

厦门大学



高效液相色谱讲座

主讲：张博

厦门大学化学系

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0592-2188691, 15960817368

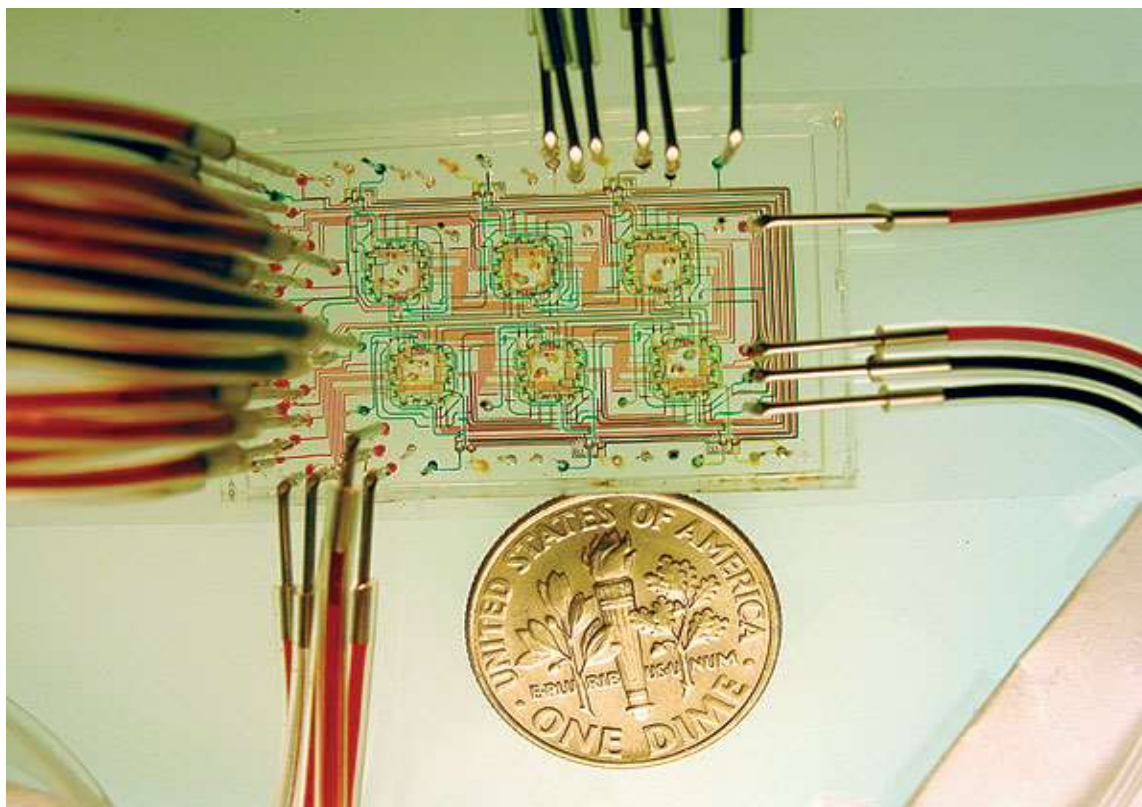
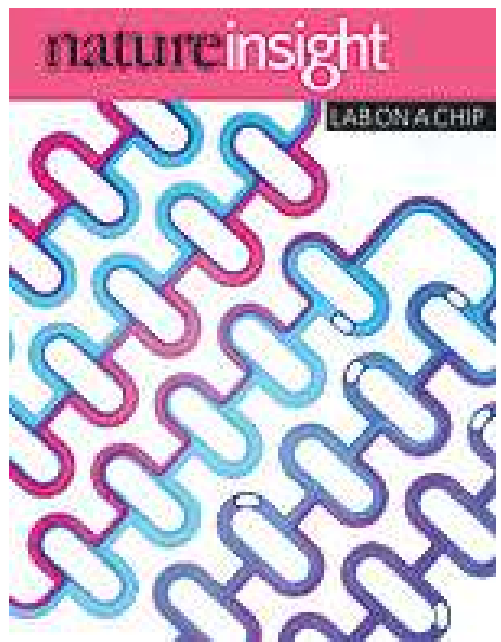
- *An essential condition for all fruitful research is to have at one's disposal a satisfactory technique. "Tout progres scientifique est un progres de methode"* as somebody once remarked. Unfortunately the methodology is frequently the weakest aspect of scientific investigations.*

—M. S. Tswett



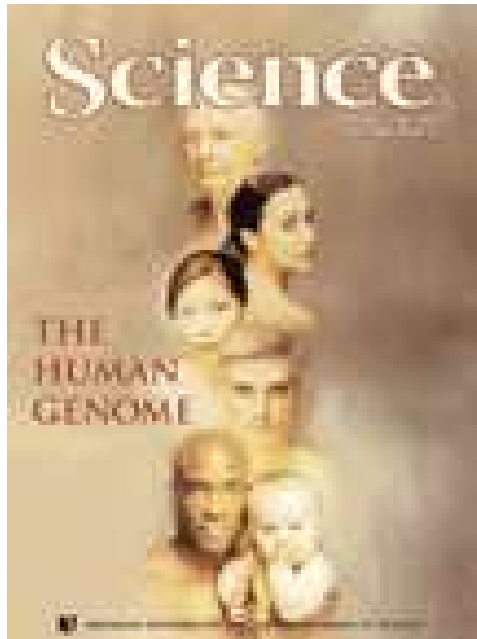
- * "All scientific progress is progress in a method." This statement is attributed to the French philosopher Rene Descartes (1596-1650), the author among others of the *book Discours de la methode*.

现代分析化学的特点和趋势：微型化

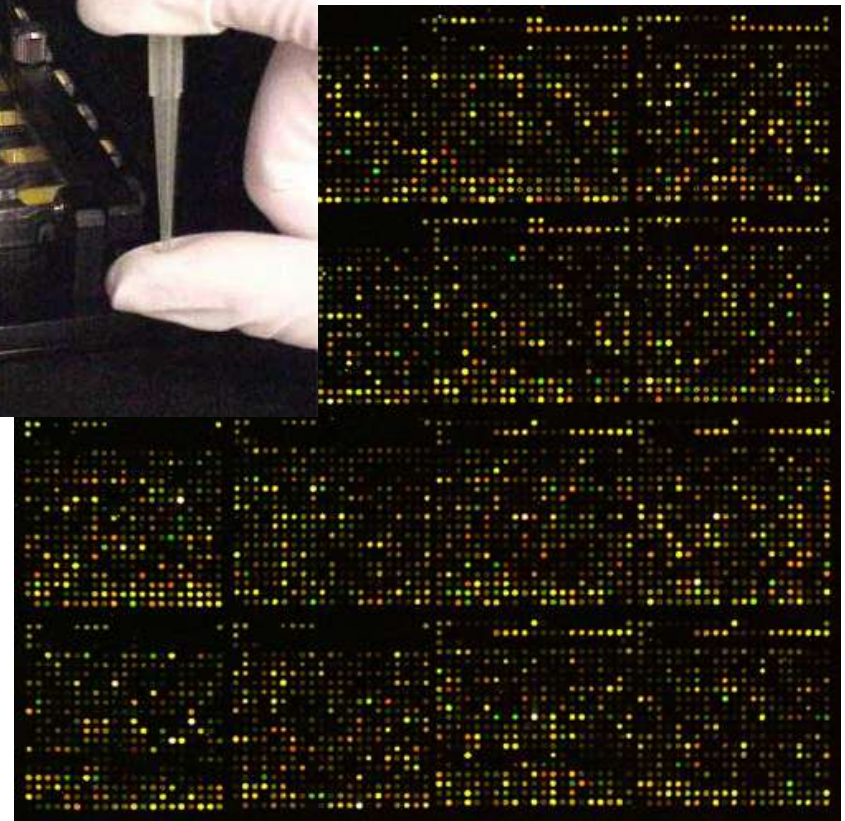
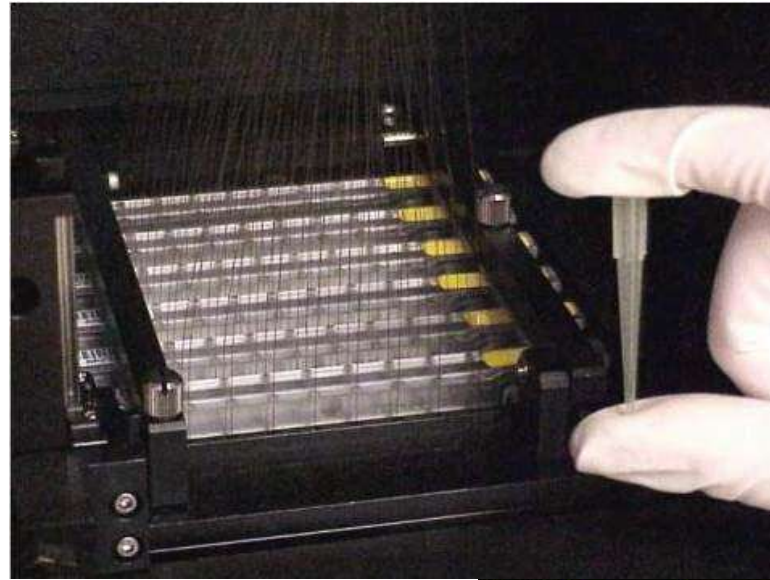


Lab-on-a-chip
微流控芯片技术
微全分析系统

现代分析化学的特点和趋势：高通量



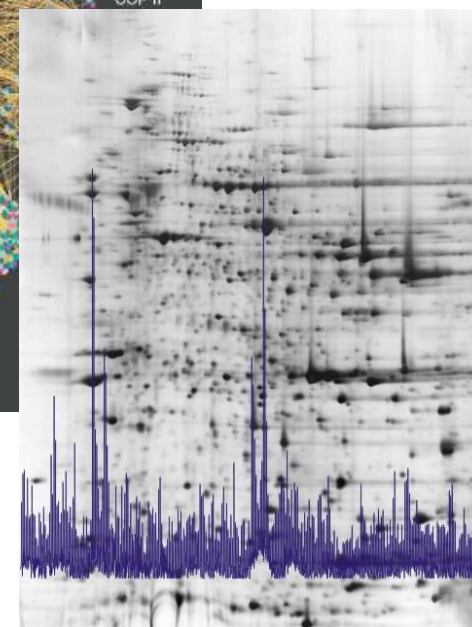
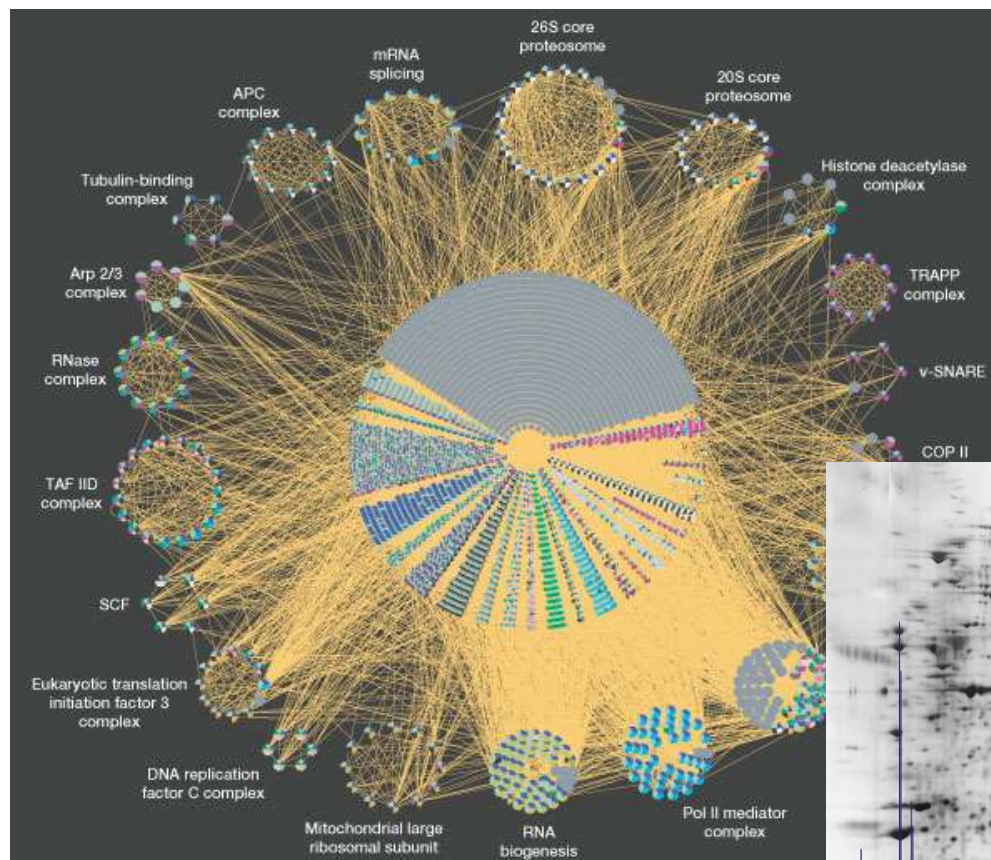
Human Genome Project
基因组计划



