Monoliths in separation science
1st part

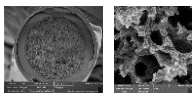
Dana Moravcová

Monolith???

A single block or piece of stone of considerable size.

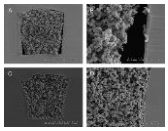
Gustav Vigeland Sculpture Park, Oslo, Norway

A continuous stationary phase cast as a homogeneous column in a single piece.



Monolithic capillary column.

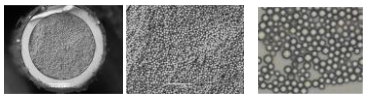
Monolithic pipette tip.



Monolithic chip
(A, B) - unmodified
(C, D) modified wall of chip. *(D. A. Mair, Lab Chip 9 (2009) 877-88.)*

Why monoliths?

- An alternative to particle packed columns



Particle packed column, 5 µm particles.

History

- 1967 - poly(ethylenglycol methacrylate) column - separation of proteins - gel filtration, low permeability and separation efficiency (M. Kubín).
- 1989 - compressed hydrophilic polyacrylamide gels - ion-exchange chromatography of proteins (S. Hjertén).

SDS-gel | Wet state | Dry state

Rigid monoliths

- The 1990s - macroporous rigid monolithic materials based on methacrylate and polystyrene-divinylbenzene copolymers suitable for separation of proteins (F. Švec, J. M. J. Fréchet); silicagel-based monolithic materials suitable for separation of small molecules (K. Nakanishi, N. Soga, N. Tanaka).

Nowadays

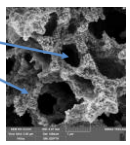
- Monolith = a rigid material with appropriate chemical, physical, and mechanical properties (stability in a wide pH range, permanent porosity).
  - Characteristic well-organized and highly porous structure
  - Variable surface area, pore texture, surface chemistry
  - Polymer-, inorganic-, and hybrid-monoliths
    - Acrylamide-, methacrylate-, and polystyrene-based monoliths
    - Alkoxysilanes - tetramethoxysilane, tetraethoxysilane
    - Alkyltrialkoxysilanes or polysilsesquioxanes as 1,2-bis(trimethoxysilyl)ethane

Monolithic stationary phases

- The desired monolithic stationary phases can be prepared utilizing one-step or multiple-modification preparation procedure.
  - One-step preparation procedure - methacrylate monolithic capillary columns
    - butylmethacrylate **BMA** + ethylenedimethacrylate **EDMA**
  - Multiple-modification preparation procedure - silicagel monolithic capillary columns
    - C18-stationary phases
    - Sulfobetaine stationary phase
    - Phosphonium ionic liquid stationary phase
    - Liposome stationary phases

Characterization of monolithic materials

- Monolith - porous material
  - Macropores > 50 nm, flow-through pores
  - Mesopores 2-50 nm, surface area
  - Micropores < 2 nm



- Material engineering
  - Pore volume - mercury intrusion porosimetry
  - Specific surface area - gas adsorption (BET)
  - Infrared spectroscopy - presence of functional groups
  - Elemental analysis
  - Electron microscopy (SEM)
- Chromatography
  - Permeability, porosity
  - Separation efficiency
  - Separation selectivity
  - Inverse size-exclusion chromatography (ISEC)

Characterization of monolithic materials

- Permeability
  - Kozeny-Carman equation

$$K_F = \frac{F \cdot \eta \cdot L}{\Delta p \cdot \pi \cdot r^2} \qquad d_p = (1-\varepsilon_0)\sqrt{\frac{180 K_F}{\varepsilon_0^3}} \qquad d_p = \sqrt{1000 \cdot K_F}\,(ODS, \varepsilon_0 \cong 0.4)$$

F - mobile phase flow rate, η - viscosity of mobile phase, Δp - pressure drop, *L* - column length, *r* - column radius, ε_0 - interstitial porosity, d_p - „equivalent permeability particle diameter".

- Porosity
  - Column total porosity determined with uracil as a non-retained marker compound

$$\varepsilon_T = \frac{V_{TO}}{V_C}$$

  - Interstitial (through pore) porosity $\varepsilon_0 = \frac{V_0}{V_C}$
  - Inner (mesopore porosity) $\varepsilon_i = \frac{V_{TO} - V_0}{V_C} = \varepsilon_T - \varepsilon_0$

The interstitial (through pore) and inner (mesopore) porosities calculated for polystyrene standard with M_r = 2 700 000 in 100% THF.

