Von Palo Alto in den Schwarzwald.
Eine Kurzgeschichte von „Low“- bis „Ultra“-HPLC

Gerard Rozing,
Agilent Technologies (ret.)
Waldbronn, Germany
http://www.rozing.com

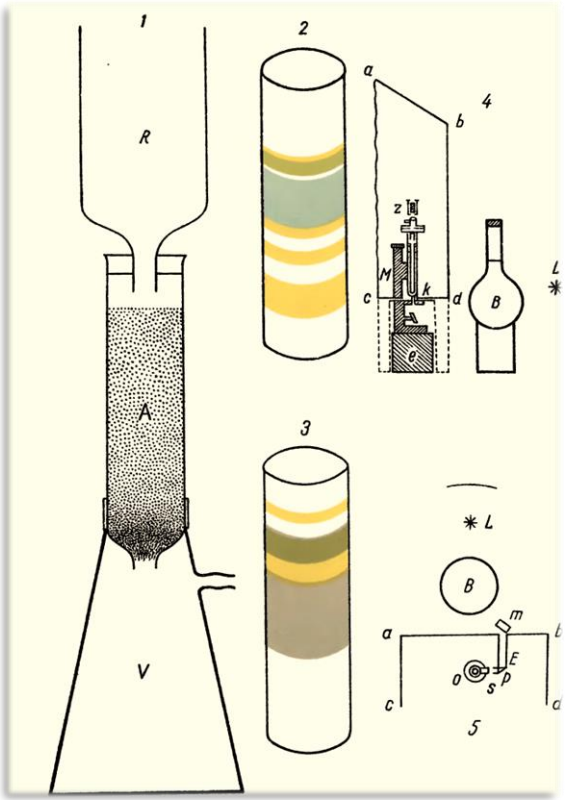
AGILENT
FORUM
ANALYTIK

12.-13. FEBRUAR 2013
AUSTRIA TREND HOTEL WIEN
SCHLOSS WILHELMINENBERG

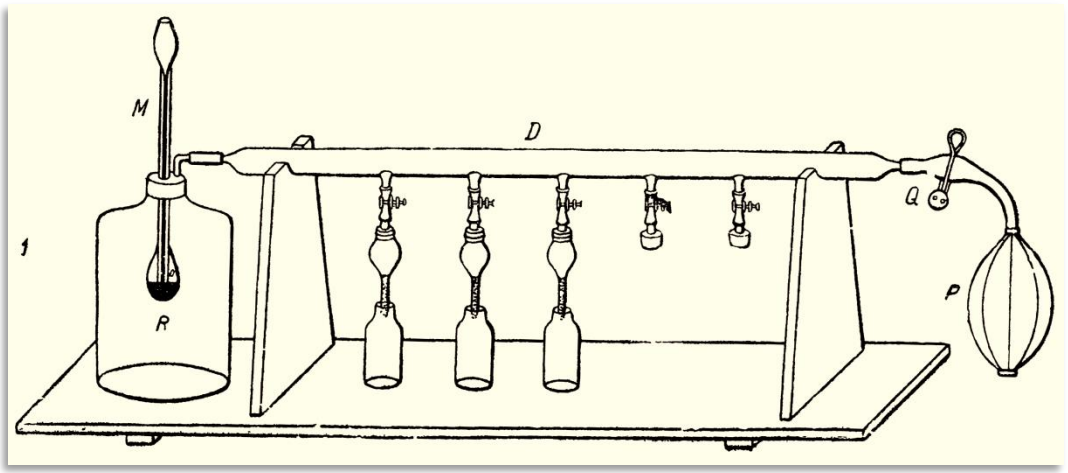
Scope of this Presentation

- Timeline of Liquid Chromatography
- Black Forest Roots
- Hewlett-Packard Culture → HPLC System Technology
- Modern Times: towards Ultra High Performance Liquid Chromatography

Dawn of Chromatography



Drawings: Mikhail S. Tswett, publications in 1903-1910



- Sample: solvent extract of plant leaves
- Column filled with chalk and alumina particles
- Solvent same as sample solvent
- Identification ("eye" colorimeter)
- Gravity flow, no control, air (gas) pressure driven
- Partition chromatography ?

M.S. Tswett proposed the name chromatography [from the Greek words *chroma*, meaning color, and *graph*, meaning writing → literally, color writing. Curiously though, the Russian word "tswet" means *color*]

Martin & Synge, 1941

Nobelprize for chemistry in 1952 for "*their invention of partition chromatography*"

151. A NEW FORM OF CHROMATOGRAM EMPLOYING TWO LIQUID PHASES

1. A THEORY OF CHROMATOGRAPHY

2. APPLICATION TO THE MICRO-DETERMINATION OF THE HIGHER MONOAMINO-ACIDS IN PROTEINS

By A. J. P. MARTIN and R. L. M. SYNGE

From the Wool Industries Research Association, Torridon, Headingley, Leeds

(*Received 19 November 1941*)

Biochem J., 35(12): 1358–13 (1941)

Introducing the Plate Theory of Chromatography:

A theoretical plate in many separation processes is a hypothetical zone or stage in which two phases, such as the liquid and vapor phases of a substance, establish an equilibrium with each other.

