Recent Advances of Capillary Electrokinetic Separation Technologies and their Applications in Pharmaceutical and Biochemical Analyses

Qing Liu[1], Soumia Cheddah[1], Yuanyuan Liu[1,2], Weiwei Wang[1], Yan Wang[1],

Chao Yan[1,2*]

[1]*Shanghai Jiaotong University, Shanghai, China*

[2]*Unimicro Technologies, Inc., Pleasanton, CA, USA*

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Contents

Part 1

Background

Quest on powerful analytical tools

Life Science

- Proteomics
- Metabolomics
- Systems Biology

Pharmaceutical

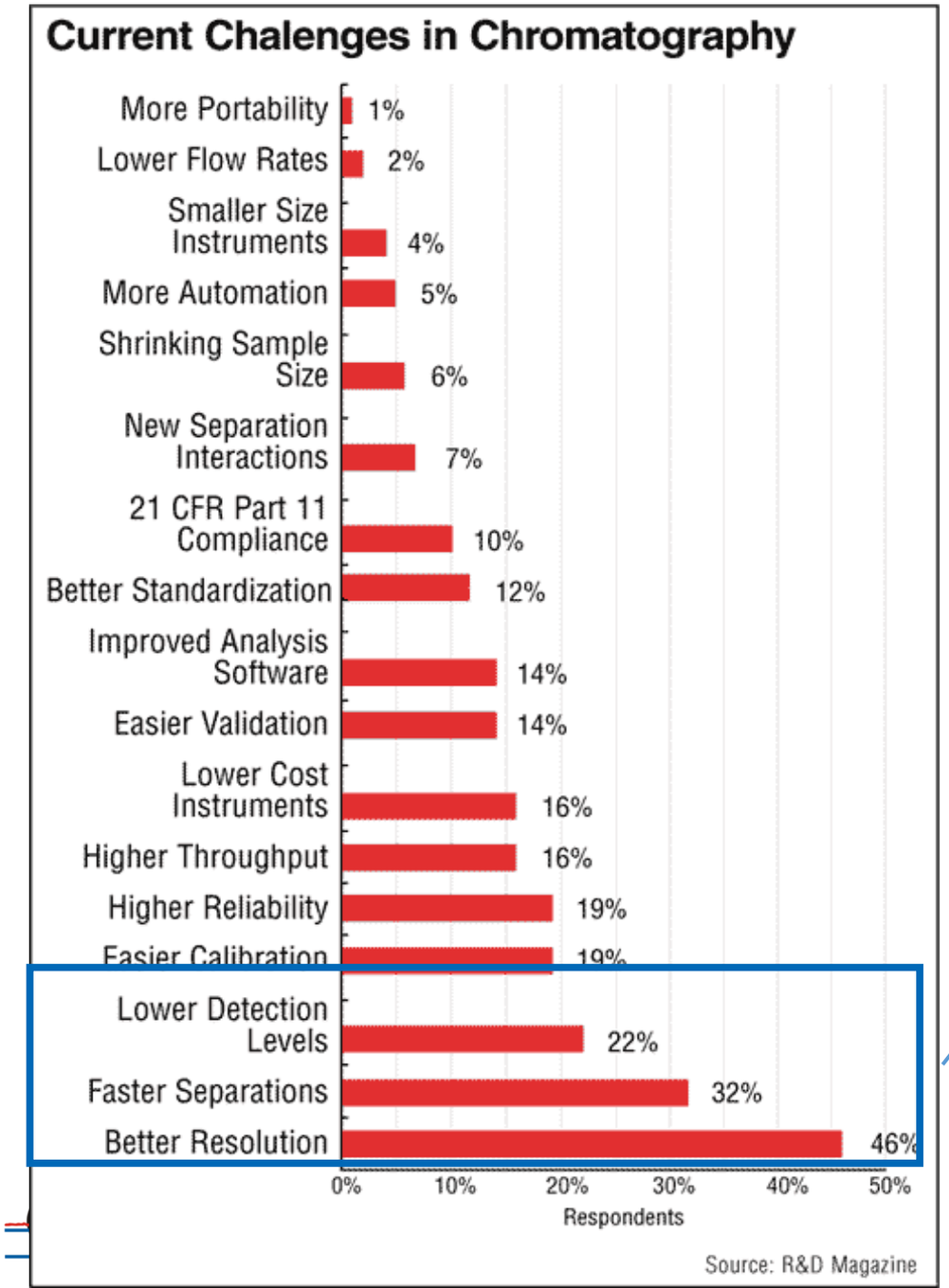
- CTM
- QC/QA
- Chiral Separation

Agriculture/ Food

- Pesticides
- Additives

Environmental

- Pollution in air
- Water
- Soil



Top three chalenges

Resolution 分辨度

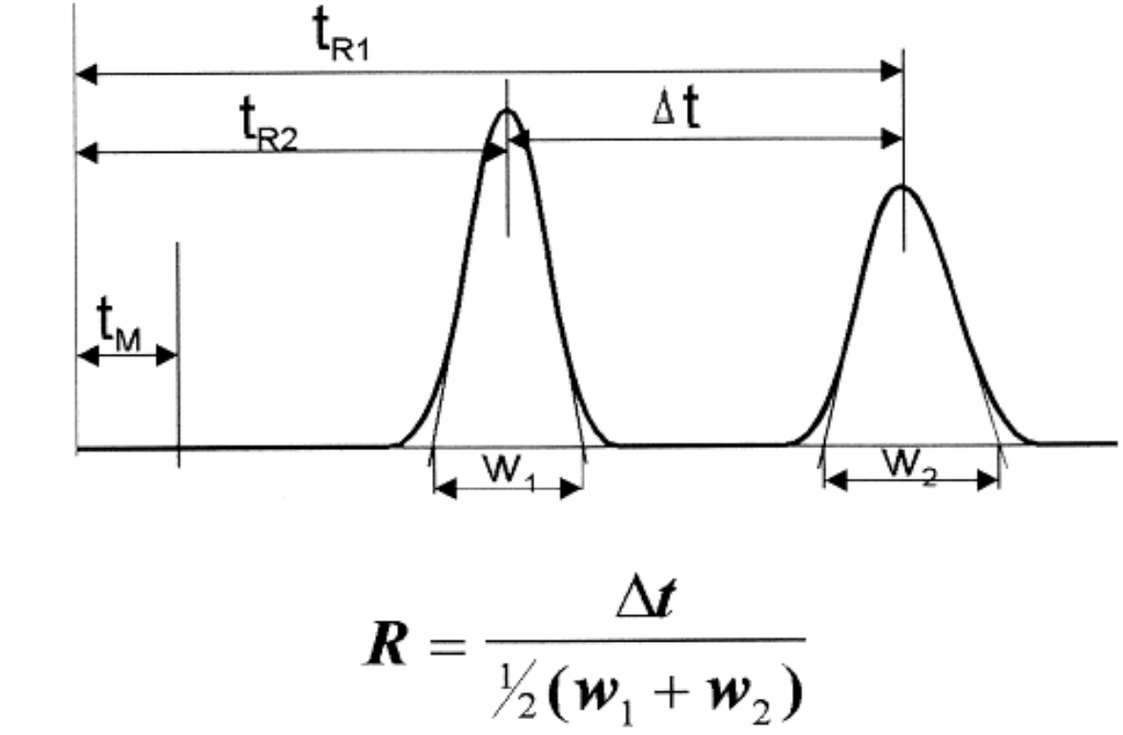
Speed 分离速度

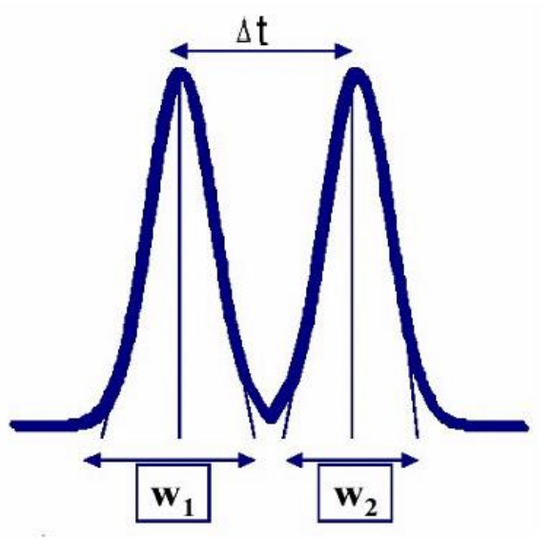
Sensitivity 灵敏度

Source： R&D Magazine

As chromatographers, what do we care?

--- Resolution (R)





$$R = \frac{\Delta t}{\frac{1}{2}(w_1 + w_2)}$$

When R=1, Peak 1 and peak 2 are separated basically.

When R=1.5, peak 1 and peak 2 are baseline separated.

Resolution (R)

$$R = \frac{1}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\sqrt{N}\right)\left(\frac{k'}{1 + k'}\right)$$

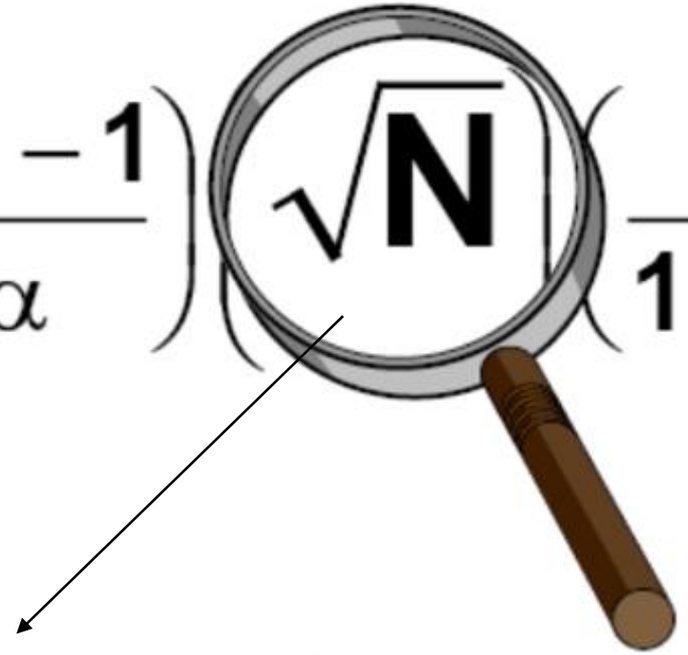
Separation factor

Column efficiency

Retention factor

Column efficiency (N)

$$R = \frac{1}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\sqrt{N}\right)\left(\frac{\kappa'}{1+\kappa'}\right)$$



N = L/H

(where L is the column length and H is the plate height.)

van Deemter equation (1956)

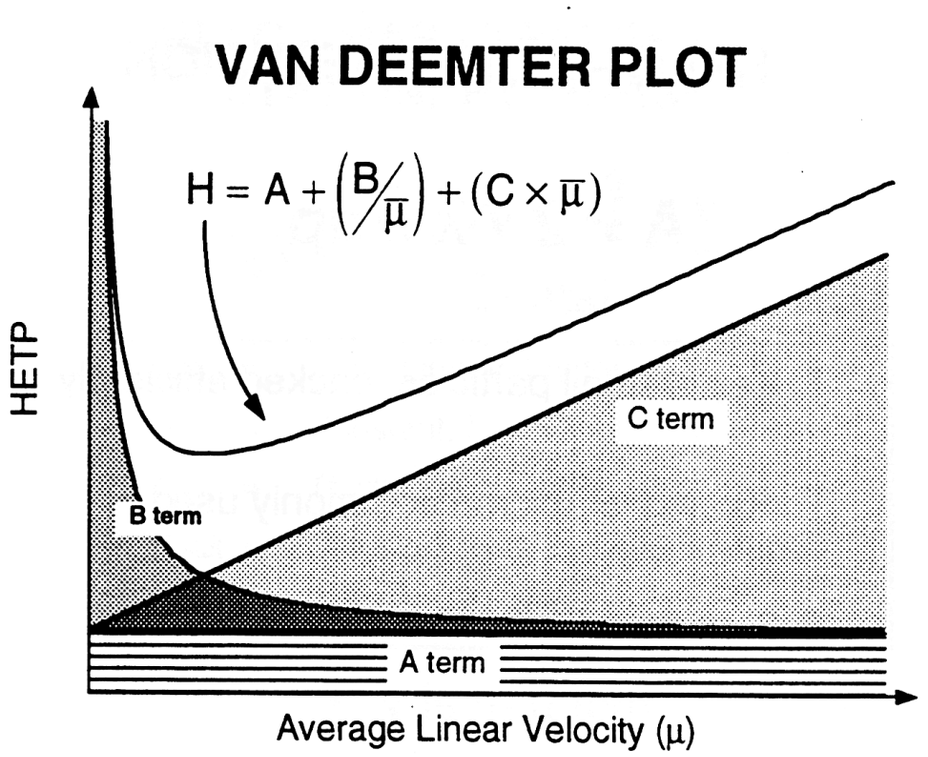
$$H = A(d_p) + \frac{B}{v} + C(d_p)^2 v$$

A term + B term + C term

A eddy diffusion

B longitudinal diffusion

C resistance to the mass transfer



$$\Delta P = \varphi \eta L \mathrm{u} / \mathrm{d}_p^2$$

φ is the flow resistance factor,

η is the solvent viscosity;

L is the packed length;

u is the mobile-phase linear velocity;

d_p is the diameter of the packed particles.

