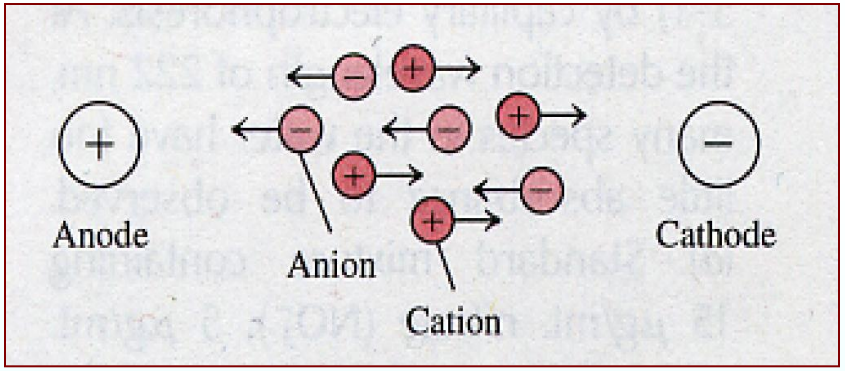
第8章-4 毛细管电泳法

(Capillary Electrophoresis, CE)

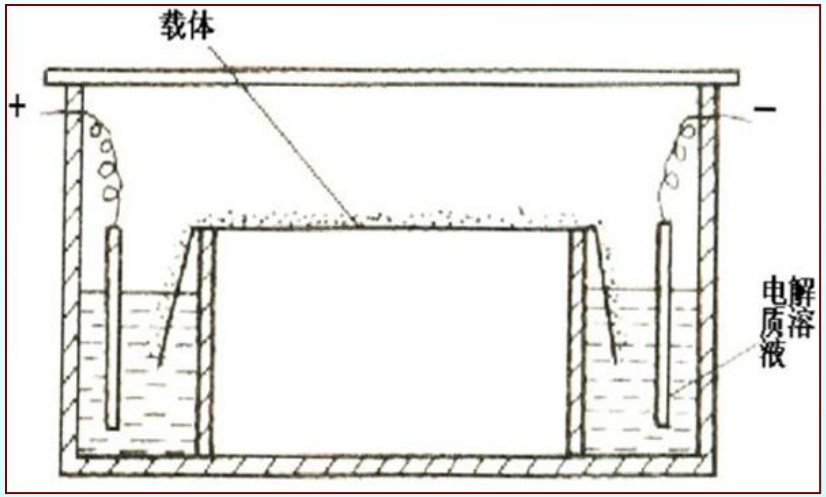
一、电泳现象和平板电泳

$$v_{ep} = \mu_{ep} \cdot E = \mu_{ep} \frac{U}{L}$$

μ_{ep} ：离子的迁移率



又称离子的有效淌度，是离子在单位时间内、单位场强下移动的距离，是离子的特性常数，与离子的电荷、体积等性质有关



平板凝胶电泳在生命科学中非常广泛的应用于核酸、蛋白质等生物大分子的分离

平板凝胶电泳的创始人： Arne Tiselius –1902-1971

Swedish Scientist in Chemistry

The Nobel Prize in Chemistry 1948
Arne Tiselius

The Nobel Prize in Chemistry 1948
Arne Tiselius

Arne Wilhelm Kaurin Tiselius

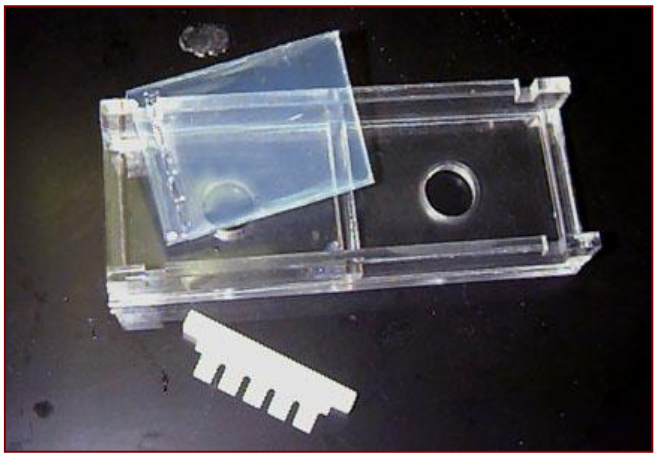
The Nobel Prize in Chemistry 1948 was awarded to Arne Tiselius *"for his research on electrophoresis and adsorption analysis, especially for his discoveries concerning the complex nature of the serum proteins".*

Nobel Lecture, December 13, 1948
Electrophoresis and Adsorption Analysis as Aids in Investigations of Large Molecular Weight Substances and Their Breakdown Products

"A new apparatus for electrophoretic analysis of colloidal mixtures" Transactions of the Faraday Society, 33 (1937) 524. Citation: 723

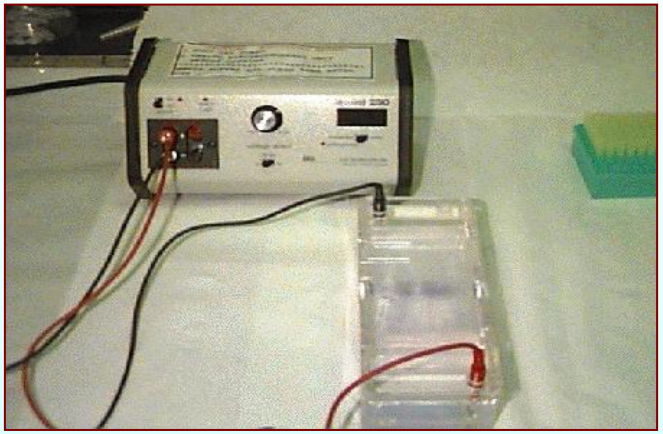
There has always been a close contact between the methodological work and the research into special problems where the methods find their application.

制胶

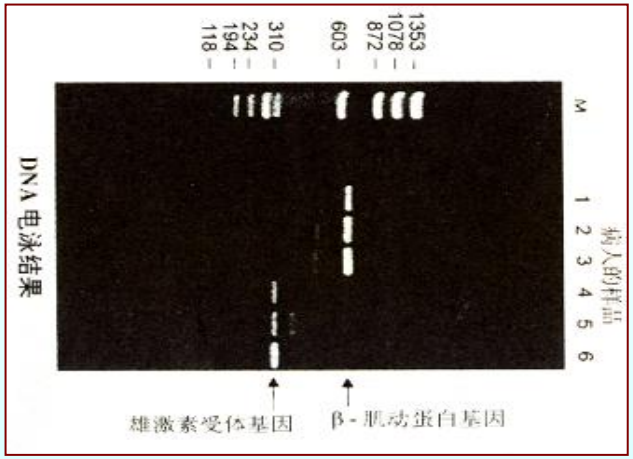


点样

分离



显示



平板凝胶电泳的缺点：由分离电压低所引起的：

1. 分离效率低
2. 分离速度慢

average of analyte concentrations determined from all six curves (1 and 5e–5i).

Individual element concentrations ranged from 0.22 to 1990 ppm. A single set of mixed standards was used covering a range of 2 orders of magnitude for each element. All elements, except Ca, were analyzed simultaneously from a single atomization in a lean air–acetylene flame at a height just above the burner slot. At this height in the flame, Ca yielded only 85% recovery. However, at a position 9 mm higher in the flame, 100% recovery was obtained for Ca. Consequently, it was necessary to run Ca separately from the other seven elements.

ACKNOWLEDGMENT

The authors would like to thank J. D. Messman for the use of his data for the detection limits of the Model 5000 AAS.

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Zone Electrophoresis in Open-Tubular Glass Capillaries

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Department of Chemistry, University of North Carolina, Chapel Hill, North Carolina 27514

A system for performing zone electrophoresis in open-tubular glass capillaries of 75 μm inside diameter and with applied voltages up to 30 kV is described. The small inside diameter of these capillaries allows efficient dissipation of the heat generated by the application of such high voltages. However, the small inside diameter also necessitates the use of a sensitive on-column fluorescence detector to record the separation of solute zones. With this system, separation efficiency is proportional to the applied voltage, with efficiencies in excess of 400 000 theoretical plates demonstrated. Strong electroosmotic flow in the capillary allows both positive and negative ions of a variety of sizes to be analyzed in a single run with relatively short analysis times. High-efficiency separations of fluorescent derivatives of amino acids, dipeptides, and amines as well as separation of a human urine sample were obtained with analysis times of 10–30 min.

Several important causes of zone broadening may be identified when considering separation efficiency in zone electrophoresis. Molecular diffusion will certainly cause zone broadening, although its effects are generally negligible. More serious difficulties often arise from convection currents in the electrophoretic medium. These are usually minimized through the use of gels, paper, or other stabilizers. However, this approach may introduce additional zone-broadening problems such as adsorptive interactions between the solutes and stabilizer and "eddy migration" in the channels created by some stabilizers (*1*). Mikkers, Everaerts, and Verheggen (2) sought to solve these convection problems through the use of the "wall effect" by performing zone electrophoresis in narrow-bore Teflon tubes. This approach appeared to solve the problem of convection in a simple way, avoiding the difficulties associated with stabilizers. They found that the concentration of sample ions must be kept well below the concentration of carrier electrolyte in order to achieve symmetric peak shapes. When the sample concentration is too high, the sample alters the conductivity of the medium in its own vicinity, resulting in a distorted electric field gradient and an asymmetric peak shape. If zone electrophoresis is performed in narrow-bore tubes using low concentrations of sample relative to carrier electrolyte, conditions arise where molecular diffusion, originally negligible, may become the predominant cause of zone broadening. The difficulty with this approach is in finding any suitable detection system capable of detecting minute quantities of solutes in small capillary tubes. In this study, zone electrophoresis was attempted in glass capillary tubes. Detection of solute zones was accomplished with an "on-column" fluorescence detector which detects fluorescent solutes while they are still in the glass capillary tube.

THEORY

Consider an electrophoresis system consisting of a tube filled with a buffering medium across which a voltage is applied. Charged species introduced at one end of the tube migrate under the influence of the electric field to the far end of the tube. If a suitable detection device is placed at the far end of the tube, the passage of each solute zone may be recorded, yielding an electropherogram.

The migration velocity of a particular species is given by

$$v = \mu E = \mu V/L \qquad (1)$$

0003-2700/81/0353-1298$01.25/0

毛细管电泳创始人

Prof. James W Jorgenson
Department of Chemistry
University of North Carolina

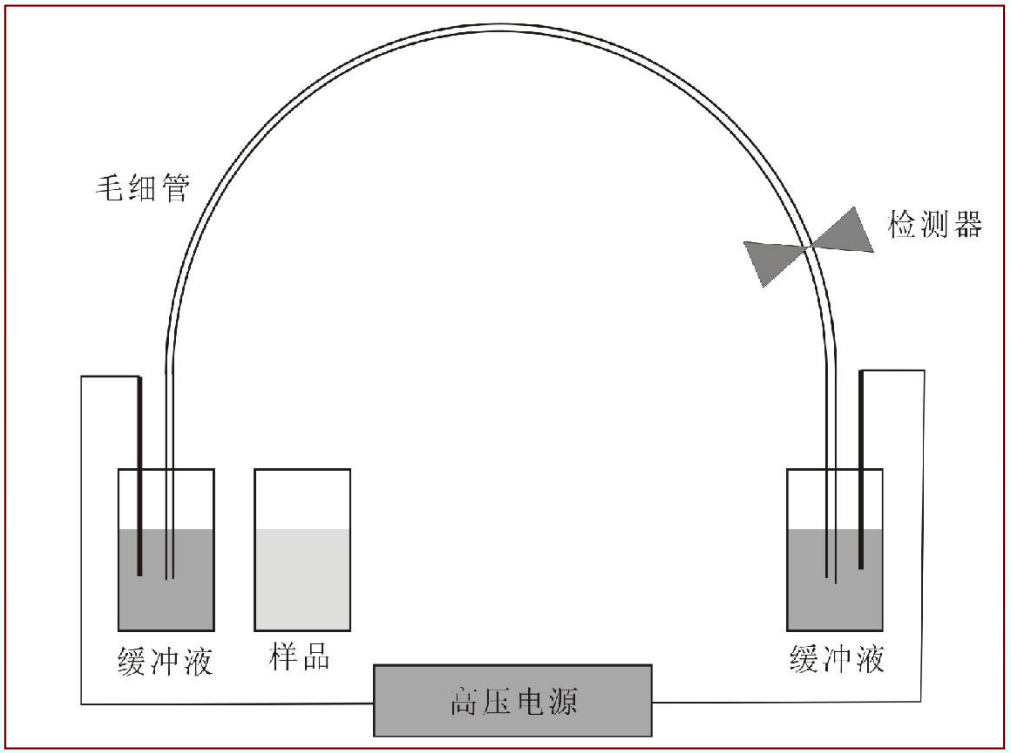
Jorgenson and Lukacs
Anal. Chem. 1981, **53**, 1298
Citation: **2008**

2. 毛细管电泳法的特点

- 分离效率高，几十万-百万塔板数/米；
- 分离速度快；
- 分离模式多；
- 应用范围广；
- 绝对检测量小，样品用量少；
- 仪器简单，分析成本低

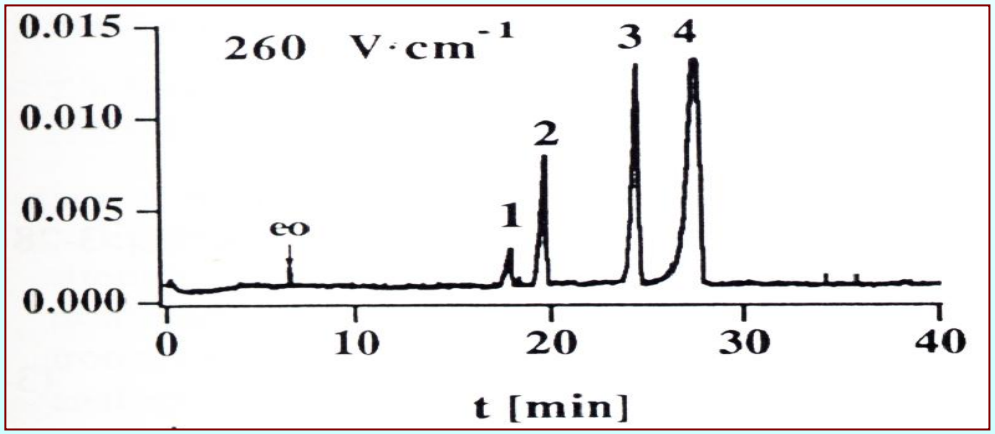
二、 毛细管电泳分离的基本原理

1. 基本装置

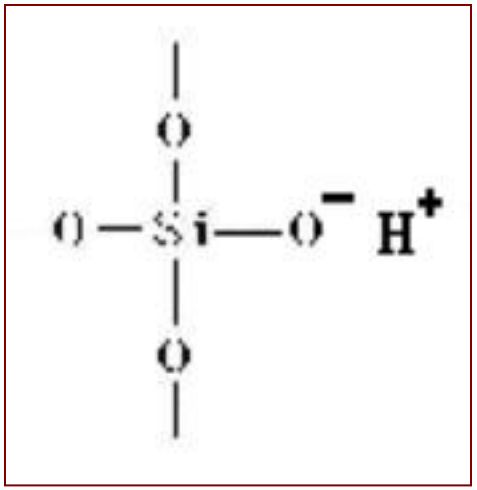


定性：迁移时间

定量：峰面积/峰高



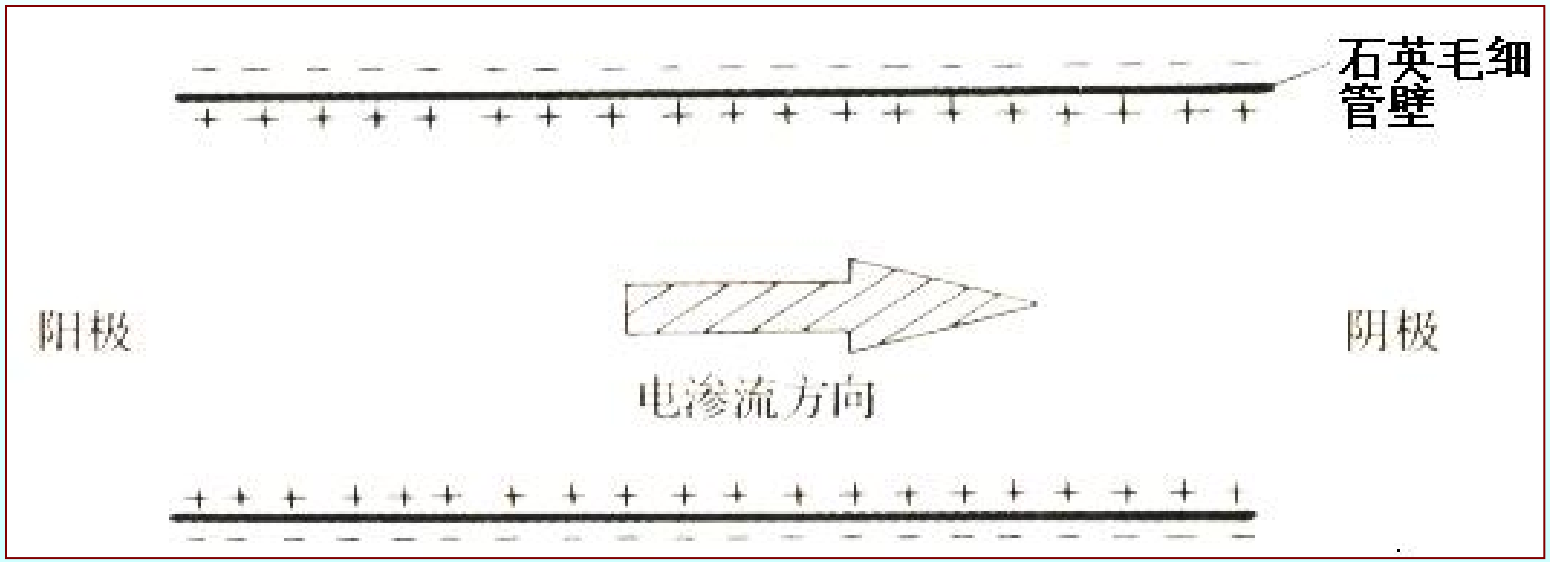
2. 电渗现象 (Electroosmosis)