现代分析化学的特点和趋势：全息化



Proteomics 蛋白质组学



色谱100年：我们身在何处？

历程

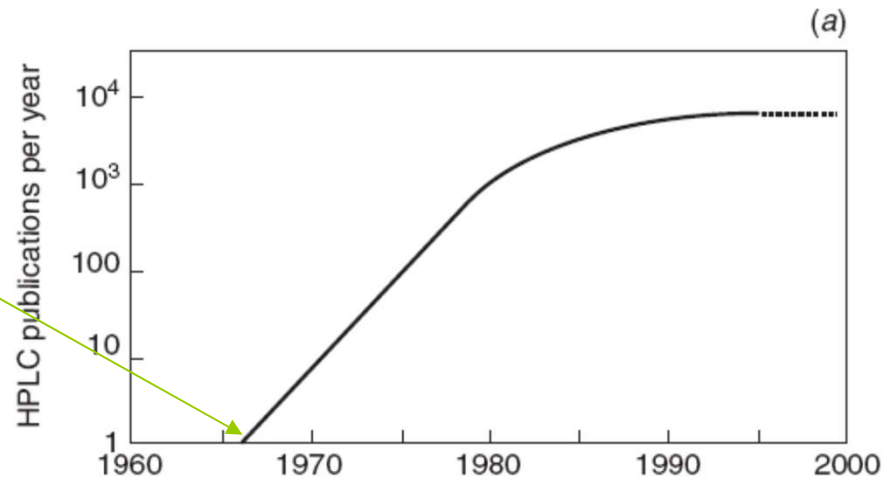
- 1900s-1940s 最初的发现、理论的建立
- 1940s-1960s 各种色谱技术相继建立
- 1970s-1980s 黄金发展阶段
- 1980s-1990s 毛细管电分离
- 1990s-2000s 微流控芯片、纳流液相色谱
- 2000s-2010s 多维分离、整体柱、超高压
- 2010s- 全新的分离概念、全新的分离方式、超高分辨率、超高信息量

对象

- 无明确对象
- 石化工业
- 药物分析与生化分析
- 人类基因组计划
- 蛋白质组计划，中药现代化
- 系统生物学最关键技术
- 推进分析科学进入全息分析时代

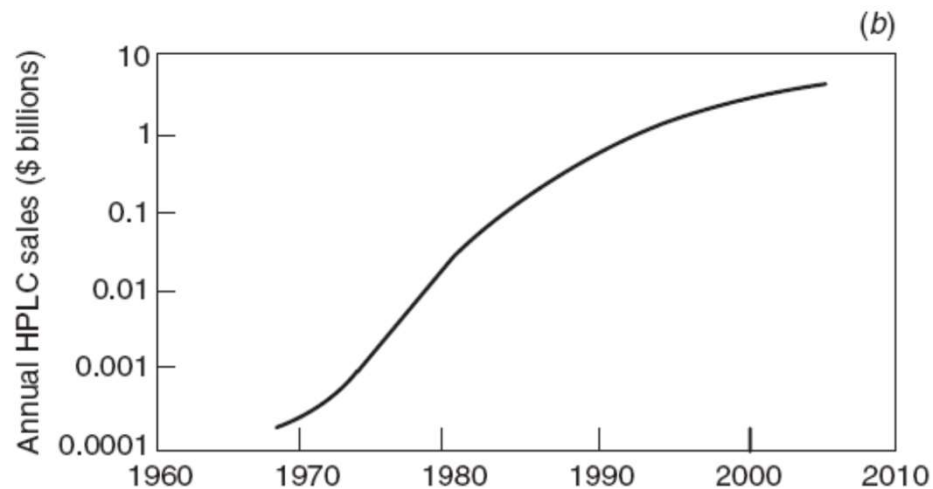
高效液相色谱的重要地位

C. G. Horvath, S. R. Lipsky,
Nature, 211 (1966) 748.



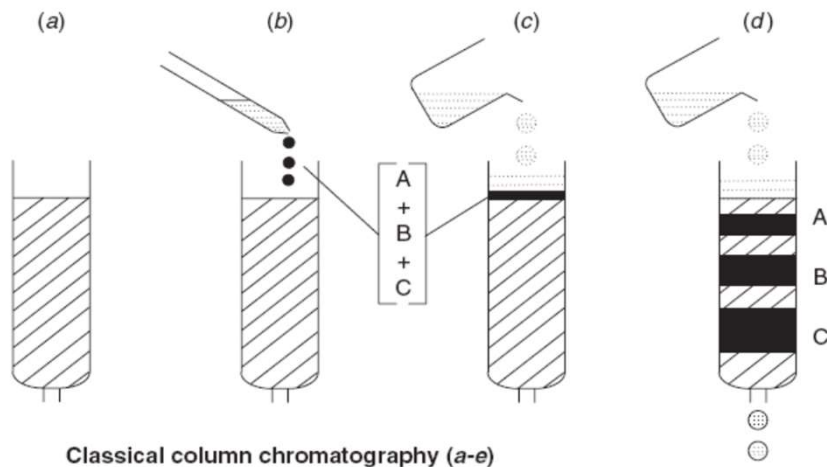
“The money spent annually on HPLC at the present time exceeds that for any other analytical technique.”

---L.R. Snyder, 2010.

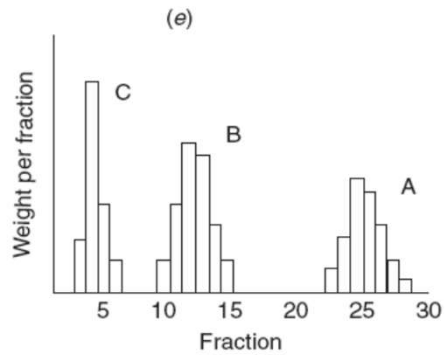


- The expanding importance of HPLC research and application since 1966. (a) Number of HPLC-related publications per year; (b) total sales of HPLC equipment and supplies per year (approximate data compiled from various sources).

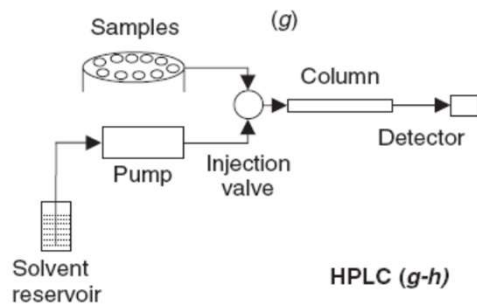
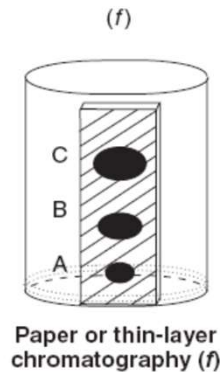
高效液相色谱的发展



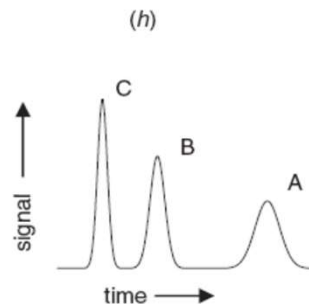
1900s
Classical Liquid Chromatography



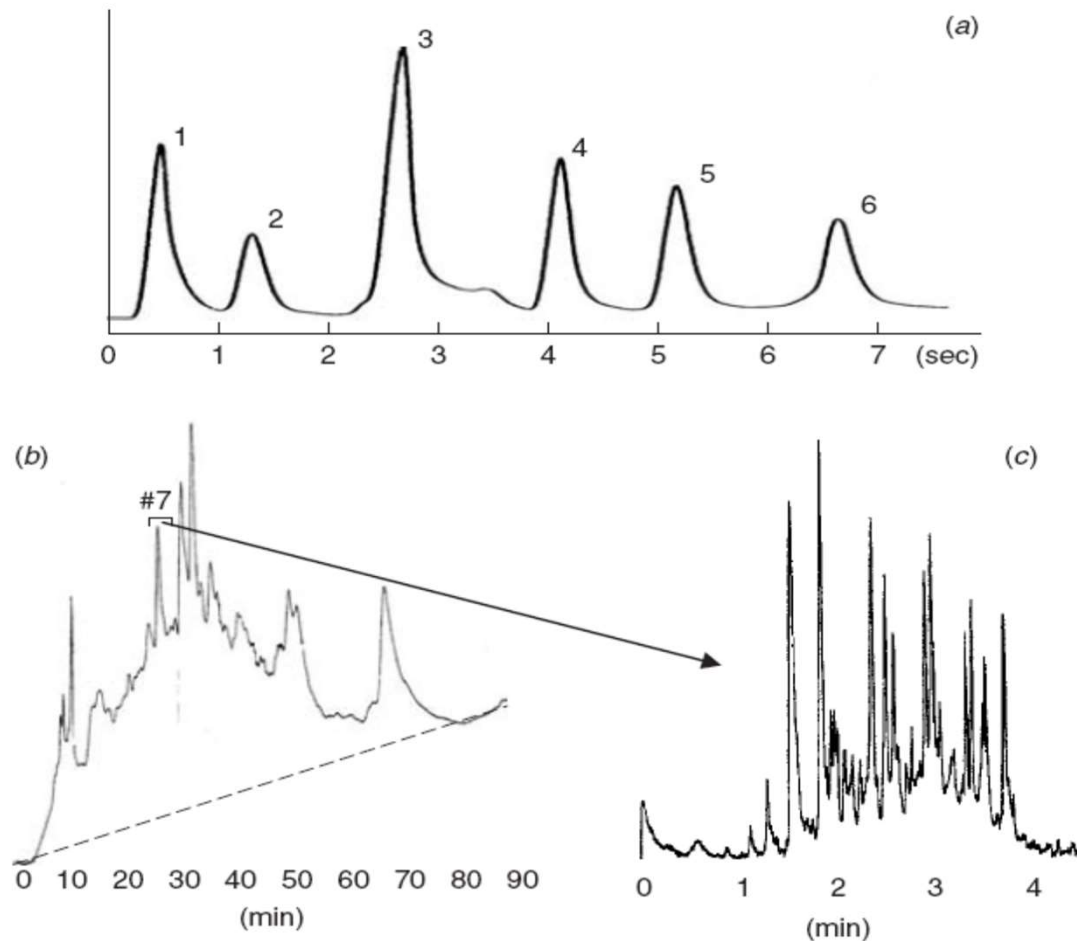
1940s
Paper / Thin Layer Chromatography



1960s
High Performance Liquid Chromatography



高效液相色谱的发展



Recent examples of HPLC capability. (a) Fast separation of six proteins, using gradient elution with a 150×4.6 -mm column packed with 1.5- μm -diameter pellicular particles; (b) initial separation of peptides and proteins from human fetal fibroblast cell by gradient cation-exchange chromatography; (c) further separation of fraction 7 (collected between 24–28 min) on a second column by gradient reversed-phase chromatography.