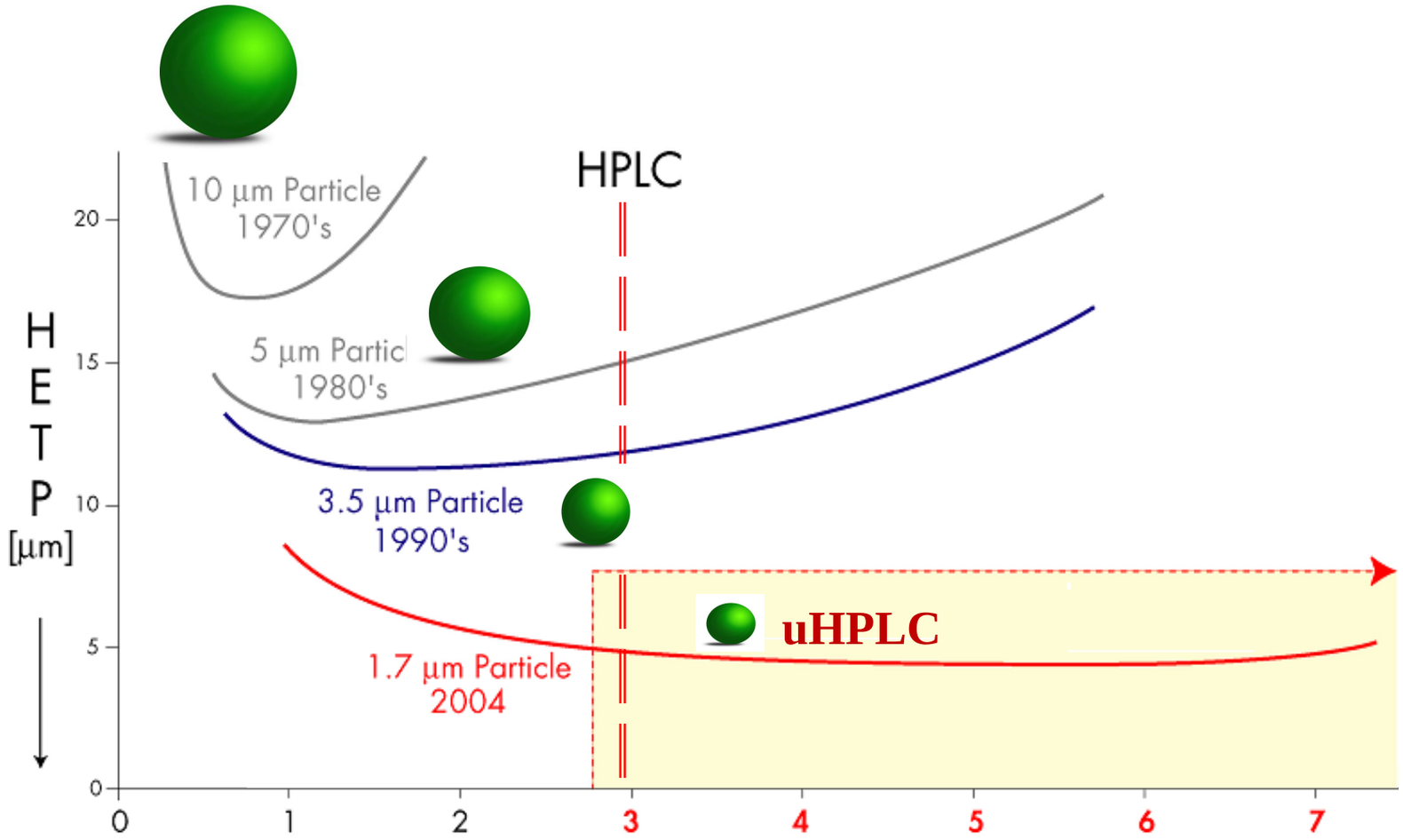
Column efficiency

$$\Delta P = \frac{\varphi \eta L v_{PR}}{d_p^2}$$

1.7 μm

N≈250 000 plates/m

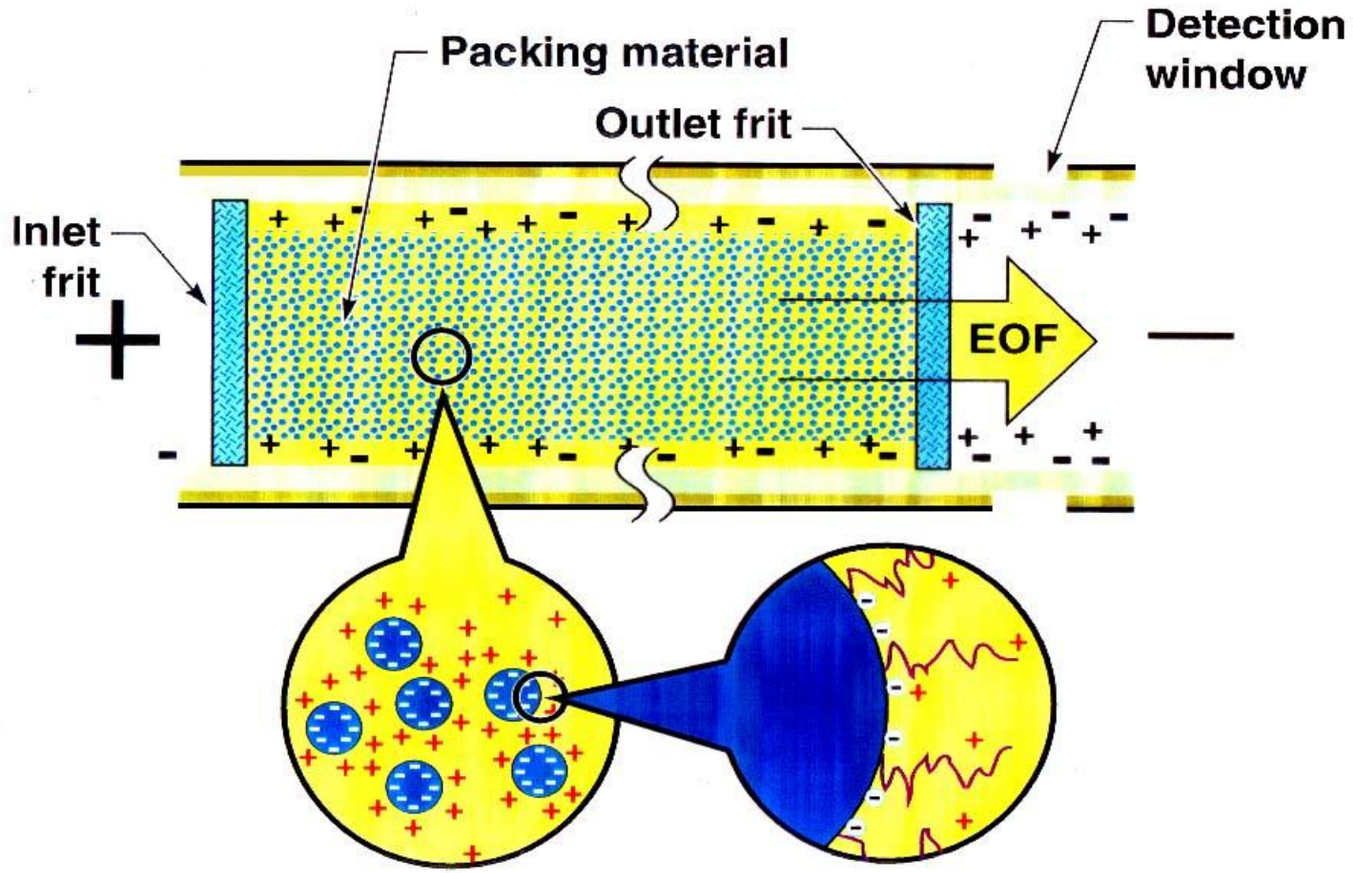
Trend in liquid chromatography in the past 50 years



Where do we go from uHPLC?

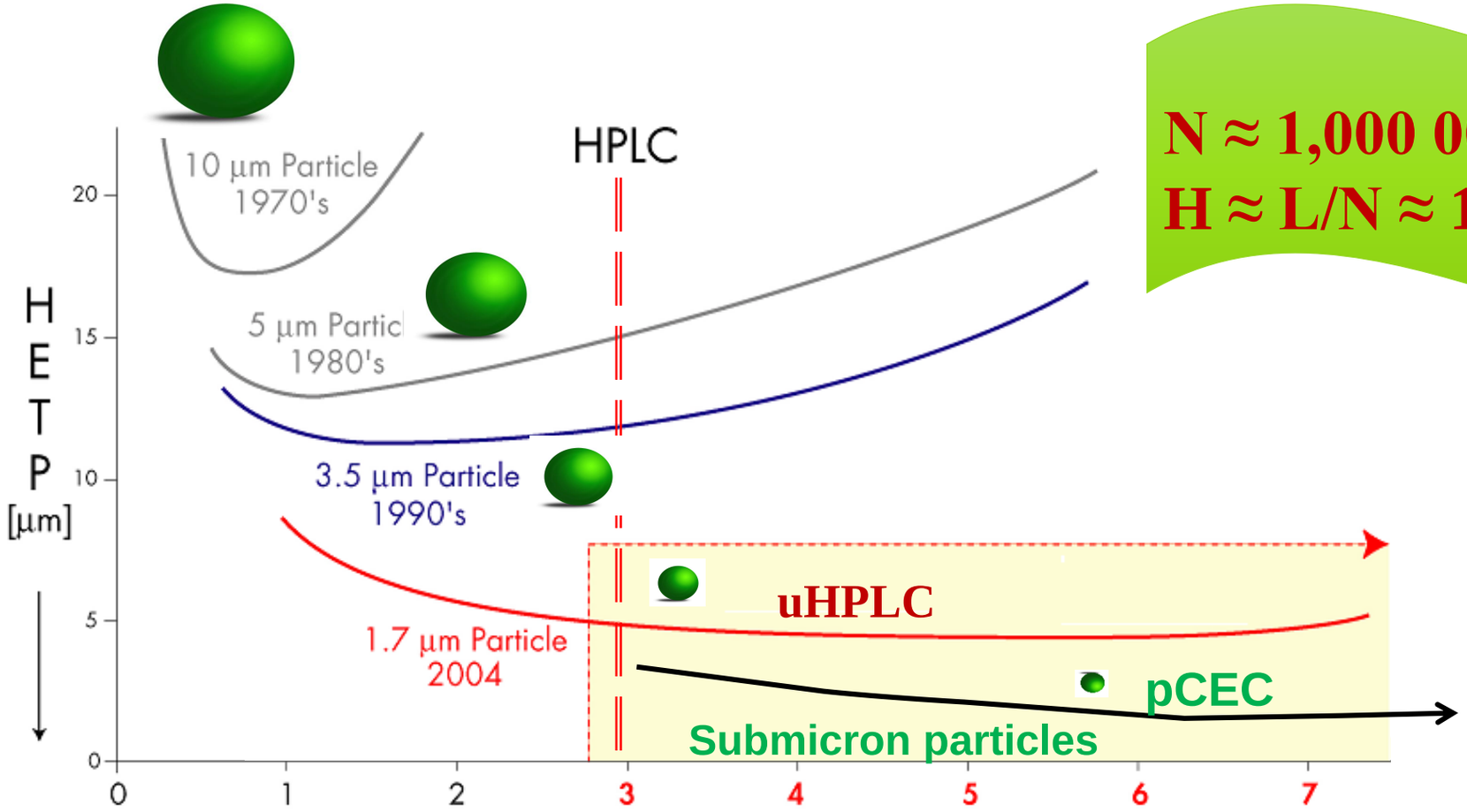
1. uuHPLC?
2. sfHPLC (slip flow HPLC)?
3. eHPLC (pCEC)?

Capillary ElectroChromatography (CEC) with submicron particles



A chromatographer's dream

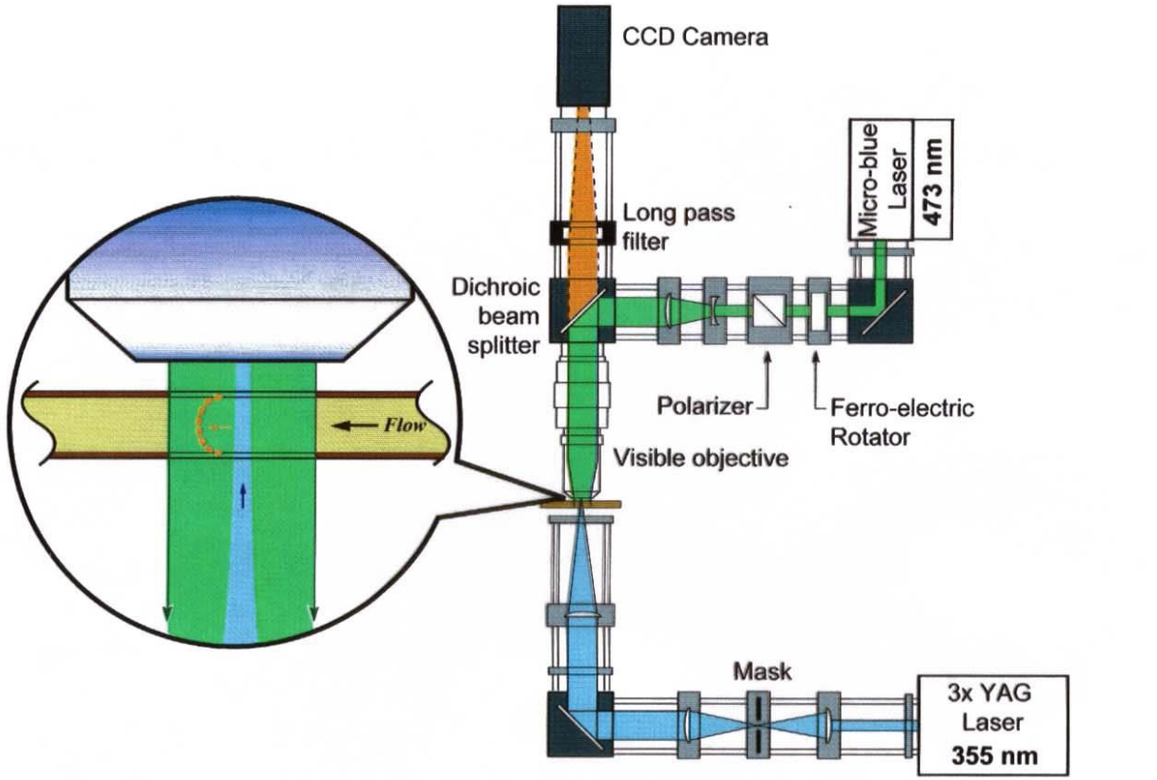
色谱梦



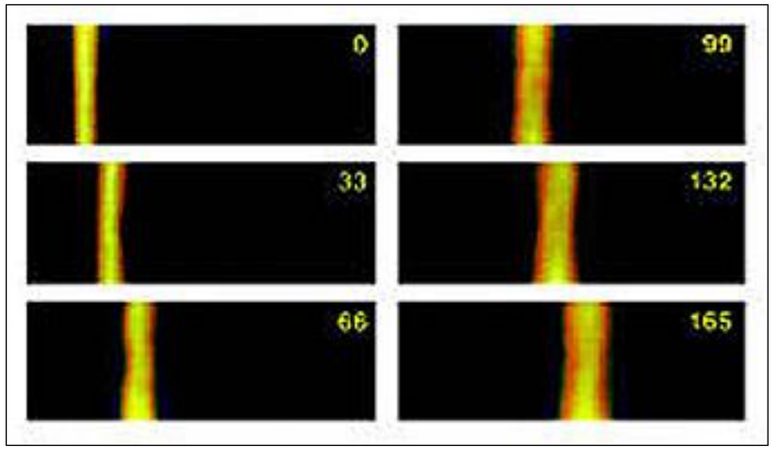
N ≈ 1,000 000 plates/m

H ≈ L/N ≈ 1 µm

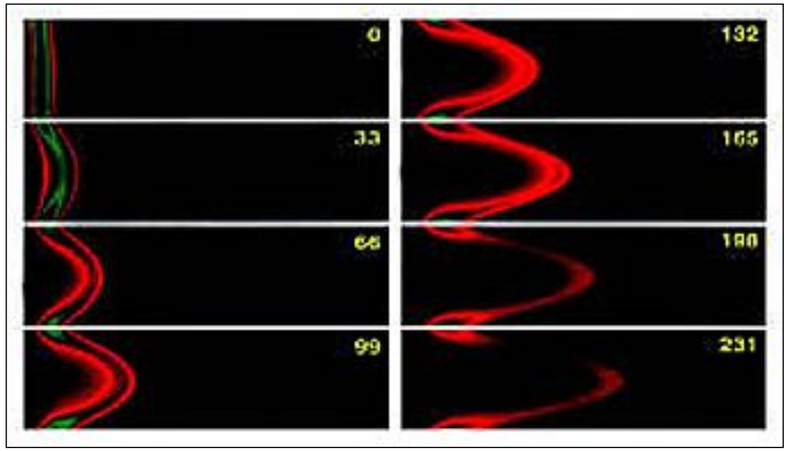
Micro-Flow Imaging Instrument



Comparison of flow profiles in CE and LC



Electroosmotic Flow



Hydrodynamic Flow

High Efficiency Separation in CEC: 600,000 p/m

Separation of 4 PAHs on 1.5 μm non-porous ODS .

Column: 100 μm i.d. x 28 cm packed length.

Mobile phase: 70% CH3CN/30% 4 mM sodium tetraborate (pH 9.1).

Voltage: 20 kV.

Injection: 5 kV/2s.

Detection: LIF, ex: 257 nm, em: 400 nm.

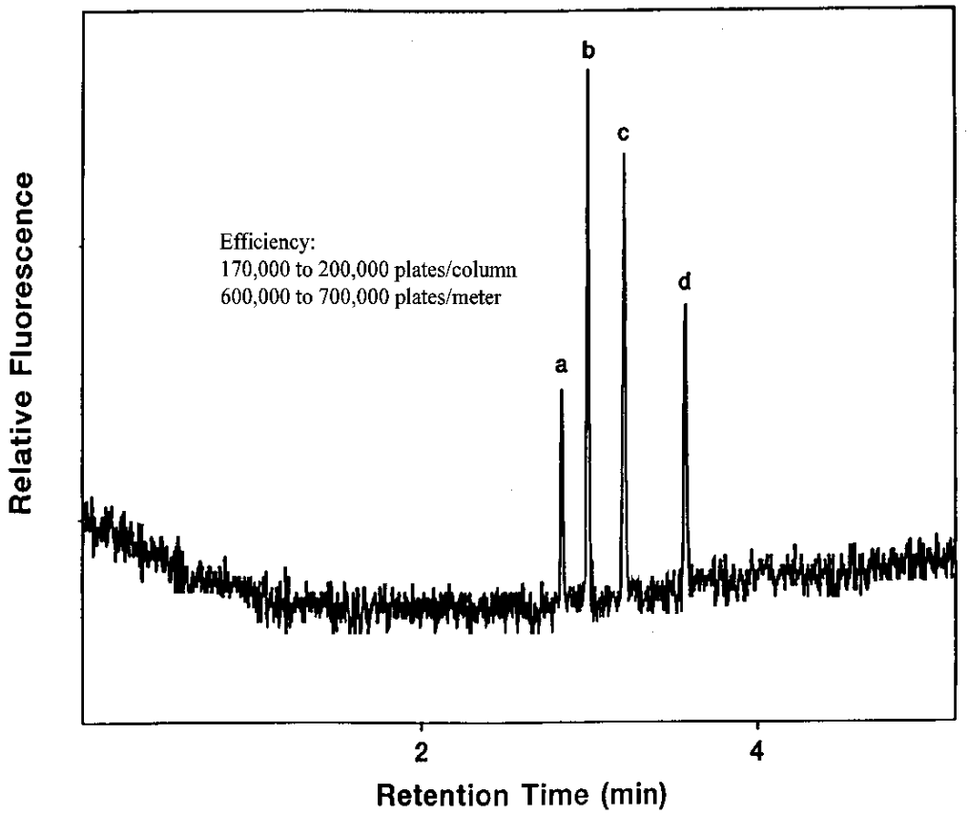
Sample:

a) Fluoranthene,

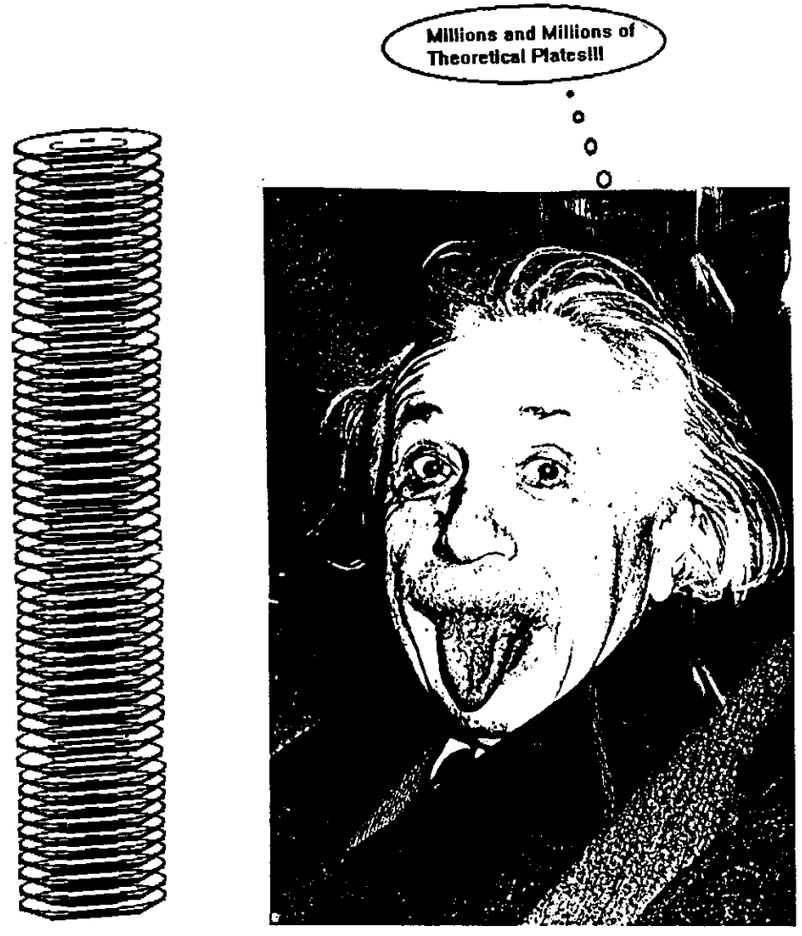
b) Benz[a]anthracene,

c) Banzo[k]fluoranthene，

d) Benzo[ghi]perylene.