（1）石英毛细管的表面结构和电渗流的形成 Electroosmotic flow (EOF)

$$v_{eof} = \mu_{eof} \cdot E = \mu_{eof} \frac{U}{L}$$

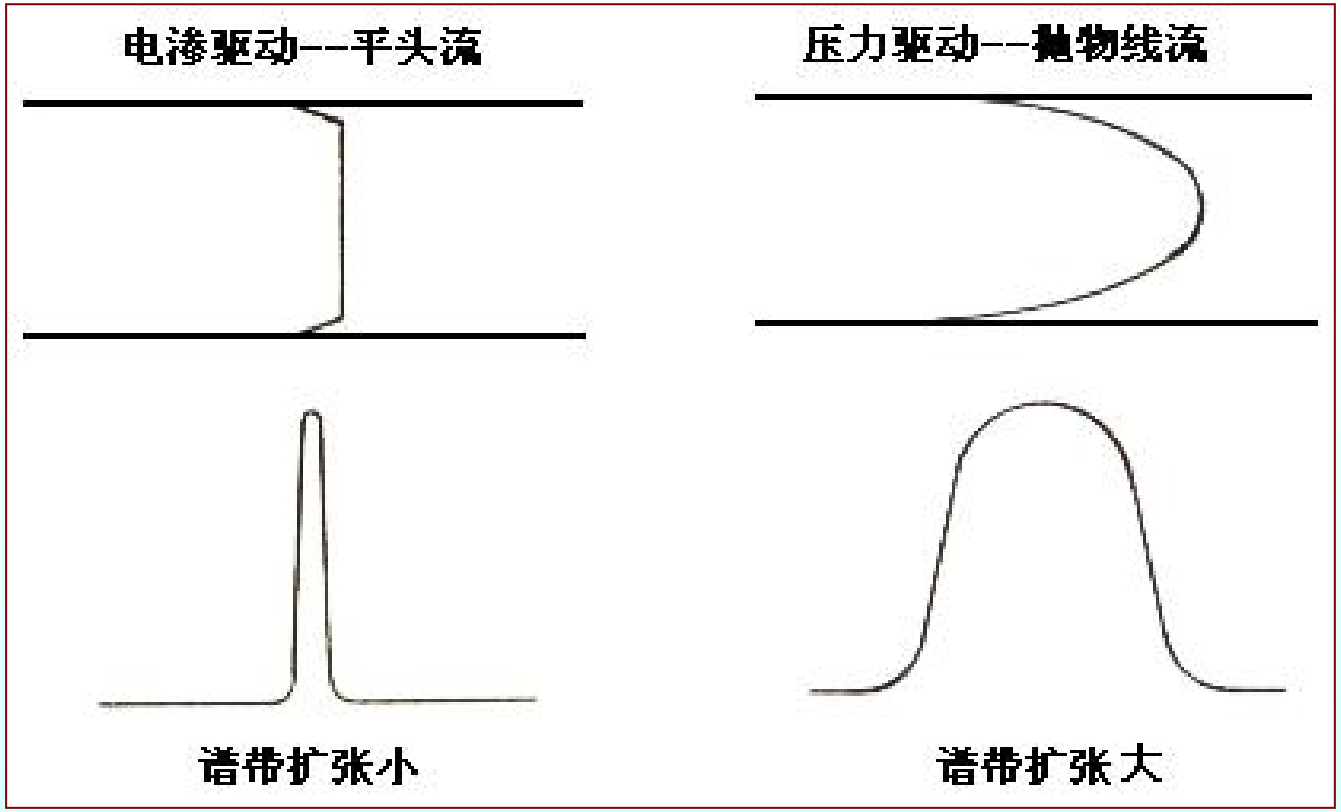
μ_{eof} ： 电渗迁移率(电渗淌度) Electroosmotic mobility



（2）电渗流的特点

平头流

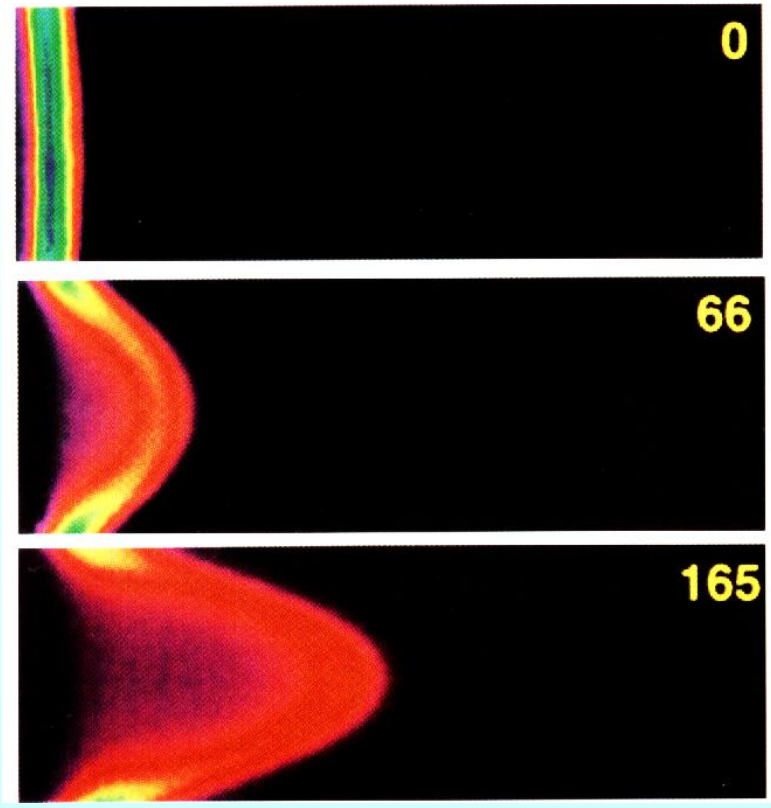
电渗流和压力流的流型对比



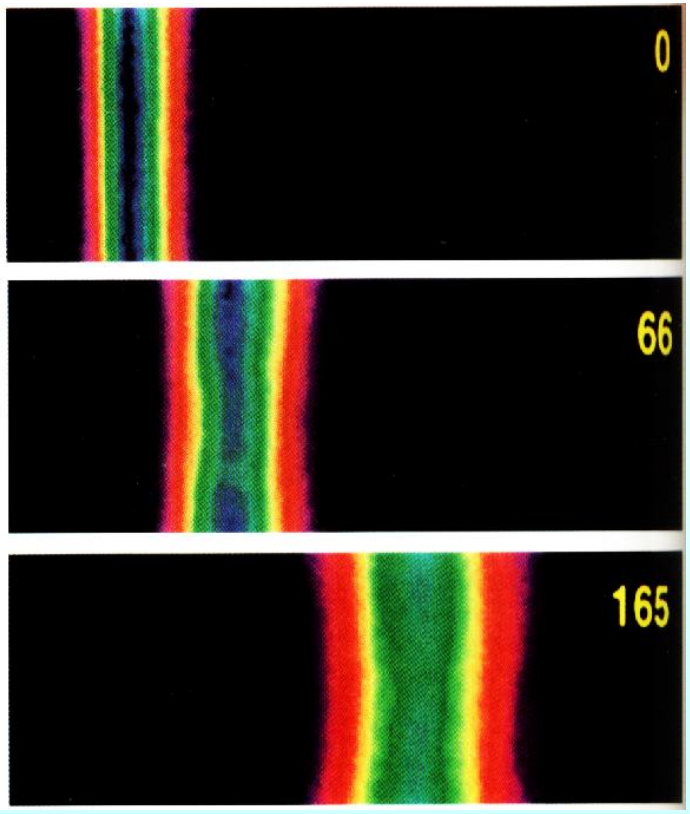
这就是为什么CE比HPLC柱效高的主要原因

流动开始后0、66、 165毫秒时荧光染料在毛细管中的谱带分布图象。兰色处染料的浓度最高，红色处染料的浓度最低。（P.H. Paul et al, Analytical Chemistry, 1998, 70, 2459）

压力流



电渗流

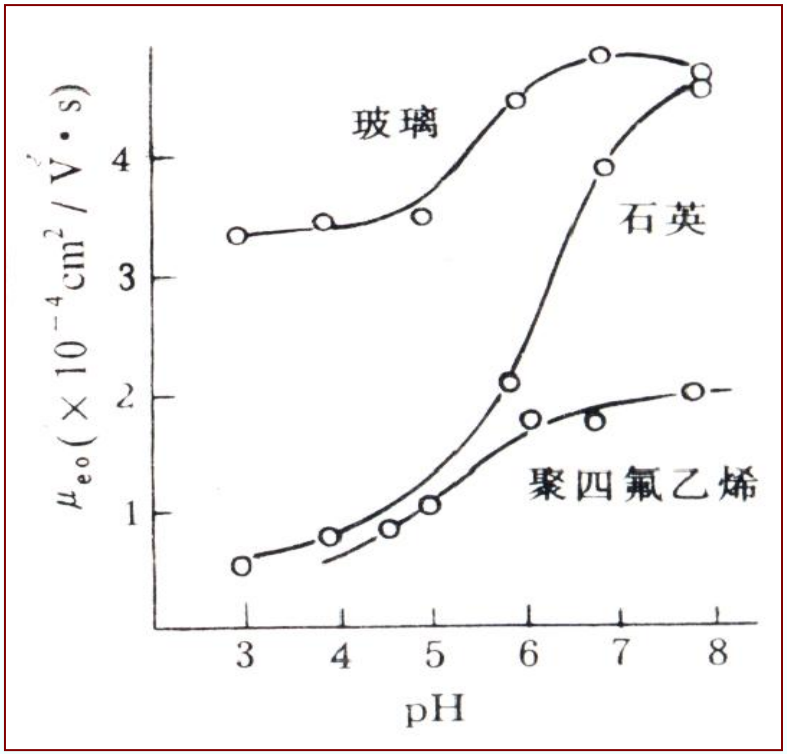


（3）影响电渗流因素

- 与电场强度有关

$$v_{eof} = \mu_{eof} \cdot E = \mu_{eof} \frac{U}{L}$$

- 毛细管的材料有关
- 与缓冲液的pH有关

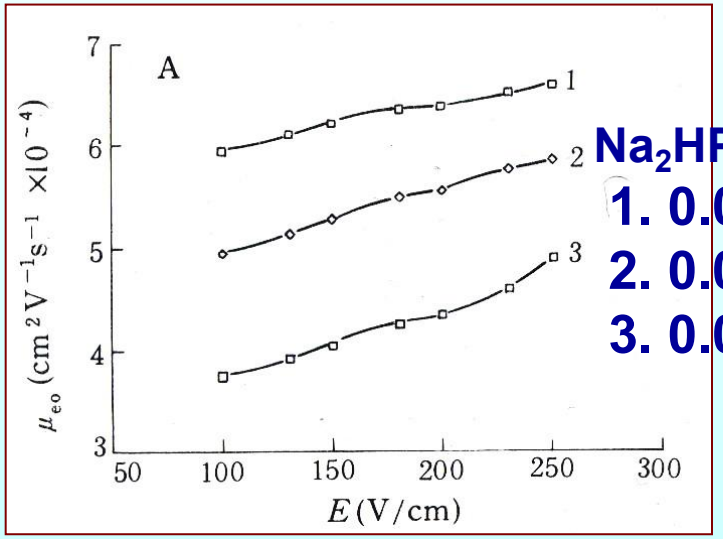


- 与缓冲液的离子强度有关

  浓度大，电渗流小

- 与毛细管表面的性质有关

  污染后，电渗流明显减小



Na_2HPO_4缓冲液：

1. 0.01 mol/L
2. 0.02 mol/L
3. 0.05 mol/L

电渗流在毛细管电泳中的重要意义：

- 驱动作用：使正离子、中性分子、负离子一起向阴极迁移。 但对不同组分不具分辨能力。
- 调节EOF，影响分离速度、柱效和分离度
- 毛细管内表面状态对EOF有非常大的影响，EOF的微小变化会显著地影响分离和分析的重现性。

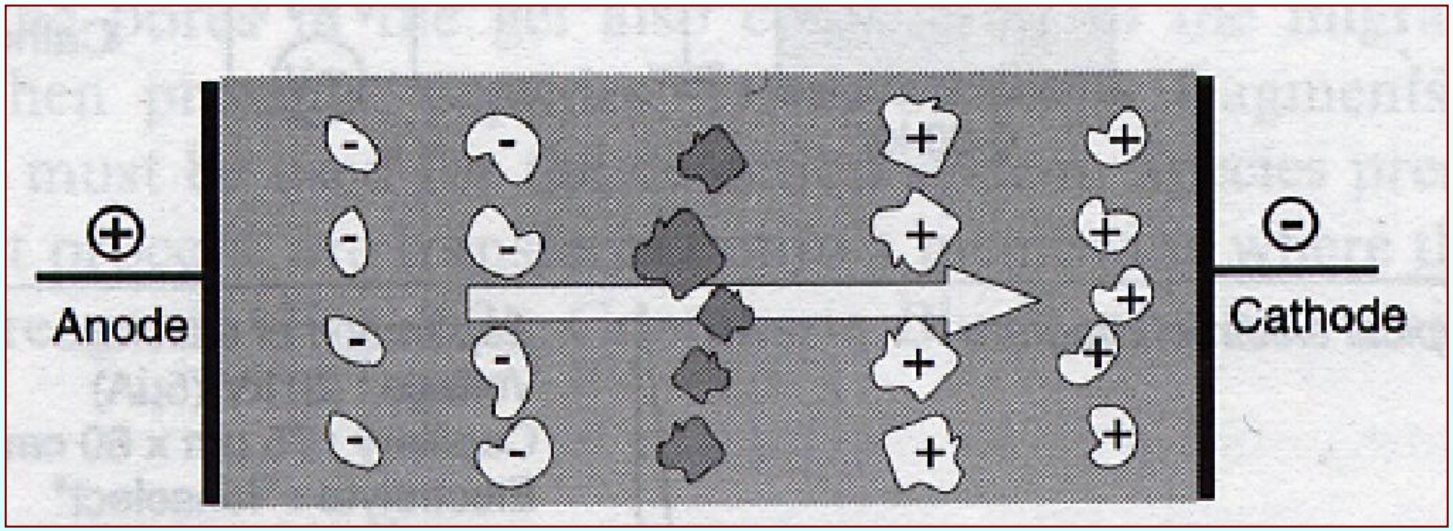
3. 毛细管电泳分离的基本原理

$$v = v_{ep} + v_{eof} = \left(\mu_{ep} + \mu_{eof}\right) \cdot E = \left(\mu_{ep} + \mu_{eof}\right)\frac{U}{L}$$