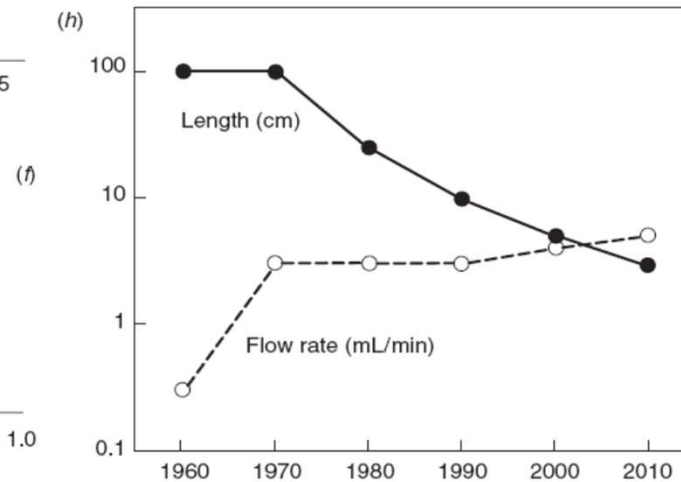
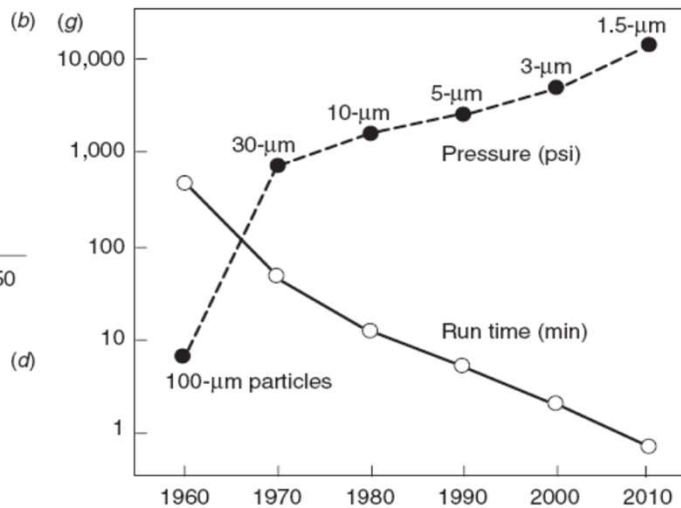
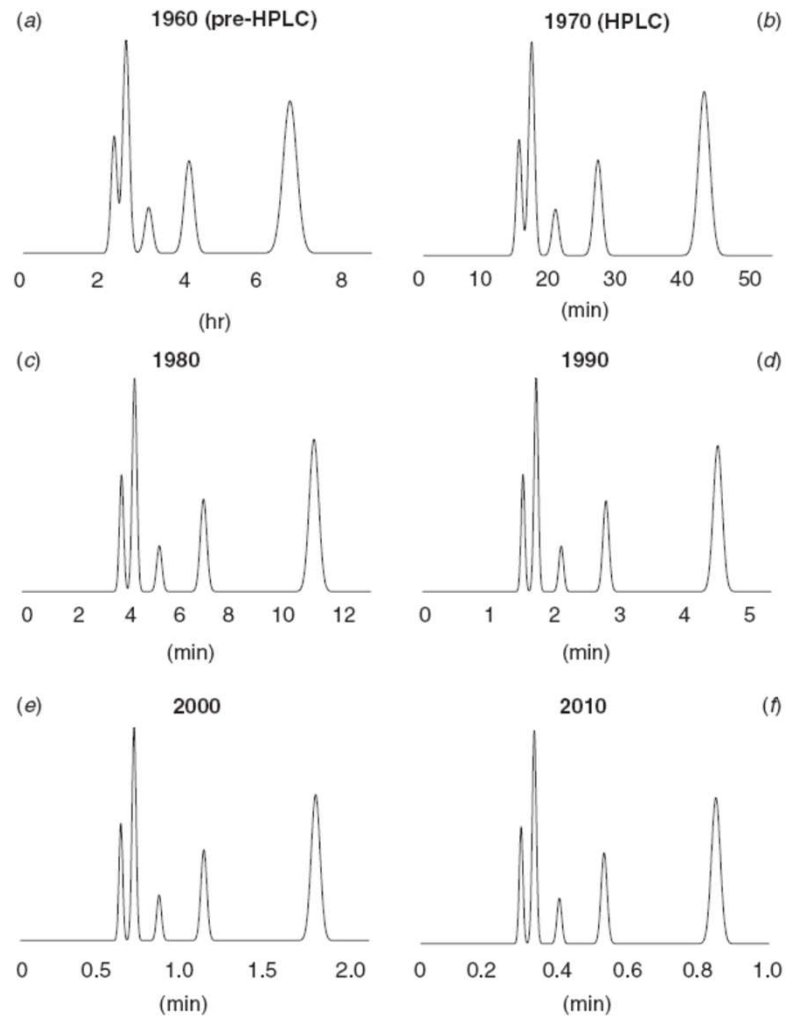
高效液相色谱简史

- Early 1900s M. S. Tswett 第一次柱色谱实验
- Early 1940s A. J. P. Martin's 分配色谱理论与纸色谱
- Late 1950s S. Moore, W. S. Stein 氨基酸分析仪
- 1966 两个课题组分别独立报道了HPLC



- 耶鲁大学 Csaba Horvath 维也纳大学 Josef Huber
- 1960s 两家公司推出液相色谱仪
- Waters Associates DuPont

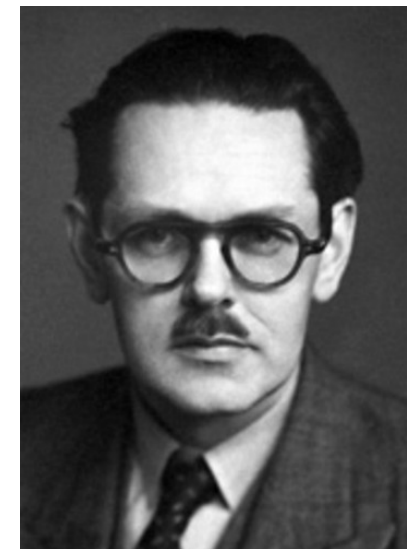
50 Years of HPLC: 1960-2010



分离时间
分离效能



填料尺寸
色谱仪器



*“the most efficient columns. . . should be obtainable by using very **small particles** and **high-pressure** differences across the length of the column...”* --- A. J. P. Martin, 1941

与液相色谱相关的分离技术

- 1960s以前

- 气相色谱, 1950s (Gas Chromatography, GC)

- 气体样品, 高柱效

- 薄层色谱, 1940s (Thin Layer Chromatography, TLC)

- 多样品, 显色直视检测, 低效, 高效薄层色谱(HP-TLC)

- 1970s以后

- 超临界色谱, 1970s (Supercritical Fluid Chromatography, SFC)

- 仪器与HPLC类似, 高温高压超临界流体 (如CO₂), HPLC与GC杂交技术, 天然产物分析

- 毛细管电泳, 1980s (Capillary Electrophoresis, CE)

- 非色谱技术, 高柱效, DNA 测序, 手性分离 (高效, 手性选择剂), 模式多样, 绿色

- 毛细管电色谱, 1990s (Capillary ElectroChromatography, CEC)

- 集色谱与电泳性质于一身 (HPLC与CE杂交), 电渗驱动, 高柱效, 优势尚未发挥, 柱技术

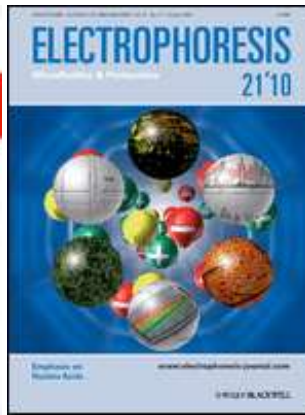
主要的色谱与分离科学专业期刊



3.6



2.9



3.6



2.6



0.9



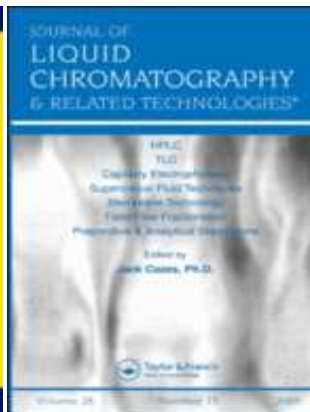
0.4



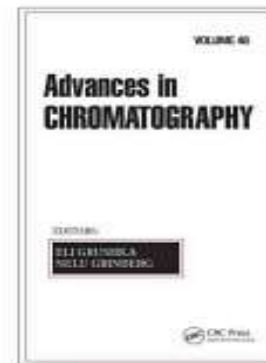
1.1



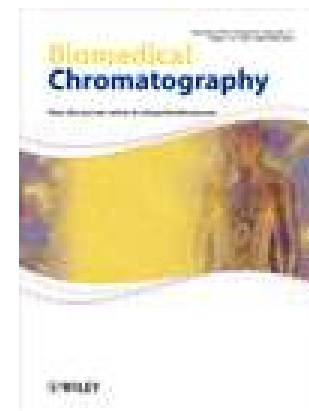
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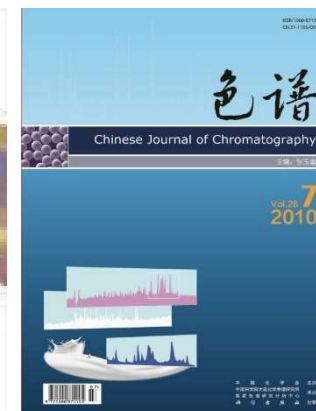
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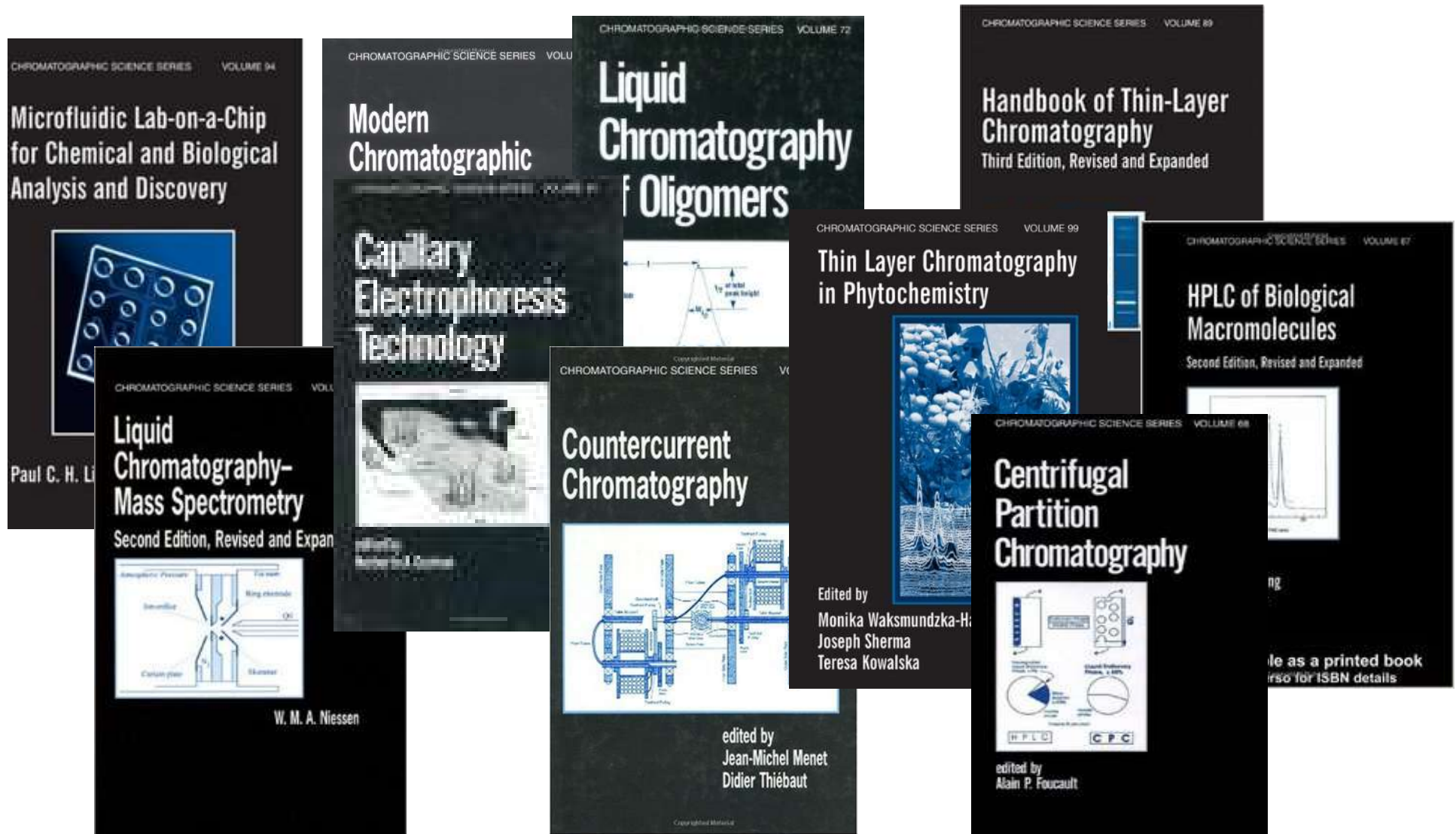
JCR IF 2007