Anal. Chem., 70(22), 787, 1998, Dadoo. R.; Zare R..; Annex. D ; Yan, C


Millions and Millions of
Theoretical Plates!!!

5 second separation of 5 PAHs by CEC

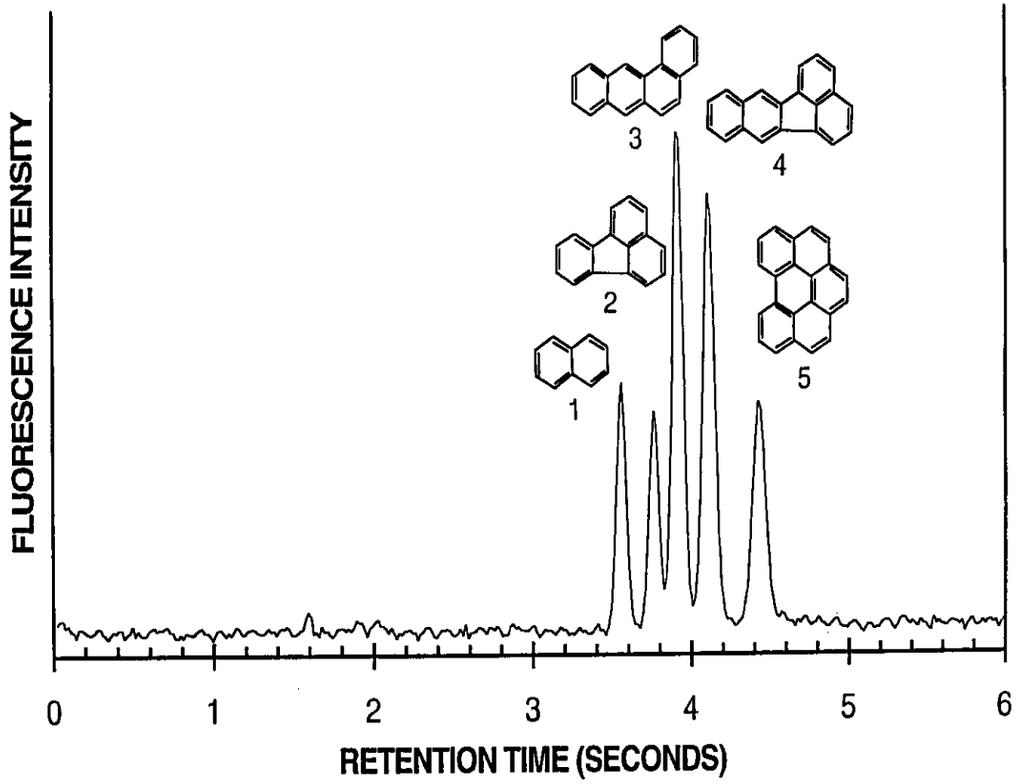
Column: 100 μm i.d. x 6.5 cm packed with 1.5 μm non-porous ODS.

Mobile phase: 70% CH_3CN/30% 2 mM TRIS (pH 9).

Voltage: 28 kV.

Injection: 1 kV/1s.

Detection: LIF, ex: 257 nm, em: 400 nm.



Anal. Chem., 70(22), 787, 1998, Dadoo. R.; Zare R..; Annex. D ; Yan, C

Double Separation Mechanism in CEC

Advantages of CEC

- ☺ Combination of CE & cLC

  -- double separation mechanism: suitable for both neutral & charged compounds.

- ☺ EOF-driven, no back-pressure, small particles

  -- high efficiency, high selectivity & high resolution, plus fast speed.

- ☺ Micro fluidic technique

  -- Economically attractive & environmentally friendly.

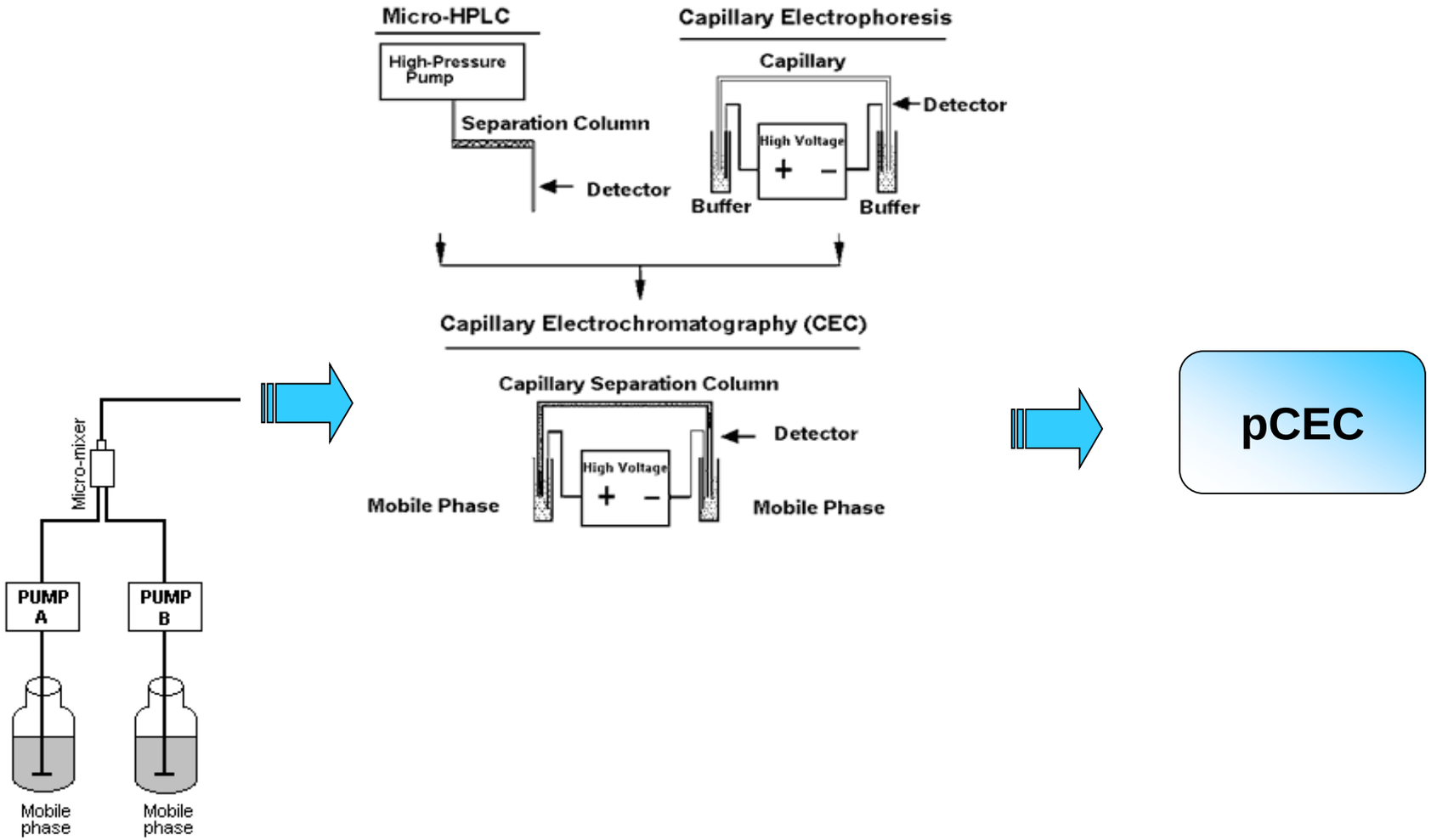
What are the bottle-neck for CEC?

1. Dedicated instrument;
2. Dedicated column;
3. Killer applications.

Part 2

高效微流电色谱
(eHPLC or pCEC)

Pressurized Capillary ElectroChromatography (eHPLC)



Advantages of pCEC（eHPLC）

1. High efficiency, resolution, selectivity and fast speed;
2. Miniaturized;
3. pCEC, capillary-HPLC and HPLC, all in one;
4. Gradient capacity;
5. Quantitative injection, fine tuning of selectivity.

Unimicro 十年磨一剑！

TriSep™-2000

TriSep™-2010GV

TriSep™-2100

TriSep®-3000 eHPLC

最新一代

LIF

ECD

eHPLC

MS

UV/Vis

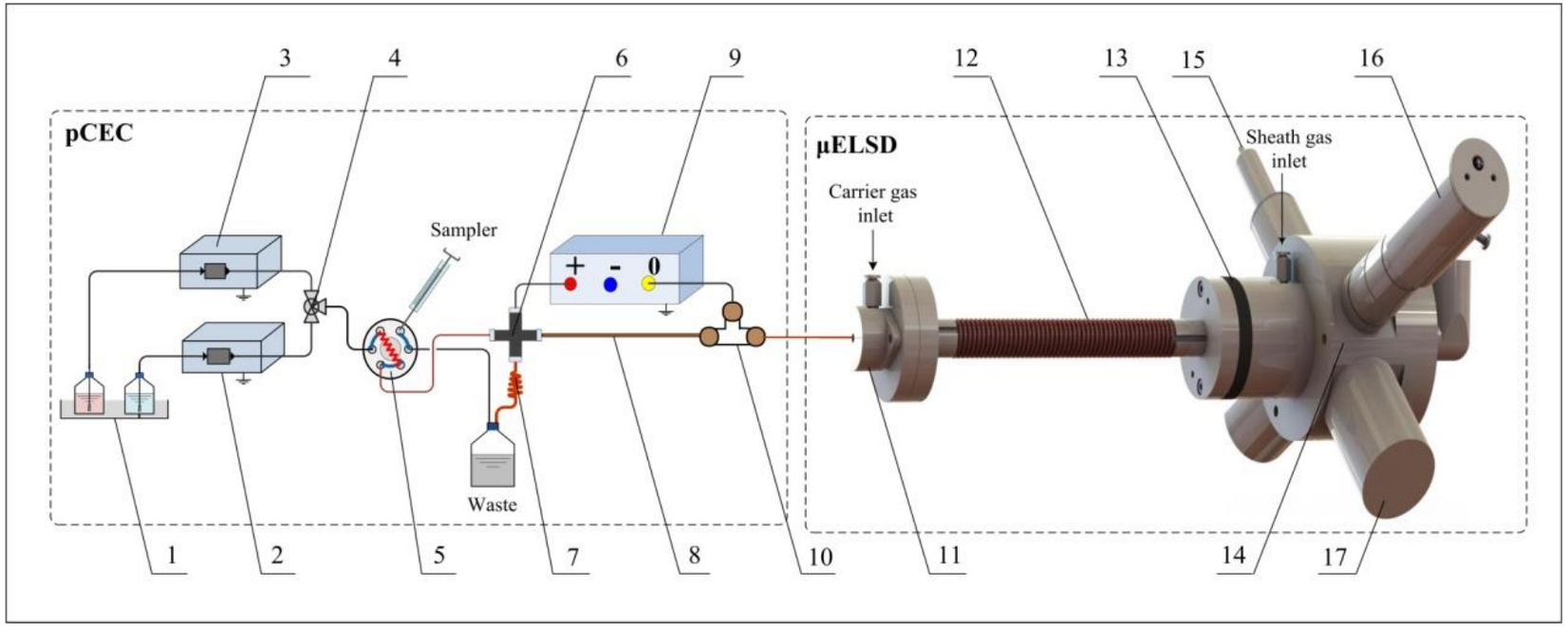
ELSD

More suitable for the separation of complex compounds!

Achievements

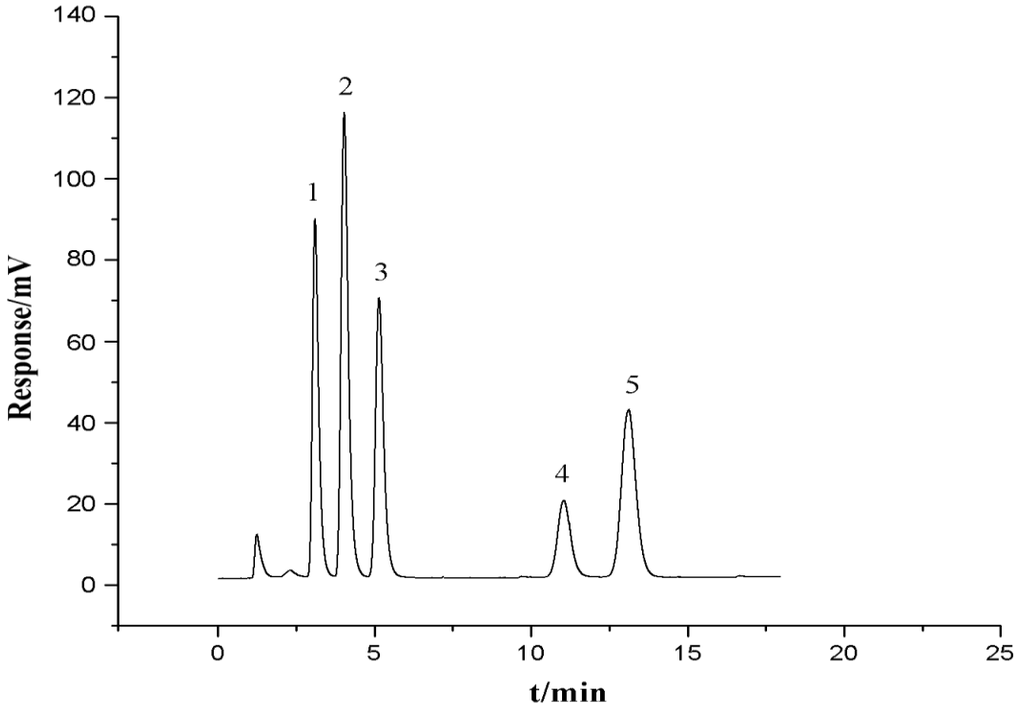
- ☆ **Program on the Development of National Key Scientific Instruments and Equipment (2011YQ150072).**
- ☆ **National Invention and Entrepreneur Award: Outstanding**
- ☆ **Shanghai Science and Technology Advancement Award: First Place**
- ☆ **National Key and New Product.**
- ☆ **BCEIA Golda Awards : 2.**
- ☆ **Patents: 83, including 4 US patents and 3 PCT.**
- ☆ **Articles: 300**
- ☆ **……**

pCEC-μELSD coupling



1. 试剂瓶；2, 3. 输液泵；4. 混合阀；5. 六通阀；6. 四通；7. 分流阀；8. 毛细管色谱柱；
9. 高压电源；10. 微型三通；11. 微流雾化器；12. 蒸发管；13. 鞘流装置；14. 光散射池；
15. 激光光源；16. 光电倍增管；17. 光阱

pCEC-μELSD separation of sugar alcohol



Column：EP-200-150-5-Amide 80 (200 μm×150 mm, 5 μm,);