正离子， $\mathbf{v} = \mathbf{v}_{ep} + \mathbf{v}_{eof}$

负离子， $\mathbf{v} = -\mathbf{v}_{ep} + \mathbf{v}_{eof}$

中性分子， $\mathbf{v} = \mathbf{v}_{eof}$



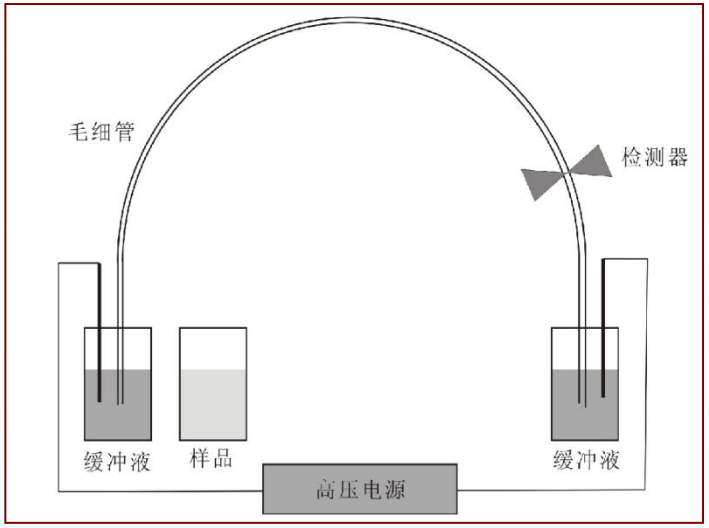
组分的迁移时间：

$$t = \frac{L_{ef}}{v} = \frac{L_{ef} \bullet L}{\left(\mu_{ep} + \mu_{eof}\right)U}$$

L_{ef}：从毛细管入口到检测窗口的毛细管有效长度

L：毛细管的总长度

U：分离电压



$\left(\mu_{ep} + \mu_{eof}\right) = \mu_{ap}$：为表观淌度，其中

μ_{ep}：有效淌度，是离子的特性

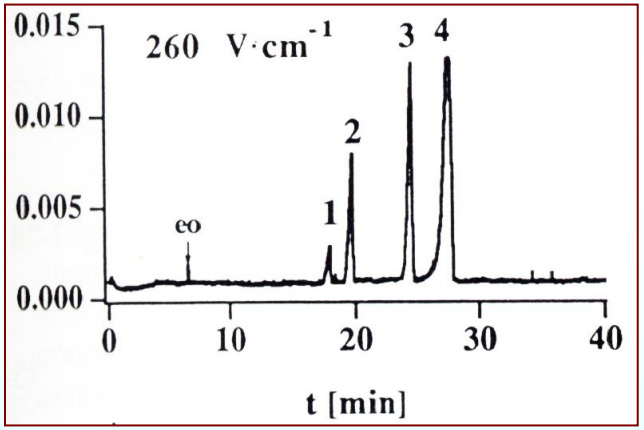
所以，不同的(离子)组分具有不同的迁移时间——定性分析的依据

4. 毛细管电泳的柱效（Column efficiency）

与色谱一样，柱效理论塔板数 *n*表示，

(1) 毛细管电泳的理论塔板数 *n*

$$n = 5.54\left(\frac{t}{W_{1/2}}\right)^2$$



毛细管电泳中理论塔板数一般 *n* 可达几十万，原因：

EOF为平头流：涡流扩散、液相传质扩散可忽略

无固定相：无固相传质阻力

(2)影响毛细管电泳分离的因素

1）纵向分子扩散引起峰变宽

——扩散系数，温度，扩散时间

2）进样引起峰变宽　　纳升级-皮升级

3）焦耳热引起峰变宽　　径向温度梯度　管径　散热

表 2.3　不同分子量分子的扩散系数（水中，25℃）

物　质	分　子　量	扩散系数（$\times 10^{-6}cm^2/s$）
HCl	36.5	33.05
NaCl	58.5	14.8
甘氨酸	75	10.6
柠檬酸	192	6.6
细胞色素 C	13 370	1.1
血红蛋白（人）	64 500	0.69
烟草花叶病毒	40 000 000	0.046

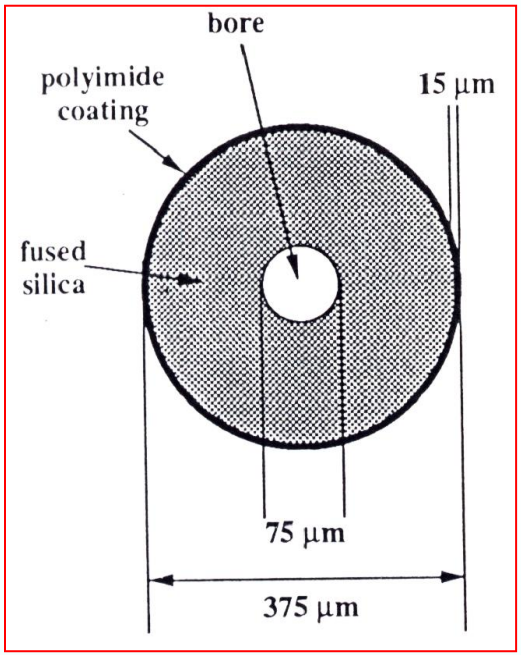
3）焦耳热

焦耳热-电流的热效应：

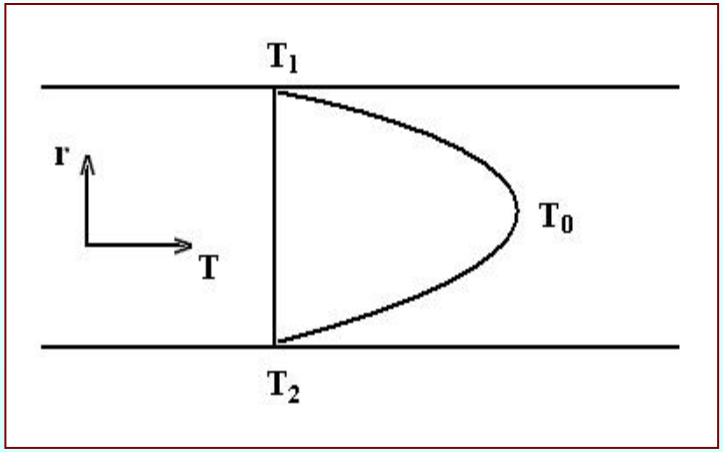
$$Q=I^2Rt$$

Q 与毛细管的尺寸、电解质的种类和浓度、分离电压和工作时间有关。

Q 直接关系到毛细管内部电解质的温度。当焦耳热通过毛细管壁向环境散逸时，在毛细管内部形成径向温度梯度。



毛细管的横截面



毛细管内温度的径向分布

表 2.5 不同内径毛细管的管壁温度及其轴心与管壁的温差[19]

内半径（μm）	壁温度（K）	温差（K）
25	299.0	0.53
50	301.2	1.39
75	304.2	3.14
100	307.7	5.58
125	311.6	8.72

径向温度梯度所造成的不良后果：

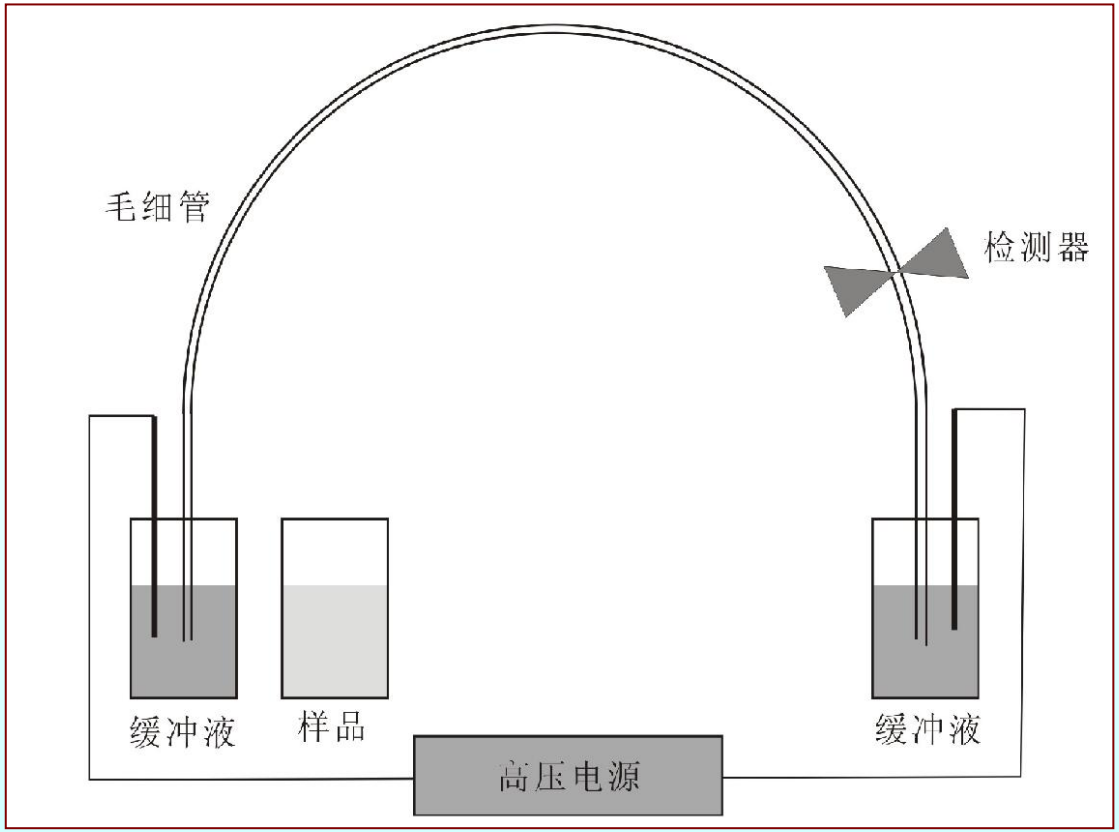
- 溶液的密度不同，引起对流，使区带扩张
- 溶液的黏度不同，使迁移率发生变化，区带扩张

温度变化1度，黏度和淌度变化 2-3 %

小内径毛细管的温度梯度小，区带扩张小，柱效高。

CE倾向于使用小内径毛细管

三. 毛细管电泳仪



- 毛细管
- 高压电源
- 缓冲液池和电极
- 进样系统
- 检测系统

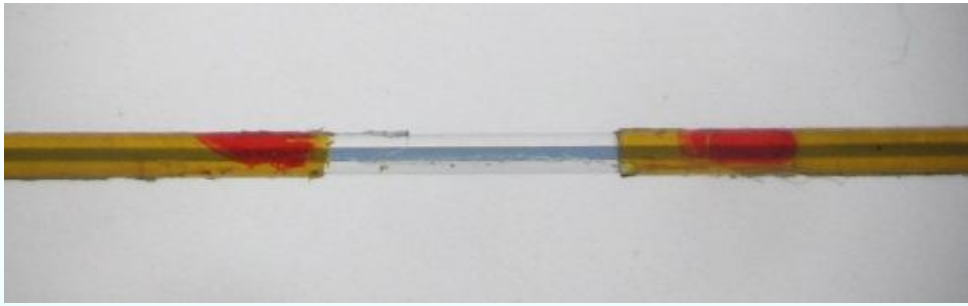
1. 毛细管

弹性石英毛细管 （fused-silica capillary）

熔硅毛细管+聚酰胺保护层

外径：350 ~ 375 微米　　内径： 25， 50， 75，100 微米

长度：10-100 厘米可选



2. 高压电源

输出电压：10~ 30 kV

最高电流：200 ~ 300 μA

3. 检测系统

——紫外检测器　灵敏度低（光程短）

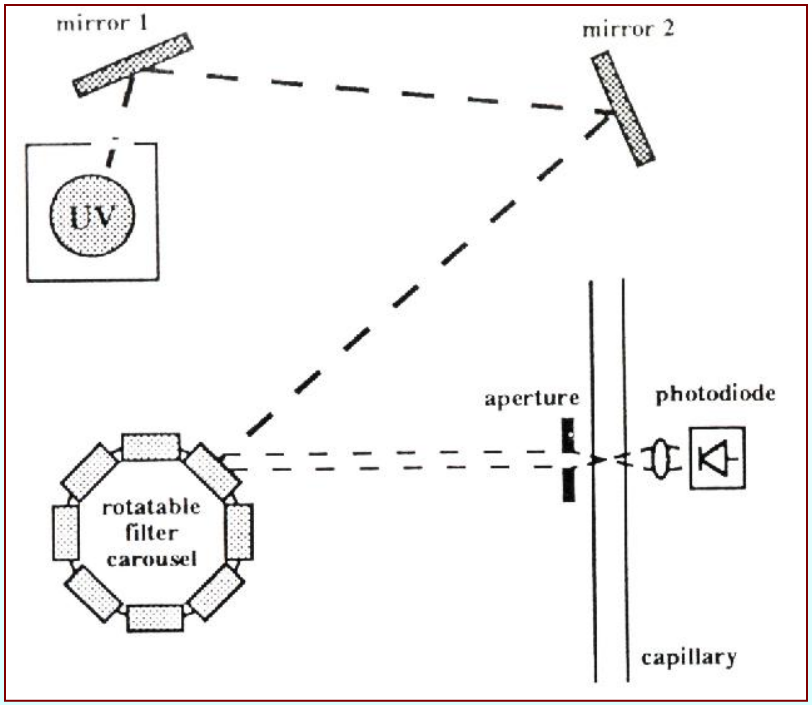
——激光诱导荧光检测器　灵敏度高, 需标记衍生

——电化学检测器　灵敏度高 适于电活性物质

紫外检测器

检测原理——郎伯-比耳定律

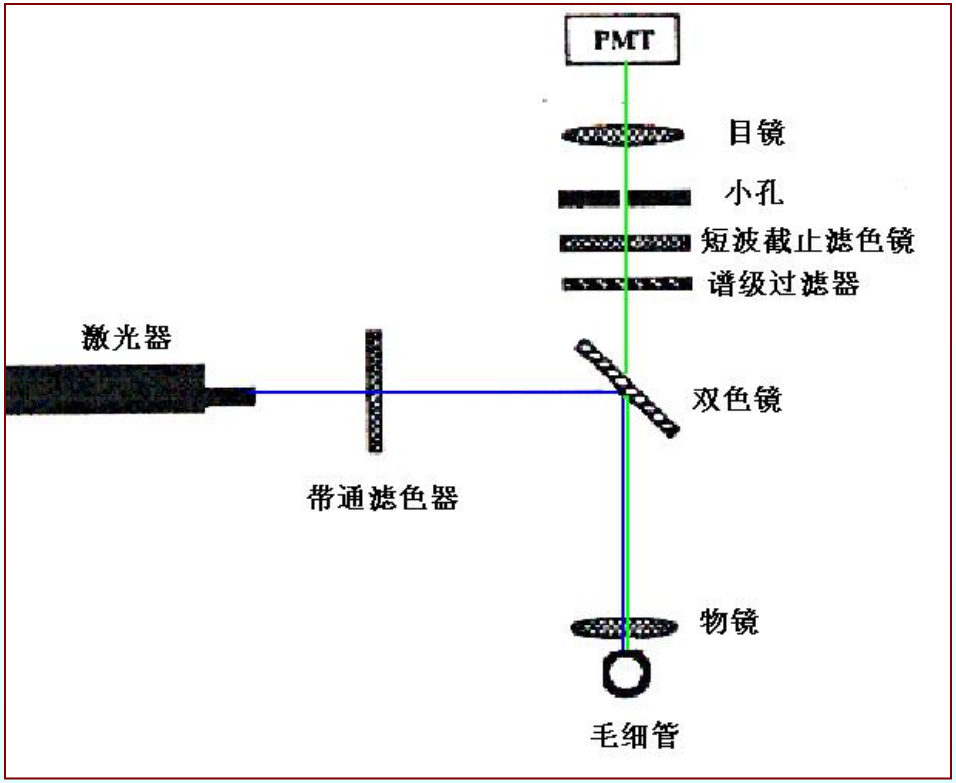
$$A = \lg \frac{I_0}{I} = \varepsilon cl$$