Characterization of monolithic materials
Separation efficiency, retention factor

- Separation efficiency
  - the number of theoretical plates (n)

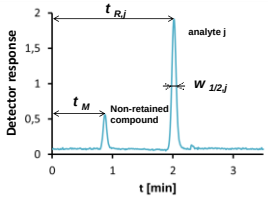
$$n = 5.545 \cdot \left(\frac{t_{R,j}}{w_{1/2,j}}\right)^2$$

- *N* - the number of theoretical plates per meter
- *H* - height equivalent to theoretical plate

$$H = \frac{N}{l}$$

l - column length

- Retention factor *k* $\quad k = \frac{t_{R,j} - t_M}{t_M}$



Monolithic methacrylate-based capillary columns

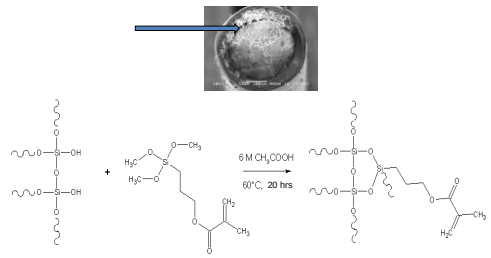
- Monomers
  - butylmethacrylate **BMA**
  - ethylenedimethacrylate **EDMA**
- Pore forming solvents
  - 1,4-butanediol **BUT**
  - 1-propanol **PROP**
  - water
- Initiator
  - azobisisobutyronitrile **AIBN**
- Thermal polymerization
  - 60°C, 24 hours
  - 0.32 mm I.D. silanized capillaries

Column	1	2	3
Porogen	60	60	60
Monomer	40	40	40
BMA	44.5	44.5	44.5
EDMA	54.5	54.5	54.5
PrOH	60	62	64
BuOH	30	28	26
Water	10	10	10

% wt.

D. Moravcová et al., J. Sep. Sci. 2004, 27, 789-800.

Silanization



Modification of inner wall of fused silica capillary

Reaction between silanol groups on the silica capillary surface and 3-(trimetoxysilyl)propyl methacrylate.

Porosity and permeability of prepared columns

- Porosity

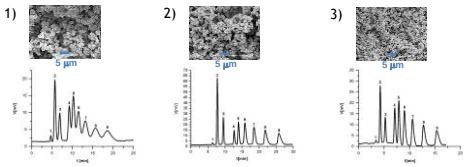
Column	1	2	3	A	B	C
ε_T	0.710	0.680	0.650	0.590	0.650	0.847
ε_o	0.490	0.470	0.410	0.310	0.290	0.680
ε_i	0.220	0.210	0.240	0.280	0.360	0.167

- Permeability

Column	1	2	3	A	B	C
K_F [cm^2]	7.79E-10	2.38E-10	3.52E-11	2.25E-10	1.47E-10	8.66E-10
d_{perm} [μm]	7.6	3.8	1.9	7.2	5.6	5.1

A - Inertsil ODS-2, 5 mm, 150 x 0.32 mm, Metachem, Torrance, USA
B - Biospher C18E, 5 mm, 141 x 0.32 mm, Labio Praha, Czech Republic
C - Chromolith CapRod RP-18e, 150 x 0.1 mm, Merck, Darmstad, Germany

Separation of alkylbenzenes



Running conditions: 70% ACN, 30% water, UV detection 254 nm, Fc = 2 μl/min, columns 0.32 mm I.D., l_1,l_2 = 240 mm, l_3 = 140 mm.

Column	1	2	3
k_{TOL}	1,19	1,13	1,30
N_{TOL}	4270	21860	30380
k_{AB}	3,03	2,81	3,47
N_{AB}	2090	9680	22110

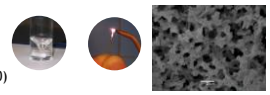
Sample:
0 - uracil (unretained compound)
1 - benzylalcohol
2 - benzaldehyde
3 - benzene
4 - toluene
5 - ethylbenzene
6 - propylbenzene
7 - butylbenzene
8 - amylbenzene

Conclusion

- The methacrylate-based monolithic columns showed comparable chromatographic performance as packed octadecylsilica capillary columns.
- The results illustrate the importance of selection of appropriate composition of the porogen solvent mixture.
- Column with 64% w/w of propanol in the porogen part showed better chromatographic performance than the columns prepared using lower propanol concentrations.

