% (v/v) methanol–water as mobile phase are summarized in Table 5 [5,11,34–38]. The general separation properties of the Synergi<sup>TM</sup> Hydro-RP stationary phase with 50% (v/v) methanol–water are similar to those of the Chromolith RP-18e and Spherisorb ODS-2 stationary phases. The *v*, *e*, *s* and *b* system constants for the Synergi<sup>TM</sup> Hydro-RP stationary phase lie within one standard deviation of the average value for the system constants of the octadecylsiloxane-bonded silica stationary phases in Table 5. The *a* system constant, however, is slightly larger than one standard deviation of the average value. The Synergi<sup>TM</sup> Hydro-RP stationary phase is associated with the lower extreme range of hydrogen-bond basic stationary phases. The alkylamidesiloxane-bonded silica stationary phase (Supelcosil LC-ABZ) with an embedded polar (amide) group close to the silica surface represents the other extreme of hydrogen-bond basicity. Here, it is assumed that the selective solvation of the silica surface by water attracted into the bonded phase by the amide group is responsible for its greater hydrogen-bond basicity [38]. One can speculate, therefore, that the polar endcapped technology incorporated into the Synergi<sup>TM</sup> Hydro-RP stationary phase, attracts on average less water into the active volume of the stationary phase, resulting in its low hydrogen-bond basicity. Although polar endcapping and embedding polar groups into the stationary phase structure are used to facilitate separations with water as a mobile phase, it is likely that this is achieved by different mechanisms.

There is another difference between the Supelcosil LC-ABZ and Synergi<sup>TM</sup> Hydro-RP stationary phases. The weak bases pyridine and quinoline were positive outliers for the solvation parameter model on the Synergi<sup>TM</sup> Hydro-RP stationary phase, which was not the case for the Supelcosil LC-ABZ

stationary phase. The solvation parameter model does not include any term to account for electrostatic interactions, which are presumed to affect the retention of nitrogen-containing bases on silica-based stationary phases [39,40]. The behavior of pyridine and quinoline on the Synergi<sup>TM</sup> Hydro-RP stationary phase provides an indication that the contribution of electrostatic interactions to retention may be different for polar endcapped and polar embedded functional group stationary phases. This aspect of the retention mechanism was not investigated further. The solutes used to characterize the stationary phase properties were selected to minimize contributions from interactions that are not explained by the solvation parameter model.

### 3.3. Response surfaces for the simultaneous variation of solvent strength and temperature

Response surface methodology was used to derive suitable equations to explain the simultaneous affect of solvent

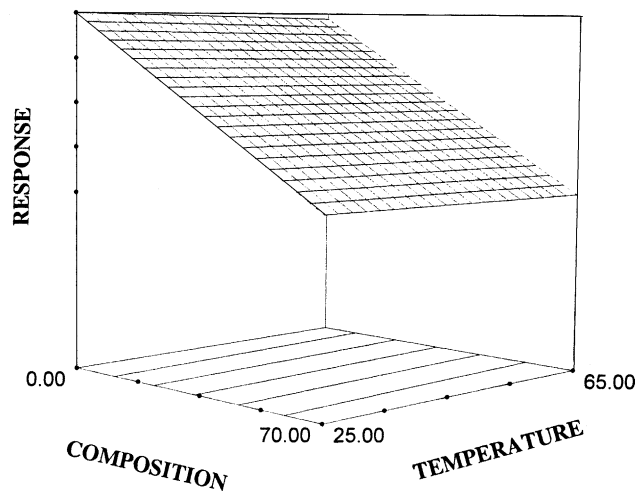


Fig. 3. Response surface for the variation of the *v* system constant with solvent strength (% v/v methanol) and temperature (°C). Chromatographic conditions as for Fig. 1.

strength and temperature on the variation of each system constant and the model constant of the solvation parameter model. These models for the response surface fall into two groups. For  $v$ ,  $b$  and  $c$ , and to a lesser extent  $s$ , the range of the variation of responses over the factor space is large compared to the average standard error for all responses. This should lead to stable quantitative models. For the  $e$  and  $a$  system constants, the range of variation of responses over the factor space is small compared to the average standard error for the responses, and the likelihood of obtaining a stable quantitative model is less certain. For the  $e$  system constant the range in response over the factor space is 0.39–0.22 (average standard error 0.08) and for the  $a$  system constant  $-0.61$  to  $-0.46$  (average standard error 0.06). We begin with an interpretation of the favorable system constants.