特点

- 适合于测定有紫外吸收的化合物，通用
- 灵敏度低（光程短）

激光诱导荧光检测器



特点:

- 灵敏度高,

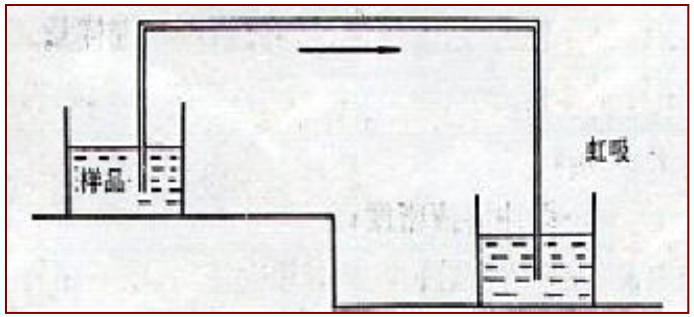
  10^{-9}~10^{-11} mol/L

- 具有天然荧光的化合物少，常用于生物大分子用荧光试剂标记后分离检测

4. 进样系统

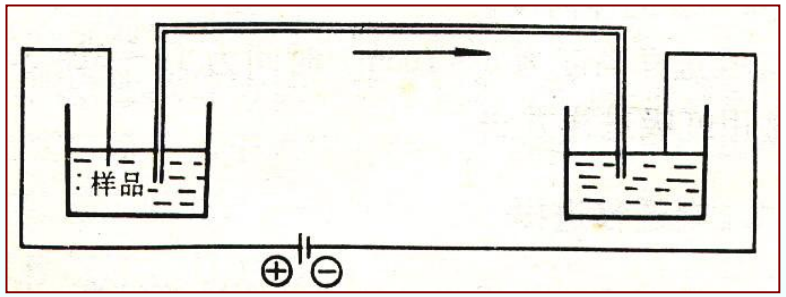
压力进样

依靠压力将组分引入毛细管

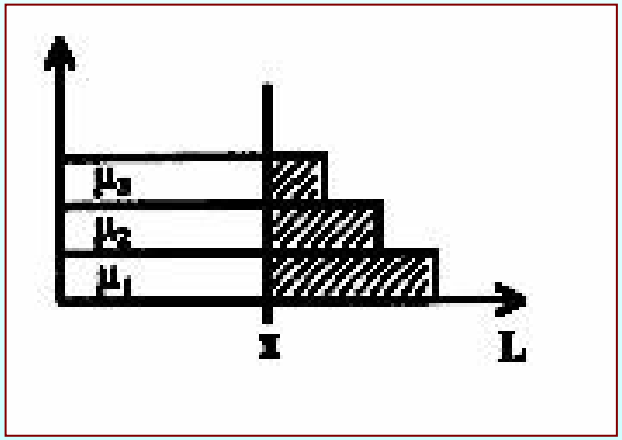


电动进样

依靠电渗和电泳作用将组分引入毛细管



对淌度不同的离子有歧视效应

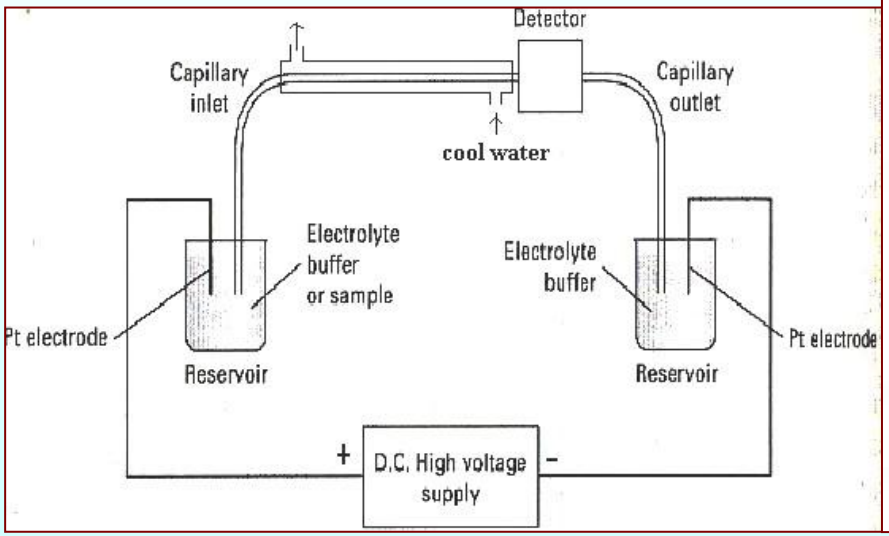


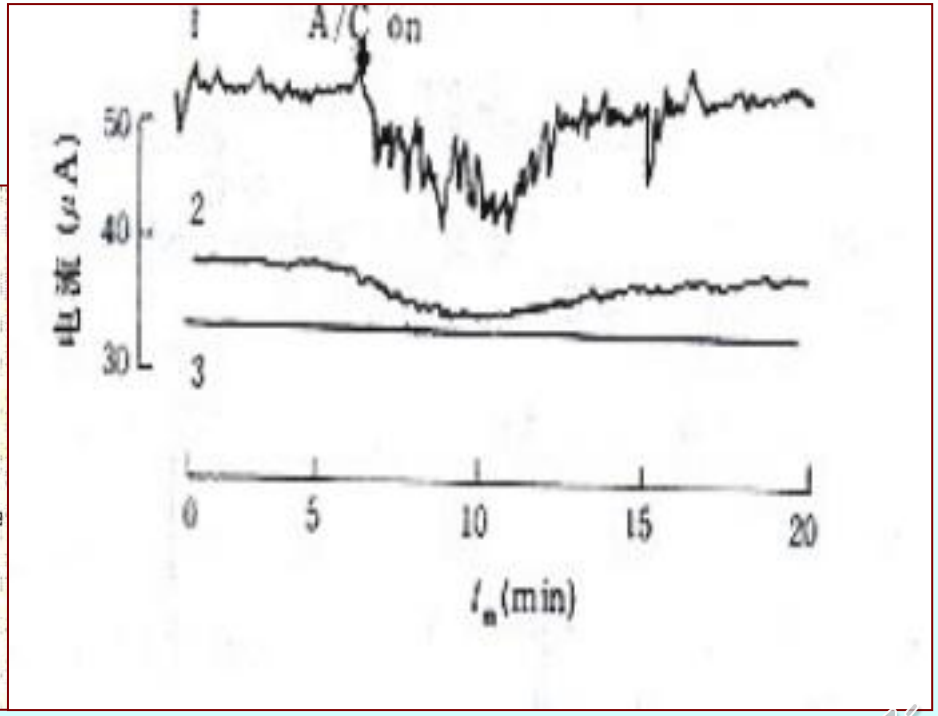
六. 温控（冷却）系统

- 自然冷却(室温)
- 风冷
- 液冷

温度对电泳电流的影响

1. 空气自然对流；2. 空气强制对流;
3. 电制冷却.





四 毛细管电泳分离模式

1. 毛细管区带电泳（Capillary zone electrophoresis, CZE）

2. 胶束电动毛细管色谱（Micelle Electrokinetic Capillary Chromatography, MEKC)

3. 毛细管凝胶电泳（Capillary Gel Electrophoresis, CGE)

4. 毛细管电色谱（Capillary Electrochromatography, CEC)

四 毛细管电泳分离模式

1. 毛细管区带电泳（Capillary zone electrophoresis, CZE）

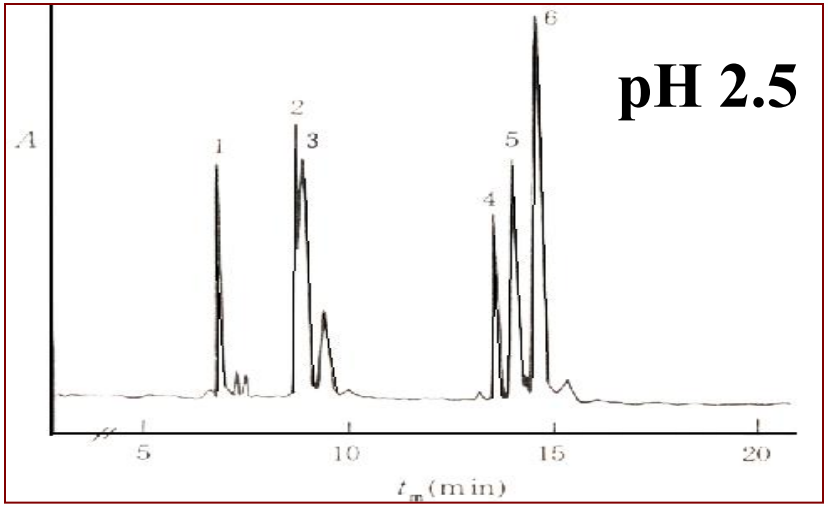
——基本分离模式，工作电解质—缓冲溶液

——分离原理——按离子组份的电泳淌度的差异实现分离

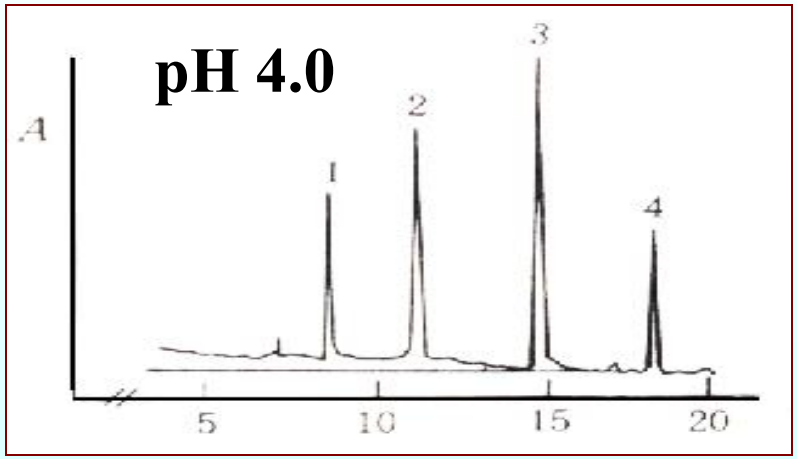
组分质荷比差异

——影响因素：缓冲液种类、pH值、浓度；场强

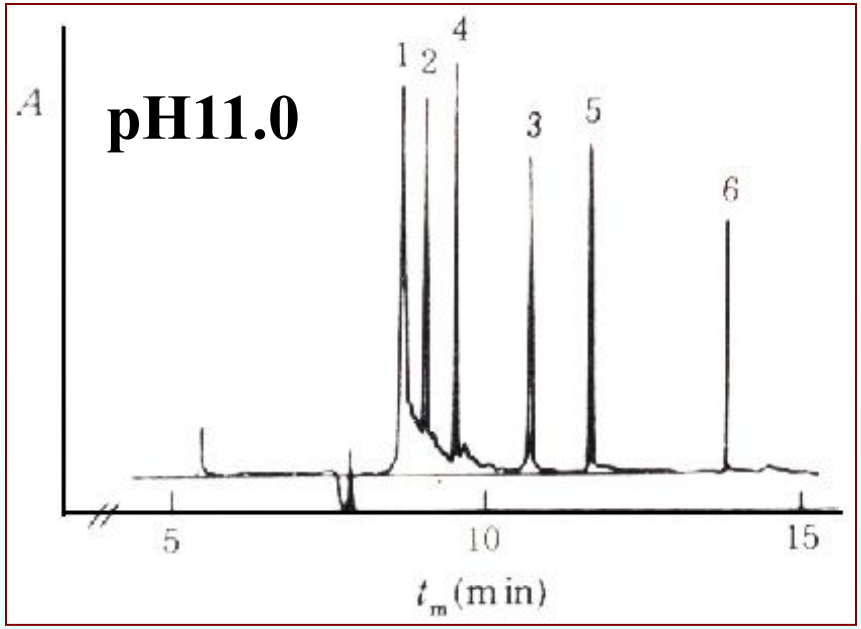
缓冲液pH值对小肽分离的影响



2-3 组分因正电荷数差别小，不能完全分离



pH 4.0 时，电渗流小。5、6 组分带负电，迁移方向与电渗流反向，不能向负极迁移而无法到达检测窗口

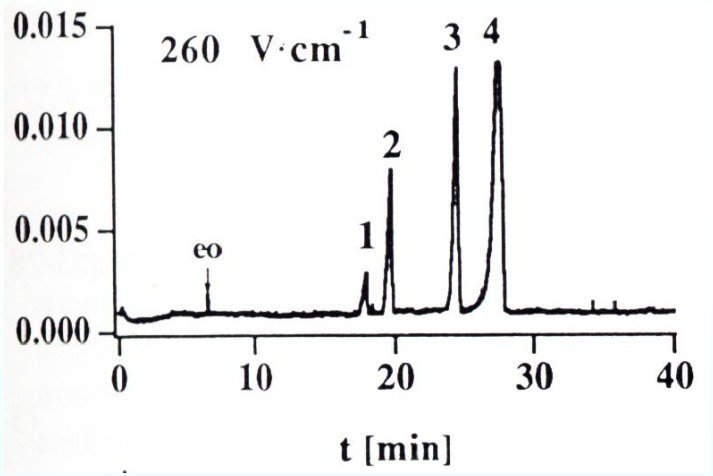


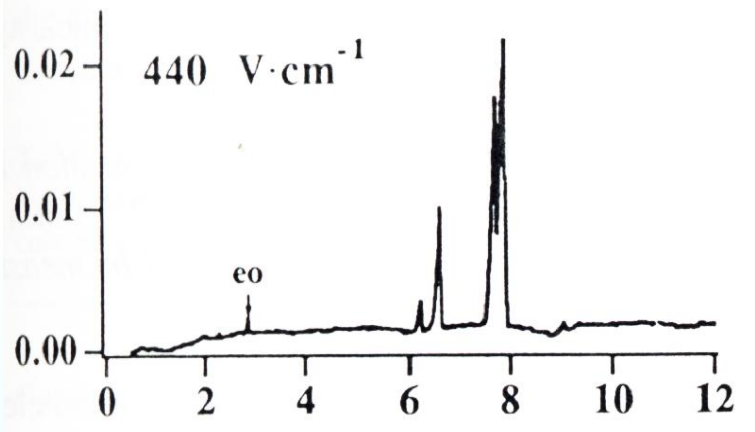
pH11时，电渗流增大，仍能使带负电荷的组分向负极迁移，组分间的电荷数差异适当，所有组分达到完全分离

