A STUDY OF BAND BROADENING EFFECTS IN HPLC

Waters

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INTRODUCTION

The van Deemter equation has been used extensively to describe the relative magnitude and sources of dispersion leading to band broadening in HPLC. The regression data from the van Deemter and Knox reduced plate height (h) equations are compared. The magnitudes of the reduced terms in the van Deemter equation are compared across bonding chemistry, particle size, and particle composition (silica versus hybrid).

In order to obtain good estimates for the *C*-term, very high flow rates are required while performing a van Deemter experiment. Small I.D. columns are need to minimize radial thermal gradients that may be generated due to frictional heating of the mobile phase at the high flow rates. Short columns are needed so that high flow rates can be reached without exceeding the pressure limits of the pump.

These requirements place aggressive demands on the chromatographic system. Ideally, high sampling rates, no signal filters, direct data acquisition, a low dispersion injector, and minimum system volumes, which require the use of small bore tubing of minimum length and a micro-bore flow cell are required. Decanophenone, a high k' (up to 16) solute, was selected for this study in order to minimize extra-column effects and to examine the relationship between k' and the *B*-term.

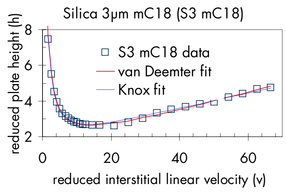
SUMMARY

- The chromatographic system and settings need to be optimized for the acquisition of reliable h-v data (see last 3 panels).
- A *B*-term value of 1.2 was obtained for the un-retained solute, thiourea, and gives an γ_m = 0.61±0.06.
- *B* parameters obtained using the van Deemter equation were consistent with those obtained by the arrested elution method.
- The relative standard errors for the coefficients using multiple linear regression analysis and the van Deemter eq. were the lowest of the methods examined.
- The value for $\gamma_s D_s/D_m$ was found to increase with decreasing carbon number in the ligand.
- *B*- and *C*-terms were lower on the 5µm packings compared to 3µm and 2µm packings. This observation needs to be confirmed and merits further investigation.

References

[1]. J.H.Knox, L. McLaren, *Anal. Chem.*,36(1964) 1477

h VERSUS LINEAR VELOCITY EQUATIONS



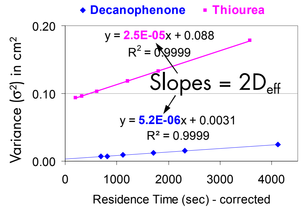
Coefficients were derived using the following methods:

1. van Deemter (VD): $h = A + \frac{B}{v} + Cv$ Eq. 1

2. Knox: $h = Av^{1/3} + \frac{B}{v} + Cv$ Eq. 2

3. Linearization (LIN) techniques:
   Given $h_{Total} = h_A + h_B + h_C$
   – $h_{Total} = B_{LIN} v^{-1} + h_{other}$ for v= 1.5 to 7
   Van Deemter eq:
   – $h_{Total} - h_{B\ LIN} = Cv + A$ for v= 10 to 60
   Knox eq:
   – $h_{Total} - h_{B\ LIN} = Cv + Av^{1/3}$ for v= 10 to 60

ARRESTED ELUTION METHOD



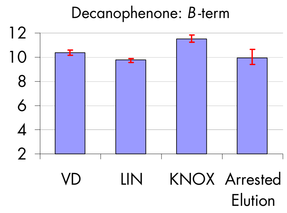
$$\sigma^2_{Total} = 2D_{eff}(\text{total residence time}) + \sigma^2_{other} \quad \text{Eq. 3}$$

Arrested elution experiments were performed to obtain an independent value for the *B* parameter [1]. The data for thiourea (using D_m=2.22E-05 cm²/sec) were used to obtain the value for *B* for an un-retained solute (*B*=1.2) and the obstruction factor in the mobile phase (γ_m=0.61).

$$B = 2\frac{D_{eff}}{D_m}(1+k') = 2\gamma_m + 2\gamma_s \frac{D_s}{D_m} k' \quad \text{Eq. 4}$$

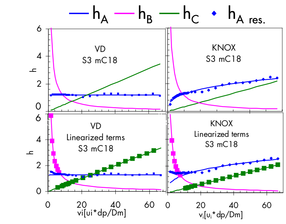
γ_m = obstruction factor in mobile phase
γ_s = obstruction factor in stationary phase
D_s = diffusion coefficient in stationary phase (cm²/sec)
D_m = diffusion coefficient in mobile phase (cm²/sec)

COMPARISON OF *B*-TERMS



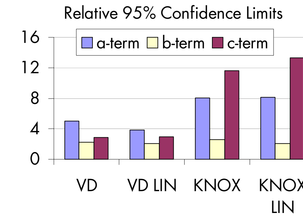
– The *B* parameter was calculated using the arrested elution method, multiple linear regression fits to the van Deemter (VD) and Knox equations, and a simple linear regression (LIN) using the linear h versus v^{-1} low flow rate region.
– All the *B* values, except that from the Knox equation, were within the 95% confidence interval of the value obtained from the arrested elution method.

COMPARISON OF *A*-TERMS



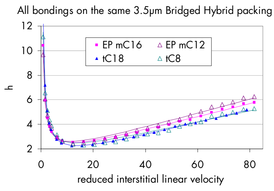
– The individual h_A, h_B, and h_C curves were constructed. Linearized terms were obtained in the velocity regions marked with large squares.
– The h_B and h_C values were subtracted from the experimental value for h_{Total}. The remaining h_A residual data are expected to coincide with the calculated h_A if all the coefficients accurately describe the data.
– The plots above show that the *A*-term is best described by the van Deemter equation. A significant residual h is introduced in the low velocity region with the *A*-term obtained from the linearized Knox eq.

