An Experimental Study of Liquid Phase Separation in Non-Cylindrical Geometry Conduits*

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* Dedicated to Csaba Horvath to honor all his contributions to separation science, to remember a friendly advisor and an excellent host on many opportunities
Trends in (Bio)Pharmaceutical Industry

- Growing demands for high throughput, high sensitive, high resolution separation based assays (e.g. proteomics).
- Ever-decreasing sample size
- Putting advanced equipment like LC-MS, in the hands of research workers who are not chromatography or MS specialist

➡️ Microfluidic Devices and Systems

- **Miniaturization** radically reduces sample size, cost and improves sensitivity.
- **Integration** increases speed and efficiency, robustness, ease of use and minimizes sample loss.
- **Systems** as tools for a much broader community than separation scientists.
Agilent Technologies HPLC-Chip MS System
Integration of µ-Fluidic Separation Column with Rotary Valve Interface

Stator

Fluid connection

Rotor groove

Sample in

LC Pump

Waste

Sample loop or trapping column

Separation channel approx. 50x95 µm

Nano electrospray
**Example: 10 fmol BSA Digest**

HPLC Chip: length 40 mm, 5µm ZORBAX 300SB-C18.

Agilent 1100 Nanoflow HPLC system, flow rate 200nL/min, gradient 5-50% in 30’, 0.5% formic acid in water/acetonitrile, sample injection 1µL

Detection Agilent 1100 Series MSD Trap, ES Voltage 2kV, base peak chromatogram.
HPLC Chip

Separation channel, 45x0.050x0.085 mm

Through holes connect to grooves

Holder approx. 4x8 cm
AIM: Systematic, experimental evaluation of the chromatographic properties of separation channels with non-cylindrical geometry.

○ Experiments with fused silica capillaries with cylindrical, square and rectangular shape

○ Experiments with LC chips
Experiments with Fused Silica Capillaries – Capillary Shape

100, 75 and 50 µm

100x100 µm
75x75 µm
50x50 µm

95x38 µm
Fused Silica Capillaries

- Permeability*

\[ v_z(r) = -\frac{q}{n} \cdot \frac{l}{U} \cdot \frac{dP}{dz} \cdot dr \]

\[ u_s = \frac{r_c^2}{8} \cdot \frac{dP}{nL} \] (Hagen-Poiseuille)

\[ \frac{q}{U} \] = Hydraulic Equivalent Radius

\[ q = \text{perimeter} \]

\[ U = \text{cross sectional area} \]

Permeability \( \kappa \) of any shape capillary

\[ u_s = \frac{r_h^2}{k_o} \cdot \frac{dP}{nL} \]

Permeability \( \kappa \) of cylindrical shaped capillary

\[ r_h = \frac{r_c}{2} \]

Compared with a cylinder, a square capillary with the same height as the diameter has the same hydraulic equivalent radius but a higher permeability; flattening of the capillary at constant height, increases the permeability.
**Experimental Results**

– Permeability Fused Silica Capillaries

\[ u_s = \frac{r_h^2}{k_o} \cdot \frac{\Delta P}{\eta L} \]

<table>
<thead>
<tr>
<th>Capillary Diameter*</th>
<th>Hydraulic radius (q/U)</th>
<th>Shape factor (k₀)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 µm</td>
<td>25</td>
<td>1.92 - 1.96 (2.0)</td>
</tr>
<tr>
<td>100 µm, square</td>
<td>25</td>
<td>1.64 – 1.68 (1.78)</td>
</tr>
<tr>
<td>75 µm</td>
<td>17.5</td>
<td>1.97 – 2.0</td>
</tr>
<tr>
<td>75 µm, square</td>
<td>17.5</td>
<td>1.87 – 1.90</td>
</tr>
<tr>
<td>53 µm</td>
<td>13.3</td>
<td>1.94 – 1.97</td>
</tr>
<tr>
<td>50 µm, square</td>
<td>12.5</td>
<td>1.55 – 1.60</td>
</tr>
<tr>
<td>38x95 µm</td>
<td>13.6</td>
<td>1.87 – 1.90 (1.94)</td>
</tr>
</tbody>
</table>

All results were obtained with an Agilent Capillary Electrophoresis System. Solvent Methanol (0.055 cP), temperature ambient. Tracer thiourea.