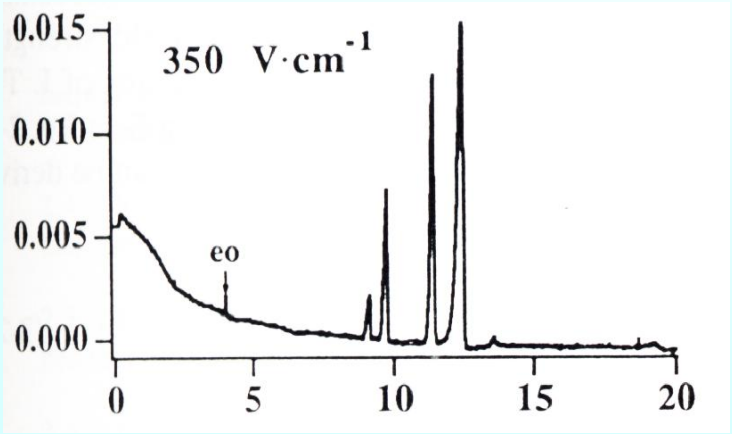
（邓延倬，高效毛细管电泳， p. 69）

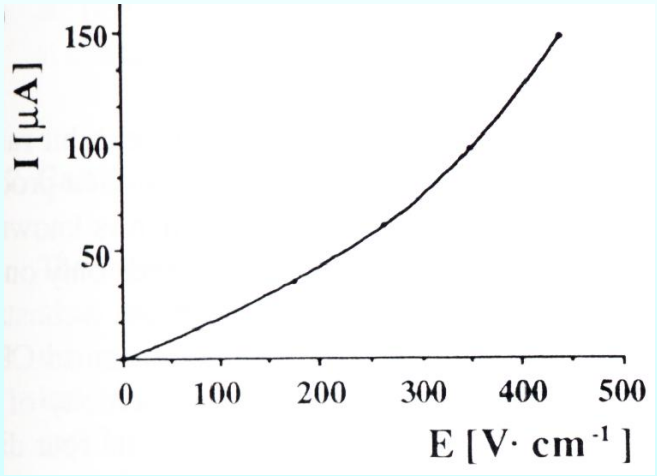
分离电压对分离效果的影响

组分：1)2,4-， 2)2,3-， 3)2,6-， 4)2,5--二羟基苯甲酸



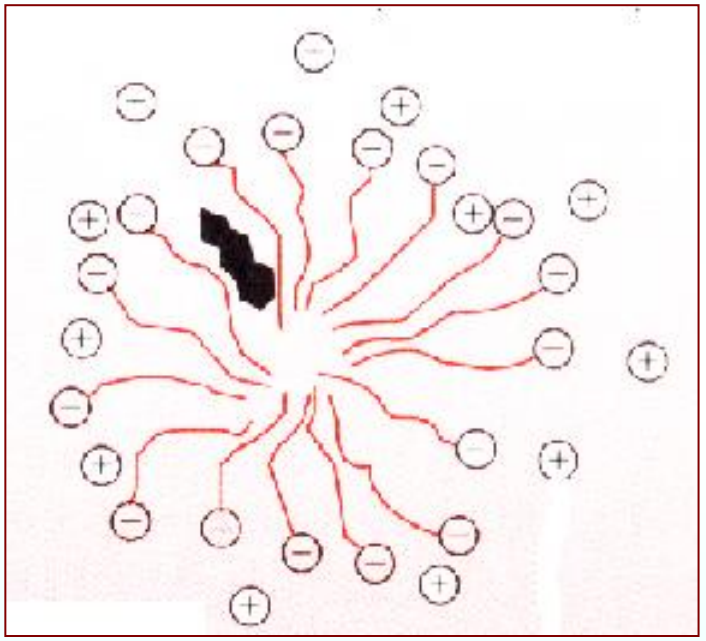




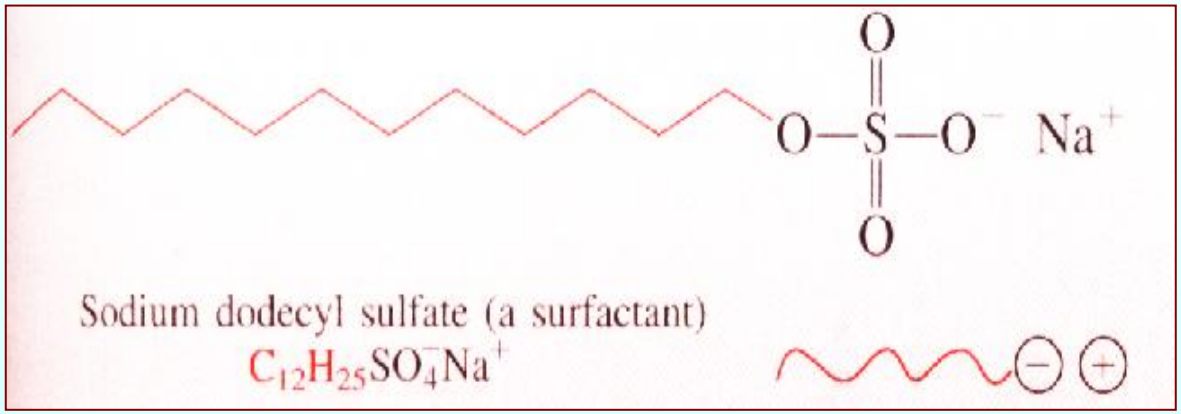


场强—电流曲线

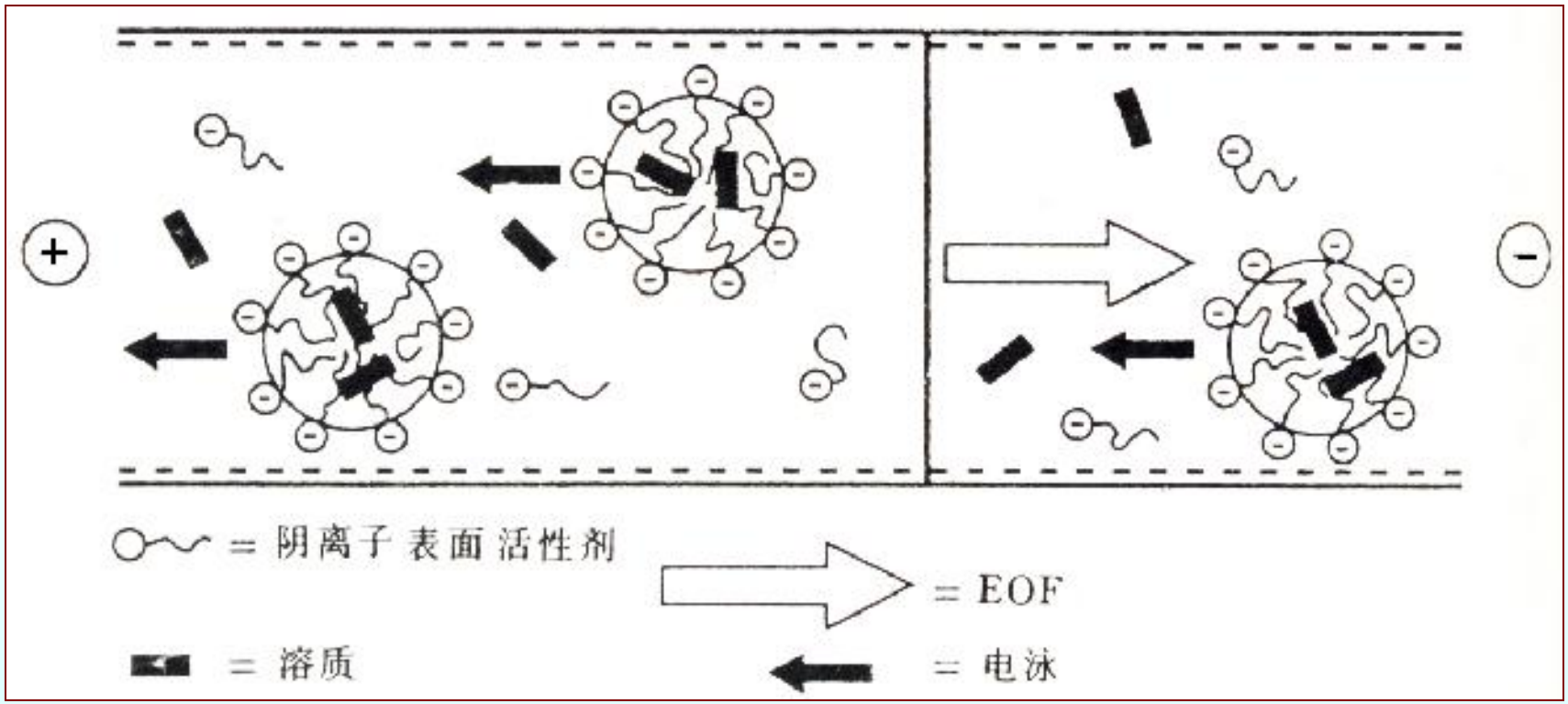
2. 胶束电动毛细管色谱（Micelle Electrokinetic Capillary Chromatography, MEKC)



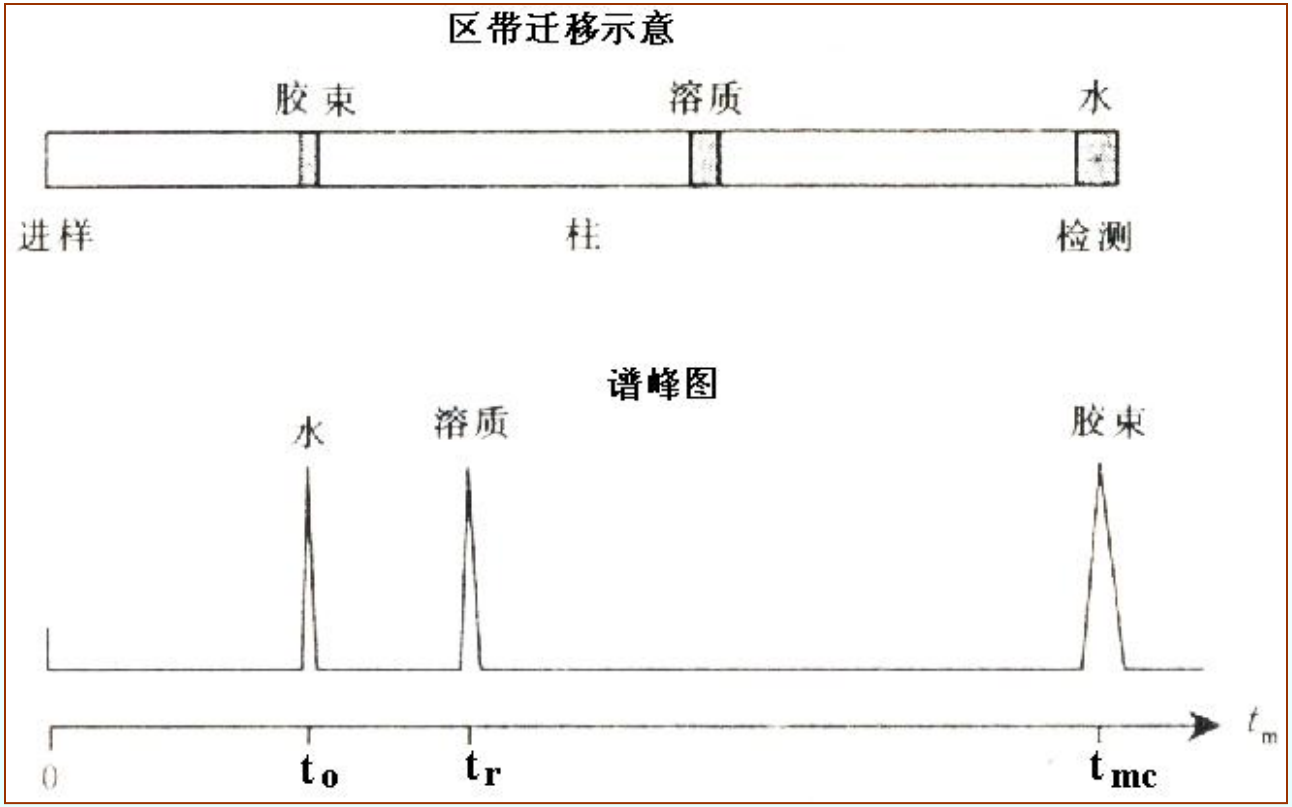
——表面活性剂胶束作为流动固定相，同时分离离子性与中性组分



MEKC分离中性化合物的原理



MEKC 的迁移时间窗口



t_o: 完全溶于水的中性溶质的迁移时间（电渗流）

t_{mc}：完全溶于胶束的溶质的迁移时间

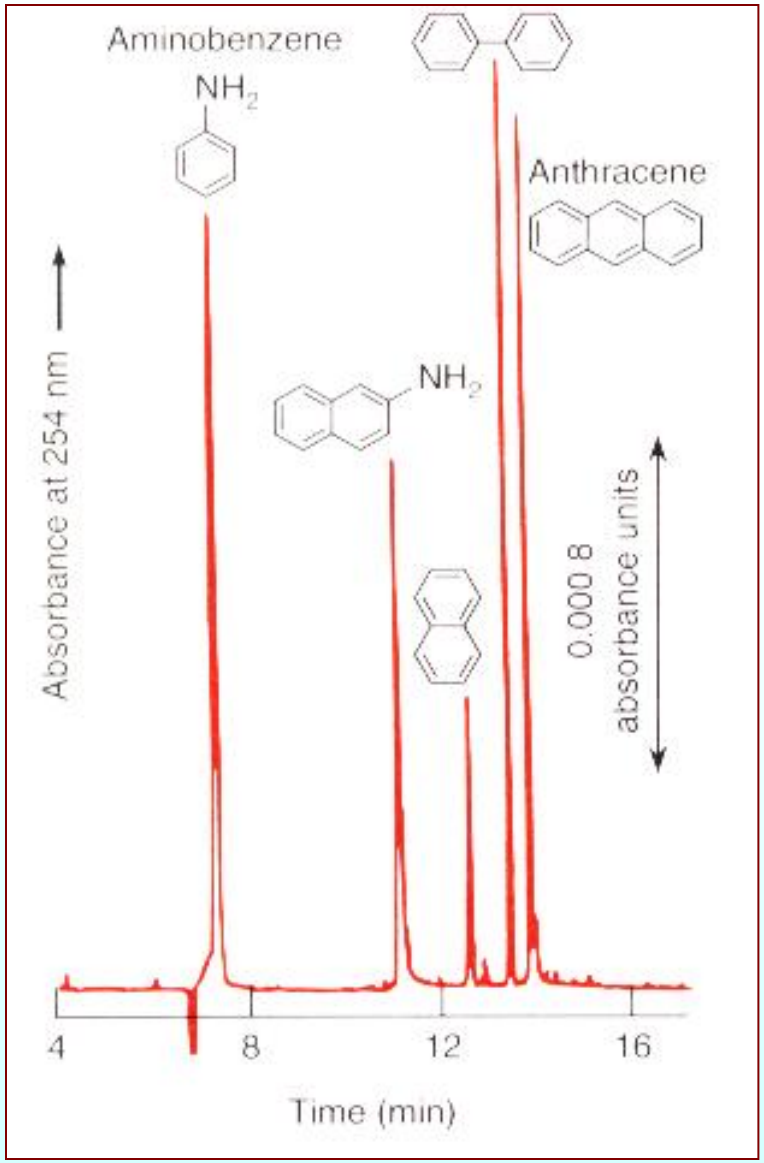
t_r：适度溶于胶束的溶质的迁移时间

t_o—t_{mc}: 时间窗口

MEKC分离芳香化合物

50 cm 毛细管，

塔板数250 000



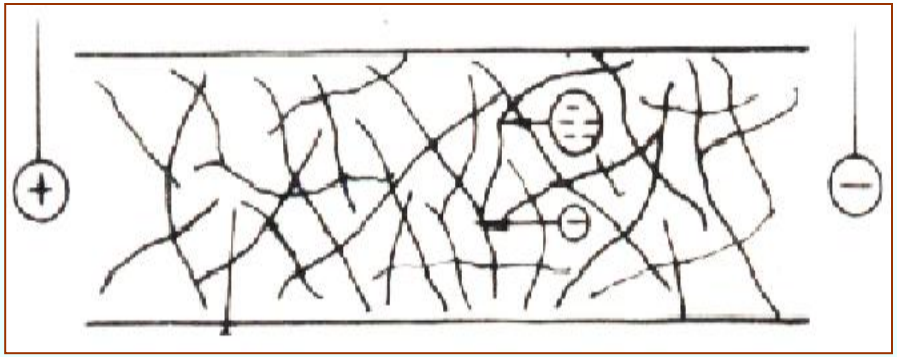
3. 毛细管凝胶电泳（Capillary Gel Electrophoresis, CGE)