Mobile phase：ACN：H_2O（40 mmoL/L TEA）=8 0: 20；

Voltage：+5 kV；

Current：5.6 μA；

Injection：50 nL；

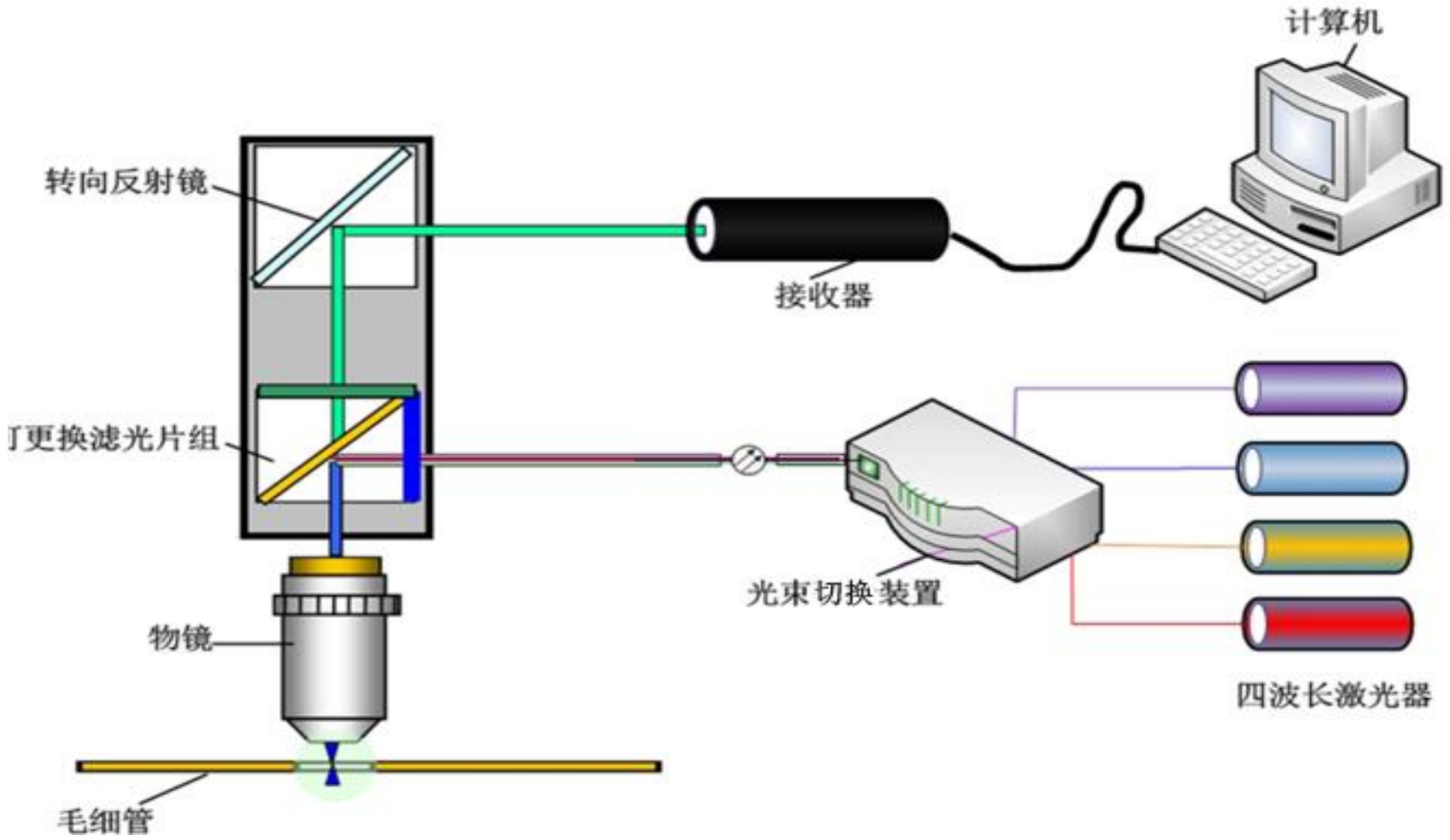
Carrier：N_2；

Evaporative Temp：120℃；，

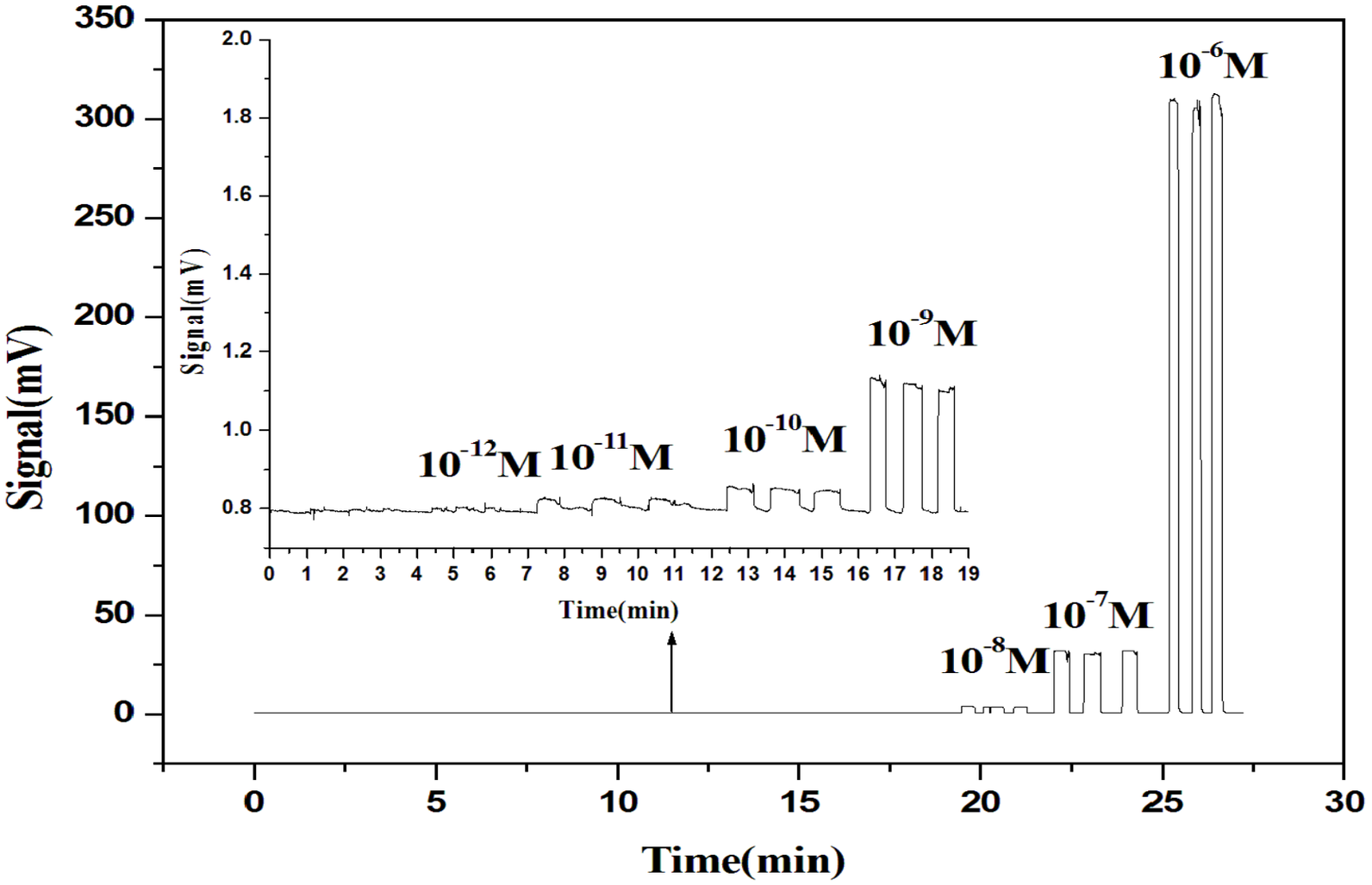
Flow rate：0.8 L/min；

Pressure：4.3 MPa

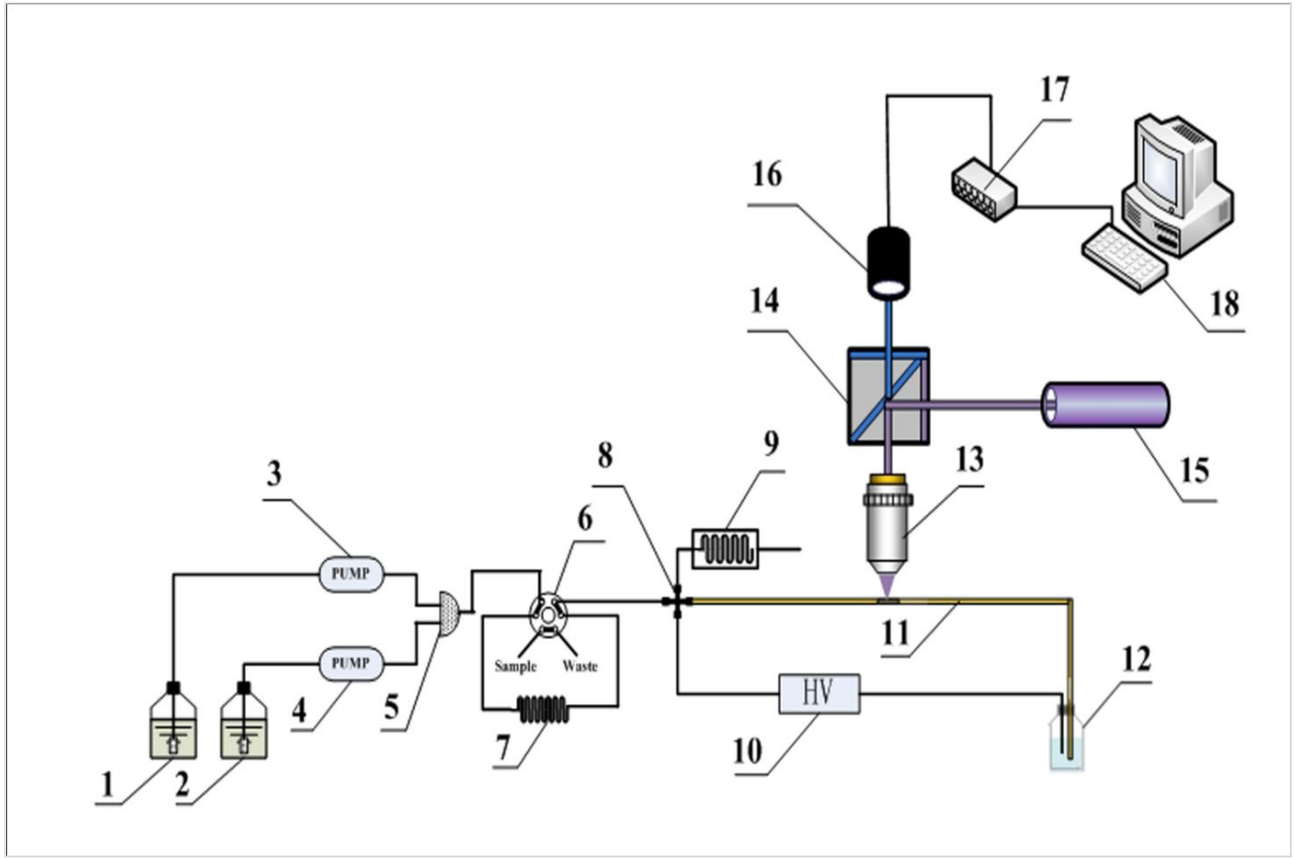
LIF with 4-lasers



Sensitivity test on LIF

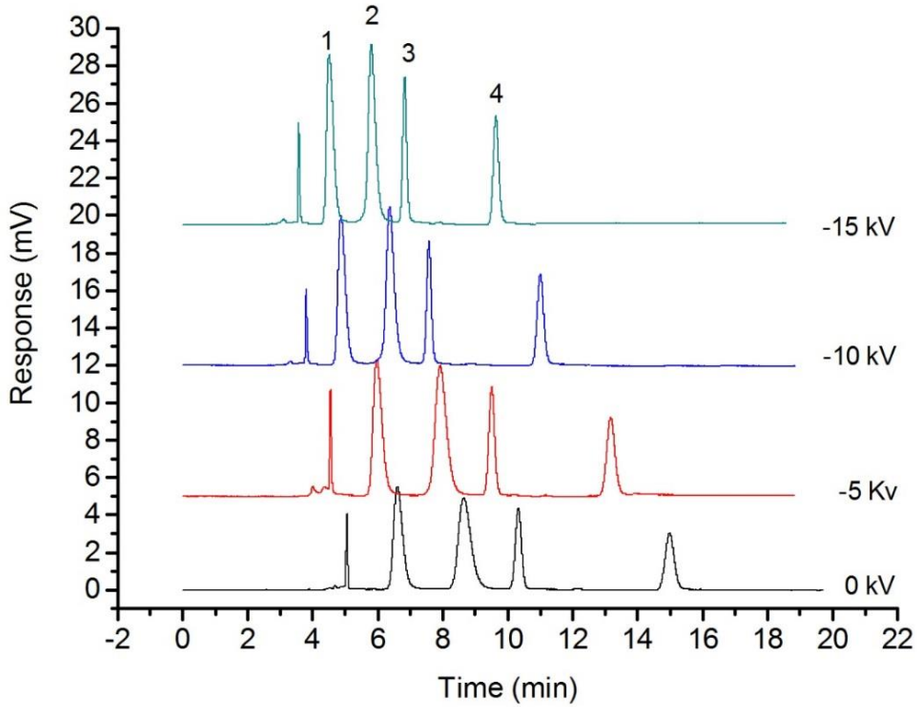


Schematic diagram of pCEC- LIF



1,2 Reagent bottle；3,4 Pump；5 Mixer Valve；6 Injection Valve；7 loop；8 splitting cross；9 Capillary restrictor；10 High Voltage；11 Capillary chromatographic column；12 waste；13 Objective lens；14 Filter system；15 Laser；16 Photomultiplier；17 Data acquisition unit；18 Data processing unit

pCEC-LIF separation of aflatoxins



Sample: 1.G_1； 2.B_1； 3.G_2； 4.B_2

μMS—eHPLC with flowrate of 150 nL/min

1. Built in vacuum pump and PC ;

2. Analyze on site;

3. Base on chip-technology, balance within 30 min;

4. Save 80% N2；

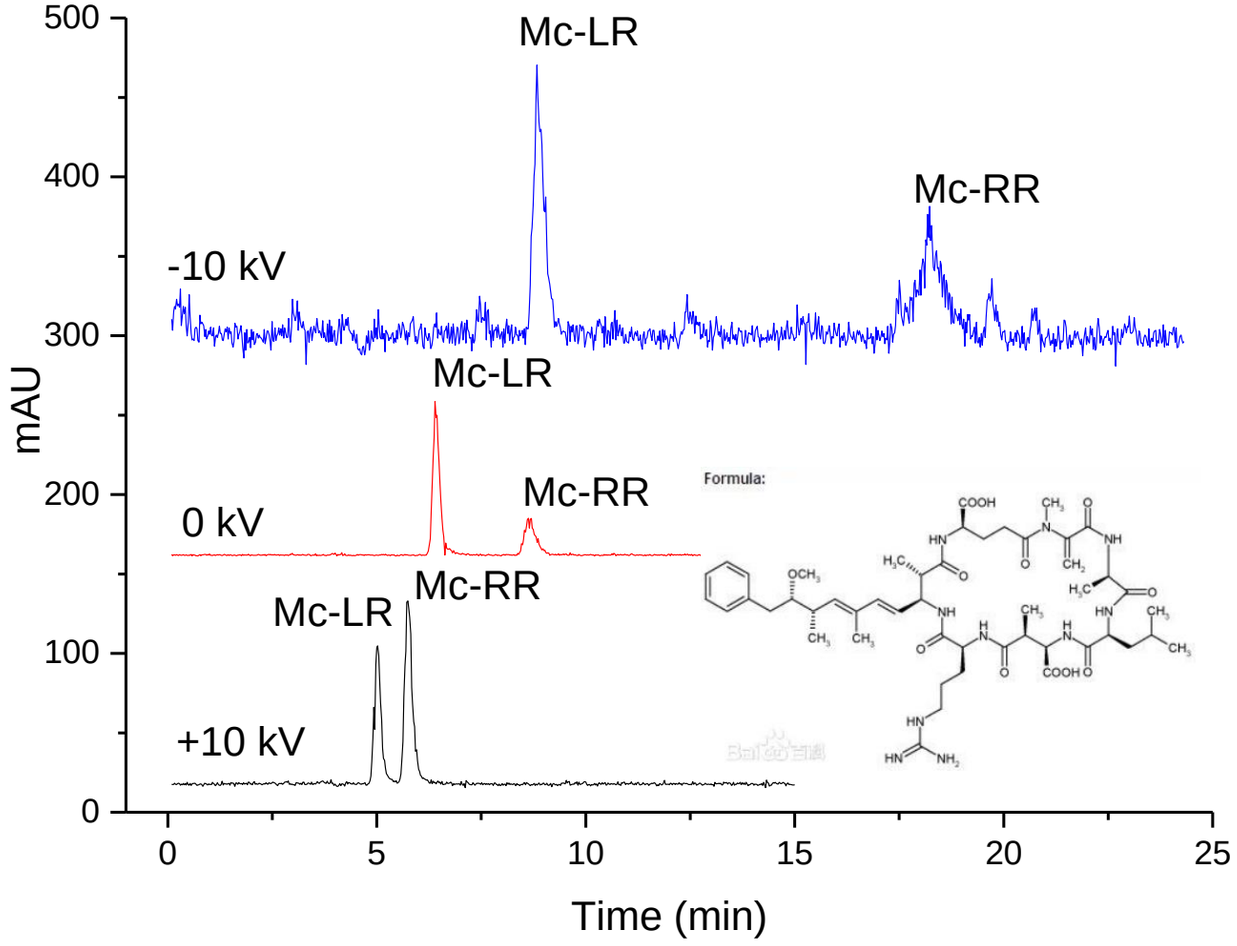
5. Easy for use and maintenance, completely tool-less；