RELATIVE STANDARD ERROR



– The *A* and *C* parameters obtained by fitting the data to the van Deemter eq. are significantly more precise (P=0.05) than those obtained from the Knox eq.
– Multiple data sets showed that the van Deemter equation gave lower standard errors than the linearized van Deemter approach and hence was selected as the method of choice.

COMPARISON OF SURFACE MODIFICATION

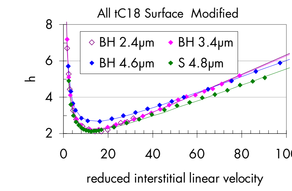


– A general trend of higher *B*-terms for tri-functional vs. mono-functional bondings were observed.

– The value for $\gamma_s D_s/D_m$ was calculated (see Eq. 4) from the *B*-terms and k', assuming γ_m=0.61. The longer ligand chain lengths have lower values for $\gamma_s D_s/D_m$. It is not known if this is generally true.

BH3	k'	A	B	C	$\gamma_s D_s/D_m$
tC18	11.63	0.8±0.1	10.0±0.2	0.054±0.001	0.38
tC8	4.38	1.0±0.2	9.6±0.7	0.051±0.004	0.96
EP mC16	7.07	1.2±0.1	7.8±0.3	0.055±0.002	0.47
EP mC12	3.73	1.3±0.2	7.1±0.5	0.059±0.005	0.80

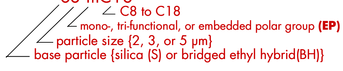
COMPARISON OF PARTICLE SIZE



tC18	k'	A	B	C	R²
BH 2.4µm	11.49	0.71±0.09	10.0±0.1	0.055±0.004	0.9998
BH 3.4µm	11.63	0.78±0.05	10.0±0.2	0.054±0.001	0.9989
BH 4.6µm	11.17	1.38±0.04	9.1±0.2	0.045±0.001	0.9995
S 4.8µm	16.31	0.94±0.07	8.5±0.4	0.045±0.001	0.9966

– The *B* and *C* parameters are the same for the 3µm and 2µm hybrid packings and are higher than those for the two 5µm packings. The 5µm silica packing has *B*- and *C*-terms that are comparable to the 5µm hybrid packing.

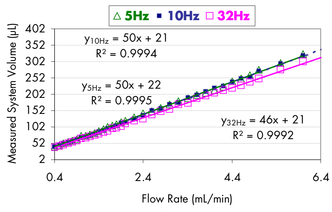
MATERIALS KEY AND EXPERIMENTAL



Experimental Conditions

– Instruments:
•Waters 2690XE for temperature and flow rate control, Waters 515 for sample delivery, Waters 2487 DUV with µbore flow cell (3µL) modified to deliver up to 32Hz data acquisition, a Valco electronically actuated 0.5µL internal loop injector, Waters two position solvent switching valves for diverting flow in the arrested elution experiments
–Chromatographic conditions:
•Flow Rate: 0.02-2mL/min, Temp: 30.0°C, Detection: 254nm, 0 Filter TC, 10-32pps, 0.5µL injection volume, on-column mass load was 1.5µg decanophenone, mobile phase: 70% acetonitrile in water (v,v)
–Columns:
•2.1x50mm, efficiencies reported at ½ peak height
–Data acquisition and processing
•Millennium[32] V3.2 or Empower V5.0
•extra-column volume and dispersion corrections
–$t_{Rcorr} = t_{Rmeas} - (\text{extra-column volume at flow rate})$
–$N_{½ht\ corr} = 5.54(t_{Rcorr})^2/(W_{0.5}^2 - W_{0.5\text{-}sys}^2)$

SYSTEM VOLUME MEASUREMENT

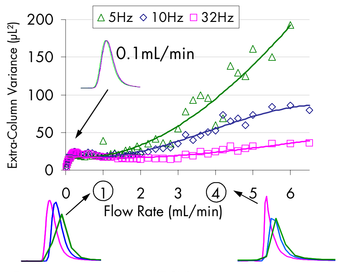


$$V_{R_{meas}} = t_R \, x \, flow$$

$$V_{R_{meas}} = t_{DELAY} \, x \, flow + V_{sys}$$

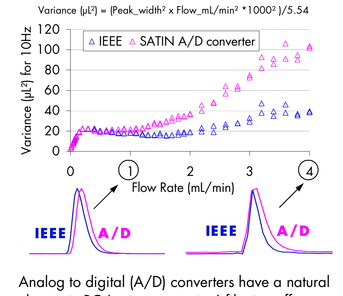
The measured retention times reveal that at high flow rates V_{Rmeas} is higher than actual. From the above, V_{sys} (~21µL) is the actual system volume.
– **All retention times should be corrected by the measured system time at each flow rate used**.

EFFECT OF SAMPLING RATE



Extra-column variance (ECV) increases with increasing flow rate when the peak is under sampled. Correcting peak width measurements using under sampled ECV leads to lower than actual *C*-terms.
– **Use the highest possible sampling rate.**

EFFECT OF RC FILTER



Analog to digital (A/D) converters have a natural electronic RC (resistor-capacitor) filtering effect on the signal. This causes peaks to be wider than they actually are.
– **Direct IEEE data acquisition maintains the best signal integrity**.