The response surface for the variation of the  $v$  system constant with solvent strength and temperature is a plane, Fig. 3, adequately explained by a first order model without interactions:

$$v = 2.59 - 1.23\phi - 0.043T, \quad r^2 = 0.996, \quad Q^2 = 0.995, \\ \text{PRESS} = 0.13, \quad \text{RMSE} = 0.055, \quad F = 4342 \quad (6)$$

where  $r^2$  is the coefficient of determination (an estimate of the fraction of overall variation in the data accounted for by the model),  $Q^2$  (the fraction of variation of the response that can be predicted by the model), PRESS the predicted residual sum of squares (a measure of how well the model fits each point in the data), RMSE the square root of the residual mean square error (an estimate of the standard deviation associated with the experimental error), and  $F$  value (a measure of the likelihood that the variance contributed by the model is significantly larger than the random error). Eq. (6) and subsequent response surface models are written in coded variables (see Table 2 and experimental). Solvent strength and temperature are independent factors which affect the  $v$  system constant in the same direction. An increase in either solvent strength or temperature decreases the contribution of the  $v$  system constant to retention. A one percent change in methanol composition, however, is about 16.4 times more effective than a  $1^\circ\text{C}$  change in temperature in reducing retention through a decrease in the  $v$  coefficient.

The response surface for the  $b$  system constant is non-linear, Fig. 4, and adequately fit by a reduced cubic model of the form:

$$b = -1.97 + 0.30\phi + 0.042T + 0.55\phi^2 - 0.022T^2 \\ + 0.028\phi T - 0.19\phi^3 - 0.055T^3 - 0.052\phi^2 T, \\ r^2 = 0.995, \quad Q^2 = 0.991, \quad \text{PRESS} = 0.031, \\ \text{RMSE} = 0.024, \quad F = 719 \quad (7)$$

All coefficients are statistically significant at the 95% confidence level. However, the terms containing temperature explain only a small fraction of the variation in the  $b$  response as indicated by stepwise regression, Table 6. Entering terms

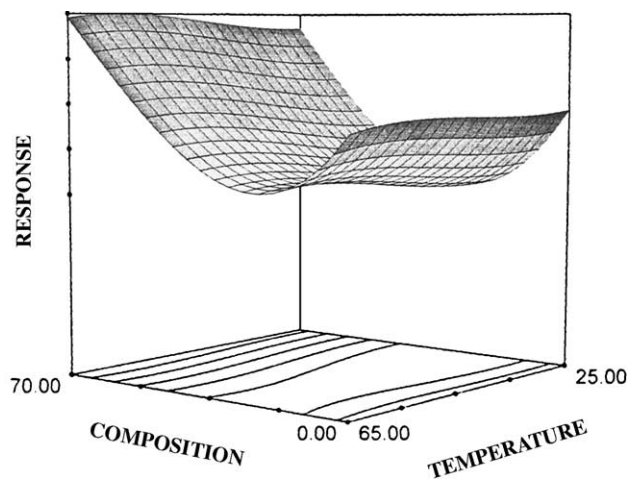


Fig. 4. Response surface for the variation of the  $b$  system constant with solvent strength (% v/v methanol) and temperature ( $^\circ\text{C}$ ). Chromatographic conditions as for Fig. 1.

containing only the volume fraction of methanol (first three variables in Table 6) accounts for 97.7% of the overall variation in the data predicted by the model. The simplest model containing temperature as a variable:

$$b = -1.98 + 0.30\phi + 0.042T + 0.55\phi^2, \quad r^2 = 0.970, \\ Q^2 = 0.961, \quad \text{PRESS} = 0.13, \quad \text{RMSE} = 0.053, \\ F = 387 \quad (8)$$

can account reasonably well for the variation of the  $b$  response, but is not as good as Eq. (7) or the cubic model containing only terms in  $\phi$  with exclusion of  $T$ . From Eq. (8) a 1% change in methanol composition is about 123 times as effective as a  $1^\circ\text{C}$  change in temperature at changing retention through a decrease in the  $b$  coefficient (i.e. the  $b$  coefficient becomes more positive).