高效液相色谱书库



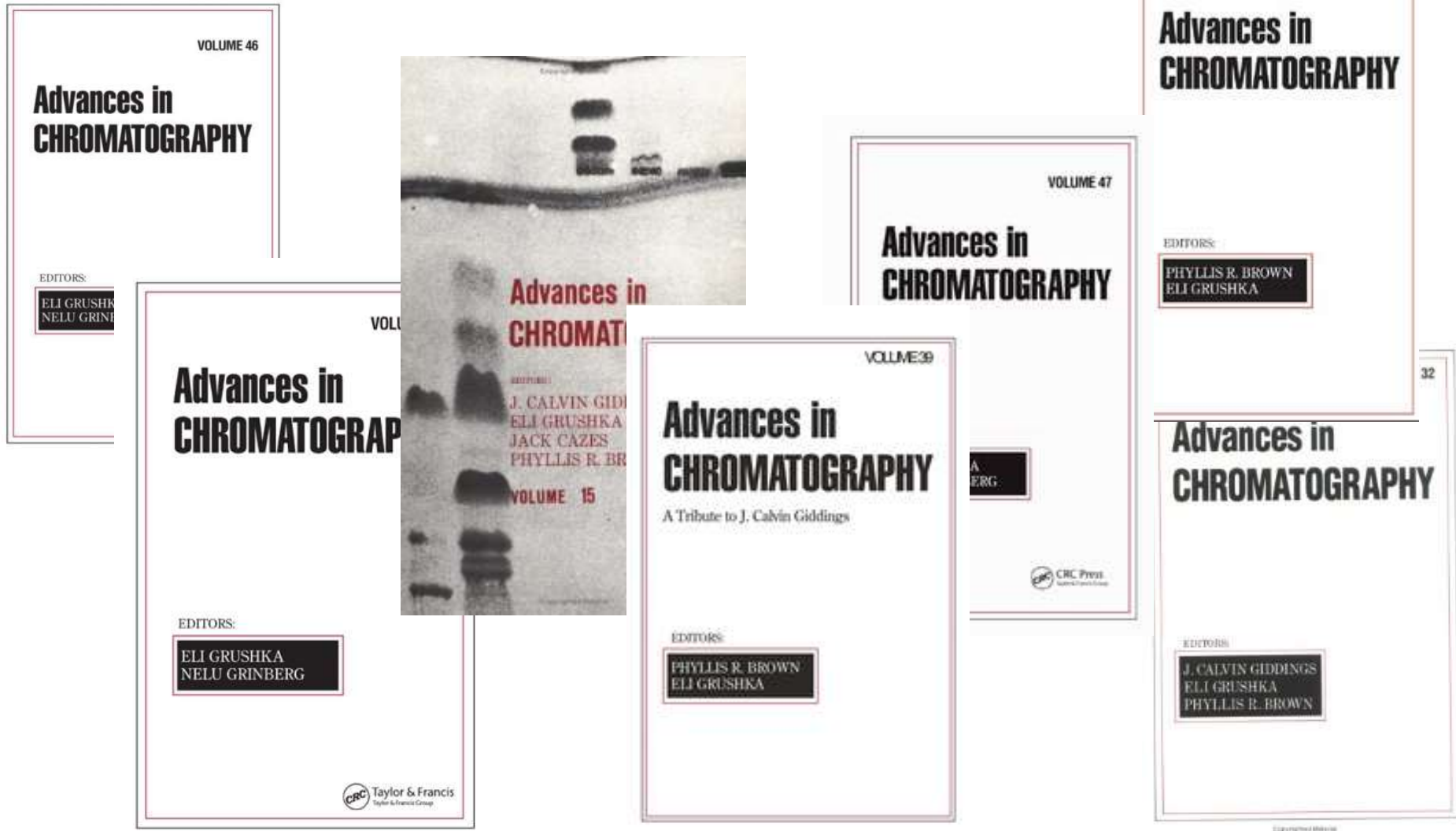
- *Journal of Chromatography Library*: Elsevier

高效液相色谱书库



Chromatographic Science Series: CRC

高效液相色谱书库

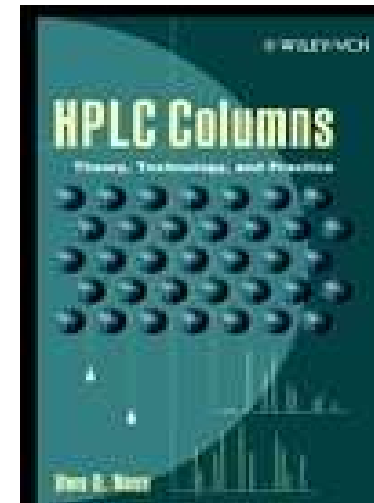
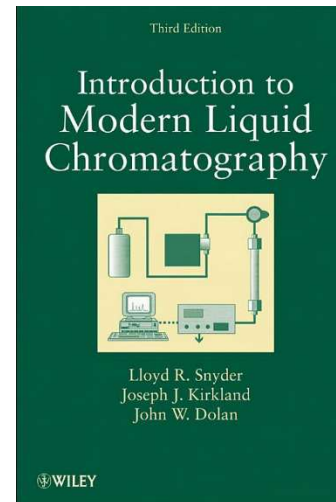
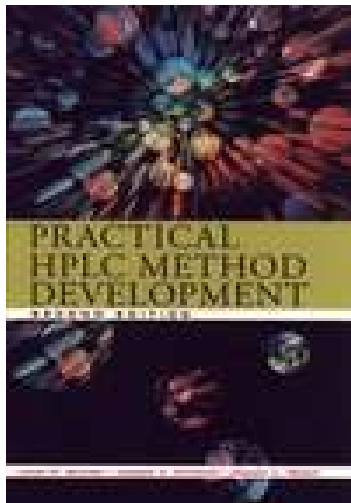


- *Advances in Chromatography*: CRC

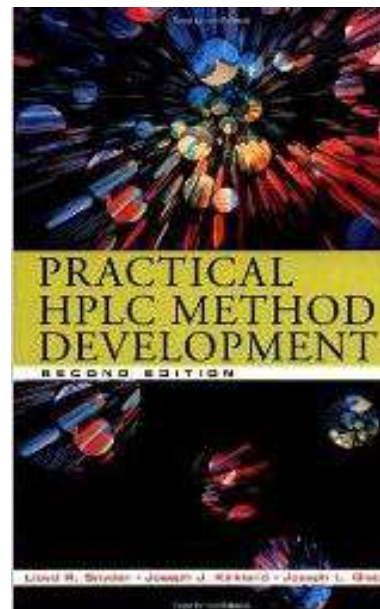
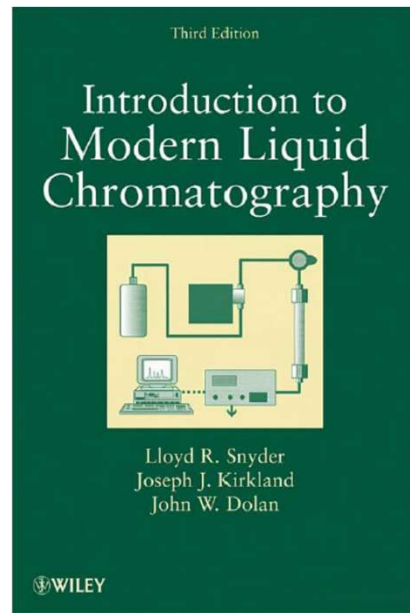
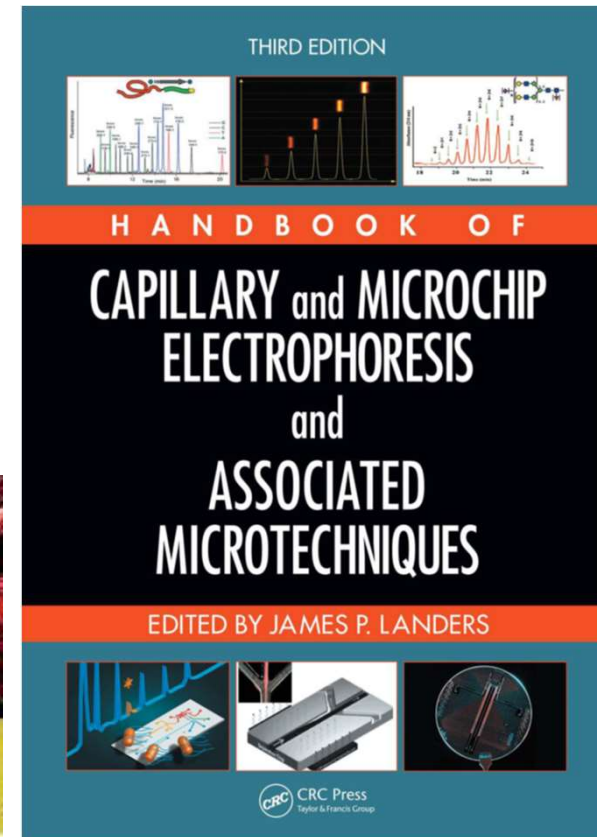
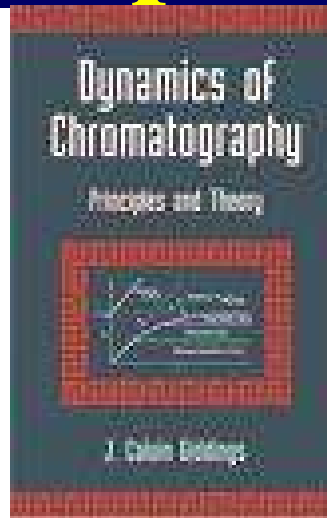
Practical HPLC Method Development, 2nd Edition
Lloyd R. Snyder, Joseph J. Kirkland, Joseph L. Glajch

HPLC Columns: Theory, Technology, and Practice
Uwe D. Neue

Introduction to Modern Liquid Chromatography, 3rd Edition
Lloyd R. Snyder, Joseph J. Kirkland, John W. Dolan



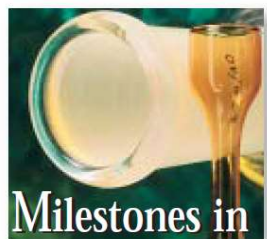
Bibles in Separation Science



*If I have seen further
it is by **standing**
upon the **shoulders**
of **giants**.*

— **Sir Isaac Newton,**
5 February 1675

128 LCGC NORTH AMERICA VOLUME 20 NUMBER 2 FEBRUARY 2002

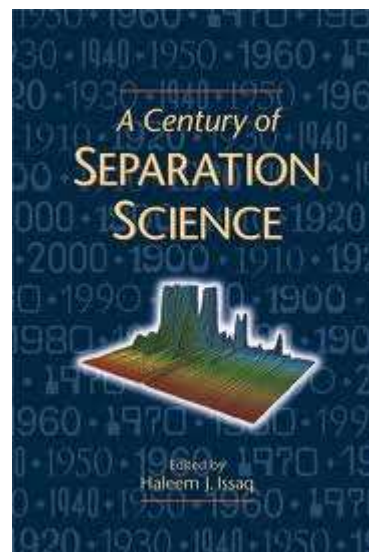


**Milestones in
Chromatography**

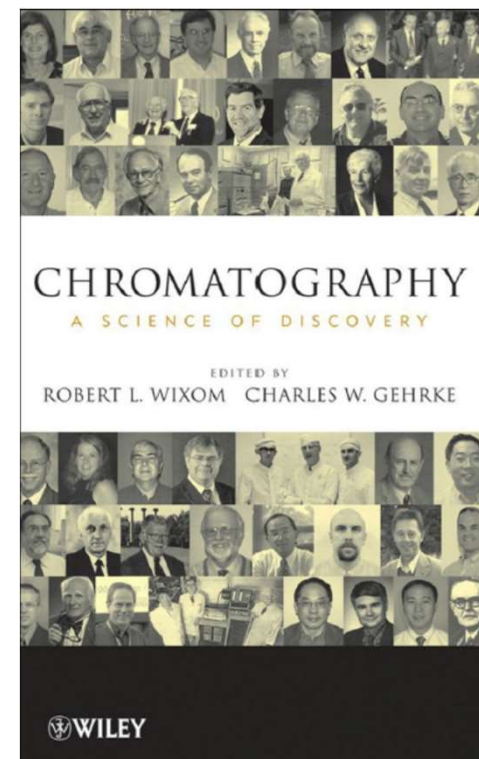
Fifty Years of Gas Chromatography — The Pioneers I Knew, Part I

The First International Congress on Analytical Chemistry was held in September 1952 in Oxford, United

Kingdom, and I was no longer among us. In the first three decades of GC they were most active in the development of the technique and its applica-



www.chromatographyonline.com



Reviews

K. K. Unger et al.

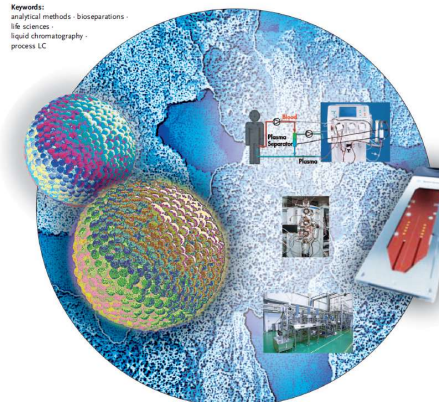
Liquid Chromatography

DOI: 10.1002/anie.200900976

Liquid Chromatography—Its Development and Key Role in Life Science Applications

Klaus K. Unger,* R. Ditz, E. Machtejevas, and R. Skudas

Keywords:
analytical methods · bioseparations ·
life sciences ·
liquid chromatography ·
process LC



Chromatography: the Separation Technique of the 20th Century

L. S. Ettre

Chemical Engineering Department, Yale University, New Haven, CT 06520, USA*

Centennial Review

Chromatographia Vol. 51, No. 1/2, January 2000

Angewandte
Chemie

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Angew. Chem. Int. Ed. 39(1): 1210–1211

今天的色谱学界在哪里？

The screenshot shows a web browser window displaying the homepage of the CASSS (An International Separation Science Society). The browser's address bar shows the URL <http://www.casss.org/>. The website features a blue header with the CASSS logo and a world map. Below the header is a navigation menu with links for "About CASSS", "Meetings & Events", "Member Center", "Partners & Products", "Career Center", and "Retention Times®".