6. Compatible with eHPLC，qCE，nano-LC, HPLC, etc;

7. On line dilution and injection to monitor a reaction.

eHPLC- μMS coupling system

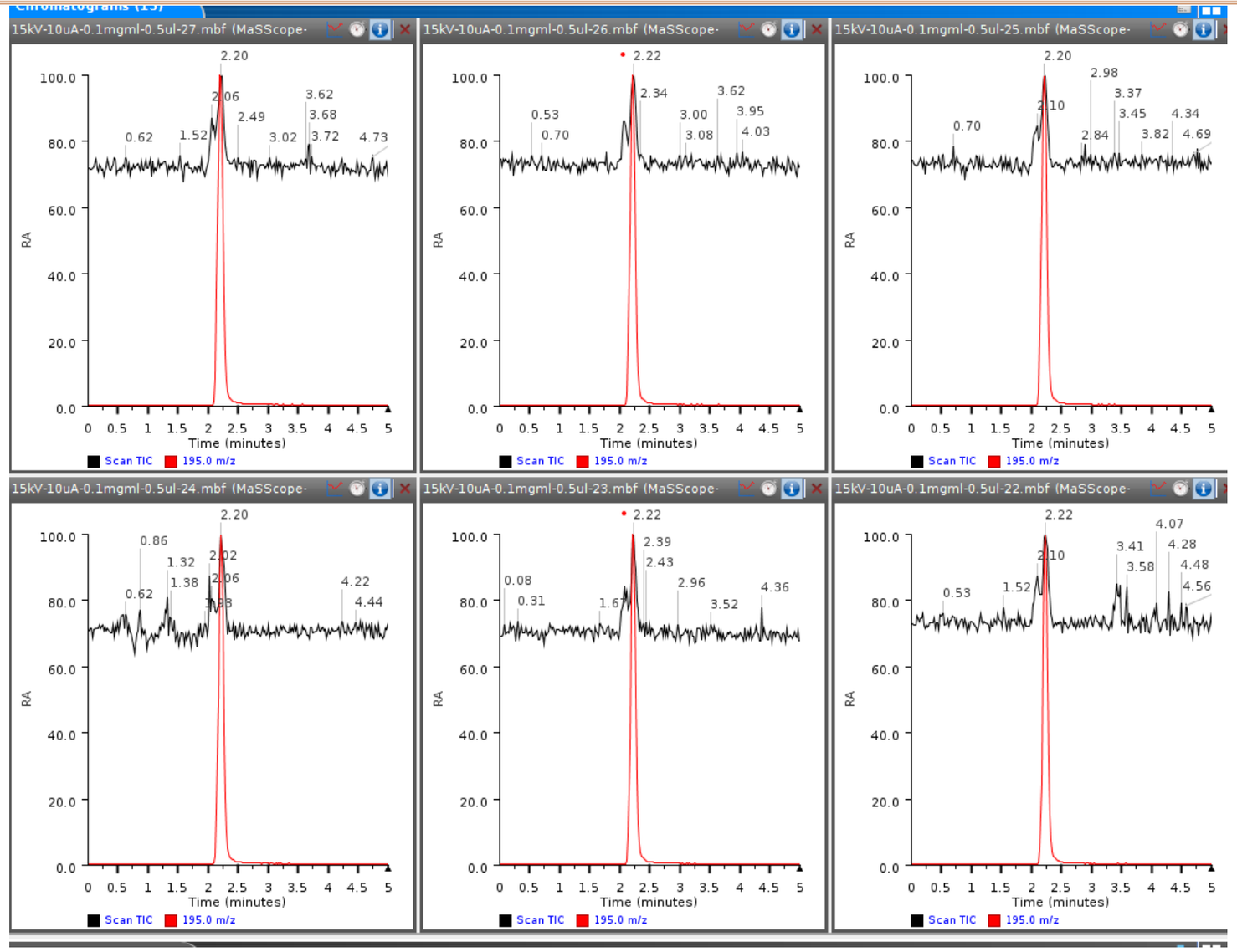
eHPLC-MS Separation of cyclic hepta-peptides （Microsystin）



Reproducibility of eHPLC-MS separation of Mc

Run	Retention Time	Peak area
1	5.55	153520
2	5.59	159197
3	5.57	151534
4	5.57	157320
5	5.59	153974
6	5.59	154009
RSD%	**0.29**	**1.8**

Reproducibility of eHPLC-MS



Condition：

Column：100 μm i.d. x 20 cm, 3.0 μm C18, +50 μm x 5 cm (decoupler)+15 cm (empty);

Moble：80/20:ACN/H2O+0.1% FA;

Flow：166 nL/min;

Sample：0.01 mg/ml Caffeine;

Injection v：1.7 nL;

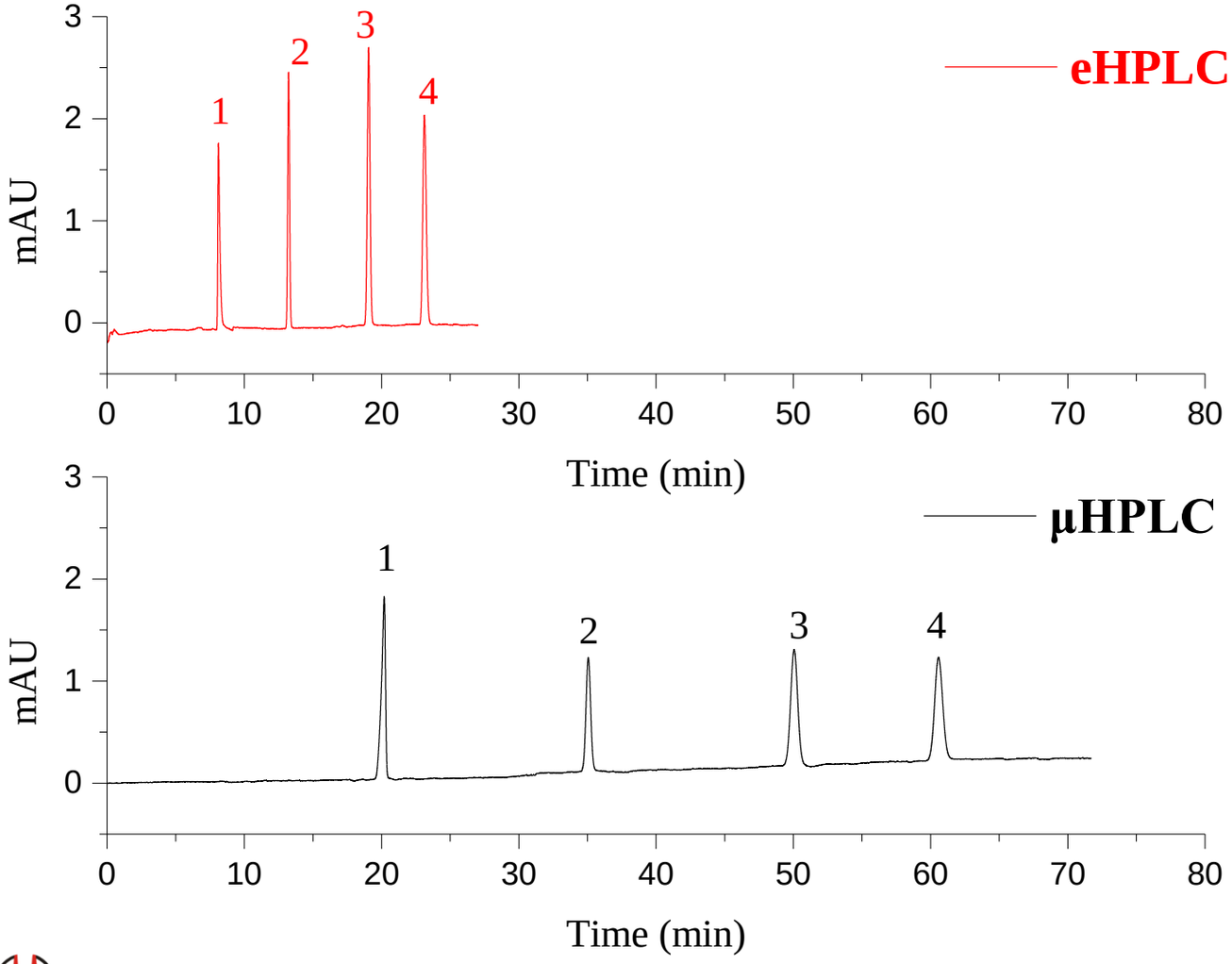
Temp：15℃。

	时间	峰面积
1	2.2	1109616
2	2.22	1064812
3	2.2	1070160
4	2.2	1075024
5	2.2	1080975
6	2.22	1103332
RSD%	0.4	1.6

Part 3

eHPLC with HALO and sub 2-micron particles

eHPLC vs nanoHPLC with 1.8 μm particles



eHPLC vs μHPLC

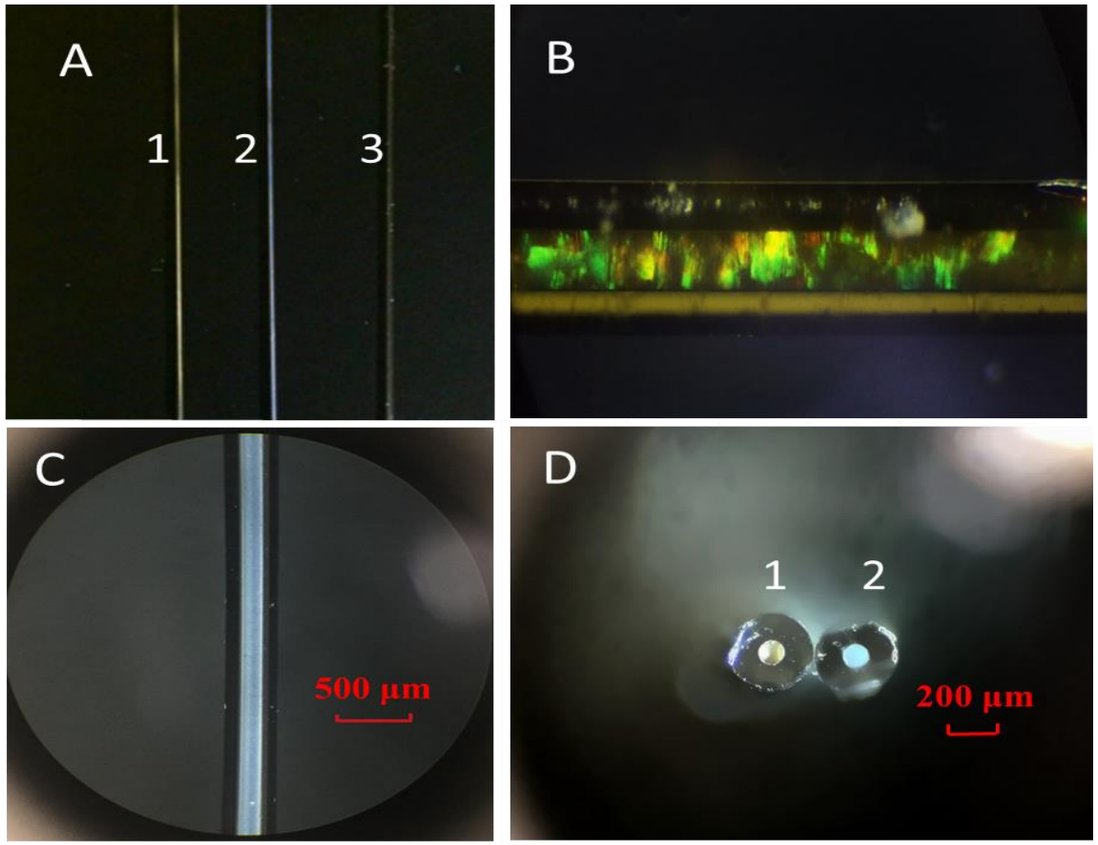
	eHPLC	μHPLC
Run Time	25 min	60 min
Solvent Consumed	4.5 mL	10.8 mL
Sample Consumed	1.0 μl	1.0 μl
Column Efficiency (naphthanlene)	200000	92000
Resolution(3/4)	9.5	9.7

eHPLC相比较μHPLC的优势：

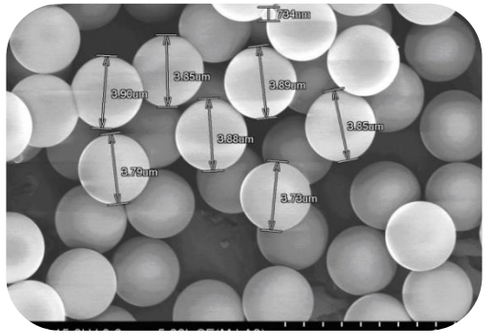
Efficiency: 2X，Speed: 2.5X，Sensitivity: 3X.