The response surface for the  $s$  system constant is non-linear, Fig. 5, and fit reasonably well by a reduced cubic model of the form:

$$s = -0.67 + 0.096\phi + 0.21\phi^2 - 0.024T^2 + 0.048\phi T \\ - 0.098\phi^3, \quad r^2 = 0.924, \quad Q^2 = 0.898, \\ \text{PRESS} = 0.032, \quad \text{RMSE} = 0.026, \quad F = 82 \quad (9)$$

Table 6  
Contribution of the terms of the reduced cubic model in order of entry to the variation of the  $b$  system constant

Model term	Coefficient	$r^2$	RMSE
Intercept	-1.97		
$\phi^2$	0.55	0.505	0.210
$\phi$	0.30	0.960	0.061
$\phi^3$	-0.19	0.977	0.046
$T$	0.042	0.988	0.034
$\phi^2 T$	-0.052	0.990	0.032
$\phi T$	0.028	0.992	0.029
$T^3$	-0.055	0.994	0.026
$T^2$	-0.022	0.995	0.024

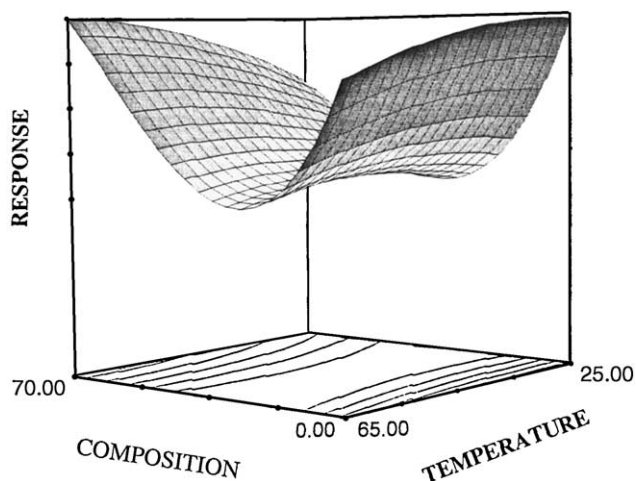


Fig. 5. Response surface for the variation of the *s* system constant with solvent strength (% v/v methanol) and temperature (°C). Chromatographic conditions as for Fig. 1.

The  $\phi T$  term is important in explaining the variation of the response and indicates a cooperative interaction between the solvent strength and temperature. The absence of a term in  $T$  and the small coefficient for the term in  $T^2$  suggests that temperature is not as important as solvent strength in regulating the *s* system constant.

The response surface for the *a* system constant is non-linear, Fig. 6, and fit approximately by a reduced cubic model of the form:

$$a = -0.57 + 0.026T^2 + 0.057\phi^3 + 0.024T^3, \quad r^2 = 0.850, \\ Q^2 = 0.809, \quad \text{PRESS} = 0.007, \quad \text{RMSE} = 0.013, \\ F = 58 \quad (10)$$

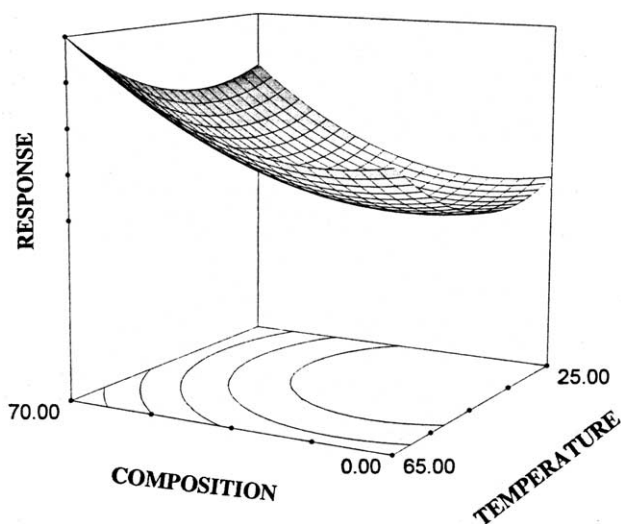


Fig. 6. Response surface for the variation of the *a* system constant with solvent strength (% v/v methanol) and temperature (°C). Chromatographic conditions as for Fig. 1.

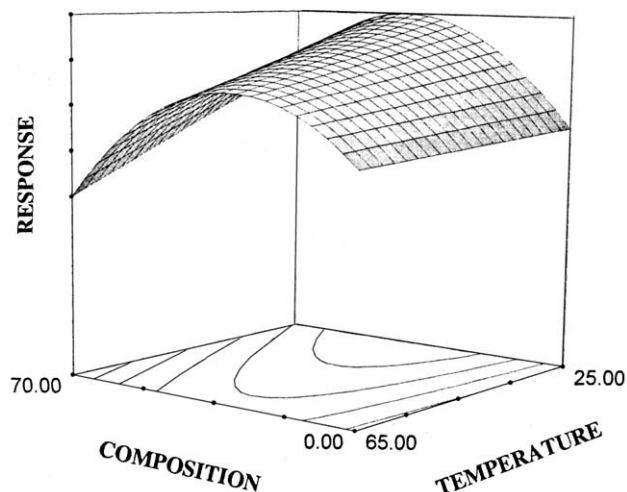


Fig. 7. Response surface for the variation of the *e* system constant with solvent strength (% v/v methanol) and temperature (°C). Chromatographic conditions as for Fig. 1.