——毛细管内填充聚合物凝胶, 多孔，亲水

——适于分离蛋白质核酸等大分子物质　第一代DNA测序

毛细管凝胶电泳的特点

- 凝胶流动性差,无对流扩散
- 基本无电渗流,靠电泳分离
- 分子扩散和管壁吸附小



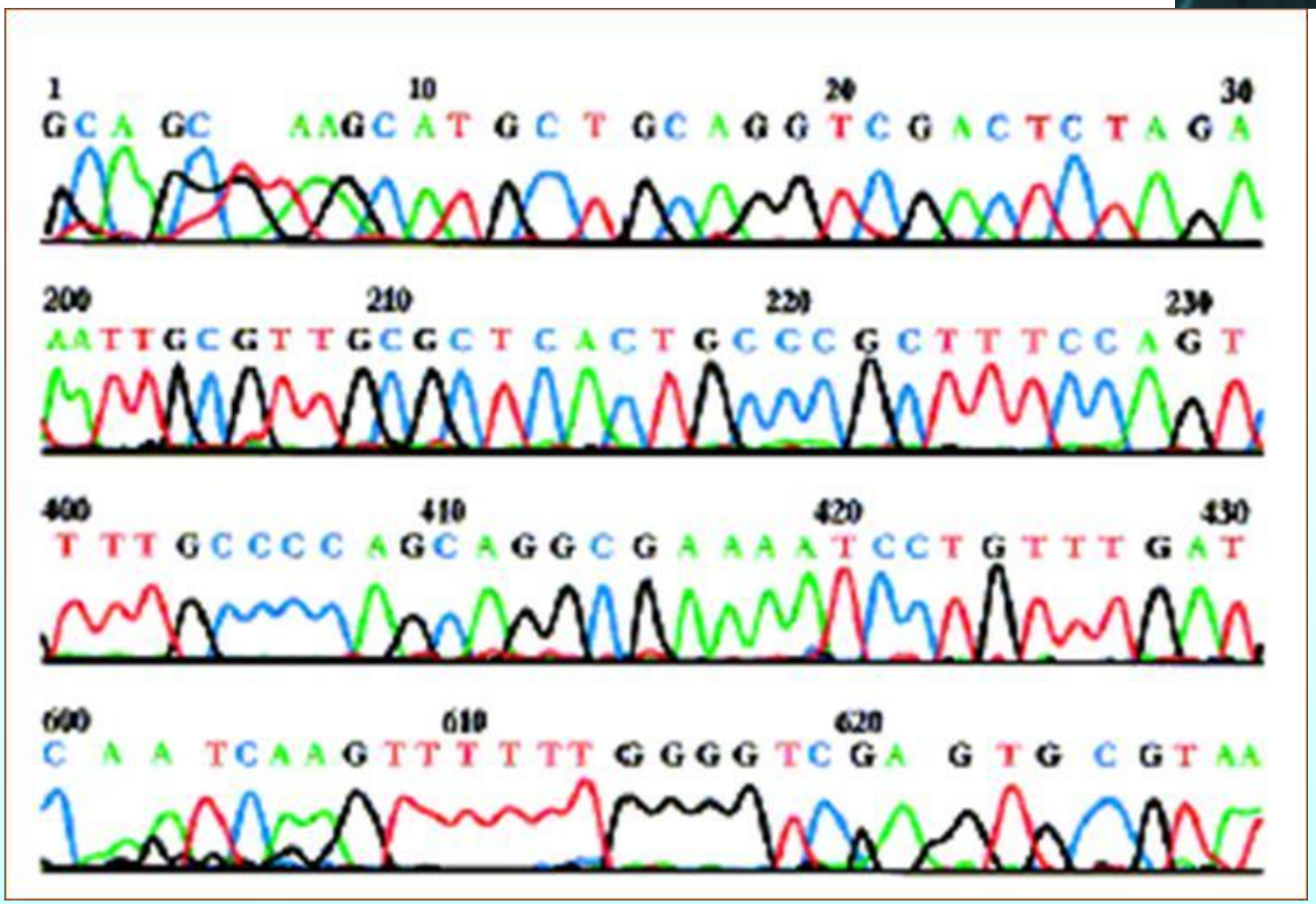
分离效率非常高　$N > 10^6$

常用于生物高分子，如DNA的分离

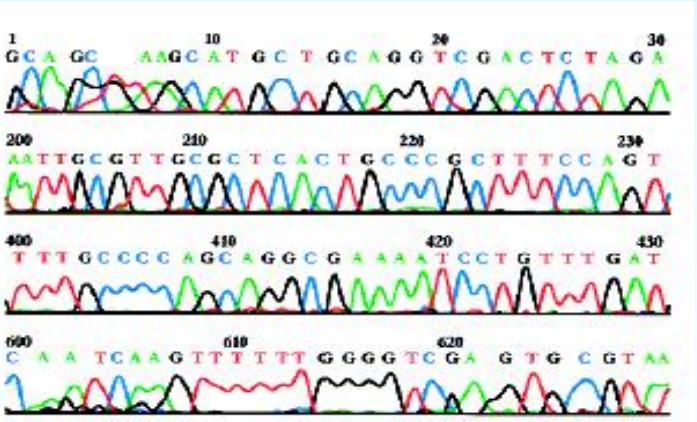
Dovichi

Kambara

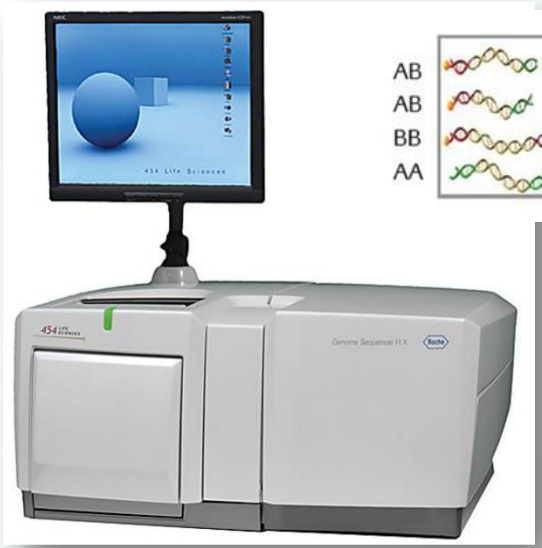
毛细管电泳DNA测序分离谱图

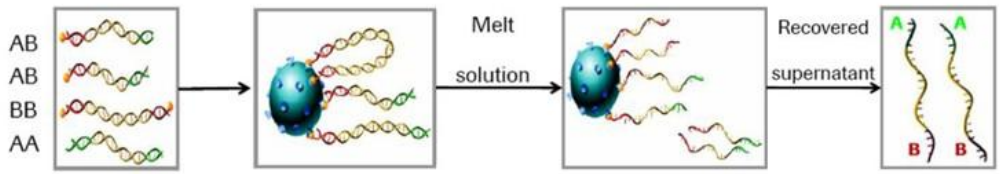


第一代测序仪



第二代测序仪





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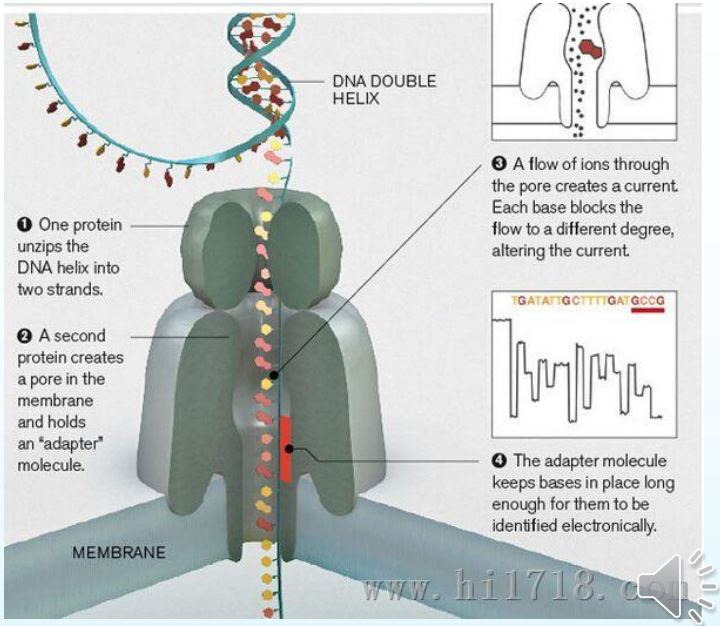


4. 毛细管电色谱（Capillary Electrochromatography, CEC)