OUR MEETINGS

- CMC Forum Europe 2011**
March 21-23, 2011
Barcelona, Spain
- Bay Area Separation Science Forum-PTMs**
April 26, 2011
South San Francisco, CA USA
- Sino-USA Symposium**
April 30 - May 1, 2011
San Diego, CA USA
- MSB 2011**
May 1-5, 2011
San Diego, CA USA

About CASSS

Headquartered in Emeryville, California, CASSS is a not-for-profit 501(c)(6) professional society made up of over 4,000 industry, academic and public sector professionals in the field of separation science and related fields.

CASSS provides outstanding networking opportunities and high-quality scientific symposia that focus on a variety of topics and techniques in separation science and related fields. The excellent scientific programs are made possible by the dedication and work of a large pool of volunteers who contribute their time and talents as a way of giving back to and improving the field of separation science.

Rewards of active participation in CASSS include great networking opportunities, career advancement and access to outstanding scientific symposia. We appreciate your interest and participation in CASSS and would be happy to discuss volunteer opportunities with you. Click on "About CASSS" to learn more.

CASSS News

- [Perspectives Paper on Effector Functions of Therapeutic Antibodies Now Available](#)
- 2011 Bay Area Discussion Group Topics Announced! Click [here](#) for details.
- Visit the Career Center to view available jobs post

The browser's taskbar at the bottom shows the Windows Start button, several open applications, and the system tray with the time 22:45.

European Society for Separation Science - 傲游(Maxthon) 2.5.12

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European Society for Separation Science

Separation Science in Europe and for Europe



Mission

Europe will thrive when its human capital, its scientific innovative potential, is utilised to its best advantage. This is the general mission of EuSSS.

EuSSS is a non-profit organisation whose target is to advance and harmonise Separation Science and to promote its recognition in the member Countries.

Individual scientists are warmly encouraged to participate in the promotion of this Society and to come up with suggestions of topics for discussion.

Main Menu


- Home
- Memorandum
- Statutes
- Protocols, Archive
- EuSSS-sponsored conferences
- Awards
- Conference calendar
- Poster, Flyer

EuSSS Event Calendar

<< April 2011 >>

S	M	T	W	T	F	S
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Member Countries of the EuSSS - Click!



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The Chromatographic Society

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Site Menu:
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Home

Welcome to the Website of the Chromatographic Society



The Society is an international organisation devoted to the promotion and dissemination of knowledge on all aspects of chromatography and related separation techniques. It was first established as the Gas Chromatography Discussion Group in 1956 with A.T. James as its chairman and D.H. Desty as secretary.

Consequently, 2006 was the golden jubilee anniversary year for the Chromatographic Society and it is still going strong.

The Year Ahead - 2011

We would like to welcome you all to our website and are pleased to announce this years meetings, starting with our Spring Symposium at Novartis (Horsham) on Wednesday 11th and Thursday 12th May 2011. The theme for the meeting is "Advances in Separation Science" with a particular focus on pharmaceutical drug discovery.

In July, we have a busy month, starting with the ever popular Reid International Bioanalytical Forum at the University of Surrey. This is followed by the 23rd International Symposium of

Register Now!

Registration

For access to the member's only pages, please register. Registration is quick, easy and free, and you don't have to be a member of the Society.

Already Registered?

If you've already registered, simply login to view the non-public areas of the site.

News & Announcements

[More](#)

Reid International Bioanalytical Forum

The latest circular for this years meeting can now be downloaded either by clicking [here](#) or via our

[More News](#)



HPLC大会系列

The image shows a screenshot of a web browser window displaying the website for the HPLC 2013 Amsterdam conference. The browser's address bar shows the URL <http://www.hplc2013.org>. The website header features the text "HPLC2013 AMSTERDAM: June 16 - 20, 2013" in red, with a silhouette of the Amsterdam skyline below it. The main content area has a background of pink and green tulips. It includes the Agilent Technologies logo, the text "Welcome to the Website of the 39th International Symposium on High-Performance-Liquid-Phase Separations and Related Techniques", the SHIMADZU logo with the tagline "Excellence in Science", the Thermo SCIENTIFIC logo, and the Waters logo with the tagline "THE SCIENCE OF WHAT'S POSSIBLE™". A button labeled "Enter the site" is positioned in the center. At the bottom of the website, there is a photograph of a building under a blue sky with white clouds. The browser's taskbar at the bottom shows the Windows Start button, several open applications including "收件箱 - Microso...", "HPLC 2013 > 39th...", "色质进展", and "Microsoft PowerP...", and the system tray with the time 10:53.

This conference originated in 1973. Advances in the field of liquid-phase separations are highlighted at each meeting. The meetings alternated between Europe and the United States with even years being in the US.

<i>Year</i>	<i>Location</i>	<i>Chair(s)</i>
2000	Seattle, USA	Edward S. Yeung
2001	Maastricht, The Netherland	Hans Poppe and Henk Lingeman
2001	Kyoto, Japan	Nobuo Tanaka and Shigeru Terabe
2002	Montreal, Canada	Irving W. Wainer
2003	Nice, France	Antoine M. Siouffi
2004	Philadelphia, USA	Mark R. Schure
2005	Stockholm, Sweden	Douglas Westerlund
2006	San Francisco, USA	John H. Frenz
2007	Gent, Belgium	Jacques Crommen and Pat Sandra
2008	Baltimore, USA	Georges Guiochon and Steven Jacobson
2008	Kyoto, Japan	Koji Otsuka and Nobuo Tanaka
2009	Dresden, Germany	Christian Huber
2010	Boston, USA	Steven A. Cohen
2011	Budapest, Hungary	Attila Felinger
2011	Dalian, China	Yukui Zhang and Peter Schoenmakers
2012	Anaheim, USA	Frantisek Svec
2013	Amsterdam, The Netherlands	Peter Schoenmakers
2013	Hobart, Australia	Paul Haddad
2014	New Orleans, USA	J. Michael Ramsey

HPCE(MSB)大会系列

MSB 2014
Pécs, Hungary

Home

General information

- Pécs & Region
- Conference venue
- Organisers
- Committee Members
- Accommodation
- Travel information
- Support & contacts

Scientific information

- Topics
- Conference programme
- Speakers

MSB 2014
27 April - 1 May, 2014

Welcome to MSB 2014 Homesite

**30th International Symposium on
MicroScale Bioseparations and Bionalyses**

27 April - 1 May, 2014
Pécs, Hungary

[more](#)

Pécs & Region

2000	Saarbrücken	H. Engelhardt
2001	Boston	B.L. Karger, W.S. Hancock
2002	Stockholm	D. Westerlund
2003	San Diego	A. Paulus, A.Guttman
2004	Salzburg	W. Lindner
2005	New Orleans	M. Ramsey
2005	Kobe	Y. Baba, K. Otsuka
2006	Amsterdam	G. Rozing
2007	Vancouver	R. Kennedy
2008	Berlin	A. Manz
2009	Boston	J. Sweedler
2009	Dalian	H-F Zou
2010	Prague	F. Foret
2011	San Diego	A. Barron
2012	Geneva	F. Kalman, G. Rozing, J-L Veuthey
2012	Shanghai	Z. Rong
2013	Charlottesville	Jeff D Chapman, James P Landers
2014	Pecs	F. Kilar, A. Guttman

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http://www.separationsnow.com/coi/cda/home.cda?chId=0

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The free online resource for the separation science community


Sample Preparation Go

 **Rooster comb overs: yielding hyaluronic acid by flow field-flow fractionation**


Electrophoresis Go

 **Journal Highlight: Analysis of amino acid neurotransmitters in hypothalamus of rats during cerebral ischemia-reperfusion by microdialysis and CE**


Gas Chromatography Go

 **Preserving cosmetic safety: nitro-containing ingredient assayed by GC to gauge risk of nitrosamine formation**


HPLC Go

 **Double peroxide attack: two HPLC methods for detecting peroxide in post-blast explosive residues**


Ion Chromatography Go

 **Would you like to interact? Exploring the intricacies of multimodal chromatography**


Proteomics & Genomics Go

 **The proteome of your pint: novel ligand capture technique uncovers more proteins**


Detectors Go

 **Journal Highlight: Charge-state-derivation ion detection using a super-conducting**

Informatics Go

 **Impure thoughts: A semi-automated process for identifying pharmaceutical impurities**

Monthly Highlight

 **FREE Agilent Technologies Sample Prep Webinar**
QuEChERS 101: The Basics and Beyond

The presenters will discuss in detail the QuEChERS extraction procedure from the point of product arrival through extraction, dispersive SPE and on to analysis. Factors that affect recovery and increase throughput will be presented in addition to future applications for QuEChERS, reaching far beyond pesticides in food products.

New users will gain a basic understanding of the QuEChERS method and its benefits; experienced users will learn about new advances and optimization of the QuEChERS methodology.

Register now to discover the unique feature and advantages of the Agilent QuEChERS kits!

Wednesday 17 November

[Find out more / register now >>>](#)

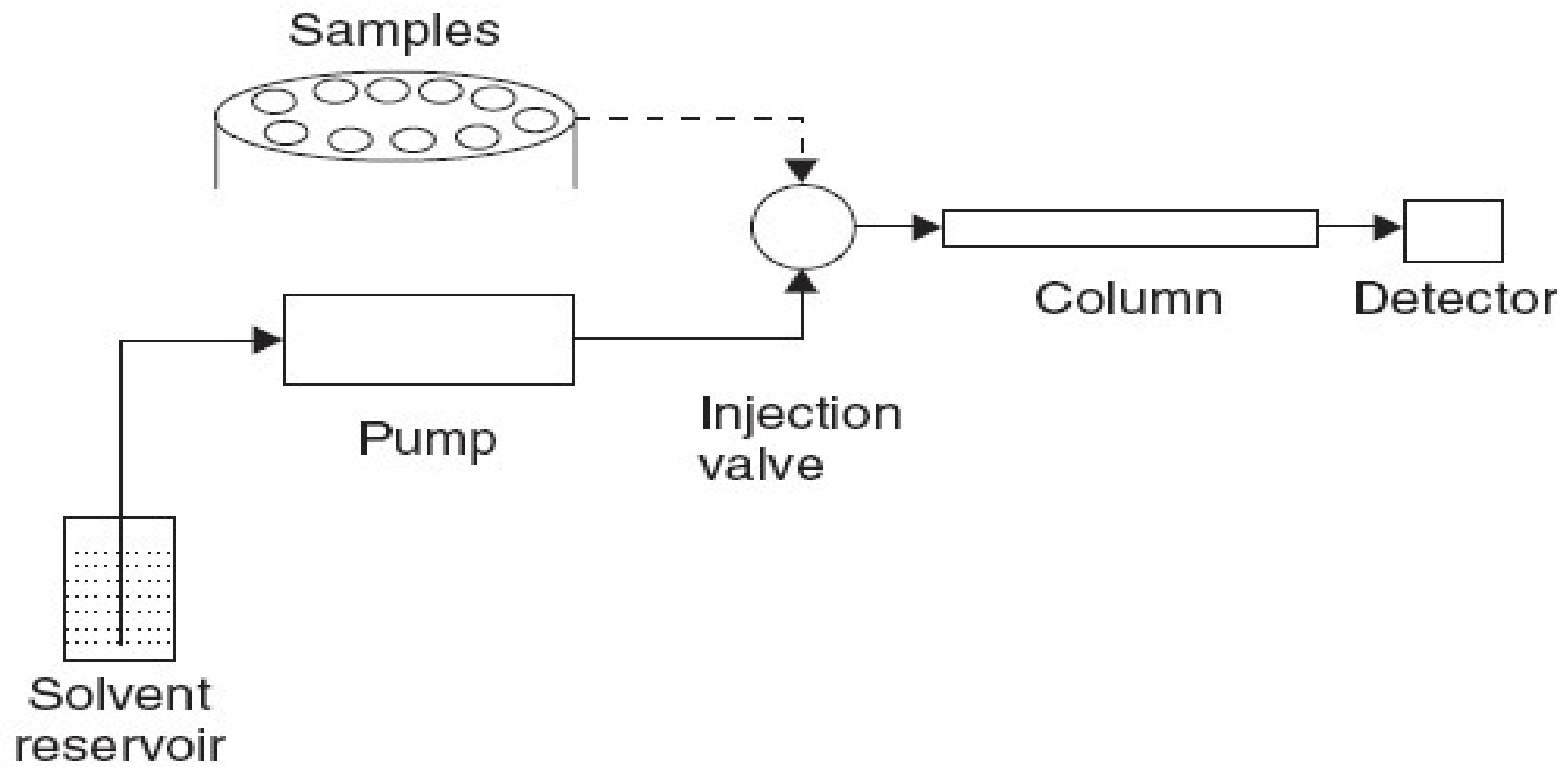
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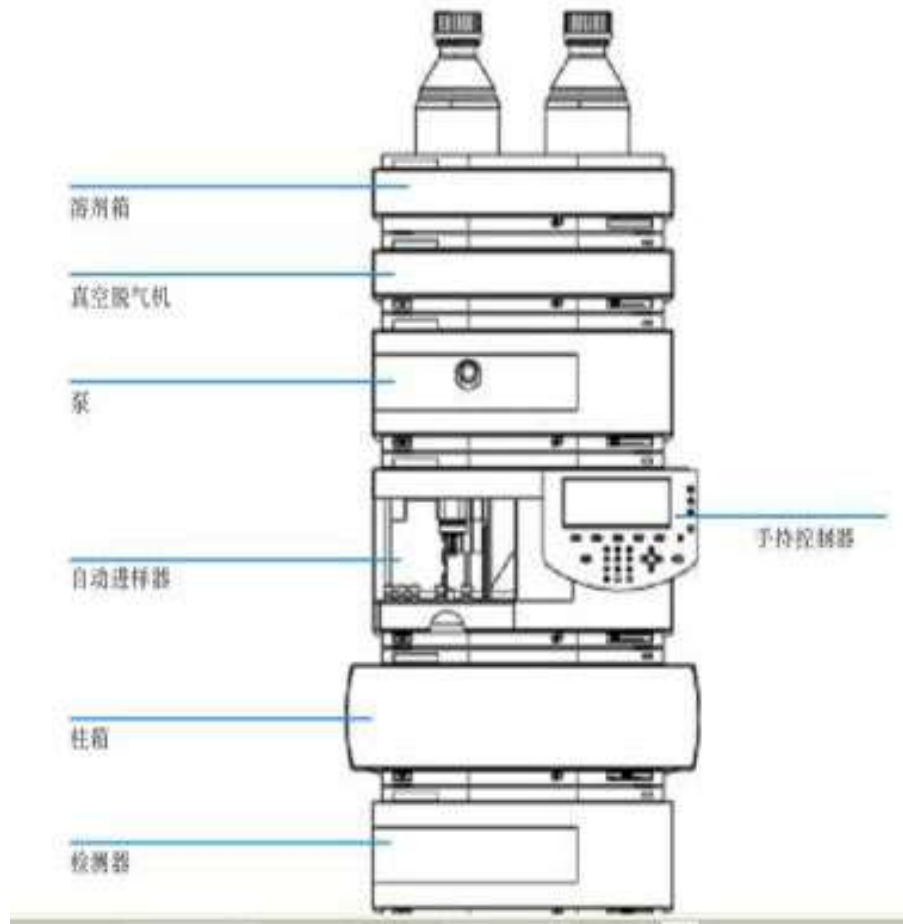
1400M