The small change in hydrogen-bond basicity with variation of the solvent strength and temperature results in poor modeling potential. Eq. (10) fits the experimental points quite well as indicated by the small PRESS and RMSE values with the modest  $r^2$  and  $Q^2$  values an indication that the range of values for the *a* coefficient is not much larger than the average standard error in the *a* coefficients. Our best estimate is that a 1% change in methanol composition is about 20 times as effective as a 1 °C change in temperature at changing the *a* system constant, but overall, the *a* system constant is not strongly dependent on the solvent strength or temperature for the factor space explored.

The response surface for the *e* system constant is non-linear, Fig. 7, and fit approximately by a reduced quadratic

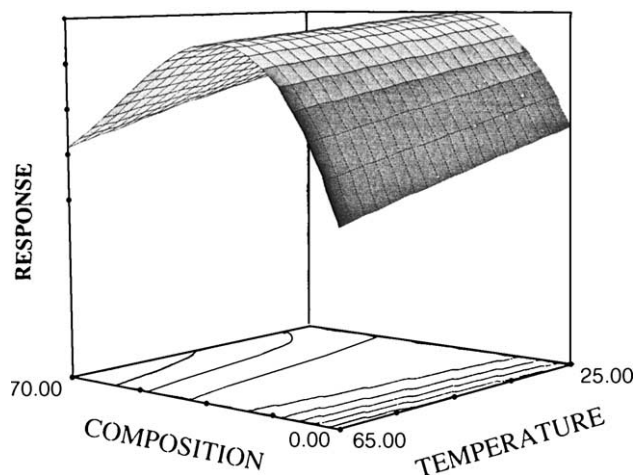


Fig. 8. Response surface for the variation of the model constant (*c* term) with solvent strength (% v/v methanol) and temperature (°C). Chromatographic conditions as for Fig. 1.



model of the form:

$$e = 0.35 - 0.026T - 0.074\phi^2 - 0.024\phi T, \quad r^2 = 0.731, \\ Q^2 = 0.658, \quad \text{PRESS} = 0.023, \quad \text{RMSE} = 0.023, \\ F = 33 \quad (11)$$

As observed for the  $a$  system constant, the model provides a good fit to the experimental values but the limited range of  $e$  values compared to their average standard deviation results in poor modeling potential. The form of the equation suggests that both temperature and solvent strength affect the  $e$  system constant on more equal terms than for the other system constants, but the limited variation of the  $e$  system constant over the factor space is an indication that lone pair electron interactions do not depend strongly on either factor.

The response surface for the model constant  $c$  is non-linear, Fig. 8, and adequately fit by a reduced cubic model of the form:

$$c = -0.31 - 0.16\phi - 0.039T - 0.37\phi^2 - 0.092\phi^2 T \\ + 0.28\phi^3, \quad r^2 = 0.969, \quad Q^2 = 0.958, \\ \text{PRESS} = 0.047, \quad \text{RMSE} = 0.032, \quad F = 212 \quad (12)$$

The  $\phi^2 T$  term is important in explaining the variation in the response and indicates a cooperative interaction between solvent strength and temperature for modeling the  $c$  term. Both solvent strength and temperature are important factors that affect the  $c$  term, but since the  $c$  term may include other contributions than the phase ratio, it is impossible to relate the form of the equation to system properties alone. The  $c$  term, though, affects absolute retention independent of the defined intermolecular interactions in both phases, and thus the shape of the response surface is an indication that the  $c$  term is a complex function of solvent strength and temperature.

#### 4. Conclusions

The solvation parameter model in combination with response surface methodology successfully explains the variation of retention with solvent strength and temperature for the Synergi™ Hydro-RP stationary phase. Differences in cohesion ( $v$  system constant), hydrogen-bond acidity ( $b$  system constant) and the model constant ( $c$  term) explain most of the variation in retention with smaller contributions from differences in dipole-type ( $s$  system constant), electron lone pair ( $e$  system constant) and hydrogen-bond basicity ( $a$  system constant) interactions. Solvent strength is many fold more effective than temperature for changing any of the system constants. The  $v$  system constant shows a linear dependence on solvent strength and temperature. For the other system constants a non-linear dependence is observed with important

contributions from factor interactions. In the case of temperature, a significant fraction of the retention variation is due to changes in the model constant ( $c$  term) without changing selectivity.

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