Monolithic silica-based capillary columns

- Silicagel-based monolith
  - Tetramethoxysilane
  - Polyethylene oxide (Mr 10 000)
  - Acetic acid, water



- Hydrolysis

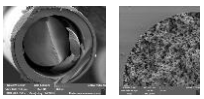
$\equiv Si\text{-}OR + H_2O \rightarrow \equiv Si\text{-}OH + ROH$

- Condensation of alcohole

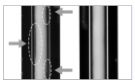
$\equiv Si\text{-}OH + RO\text{-}Si\equiv \rightarrow \equiv Si\text{-}O\text{-}Si\equiv + ROH$

- Condensation of water

$\equiv Si\text{-}OH + HO\text{-}Si\equiv \rightarrow \equiv Si\text{-}O\text{-}Si\equiv + H_2O$



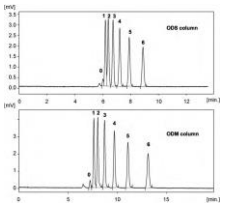
Five steps of preparation – polymerization, washing, drying, calcination, and modification to appropriate stationary phase.



Silicagel-based monolith

Monolithic silica-based capillary columns

C18-stationary phases (RPLC)



Isocratic separation of alkylbenzenes (benzene -hexylbenzene, n = 0-6) in 80% ACN/20 % water. Test mixture: 50 µL of each in 20 mL of 80% v/v acetonitrile, injection 60 nL loop, splitter, columns 150 x 0.1mm, F_c = 500 nl/min, UV detection 220 nm.

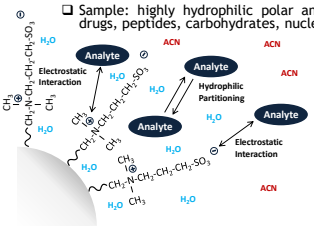
- ODS column - chemical modification

Silica monolith modified by octadecyldimethyl-N,N-diethylaminosilane

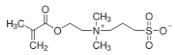
- ODM column - „grafting" – two step modification procedure
  - silanization of monolith by 3-trimethoxysilylpropyl methacrylate
  - radical polymerization of 3-trimethoxysilylpropyl methacrylate and octadecyl methacrylate

Hydrophilic Interaction Chromatography (HILIC)

- Alpert (A. J. Alpert, J. Chromatogr. A 499 (1990) 177.)
  - Mobile phase: organic solvent (40-97% ACN) in water or volatile buffer.
  - Stationary phase: silica, amino-, diol-, polyhydroxyethyl-, aspartamide-, cyclodextrin-, and zwitterion-based packings.
  - Sample: highly hydrophilic polar analytes – small molecules - drugs, peptides, carbohydrates, nucleosides, nucleotides.



The retention processes in HILIC illustrated by hydrophilic partitioning, and electrostatic interactions with either positive or negative charges.



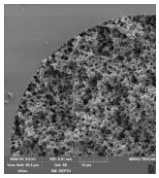
Monomer: [2-(methacryloyloxy)ethyl]-dimethyl-(3-sulfopropyl)-ammonium hydroxide (MEDSA).

Preparation of monolithic capillary columns

- Monolithic silica
  - Tetramethoxysilane, PEG 10 000, urea, 0.01M acetic acid.
- 1st modification
  - 3-Trimethoxysilylpropyl methacrylate, ethanol, acetic acid, water.
- 2nd modification
  - 15 mg/ml of [2-(methacryloyloxy)ethyl]-dimethyl-(3-sulfopropyl)-ammonium hydroxide (MEDSA) in methanol (30% v/v) and xylenes (70% v/v).

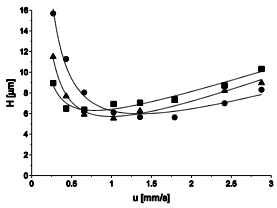
⇨ thermal polymerization

- 80°C, 3 hours.



The prepared monolithic column (SEM).

Separation efficiency



Van-Deemter dependency of monolithic capillary column (150 mm × 0.1 mm).

Mobile phase: 90% (v/v) ACN/ 10% 5mM ammonium acetate pH=6; UV detection 210 nm.

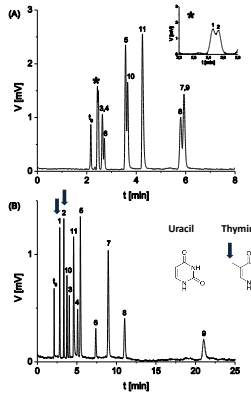
● – toluene
■ – uracil
▲ – cytosine
u – mean linear velocity of mobile phase

Compound	H [µm]	N [tp/m]	k	u [mm/s]
Toluene	5.7	175 500	---	1.5
Uracil	6.4	156 000	0.29	0.7
Cytosine	5.5	182 000	1.01	1.0

Separation efficiency of sulfobetaine monolithic capillary column.