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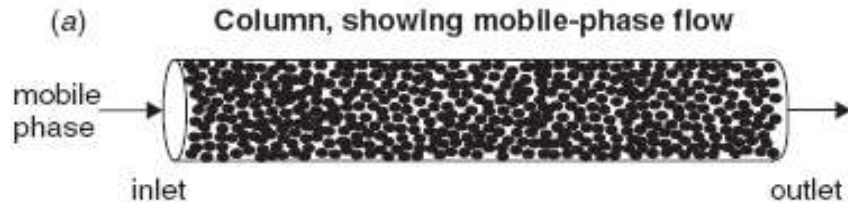
现代高效液相色谱系统



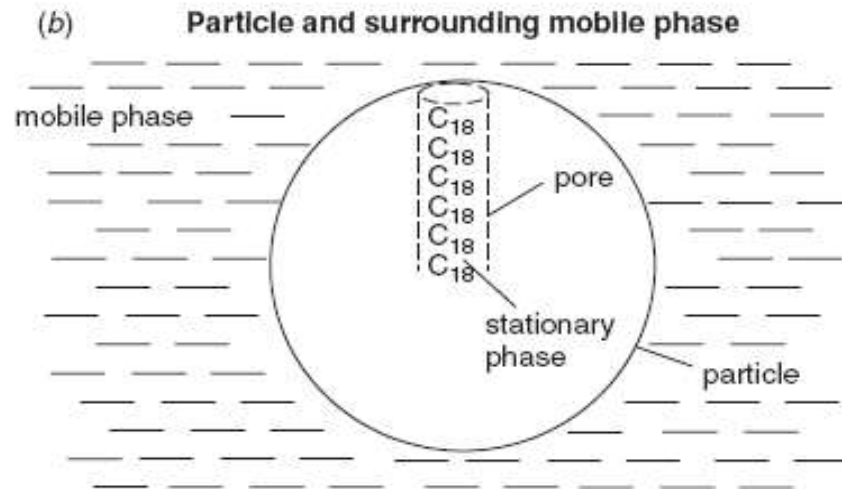
现代高效液相色谱系统



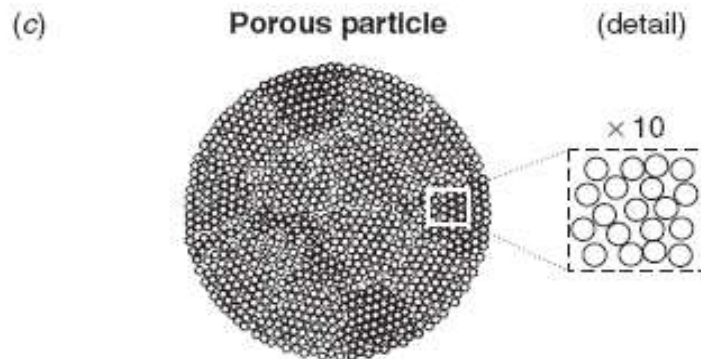
高效液相色谱柱



(a) Column packed with spherical particles;

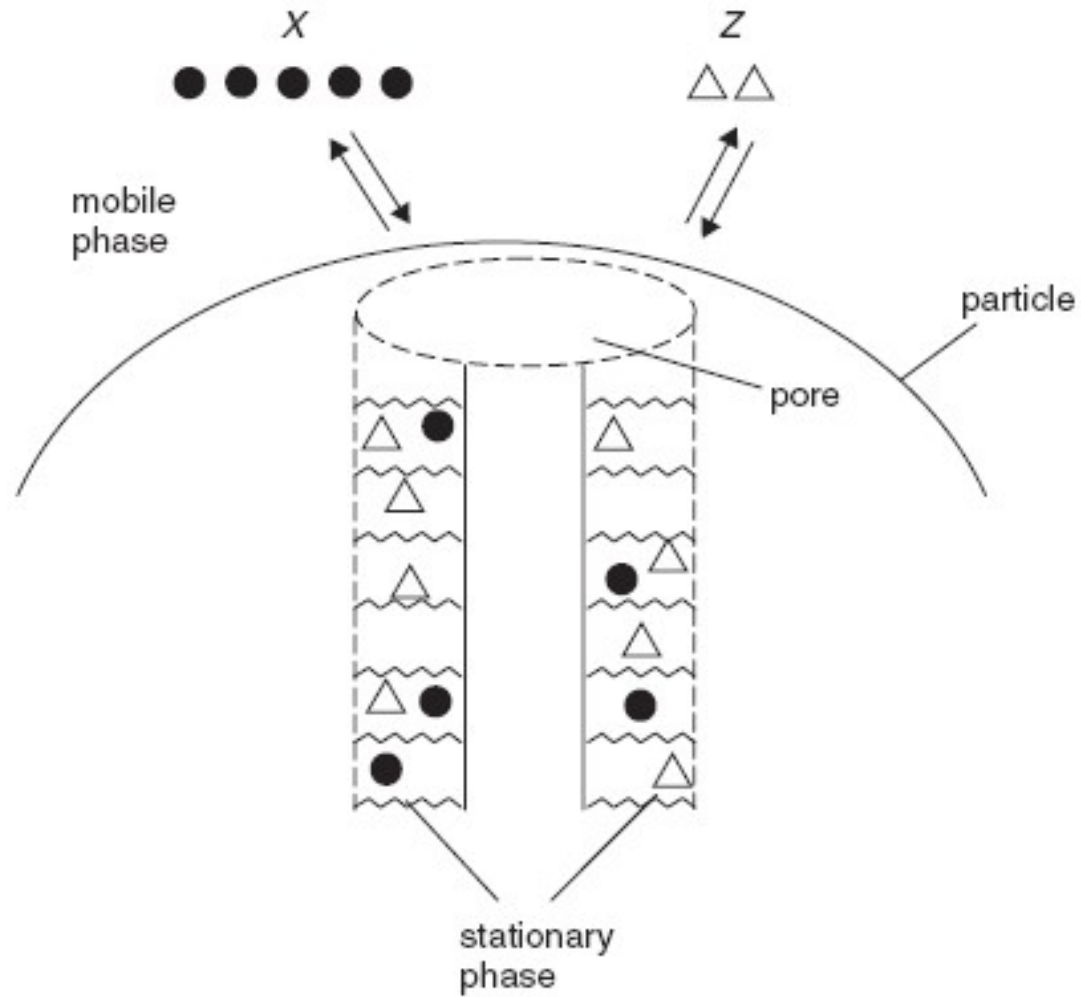


(b) Schematic of an individual particle, showing an idealized pore with attached C18 groups;

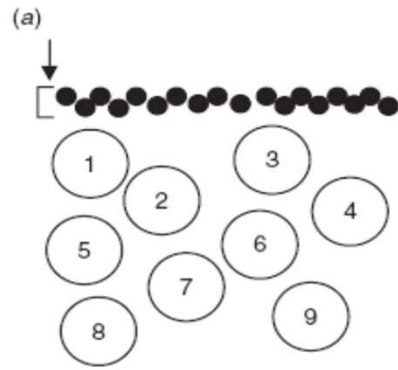


(c) More realistic picture of a spherical, porous particle, showing detail (10× expansion).

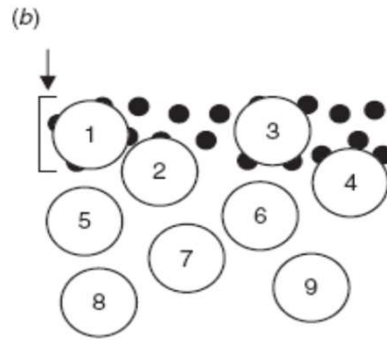
高效液相色谱过程



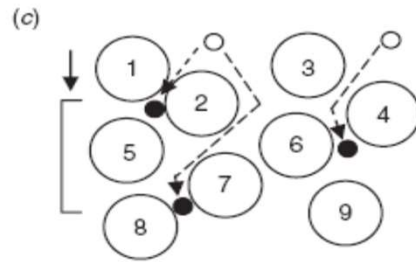
高效液相色谱过程与峰展宽



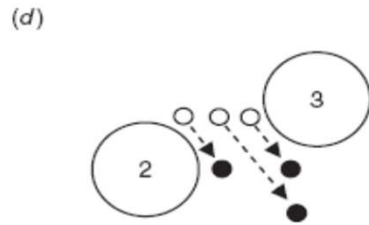
(a) Sample injection
(extra-column band broadening)



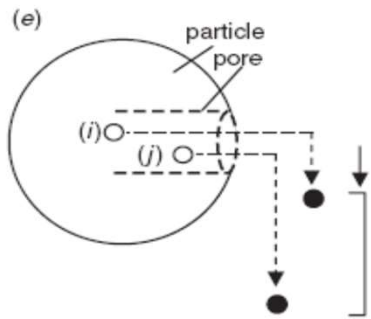
(b) Longitudinal diffusion
(time dependent)



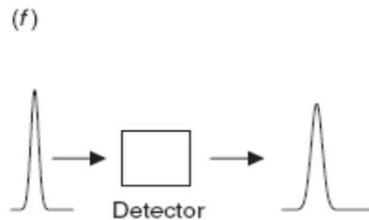
(c) Eddy diffusion
(flow independent)



(d) Mobile-phase mass transfer
(flow dependent)



(e) Stationary-phase mass transfer
(flow dependent)



(f) Flow through detector +
connecting tubing
(extra-column peak broadening)