Part 4

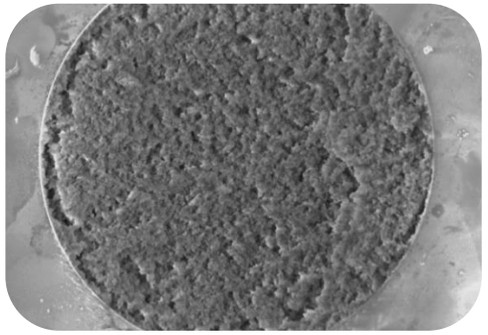
eHPLC with Submicron particles



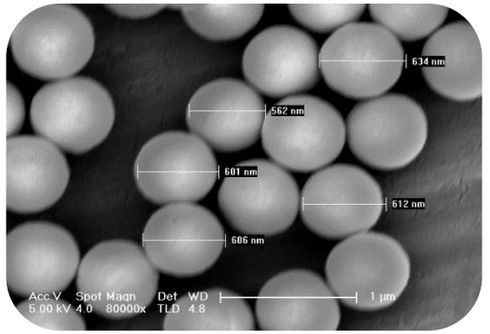
Capillary columns packing material



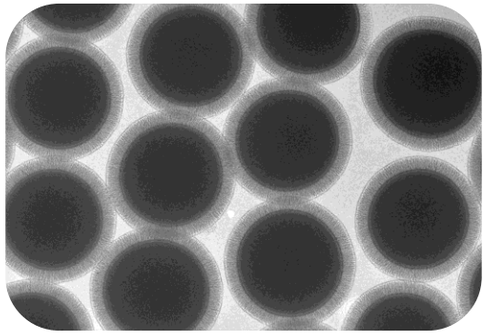
Submicron



Monolithic



NPS

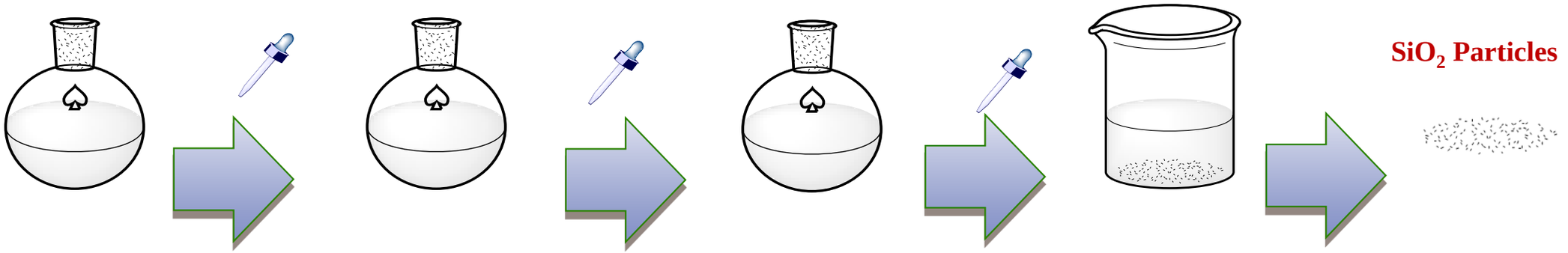


Core-Shell

Preparation

Preparation of Submicrometer silica particles：

Improved Stöber method



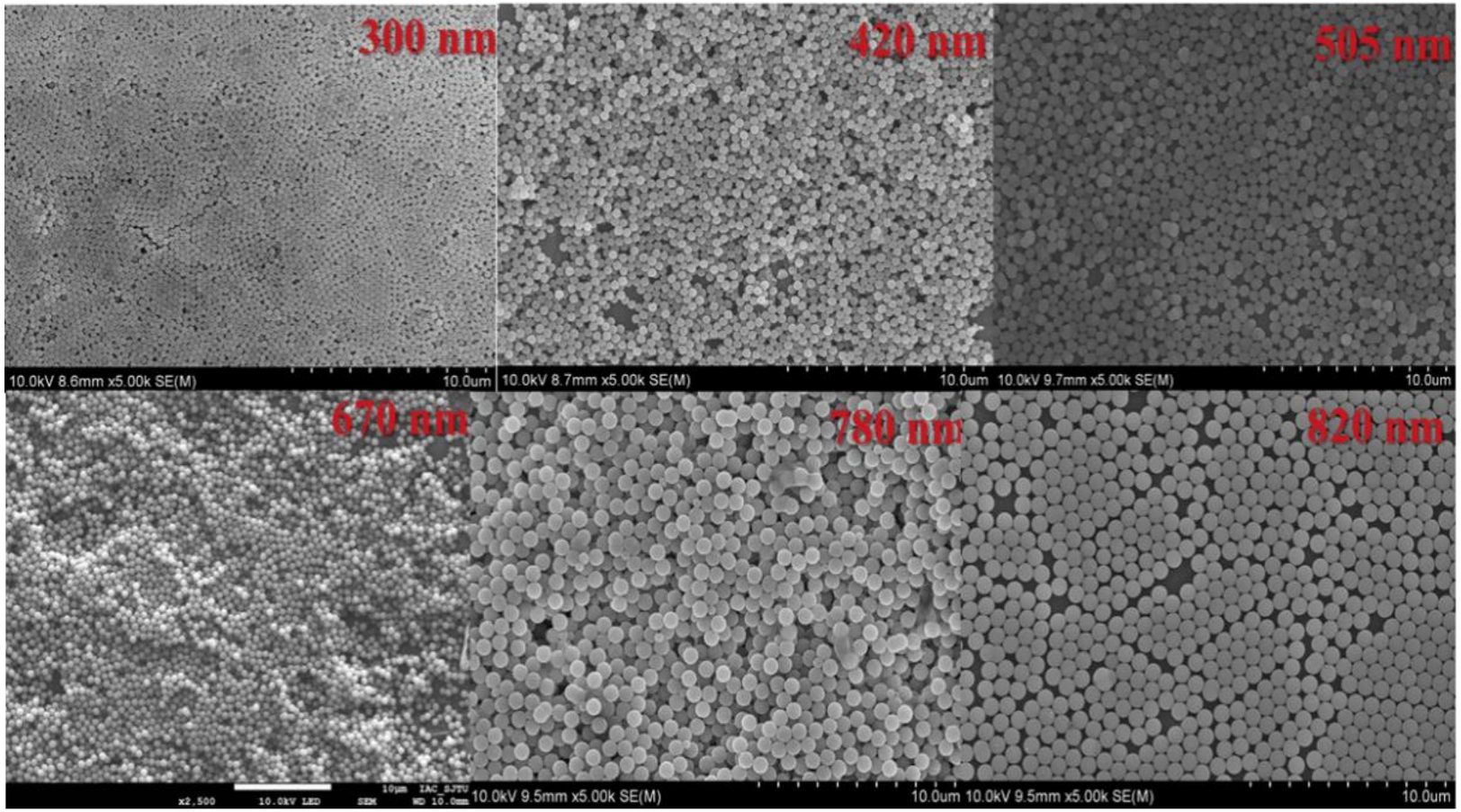
modification：

Silica particles were calcined at 600° C for 6 h for three times

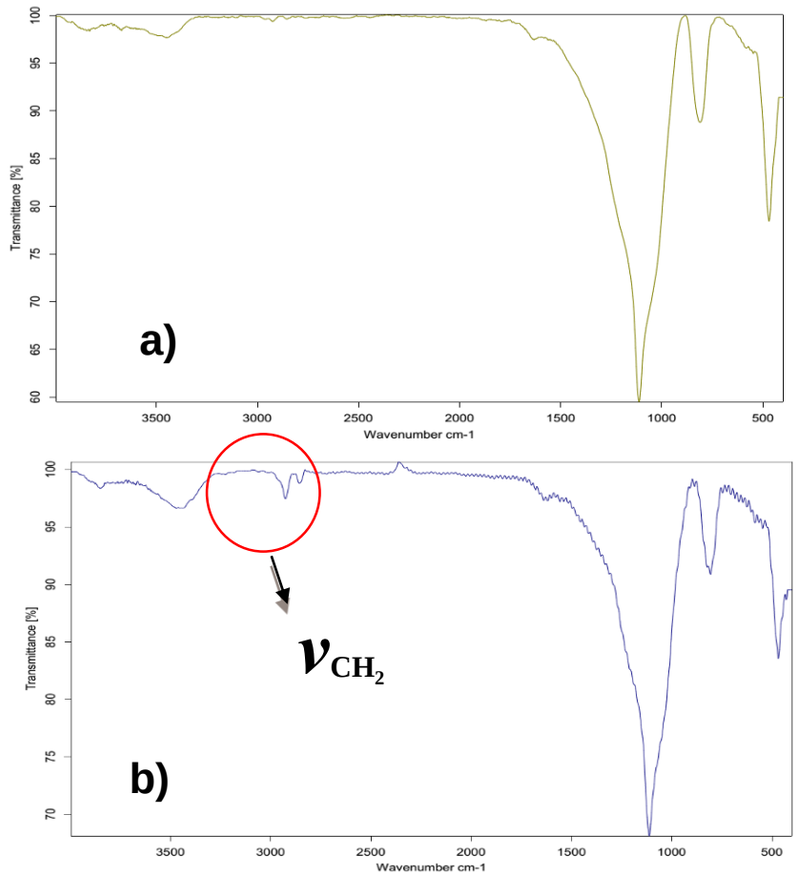
Rehydroxylated in 50/50% (v/v) nitric acid/water overnight

reacted with 16% n-octade--cyltrichlorosilane and 2% methyl trichlorosilane respectively in dry toluene with the protection of nitrogen

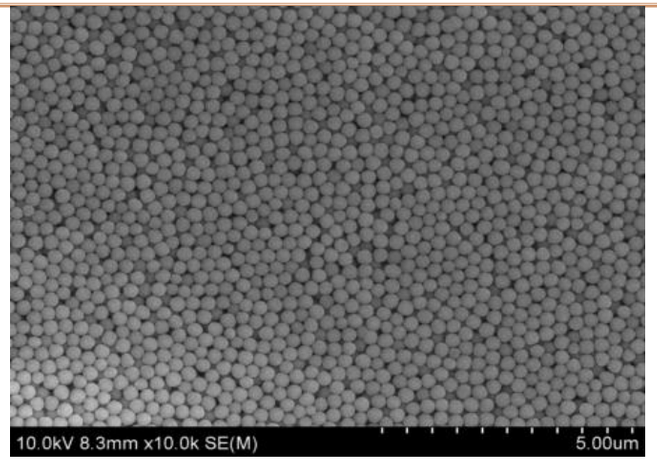
The SEM photos of 300-800 nm SiO_2 particles



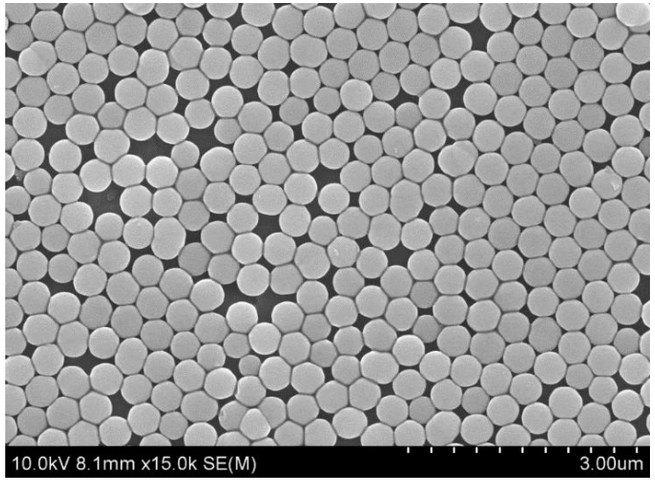
Submicron SiO2 modified with C18



Infrared spectra for a colloidal crystal (a) before and (b) after modification.

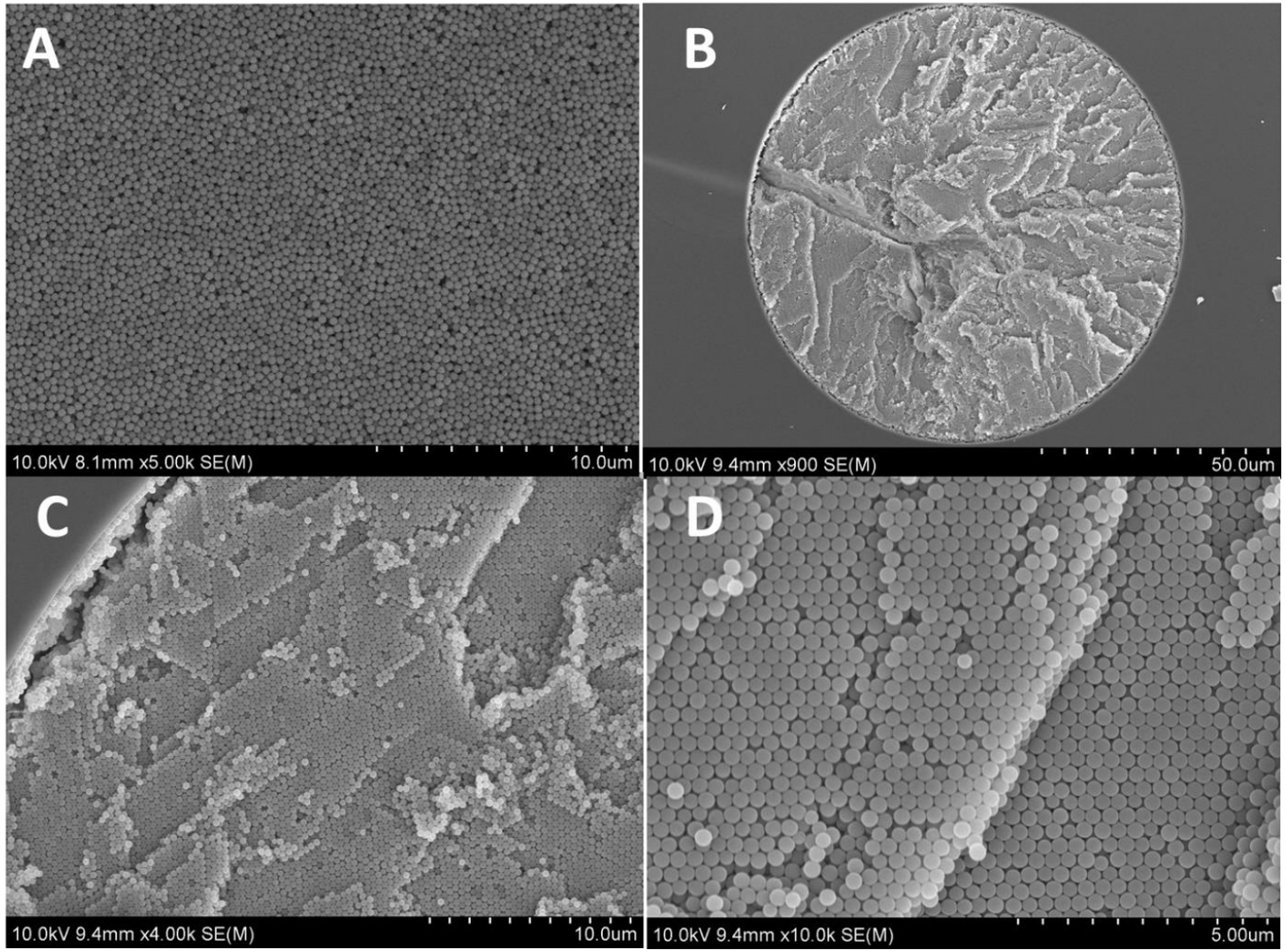


SEM micrograph of 343 nm SiO_2 particles.



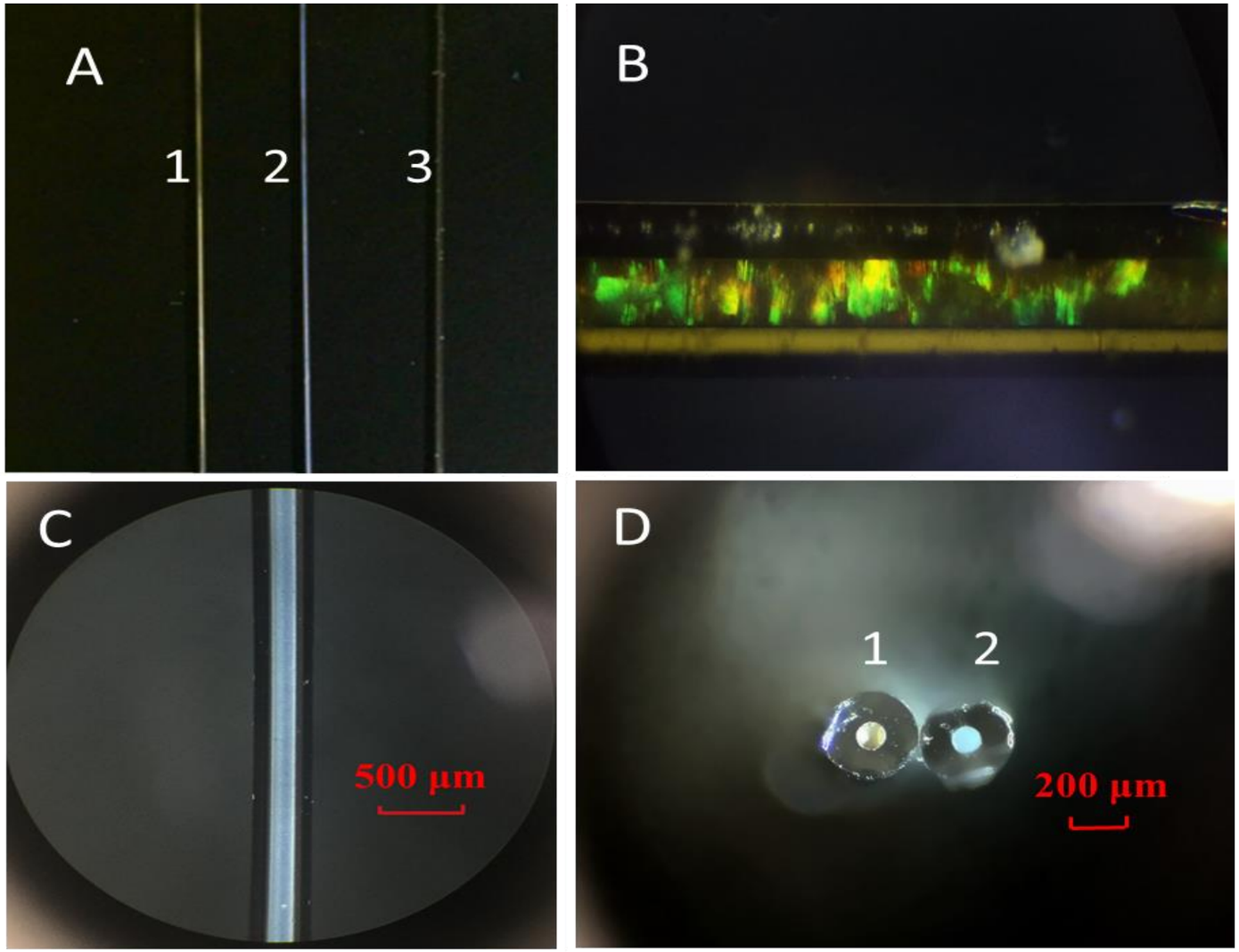
SEM micrograph of 454 nm SiO_2 particles modified with C18.