D. Moravcová et al., J. Chromatogr. A 1270 (2012) 178– 185.

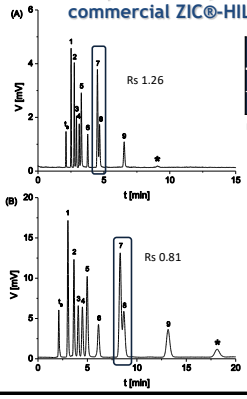
Isocratic separation



Comparison of isocratic elution on bare silica monolithic (A) and sulfoalkylbetaine monolithic (B) capillary columns.
Mobile phase: 95% (v/v) ACN/ 50 mM ammonium formate, pH = 4.5, flow rate 0.5 µl/min; Detection: UV 210 nm.

Sample:
toluene (t_0 marker)
(1) Thymine
(2) Uracil
(3) 2-Deoxyuridine
(4) 5-Methyluridine
(5) Adenosine
(6) Uridine
(7) Cytosine
(8) 2-Deoxycytidine
(9) Cytidine
(10) 2-Deoxyadenosine
(11) Adenine

Comparison of synthesized capillary column with commercial ZIC®-HILIC column



	ZIC®-HILIC column	Capillary column
Permeability	$3.16 \times 10^{-14} m^2$	$1.68 \times 10^{-14} m^2$
Porosity	0.74	0.79

Permeability and porosity of tested columns.

HILIC separation.
Mobile phase: 90% ACN/10% 25 mM ammonium formate (v/v) , pH = 4.5. UV detection 210 nm.

(A) synthesized monolithic capillary column (150 mm x 0.1 mm), F_c = 0.5 µl/min*;

(B) ZIC®-HILIC column (150 mm × 2.1 mm, 5µm), F_c = 200 µl/min*;

*mean linear velocity of the mobile phase is (A) 1 mm/s and (B) 1.2 mm/s.

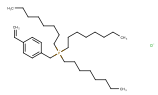
Sample:
toluene (t_0 marker), (1) Thymine, (2) Uracil, (3) 2-Deoxyuridine, (4) 5-Methyluridine, (5) Adenosine, (6) Uridine, (7) Cytosine, (8) 2-Deoxycytidine,(9) Cytidine, (★) Guanosine.

Conclusion

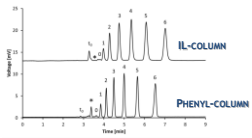
- The simple two-step modification of silica-based monolithic capillary columns provides stable sulfoalkylbetaine stationary phase suitable for separation of polar analytes.
- The high separation efficiency of original silica monolithic columns is preserved even after modification by MEDSA.
- The synthesized column shows a long-term stability under the separation conditions when the relative standard deviations for the retention times of tested solutes were lower than 2% under the isocratic conditions and lower than 3.5% under the gradient conditions.
- The ability of synthesized columns to separate modified nucleobases and nucleosides such as thymine and uracil or 5-methyluridine and uridine extends the application range of these columns to the field of proteomics where separation of similar compounds with different levels of methylation is required.

Phosphonium-based ionic liquid as stationary phase in HPLC

- Silicagel-based monolith modified by trioctyl(4-vinylbenzyl)phosphonium chloride via 3-trimethoxysilylpropyl methacrylate

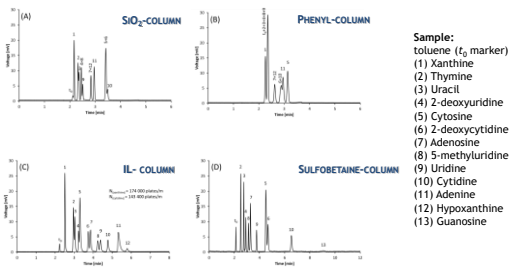


Trioctyl(4-vinylbenzyl)phosphonium chloride



RP separation of alkylbenzenes on IL- and phenyl-columns.
Mobile phase: 60%ACN/40% ammonium chloride (2mM in mobile phase); flow rate 500 nL/min; UV detection at 210 nm. Sample: t_0 - uracil, (0) benzene, (1) toluene, (2) ethylbenzene, (3) propylbenzene, (4) butylbenzene, (5) pentylbenzene, (6) hexylbenzene, (-) impurities.