柱前峰展宽：仪器与柱尺寸

纵向扩散：与液流是否流动无关，流速越大影响越小

涡流扩散：与液流无关，取决于颗粒大小与排列，即依赖于柱子填充质量

流动相传质：流路差异导致，流速越大越显著

固定相传质：在多孔填料内扩散差导致，流速越大越显著

柱后峰展宽：检测器体积，连接流路等

峰展宽的描述

$$H = A + \frac{B}{F} + CF$$

eddy diffusion longitudinal mass transfer mobile-phase plus stationary-phase mass transfer

Van Deemter equation $H = A + \frac{B}{u} + Cu$

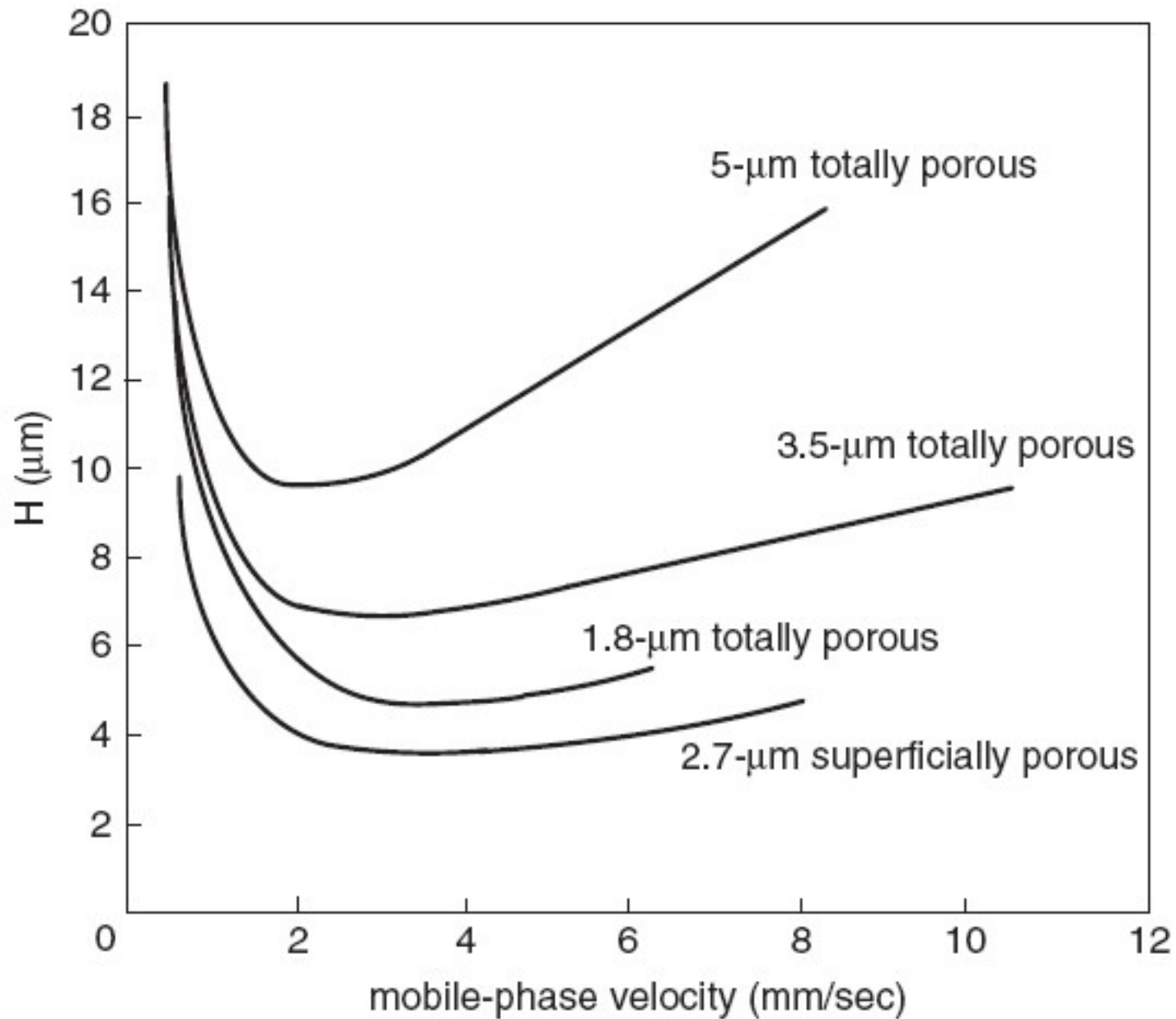
$$H = \frac{B}{F} + AF^{1/3} + CF$$

longitudinal diffusion eddy diffusion + mobile-phase mass transfer stationary-phase mass transfer

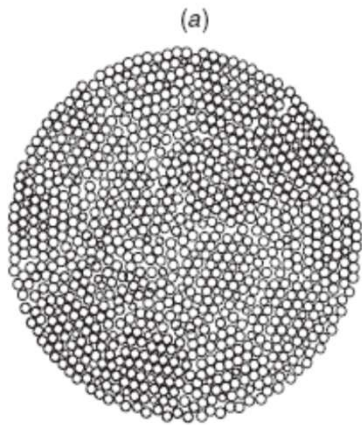
Knox equation $h = Av^{0.33} + \frac{B}{v} + Cv$

A, B, C与分析物，色谱柱以及实验条件有关

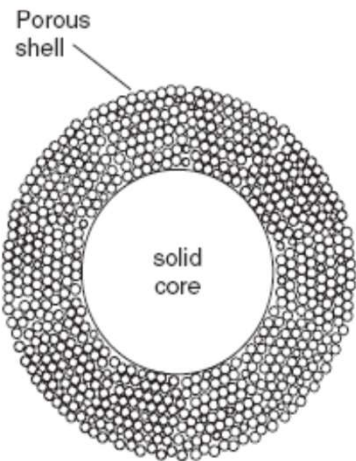
色谱填料的发展



色谱填料的构造

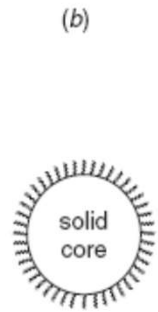


Porous particle

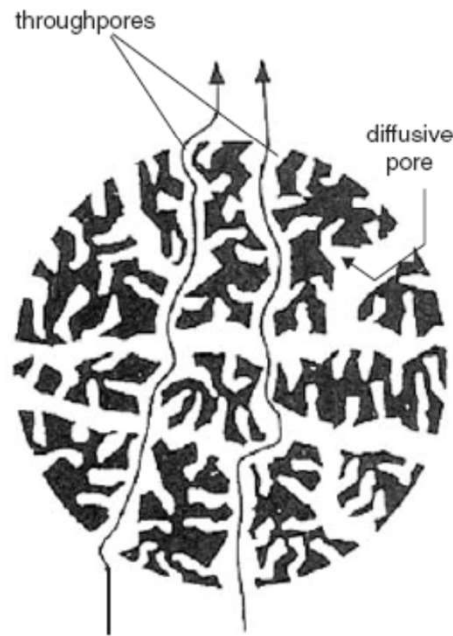


Porous-shell particle

(c)



Micro-pellicular particle



Perfusion particle

(d)

全多孔型：最常用，高柱容量。1.5-5 微米，由小颗粒聚集而成。

薄壳型：内部硬核，外部一层薄薄的固定相。以前粒径为几十微米，现在1.5-2.5微米。对生物大分子分离有利（传质阻力小），柱容量小，主要用于生物大分子分离。

表面多孔（熔核）型：内部硬核，外部多孔层。粒径2-5微米，其中多孔层厚0.25-0.5微米。表面积比薄壳型显著大（是全多孔型表面积的3/4），柱容量大。同时又有利于生物大分子的分离。且柱效高。

灌注型：贯穿的大孔（400-800纳米）通道（对流）和非贯穿的多孔（30-100纳米）网络（扩散）组成。有利生物大分子的高速分离。通常粒径较大（10微米），常用于蛋白质的制备分离。

常用色谱填料尺寸：昨天、今天和明天

1980-2000

5微米颗粒填充柱为主

柱效，压力，操作方便，仪器要求，柱寿命等因素的最佳平衡。



2000-2010

3微米颗粒填充柱已逐渐成为主流

缩短分析时间，提高分离通量。



自从**2005**

亚2微米颗粒填充柱开始出现

超高压（15,000 psi），快速，高分离度。

常用色谱填料尺寸

Separation Characteristics of Particles for HPLC Columns					
Particle Type	Separation Speed	Pressure	Ruggedness	Operator Convenience	Column Stability ^a
5- μm totally porous	1	4	4	4	4
3.5 totally porous	2	3	4	4	4
2- to 3- μm totally porous	3	2	2	3	3
<2- μm totally porous	4	1	2	1	3
5- μm superficially porous	2	4	3	3	3
2- to 3- μm superficially porous	4	2	4	3	4
1.5- μm pellicular (nonporous)	4	1	2	1	1
Pore diameter					
7- to 12-nm pores (150–400 m^2/g)	Small-molecule separations (<10,000 Da)				
15- to 100-nm pores (5–150 m^2/g)	Large-molecule separations (>10,000 Da)				

Note: Ratings in terms of advantage from moderate (1) to high (4).

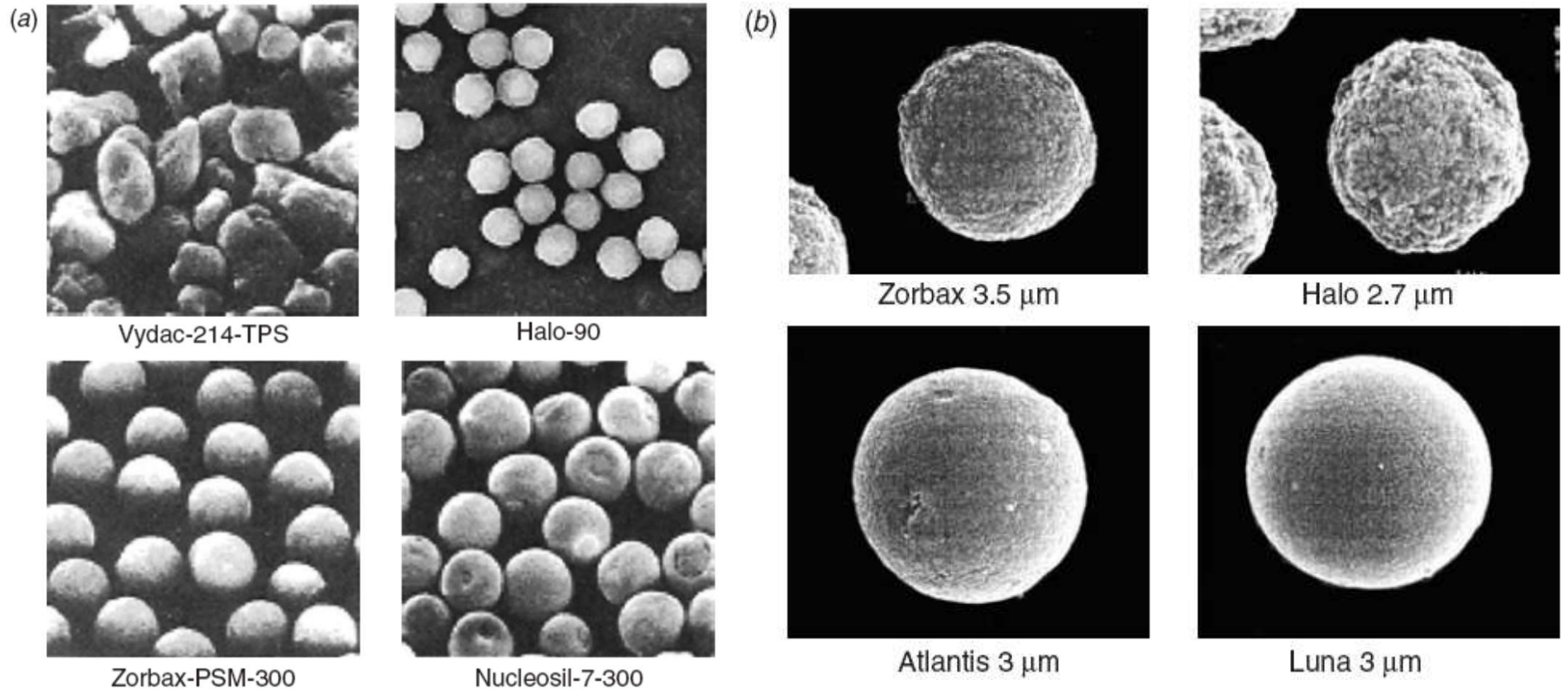
^aAbility to tolerate high pressures or a rapid change in pressure.