SEM of Photonic Crystal column



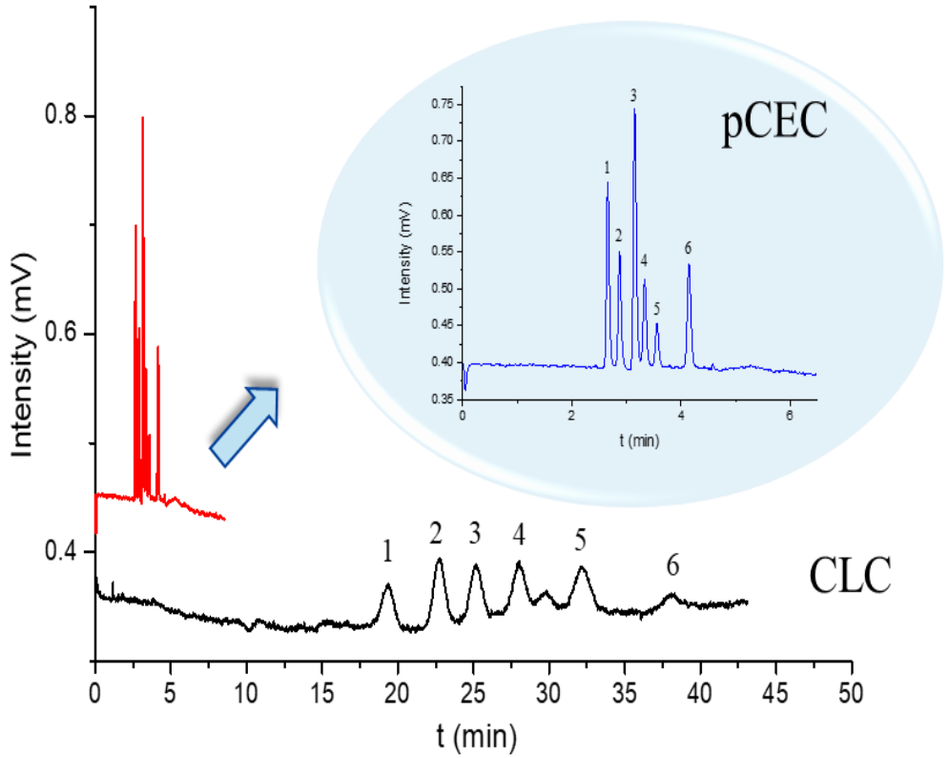
A： SEM of 320 nm ODS on a plate.
B-D： SEM of cross section of a packed 100 μm i.d. capillary,
B, X900; C, X4000; D, X10000.

Bragg diffraction (Blue color)



The most amazing photo in history of science

Separation of organic compounds with submicron particles in eHPLC and cLC



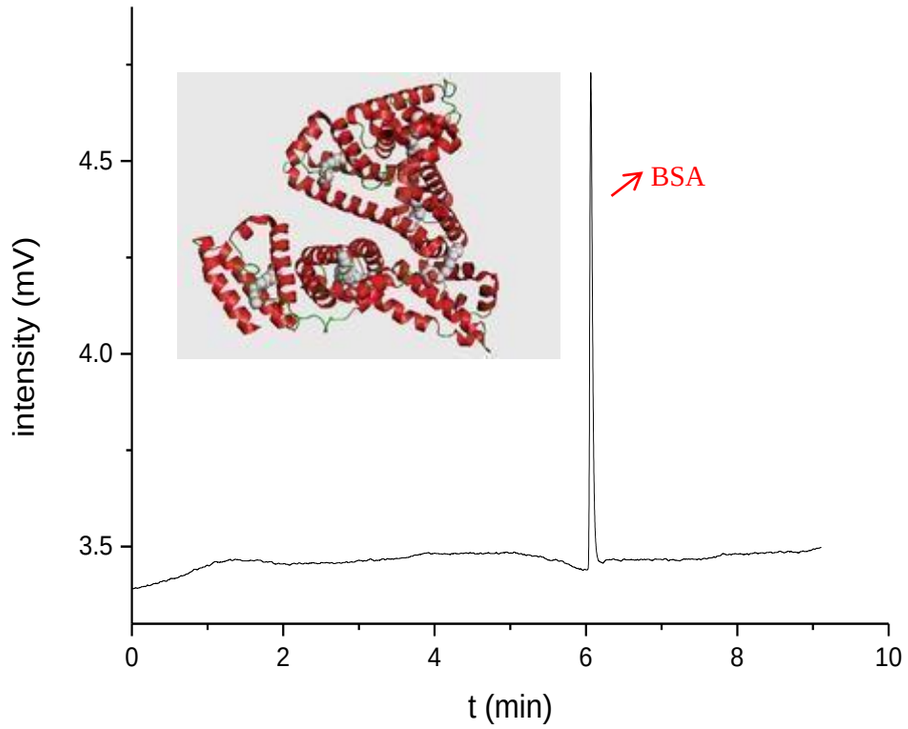
Applied voltage: 10 kV;

1) thiourea; 2)a- naphthol; 3) benzophenone; 4) naphthalene; 5) biphenyl; 6) butylbenzene.

Column efficiency N (plates/m): 11,780 to 170,000.

eHPLC separation of BSA with 603 nm C18

----N: 1,264,910 plates/m



Instrument: TriSepTM-2100 pCEC

Column: 30 cm total length (10 cm effective) 100 μm id packed with 603 nm of nonporous C18

Mobile phase: 45% v/v ACN and 55% v/v H_2O

Applied voltage: -10 kV at outlet

Applied pressure: 15.5 MP

Injection: 0.22 nL (a sample loop of 1 μL with splitting ratio of 4500:1

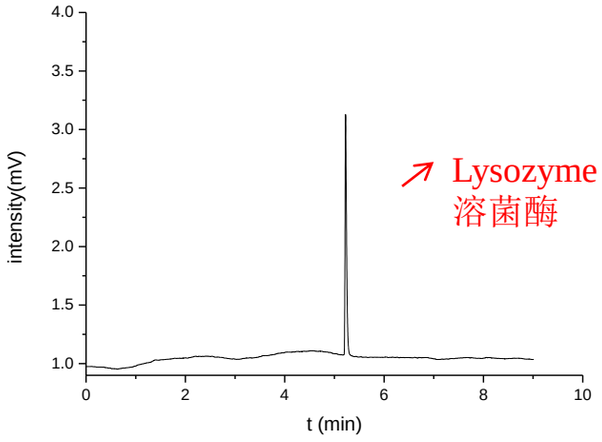
UV detection: 280 nm

Sample: 5 mg/mL BSA

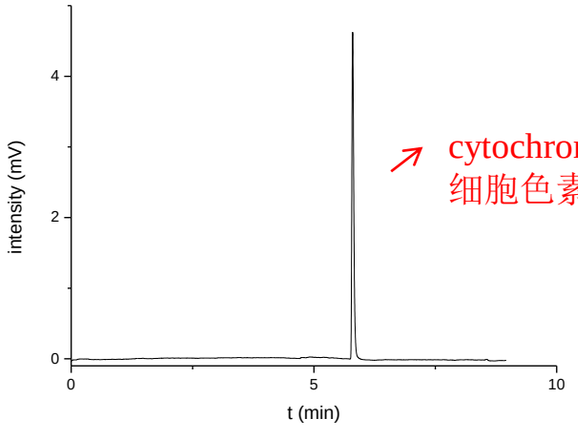
eHPLC separation of lysozyme with 670 nm C18

-----N :1,391,520 plates/m

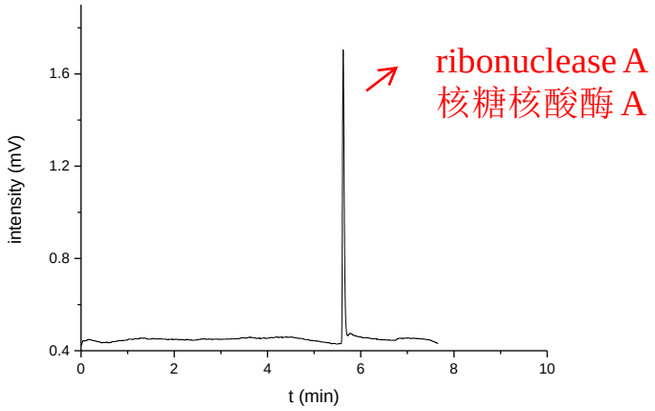
Instrument: TriSep^{TM}-2100 pCEC
Column: 30 cm total length (10 cm effective) 100 μm id packed with 603 nm of nonporous C18
Mobile phase : 42% v/v ACN and 58% v/v H_2O
Applied voltage : -10 kV at outlet； applied pressure :13.3 MP
Injection : 0.22 nL (a sample loop of 1 μL with splitting ratio of 4500:1
UV detection : 280 nm； Sample : 5 mg/mL



N (plates/m) :1,391,520.

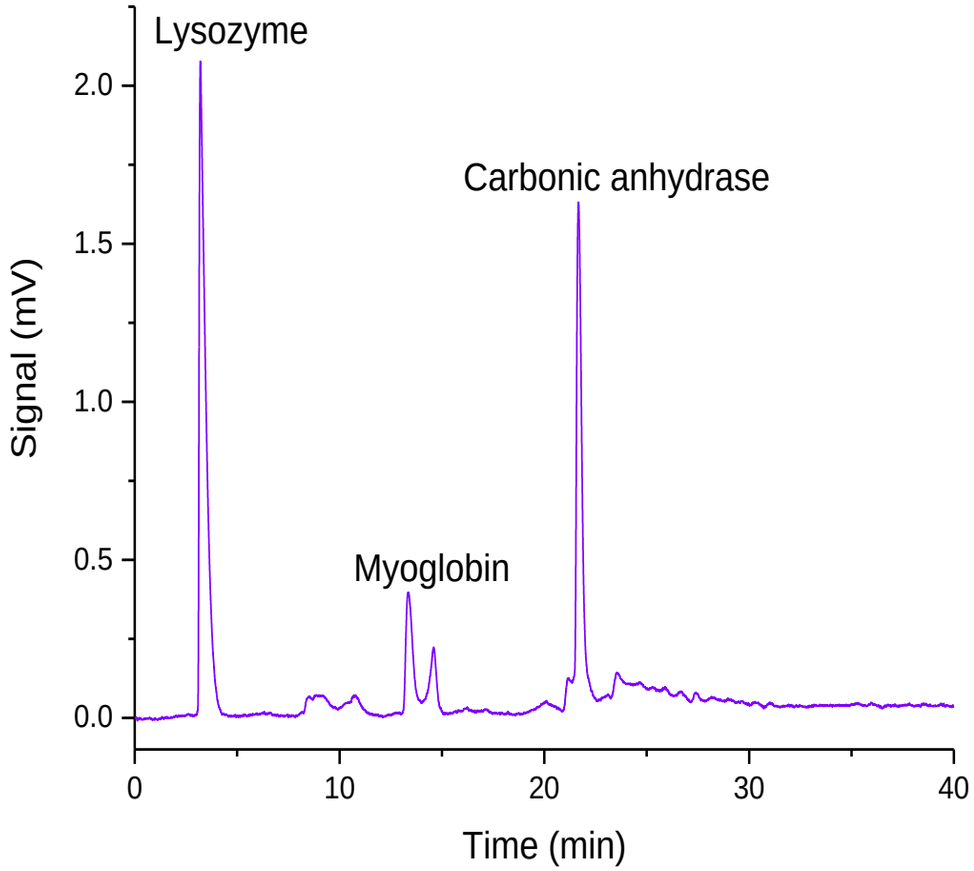


N (plates/m) :1,011,560.



N (plates/m) :989,730.

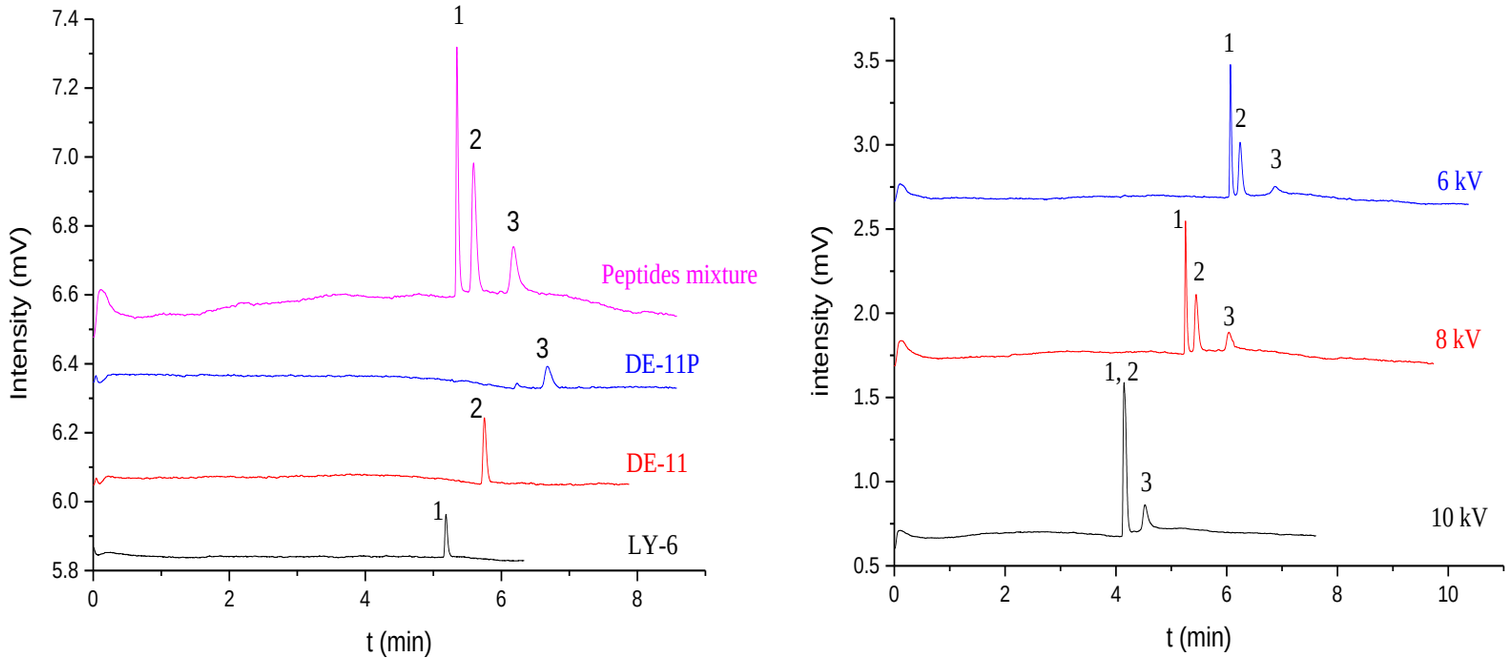
eHPLC Separation of Protein Mixture on submicron C18



Experimental conditions:

Column: 10 mm x 100 μm, 420 nm/C18-NPS
Isocratic: H2O: ACN (v:v, 57:43), +0.1%TFA
Pressure: 16.6 MPa,
Split ratio: 1/2250,
Linear velocity: 0.13 mm/s
Wavelength: 280 nm,
Applied voltage: 4 kV,
Sample: lysozyme, myoglobin and carbonic anhydrase
Efficiency: carbonic anhydrase: 280,000 p/m.

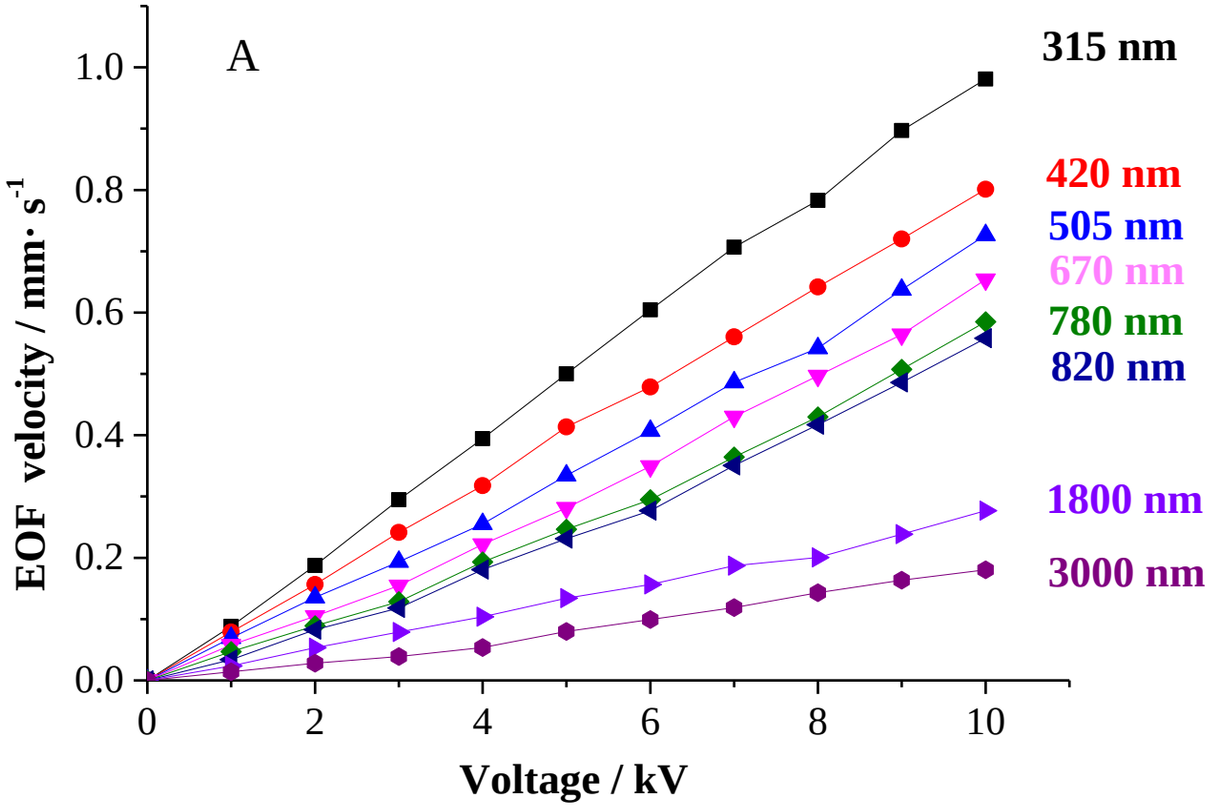
eHPLC separation of peptides with submicron particles



pCEC separation of 3 peptides with 420 nm C18-bonded silica particles;

N (plates/m) at 6 kV: LY-6, 1,752,000; DE-11, 460,000; DE-11p, 230,000.