*Nominal diameter as specified by the vendor.
Packed Fused Silica Capillaries
– Permeability

\[ u_s = K_s \cdot \frac{\Delta P}{\eta \cdot L} \]

\[ \Phi_s = \frac{d_p^2}{K_s} \]

\[ K_s^{\text{theor.}} = \frac{d_p^2}{180 \theta^2 \cdot (1 - \varepsilon)^2} = \frac{d_p^2}{1012} \]

\( u_s \) superficial velocity

\( K_s = \) permeability for superficial velocity

\( \Phi_s = \) column resistance factor based on superficial velocity

Permeability according to Kozeny-Carman

\( \varepsilon = \) interstitial porosity (0.4 typical)

\( \theta = \) shape factor (1 for spherical particles)
\[ u_s = K_s \frac{\Delta P}{\eta L} \]

Theoretical value of \( \Phi_s \) according to Kozeny/Carman equation for interstitial porosity, 0.4 is 1012

<table>
<thead>
<tr>
<th>Column</th>
<th>( K_s (cm^2) )</th>
<th>( \Phi_s )</th>
</tr>
</thead>
<tbody>
<tr>
<td>250x0.1 mm (n=4)</td>
<td>1.04 – 1.13E-10</td>
<td>799 – 863</td>
</tr>
<tr>
<td>250x0.1x0.1 mm (n=2)</td>
<td>1.05 – 1.25E-10</td>
<td>721 – 856</td>
</tr>
<tr>
<td>250x0.075 mm (n=2)</td>
<td>1.03 – 1.28E-10</td>
<td>701 – 874</td>
</tr>
<tr>
<td>250x0.075x0.075 mm (n=1)</td>
<td>0.813E-10</td>
<td>1107</td>
</tr>
<tr>
<td>250x0.05 mm (n=1)</td>
<td>1.03E-10</td>
<td>870</td>
</tr>
<tr>
<td>250x0.05x0.05 mm (n=2)</td>
<td>1.28 – 1.57E-10</td>
<td>575 – 703</td>
</tr>
<tr>
<td>250x0.036x0.098 mm (n=1)</td>
<td>1.45E-10</td>
<td>621</td>
</tr>
</tbody>
</table>

All columns packed with 3 µm CEC Hypersil C18
Experimental Results
– Permeability Packed LC Chips
<table>
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<tr>
<th>Column</th>
<th>$K_s (cm^2)$</th>
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<td>250x0.1 mm (n=4)</td>
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<td>721 – 856</td>
</tr>
<tr>
<td>250x0.036x0.098 mm (n=1)</td>
<td>1.45E-10</td>
<td>621</td>
</tr>
<tr>
<td>3.5 µm, 200 bar</td>
<td>1.9692E-10</td>
<td>622</td>
</tr>
<tr>
<td>5.0 µm, 200 bar</td>
<td>3.0889E-10</td>
<td>809</td>
</tr>
<tr>
<td>3.5 µm, 100 bar</td>
<td>1.8685E-10</td>
<td>655</td>
</tr>
<tr>
<td>5.0 µm, 100 bar</td>
<td>3.8140E-10</td>
<td>655</td>
</tr>
</tbody>
</table>

All chips are 43x0.050x0.085 mm. Packing pressure in table. Packed with 5 µm ZORBAX 300SB C18. CEC capillaries packed with 3.0 µm CEC Hypersil
Plate Height of Any Shape Open Capillaries*

\[ H = \frac{2D_m}{u_0} + \frac{F(k', \varphi)a^2u_0}{ND_m} \]

- \( D_m \): diffusion constant in the mobile phase
- \( u_0 \): mobile phase velocity (equals superficial velocity in an open tube)
- \( a \): characteristic length e.g. diameter \( d_c \) or channel height \( h \) or particle size \( d_p \)
- \( F(k', \varphi) \): function of the capacity factor and the aspect ratio \( \varphi \)
- \( N \): numerical factor depending on capillary shape
- \( f \): constants depending on capillary shape

\[ F = \frac{f_0 + f_1k' + f_2k'^2}{(1 + k')^2} \]

Experiments with Fused Silica Capillaries – Dispersion

Theory

<table>
<thead>
<tr>
<th>Pressure Drive (cyl. capillary)</th>
<th>Experiment</th>
</tr>
</thead>
</table>
| $H_{\text{theor.}} = \frac{2D_m}{u_0} + \frac{d_c^2.u_0}{96D_m} + \frac{L_{\text{inj.}}^2}{12.L_c}$ | Agilent Capillary Electrophoresis System. Solvent Methanol (0.55 cP), temperature ambient. Tracer thiourea (diff. coeff. $2.3 \times 10^{-5}$ cm$^2$/s).
Capillary length 40 cm to the detector, 48.5 cm total. |
| **Pressure Drive (square & rect. capillary)** | Pressure injection; |
| $H_{\text{theor.}} = \frac{2D_m}{u_0} + \frac{f_0.h^2.u_0}{105D_m} + \frac{L_{\text{inj.}}^2}{12.L_c}$ | P-drive 10-50 mbar |
| **Electrical Drive (acc. Aris-Taylor)** | E-drive 10-30 kV |
| $H_{\text{theor.}} = \frac{2D_i}{\bar{u}} + \frac{L_{\text{inj.}}^2}{12L_c}$ | Cylindrical capillary, $f_0 = 1$
Square capillary, $f_0 = 1.8^*$
Rectangular capillary, $\varphi = 2.5, f_0 \approx 4.0^*$ |