——毛细管内填充固定相，电场驱动流动相

——色谱与电泳的结合，理论分离效率极高。

实际实验中，容易产生气泡，影响系统工作可靠性

微流控皮升级试样引入-高速毛细管电泳

研究背景

- 高速毛细管电泳，典型代表—芯片毛细管电泳 > 4000篇

  High-Speed Capillary Electrophoresis，HSCE

主要特征

- 高分离速度（<100 s）
- 高分离效率（>1,000,000 N/m）

  （<1 μm）

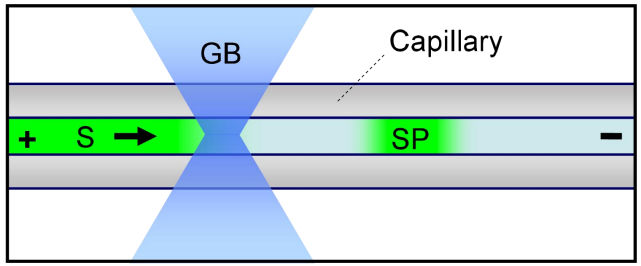
要素

- 高分离场强（>500 V/cm）
- 短分离距离（<10 cm）
- 窄进样区带（**~100 pL**）

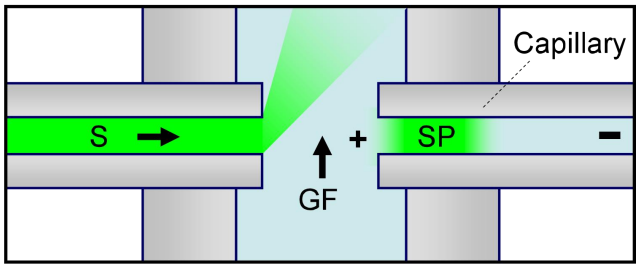
- 常规毛细管电泳进样方法（nL级）不能满足

  高速毛细管电泳对（pL级）进样的要求

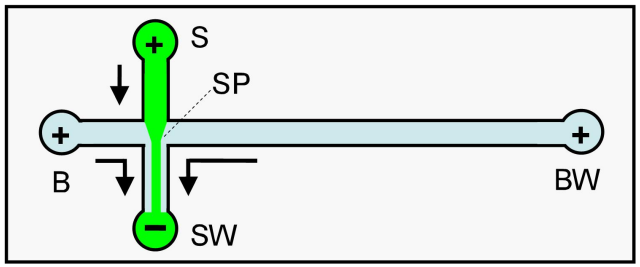
不同的高速毛细管电泳模式



光门进样



流动门进样



芯片十字通道进样

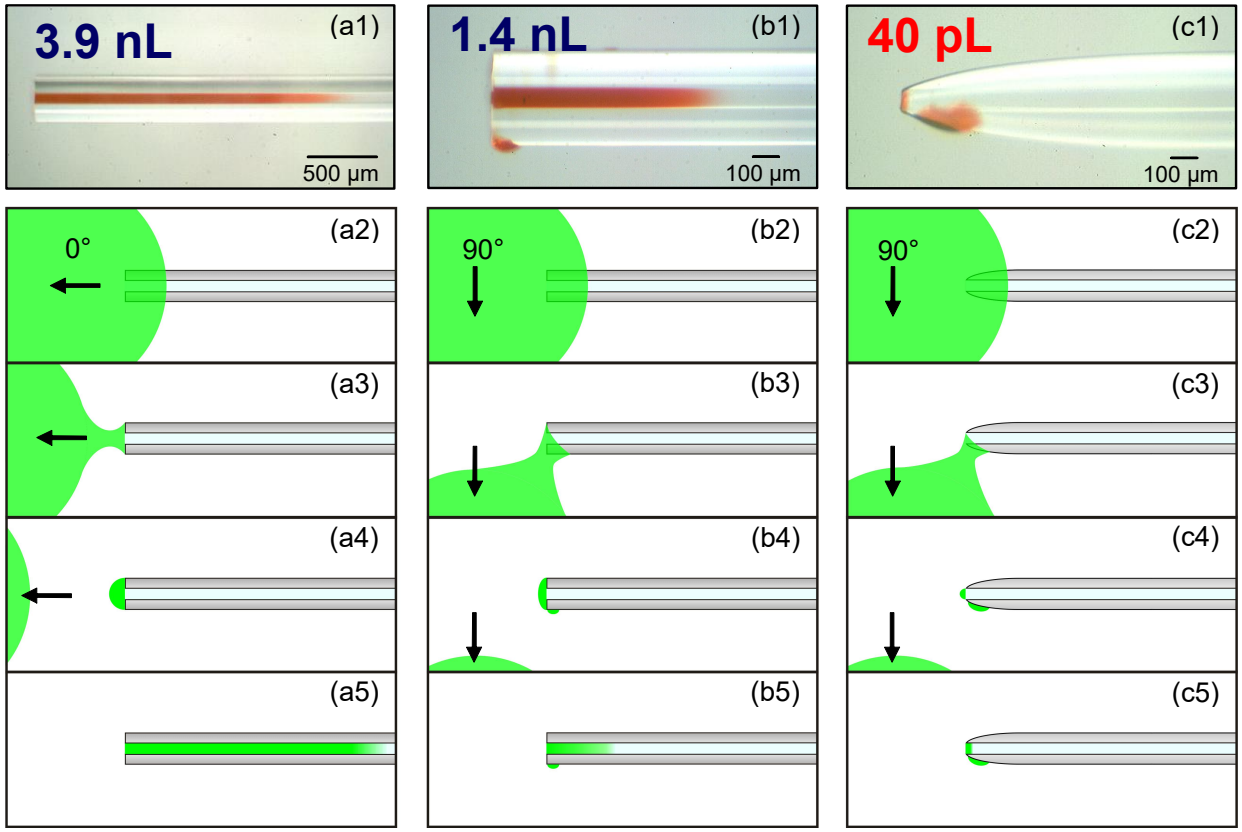
▶ 微流控芯片进样特点

- 微米级通道液流的操控，完成皮升级进样
- 开创芯片毛细管电泳新时代
- 发表论文 > 4000篇
- 加工和操作复杂，专利保护

微流控皮升级平移自发进样

新现象 → 新方法

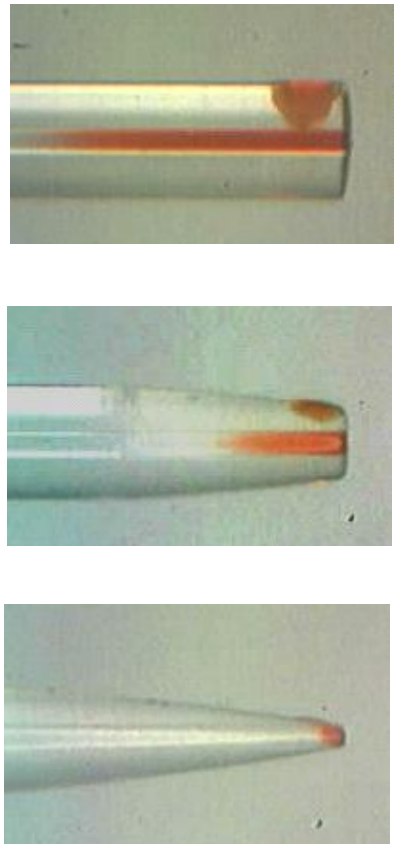
nL级自发进样　液滴分裂　pL级平移自发进样

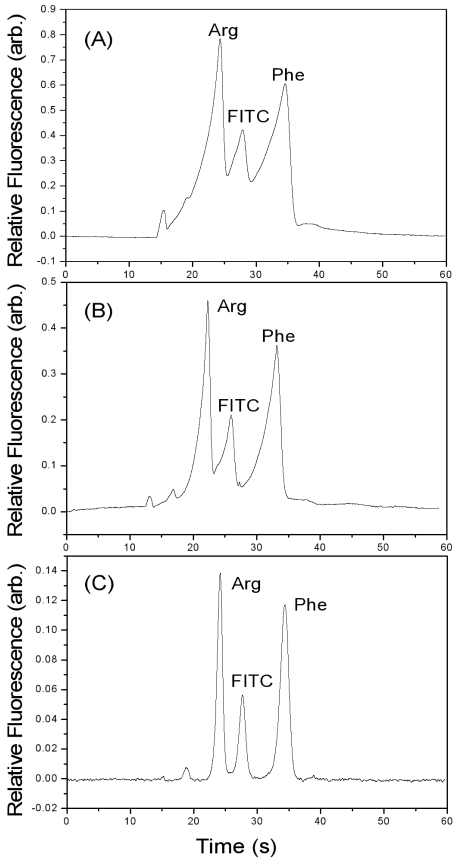


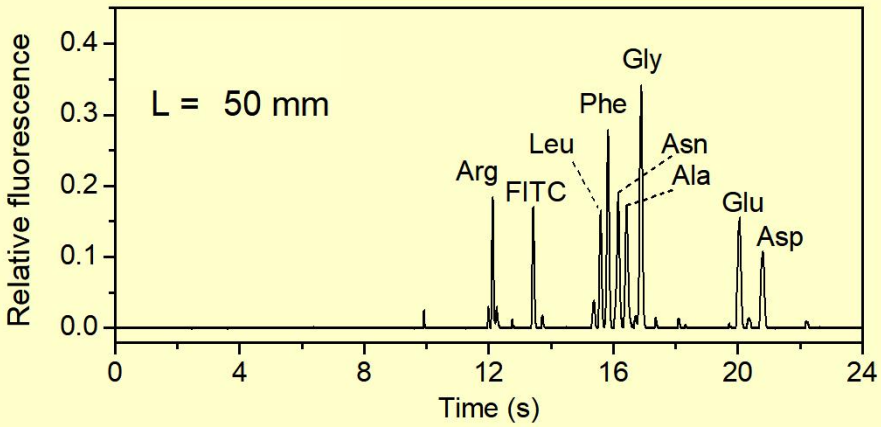
Fishman et al, Anal. Chem. 1994, 66, 2318.

Anal. Chem., 2009, 81, 3693

应用于氨基酸高速毛细管电泳分离





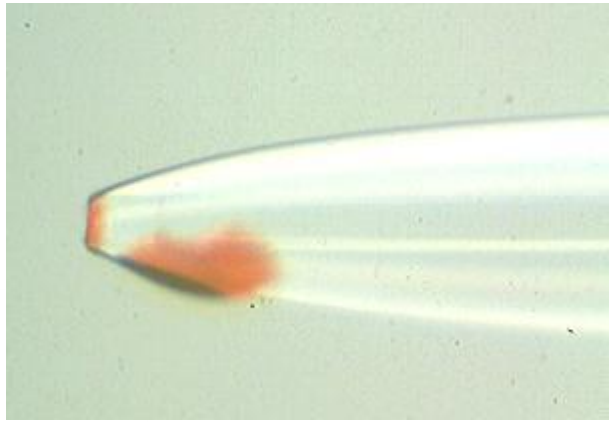


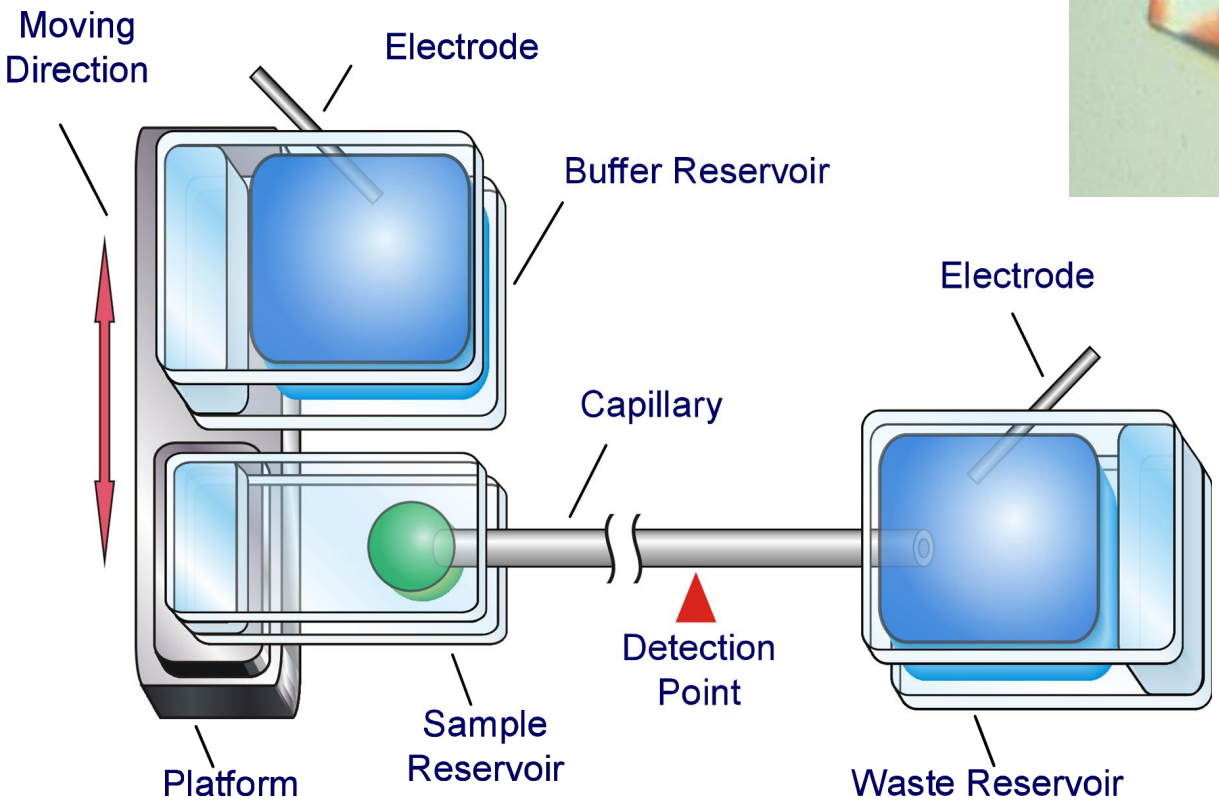
- 有效分离距离： 50 mm
- 分离时间： < 21 s
- 分离效率：0.2 - 0.3 μm 塔板高度
  3,230,000 - 5,000,000 /m
- 分辨率：163,000 - 251,000 塔板