EOF vs Voltage



Experimental conditions: ACN/H2O (70: 30, v /v); pH 7.8; 10 / 30 cm and 100 μm.

Part 5

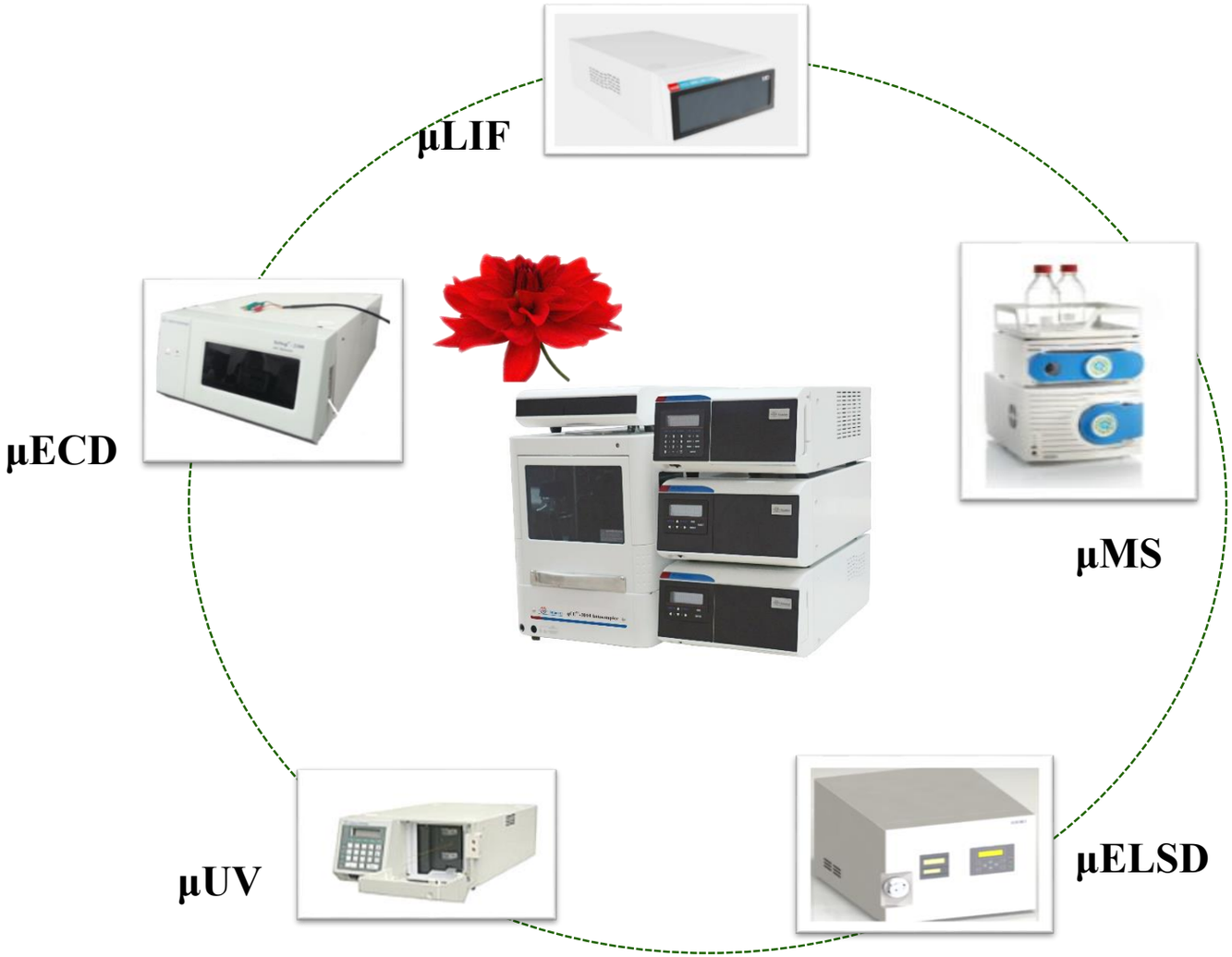
Automated qCE® -3010)

What is the bottle-neck for CE?

1. **Accuracy and precession?**
2. **Detection sensitivity?**

Automated qCE

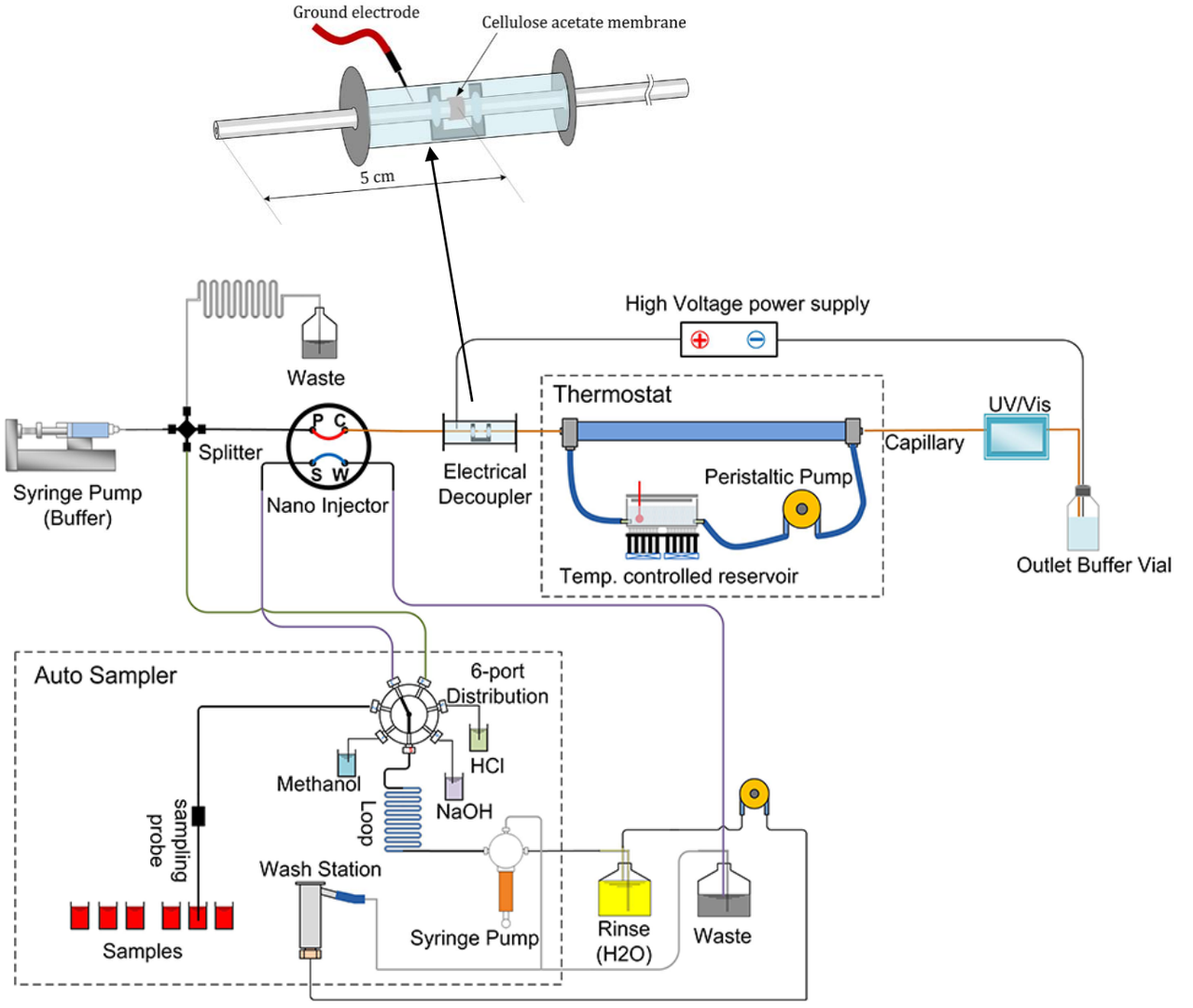
qCE®-3010 • qCE- μUV/μLIF/μMS/μELSD/μECD



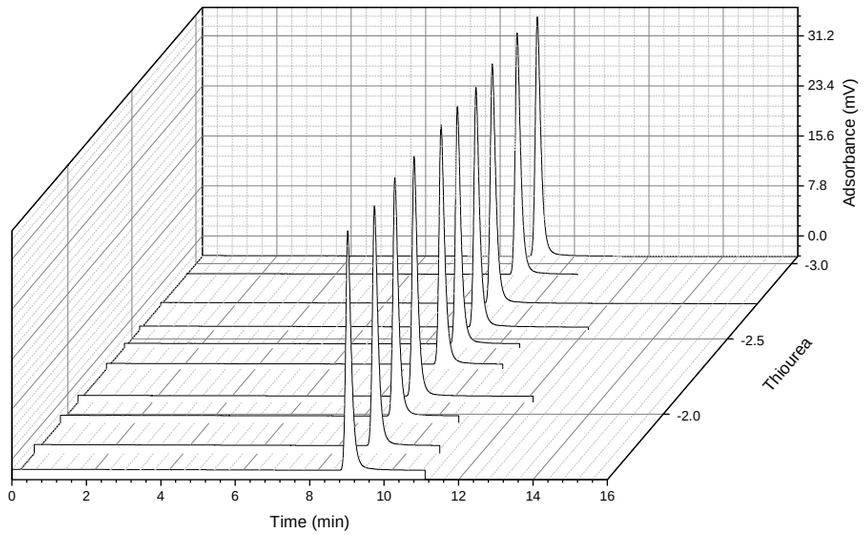
More suitable for the separation of complex compounds!

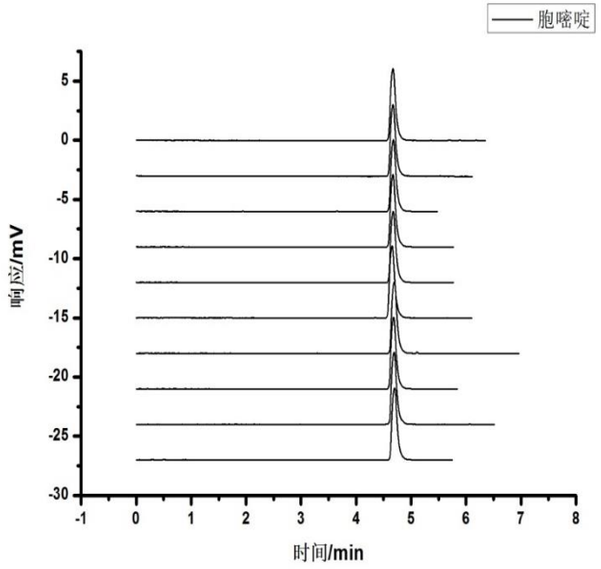
Schematic of Fully automated qCE（First in the world）

- ☺ Accurate injection with nL volume
- ☺ **Qualitative reproducibility <1% (time)**
- ☺ **Quantitative reproducibility <2% (peak area)**
- ☺ **Excellent New Products Award in the Instrument Industry.**



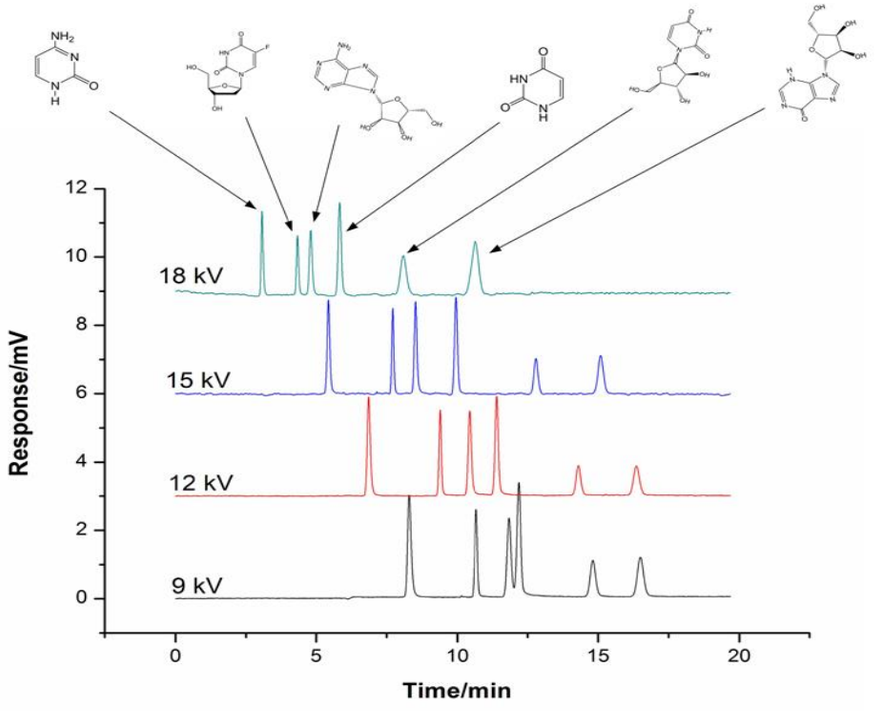
qCE reproducibility



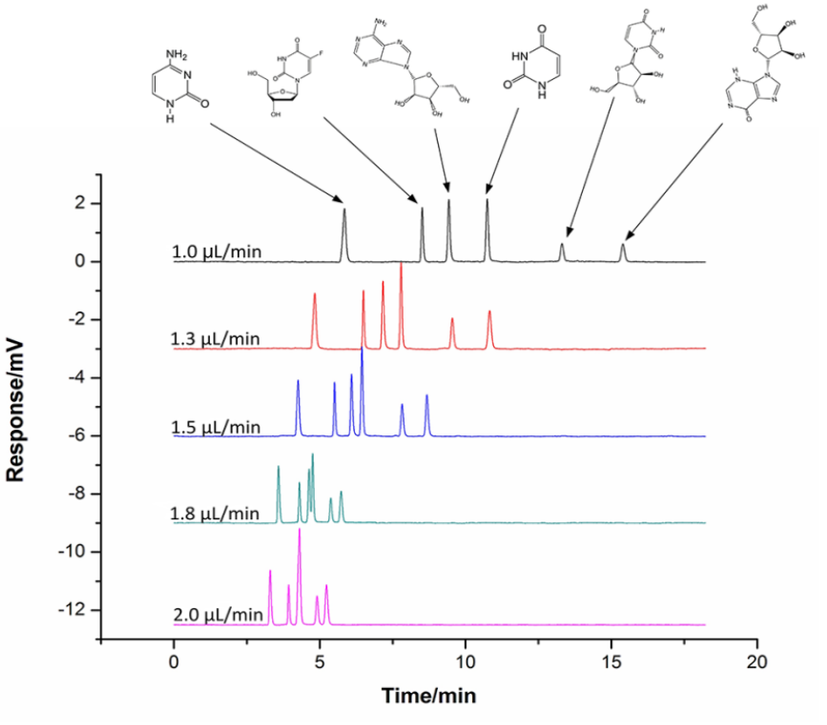


	1	2	3	4	5	6	7	8	9	10	RSD
保留时间	4.673	4.673	4.679	4.672	4.676	4.657	4.697	4.683	4.692	4.699	**0.28 %**
峰面积	41331	41015	41508	41754	41267	41831	41782	41392	41654	41576	**0.63 %**

qCE separation of six nucleosides



Constant flowrate



Constant voltage

Capillary: 40 cm x 50 μm i.d. , Sample：(a) 胞嘧啶， (b) 5-氟-2‘-脱氧尿苷，(c) 腺苷，(d) 尿嘧啶，(e) 尿苷，(f) 肌苷

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上海交通大学药学院药分和代谢组学课题组

Yan Lab, School of Pharmacy, Shanghai Jiao Tong University

上海通微分析技术有限公司团队

Unimicro Team


Thanks for your attention!