Experimental Results
- Dispersion Fused Silica Capillaries: Pressure Drive

Capillary ID 100 µm

Red – Cylindrical
Green – Square
Solid Line - Theory

HETP (µm)

Solvent Velocity (cm/s)
Experimental Results
- Dispersion Fused Silica Capillaries: Pressure Drive

<table>
<thead>
<tr>
<th>Color</th>
<th>Shape</th>
<th>Dimension</th>
<th>Label</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red</td>
<td>Cylindrical</td>
<td>50 µm</td>
<td>Red – Cylindrical 50 µm</td>
</tr>
<tr>
<td>Green</td>
<td>Square</td>
<td>50 µm</td>
<td>Green – Square 50 µm</td>
</tr>
<tr>
<td>Brown</td>
<td>Rectangular</td>
<td>35x95 µm</td>
<td>Brown – Rectangular 35x95 µm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Solid Line - Theory</td>
</tr>
</tbody>
</table>

Solvent Velocity (cm/s) vs. HETP (µm)
Experimental Results
- Dispersion Fused Silica Capillaries: E-drive
Experimental Results
– CEC of Cylindrical vs. Square Capillary

Capillary: 250x0.1 mm, CEC-Hypersil C18, 3 µm
Top, cylindrical; Bottom square capillary

Run Buffer: TRIS Buffer, 50 mM pH 8/Acetonitrile 1/4

Sample: Agilent RP Checkout Sample

Injection: Electrokinetic, 0.1' 5 kV

Detection: 250:20 nm

Temp.: 25 °C
Experimental Results
– CEC of Cylindrical vs. Square Capillary

- **Capillary**: 250x0.075 mm, CEC-Hypersil C18, 3 µm
  - Top, cylindrical; Bottom square capillary
- **Run Buffer**: TRIS Buffer, 50 mM pH 8/Acetonitrile 1/4
- **Sample**: Agilent RP Checkout Sample
- **Injection**: Electrokinetic, 0.1' 5 kV
- **Detection**: 250:20 nm
- **Temp.**: 25 °C
Experimental Results
– CEC of Rectangular Capillary

Capillary: 250x0.038x0.095 mm
CEC-Hypersil C18, 3 µm
Run Buffer: TRIS Buffer, 50 mM pH 8/
Acetonitrile 1/4
Sample: Agilent RP Checkout Sample
Injection: Electrokinetic, 0.1’ 5 kV
Detection: 250:20 nm
Temp.: 25 °C
Experimental Results

- Comparison Capillary and HPLC Chip Column

Sample: 10 fmol (1µl inj. volume) of BSA tryptic digest. Agilent 1100 Nanoflow HPLC system, flow rate 200nL/min, gradient 5-50% in 30’, 0.5% formic acid in water/acetonitrile. Detection Agilent 1100 Series MSD Trap, ES Voltage 2kV, base peak chromatogram.
Experimental Results

- Isocratic Elution of Peptides on HPLC Chip Column

Special Chip with 4 nL internal sample loop.

Column: 43x0.05x0.085 mm (approx. 180 nL!)

ZORBAX 300SB C18, 5 µm.
Solvent 0.1% formic acid in 81/19 water/acetonitrile.
Flow rate 300 nL/min

Width at half height 50 nL, approx. 500-600 plates

t₀ = 0.6 min
Experimental Results
- Gradient Elution of Simple Peptide Mix

Conditions as on previous page.
Gradient 2-52% in 25’
Conclusions

- Demonstrated coherence to theory of the permeability of open, non-cylinder geometry capillaries
- Demonstrated coherence to theory of the HETP of open, non-cylinder geometry capillaries in P-drive and E-drive mode
- Permeability of packed capillaries increases with decrease in diameter; square capillaries seem more permeable as cylindrical capillaries
- The HPLC chip columns are more ‘permeable’ than packed capillary LC columns of similar dimension
- Efficiency in rectangular shaped columns CEC mode is comparable to cylindrical capillaries
- Efficiency of HPLC chip columns and nanocolumns is about equal but lag theoretical predictions
Dr. Henk Lauer, HLCE Amsterdam, NL
Karl-Heinz Blum, LC Column Manufacturing, Agilent Technologies, Waldbronn
Polymicro Technologies for samples of the rectangular fused silica capillaries