Phosphonium-based ionic liquid as stationary phase in HPLC



Sample:
toluene (t_0 marker)
(1) Xanthine
(2) Thymine
(3) Uracil
(4) 2-deoxyuridine
(5) Cytosine
(6) 2-deoxycytidine
(7) Adenosine
(8) 5-methyluridine
(9) Uridine
(10) Cytidine
(11) Adenine
(12) Hypoxanthine
(13) Guanosine

HILIC separation of nucleobases and nucleosides.
Mobile phases: (A,B,C) 90% ACN, 10% ammonium acetate (5mM in mobile phase), pH = 4.5; (D) 90% ACN, 10% ammonium formate (2.5mM in mobile phase), pH = 4.5.
Flow rate 500 nL/min, UV detection at 210 nm.

Conclusion

- The synthesized IL-columns possess distinct separation selectivity compared to bare monolithic silica and phenyl-type as well as zwitterionic stationary phase.
- The high separation efficiency of original silica monolithic columns is preserved even after modification by phosphonium-based ionic liquid.
- These columns show mixed interactions and are suitable for multimodal chromatography.

Liposomes as stationary phase in HPLC

- Silica-based monolith in capillary format (0.1 mm × 100 mm) was used as a support for immobilization of liposomes. This new type of biomimicking monolithic stationary phase was evaluated by capillary LC and cryo-scanning electron microscopy (cryo-SEM).

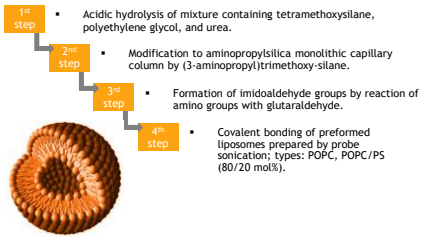
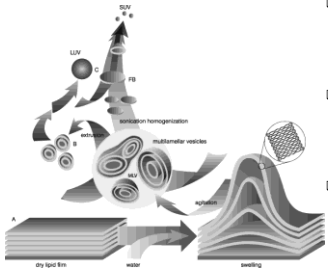


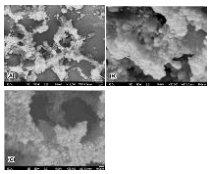
Figure 1. Liposome structure formed by phospholipids.

Preparation of liposomes



- Liposomes – wide variety of lipids can be involved in the preparation ⇒ different biomembrane models
- Preparation of liposomes – the lipid residues are hydrated in phosphate buffer → multilamellar vesicles (MLV)
- Extrusion or sonication leading to formation of unilamellar vesicles
  - < 100 nm SUV
  - > 100 nm LUV

Liposomes as stationary phase in HPLC



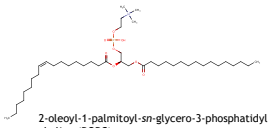
Cryo-SEM images of the prepared stationary phases.

(A) Silica-based monolith modified by POPC liposomes;

(B) Detail of silica monolith modified by POPC liposomes;

(C) Detail of aminopropylsilica monolith.

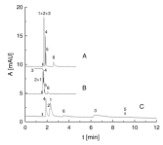
The structure of used phospholipids.



2-oleoyl-1-palmitoyl-*sn*-glycero-3-phosphatidyl choline (POPC)

1,2-diacyl-sn-glycero-3-phospho-L-serine (PS)

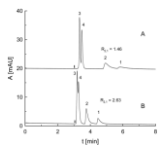
Liposomes as stationary phase in HPLC



Separation of sulfa drugs on monolithic capillary columns.

(A) bare silica monolithic capillary column
(B) aminopropylsilica monolithic capillary column
(C) POPC-modified monolithic capillary column.

Sample:
arrow – methanol (t_0 marker); 1 – sulfanilic acid; 2 – sulfacetamide sodium; 3 – sulfafurazole; 4 – sulfanilamide; 5 – sulfathiazole; 6 – sulfadimidine.
Running conditions:
Mobile phase 20 mM sodium phosphate, pH 7.4; columns (100 mm × 0.1 mm); flow rate 500 nL/min; UV-detection: 220 nm.



Separation of uric acid and its derivatives on liposome-modified monolithic capillary columns.

(A) 80/20 mol% POPC/PS column
(B) POPC column.

Sample:
arrow – methanol (t_0 marker); 1 – uric acid; 2 – xanthine; 3 – etofylline; 4 – caffeine.
Running conditions:
Mobile phase 20 mM sodium phosphate, pH 7.4; columns (100 mm × 0.1 mm); flow rate 250 nL/min; UV-detection: 220 nm.

Conclusion

- The cryo-SEM images confirmed that individual lipid vesicles persist in their fully hydrated form as spherical vesicles even after bonding to the monolithic silica back bone.
- The drug retention on the liposome-modified columns is caused by their interactions with the immobilized liposomes, where electrostatic interactions play a crucial role.
- The composition of the liposome mixture used for column preparation significantly affects the retention of solute.