分离速度和分离效率达到甚至超过多数芯片毛细管电泳系统

Anal. Chem., 2009, 81, 3693

平移自发进样—高速毛细管电泳系统

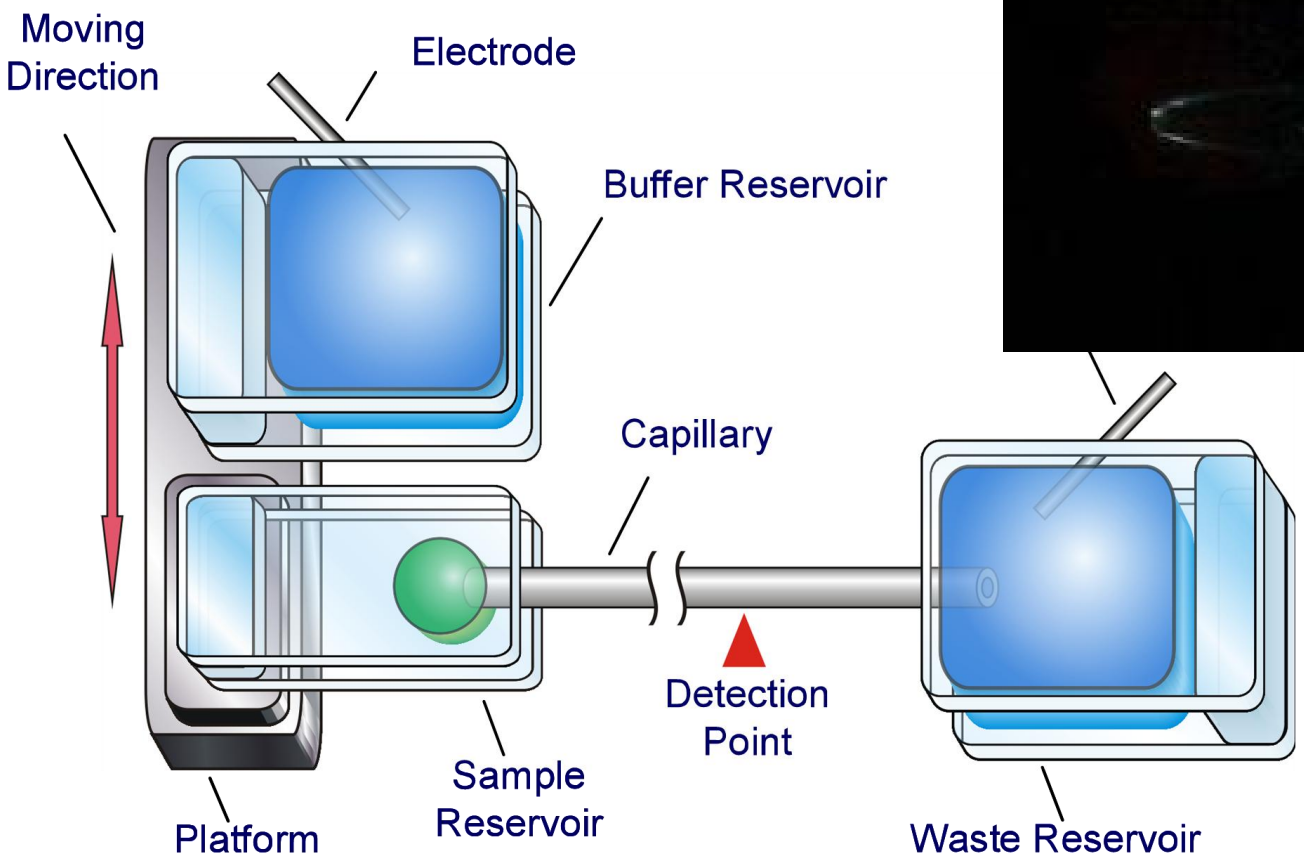




中国发明专利，专利号： ZL 200610052734.X

Anal. Chem., 2009, 81, 3693

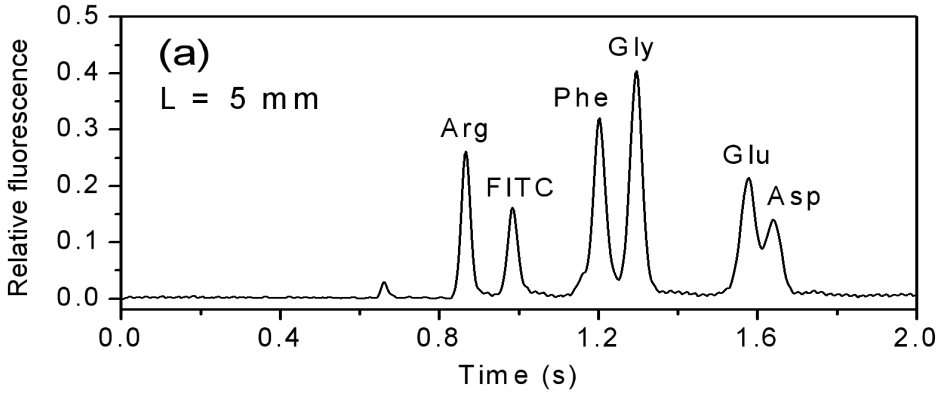
微流控平移自发进样高速毛细管电泳分离



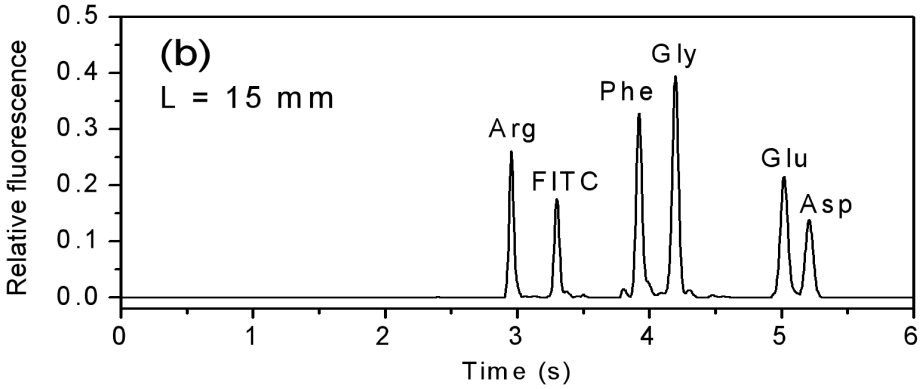
中国发明专利，专利号： ZL 200610052734.X

Anal. Chem., 2009, 81, 3693

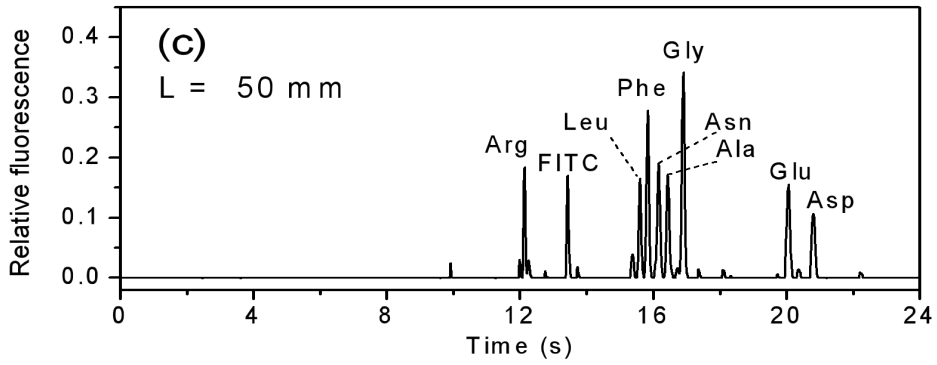
分离性能



- 进样体积：**40 pL**
- 有效分离距离： **5 mm**
- 分离时间： **< 1.7 s**
- 最高分离效率：**0.7 μm 塔板高度**

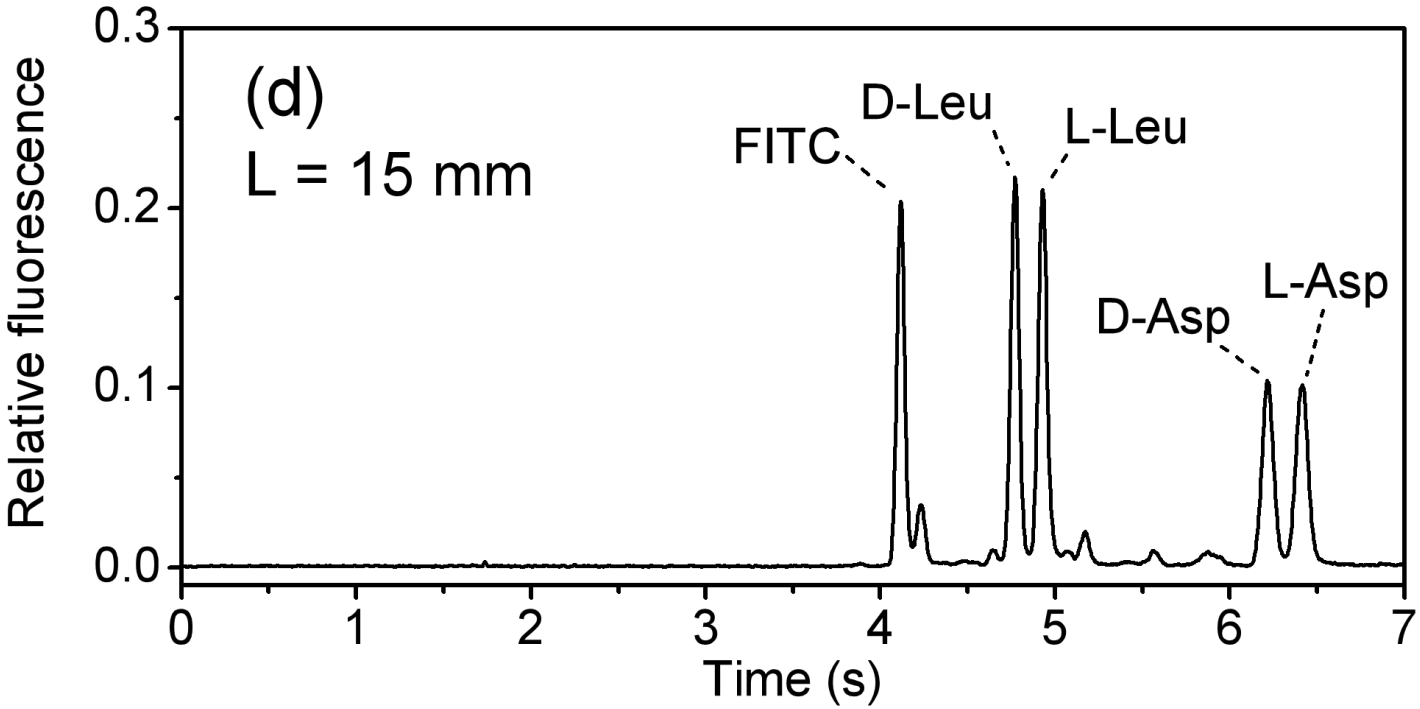


- 有效分离距离： **15 mm**
- 分离时间： **< 5.5 s**
- 分离效率：**0.4 - 0.5 μm 塔板高度**

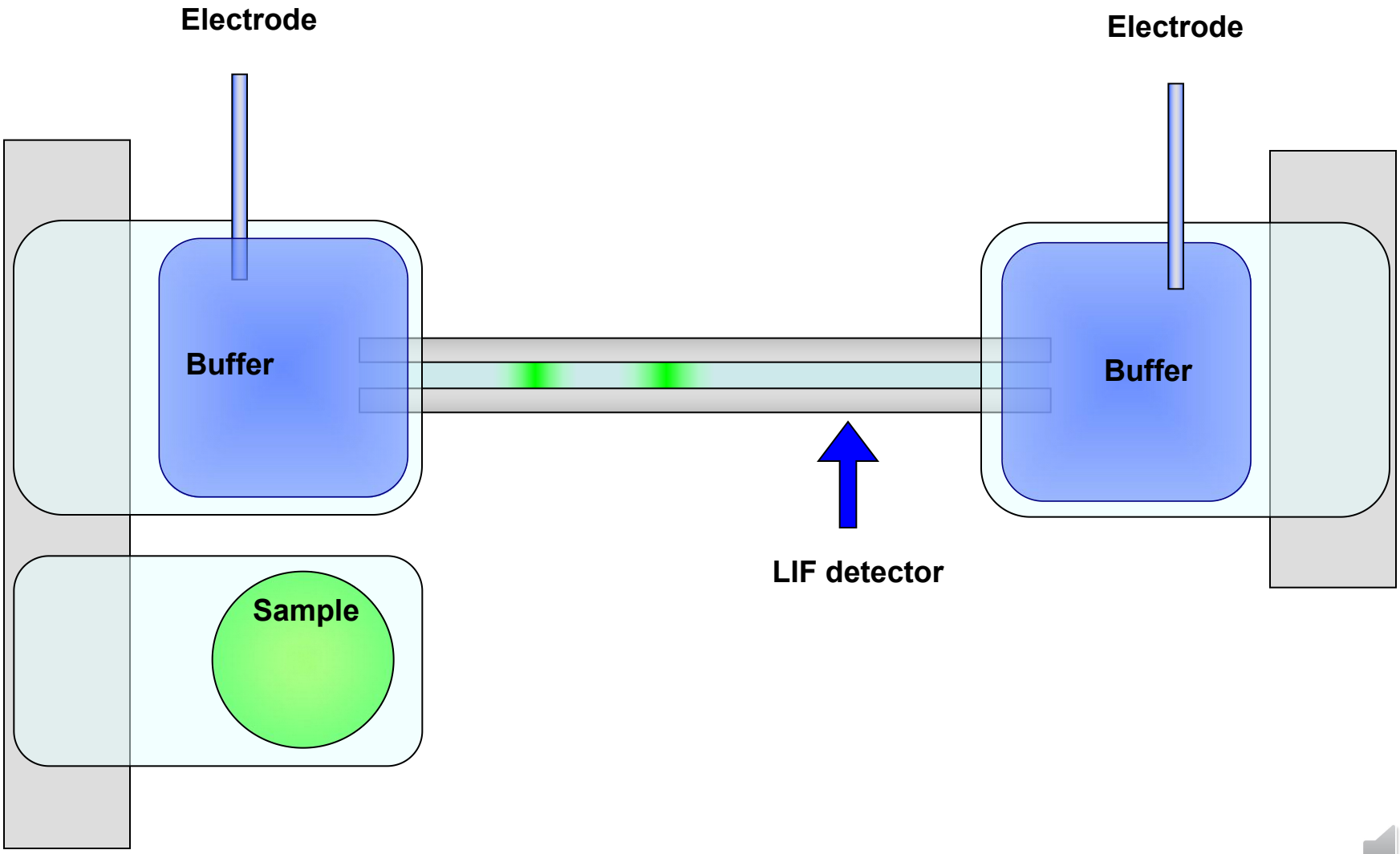


- 有效分离距离： **50 mm**
- 分离时间： **< 21 s**
- 分离效率：**0.2 - 0.3 μm 塔板高度**
- 分辨率：**163,000 - 251,000 塔板**

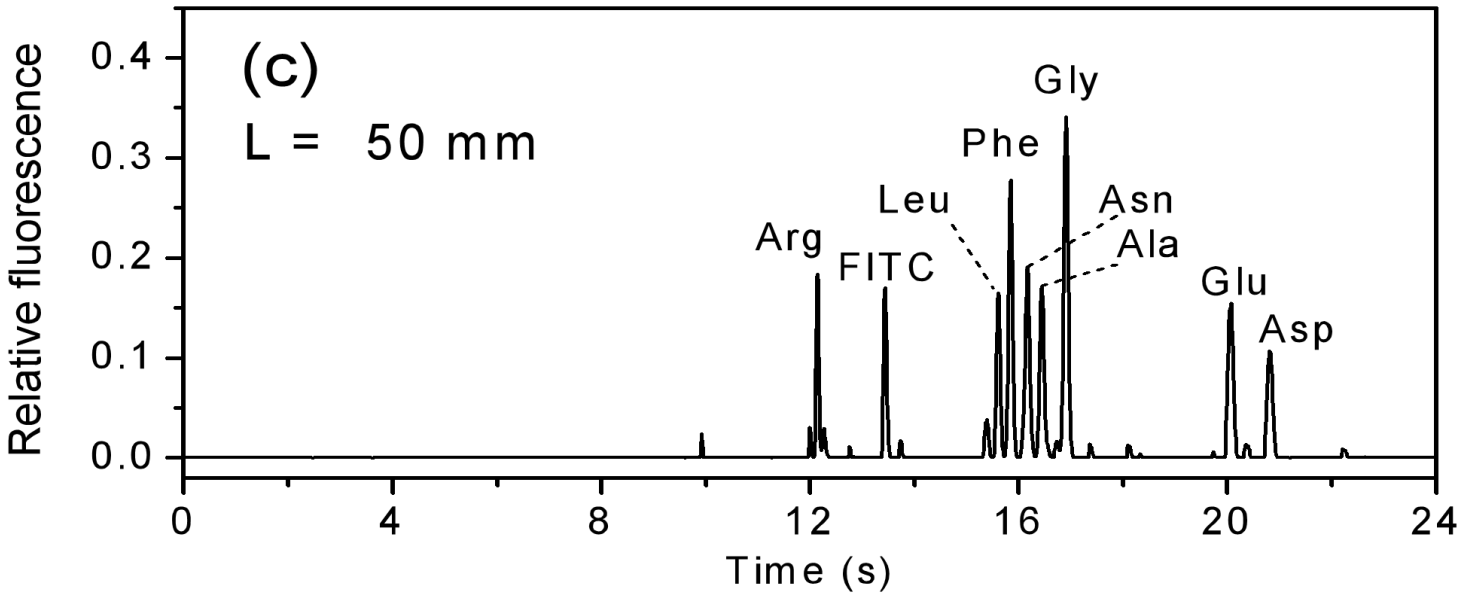
应用于手性氨基酸分离



- 有效分离距离： **15 mm**
- 分离缓冲液：**5 mM borate buffer (pH 9.2)**
  8 mM β-CD, 12 mM STC
- 分离时间： **< 7 s**
- 手性对映体分离度：**1.10-1.95**


Electrode
Electrode
Buffer
Buffer
LIF detector
Sample

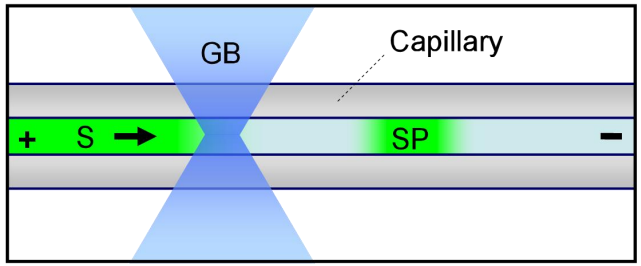
应用于氨基酸高速毛细管电泳分离



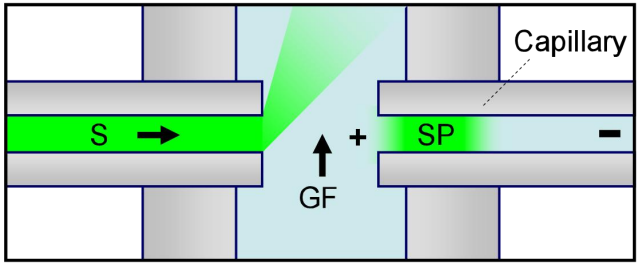
- 有效分离距离： 50 mm
- 分离时间： < 21 s
- 分离效率：0.2 - 0.3 μm 塔板高度
  3,230,000 - 5,000,000 /m
- 分辨率：163,000 - 251,000 塔板

Anal. Chem., 2009, 81, 3693

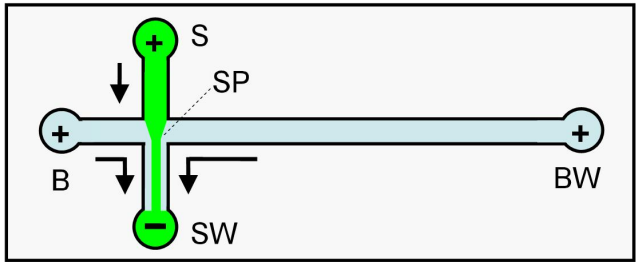
不同的高速毛细管电泳模式



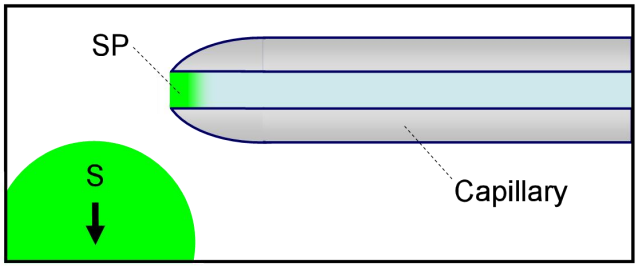
光门进样



流动门进样



芯片十字通道进样



平移自发进样

▶ 微流控芯片进样特点

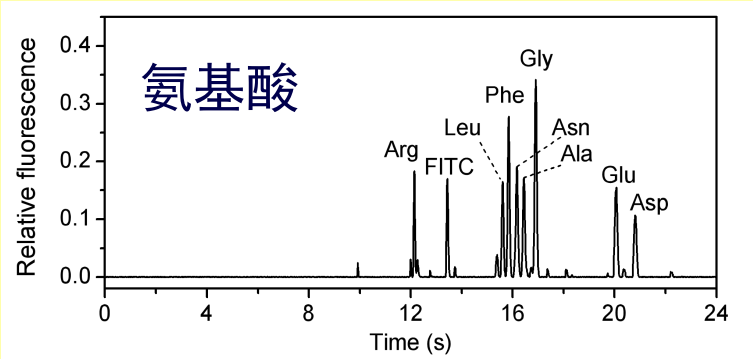
- 微米级通道液流的操控，完成皮升级进样
- 开创芯片毛细管电泳新时代
- 发表论文 > 4000篇
- 加工和操作复杂，专利保护

▶ 平移自发进样特点

- 自主知识产权
- 结构简单，容易微型化
- 操作方便，全自动化
- 通用性好

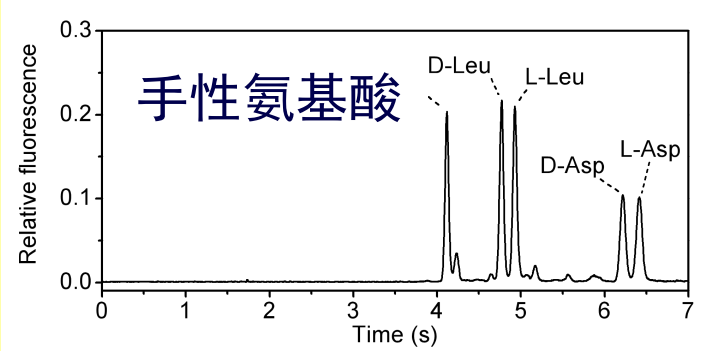
平移自发进样的应用拓展

毛细管区带电泳 (CZE)



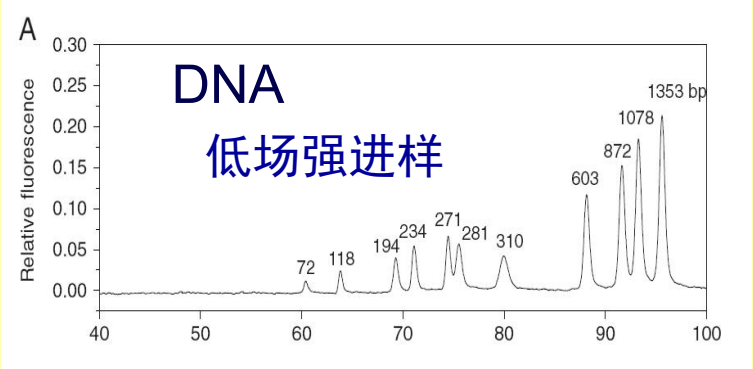
Anal. Chem., 2009, 81, 3693

毛细管胶束电动色谱 (MEKC)



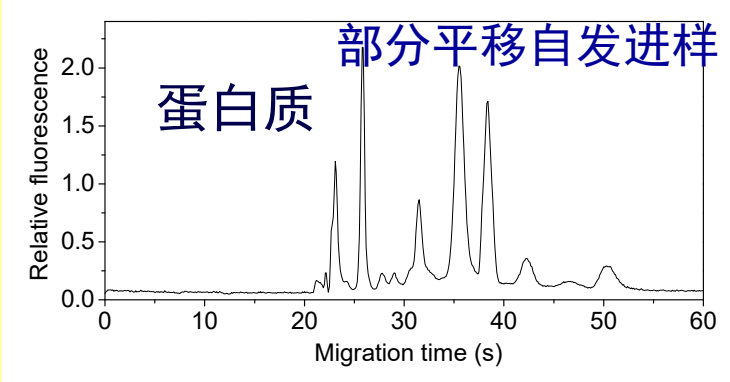
Anal. Chem., 2009, 81, 3693

毛细管凝胶电泳 (CGE)



Electrophoresis, 2010, 31, 3184

毛细管凝胶电泳 (CGE)



Electrophoresis, 2011, 32, 1

作业：

P307： 58，65