常用的色谱填料

硅基质：最常用。高机械强度，长时间承受高压。在宽范围内孔径，粒度，颗粒结构可控。

可键合多种配体（C8, C18, phenyl, cyano等）。多种溶剂兼容性好，无膨胀与收缩。适合梯度洗脱。但是，通常无法在pH>8下使用，硅开始溶解。碱性样品有拖尾。

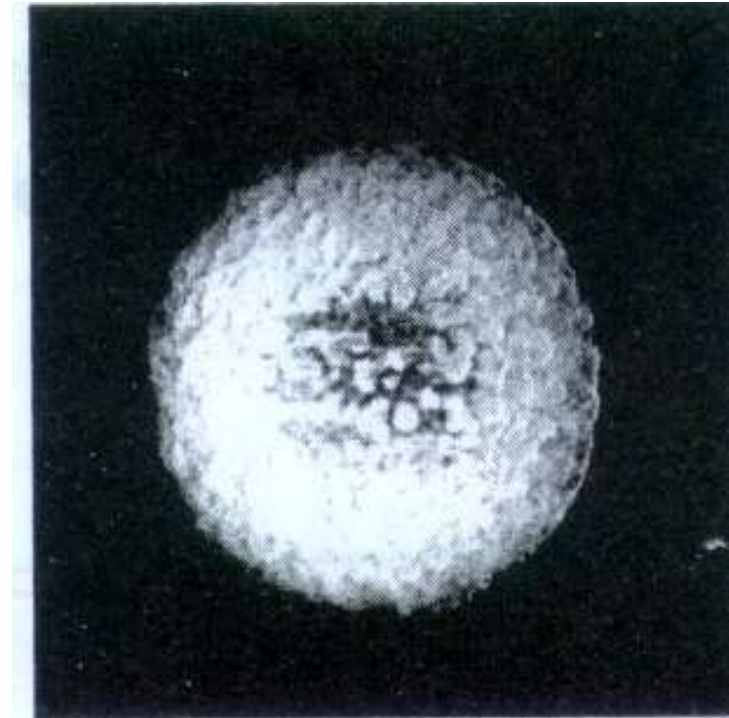
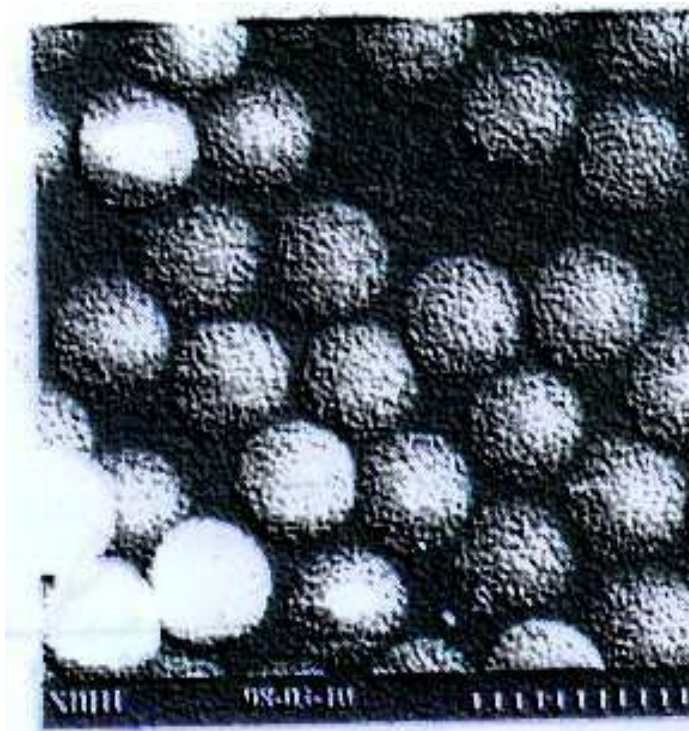
最常用的为球形硅胶。大颗粒、无定型硅胶用于制备（成本低、无需高效）。



商品化硅胶颗粒

高分子聚合物

PS/DVB, PA, PMA, 亲水聚乙烯 (TSK gel PW)



生物大分子分离介质

两个要点:

A. 孔径/分子体积适应性, 一般使用300-500Å大孔填料

B. 生物适应性 (biocompatibility) :

某些元素, 无机载体, 有机溶剂可使生物大分子失去活性

生物大分子分离介质

大孔填料

孔径比溶质分子直径大5倍以上

蛋白质MW	分子直径, nm	要求孔径, nm
10万	7.6 (25.2)	38 (150)
100万	16.3 (81.6)	81 (480)

生物大分子分离介质

灌注色谱填料 (Perfusion chromatography)

对流孔 (穿透孔) : 600~800 nm

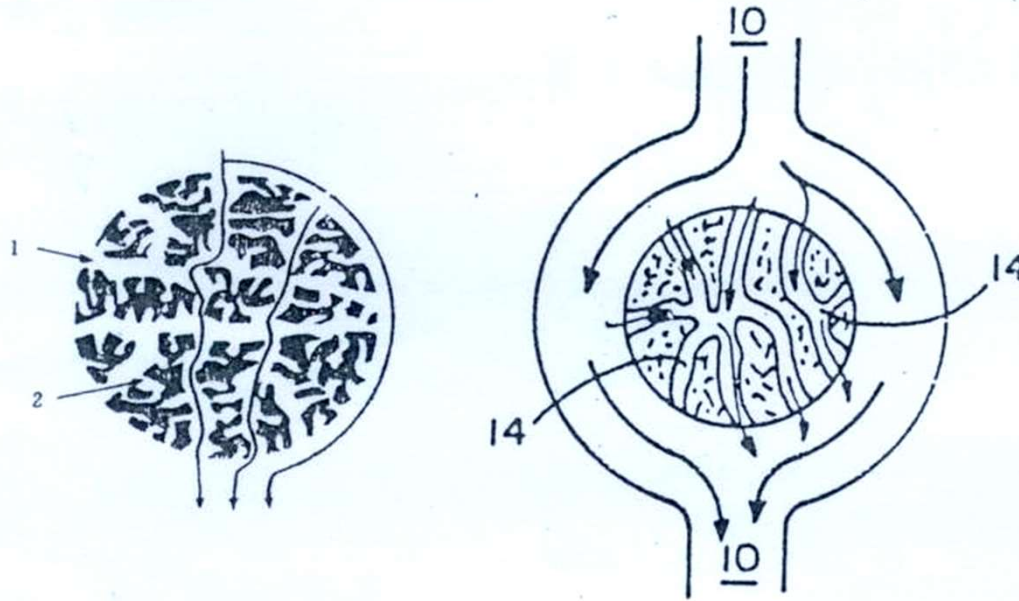
扩散孔: 50~150 nm

POROS R (反相)

POROS Q (强阴离子交换)

POROS S (强阳离子交换)

生物大分子分离介质



Schematic diagram of perfusion chromatographic peaking particle

1 throughpore 2 diffusive pore

生物大分子分离介质

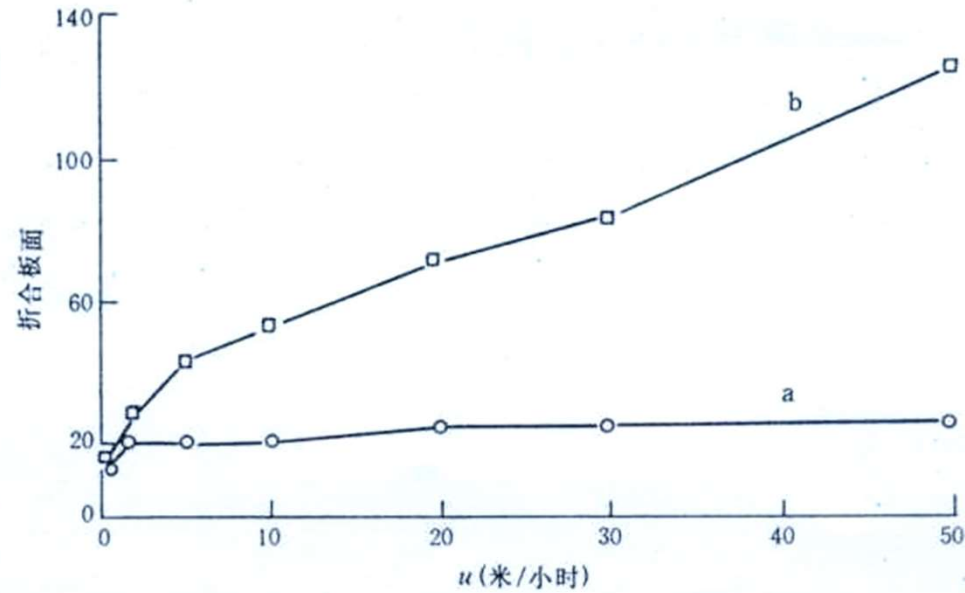
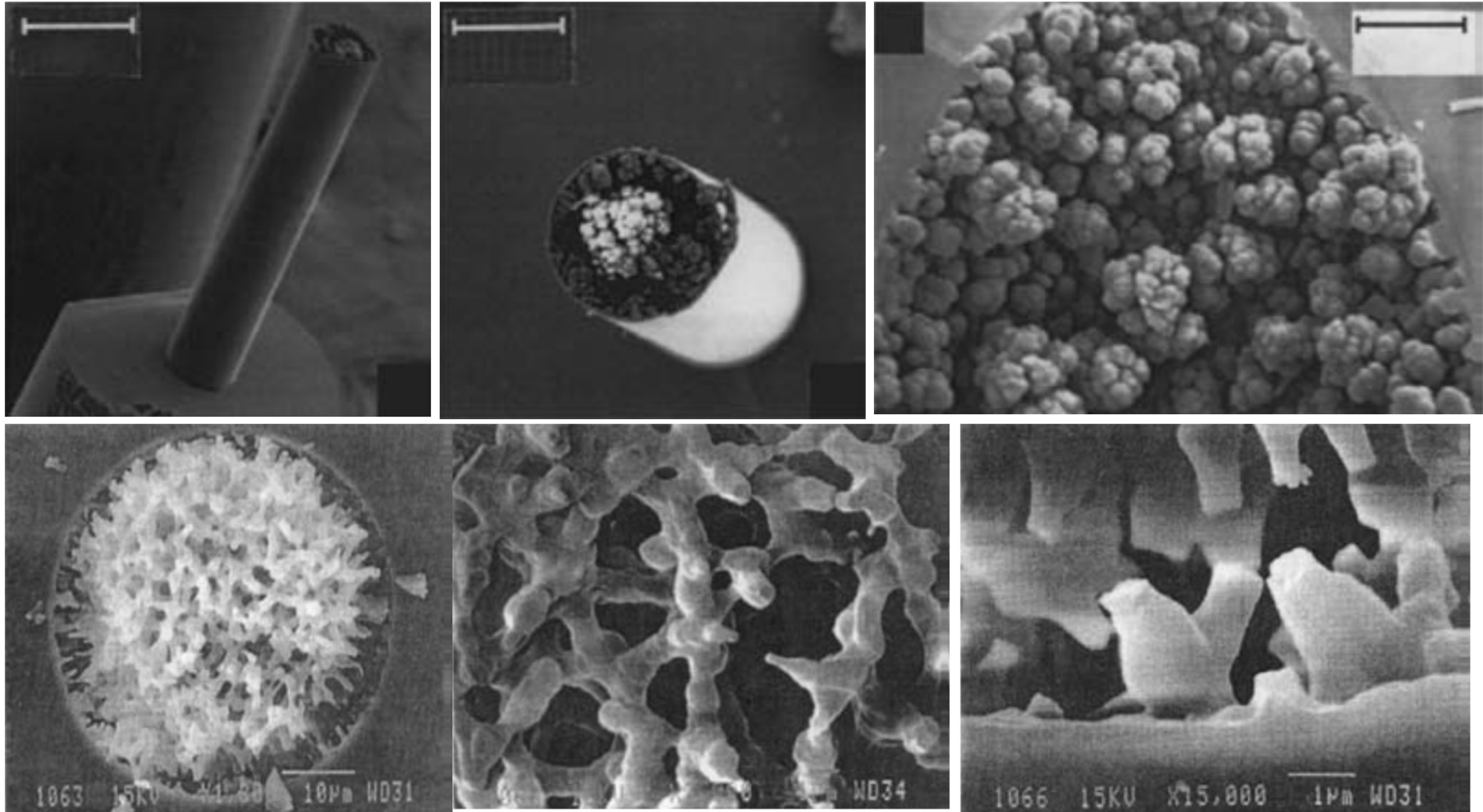


图 4-8 灌注填料 (a) 和普通大孔树脂
(b) 折合板高 h 与流速 u 关系的比较

整体柱 (Monolithic column)

把单体、添加剂、制孔剂和引发剂按一定比例加入到色谱柱管内，在一定条件下发生原位聚合反应，形成网状的连续床层的分离介质。



开管柱

固定相固载于柱内壁；
相比有限，内径小，柱
容量有限；
适于微分离与电色谱；
一般需自己制备，无商
品化柱子。