$$N = \frac{Length}{Height\ Equivalent\ to\ a\ Theoretical\ Plate}$$

HETP: dependent on slowness mass transport between mobile & stationary phase

Van Deemter et al, 1956

Proposed two further contributions to band broadening: the first arising from the complex flow pattern in a packed bed, and the second from axial molecular diffusion.

$$H = A + \frac{B}{u} + C_m u + C_s u$$

"van Deemter" equation

A	zone dispersion contribution based on flow velocity inequalities in the packed bed and "assumed to be independent of flow velocity" **(NOTE!)**
B	zone dispersion contribution based on axial molecular diffusion
C_m C_s	dispersion contribution slowness of mass transport in the mobile resp. stationary phase

J.J. van Deemter, F.J. Zuiderweg, A. Klinkenberg, Chemical Engineering Science, Vol. 5, Issue 6, 271–289 (1956)
S.J. Hawkes, J. Chem. Educ., Vol. 60, Issue 5, 393, (1983)

The 60ties

"grounding fathers"

Fundamentals of HPLC

The 60ties – Fundamentals of HPLC

J.C. Giddings, Anal. Chem., 36(10) 1891 (1964),
Comparison of the Theoretical Limit of Separating Ability in Gas and Liquid Chromatography
J.C. Giddings, Anal. Chem., 37(10) 60 (1965),
Comparison of Theoretical Limit of Separating Speed in Gas and Liquid Chromatography
J.C. Giddings, "Dynamics of Chromatography", Marcel Dekker, New York, 1965

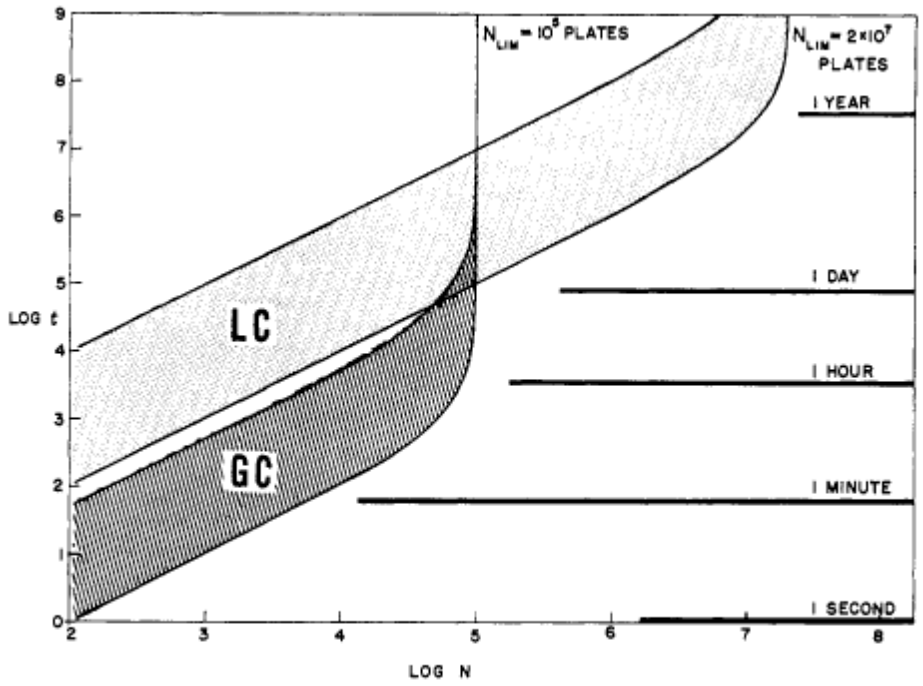


Figure 1. Comparison of separation time in gas and liquid chromatography as a function of the number of required plates

1st Kinetic Plot!!! (Desmet et al.)
A-term flow dependence

The 60ties – Fundamentals of HPLC

Josef Huber

High Efficiency, High Speed Liquid Chromatography in Columns

by **J. F. K. Huber,** Analytical Chemistry Laboratory, University of Amsterdam, Amsterdam, The Netherlands

J. of Chrom. Sci., 7, 85 (1969)

Csaba Horvath

Column Design in High Pressure Liquid Chromatography

by **Csaba Horvath*** and **S. R. Lipsky,** Physical Sciences Section, Yale University School of Medicine, New Haven, Connecticut

J. of Chrom. Sci., 7, 109 (1969)

Invented acronym:
"**High Performance Liquid Chromatography**"

John Knox

Kinetic Conditions for Optimum Speed and Resolution in Column Chromatography

by **J. H. Knox** and **M. Saleem,** Department of Chemistry, University of Edinburgh, Scotland.

J. Chrom. Sci., 7, 614 (1969)

"Knox" equation

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

The 60ties – Fundamentals of HPLC

Fifth International Symposium on Advances in Chromatography held in Las Vegas, Nevada, January 20-23, 1969.

Requirements for HPLC

- Very small particles (≤ 10 µm)
- High pressure as solvent driving force (max. 400 bar)

The 70ties - Growth

Instrumentation
Column Technology & Stationary Phases
More Fundamentals & New Methods
and Application Development

“Black Forest Roots”

1960

1962, Dr. Hupe Apparatebau, Karlsruhe, Germany

1965, 1st Generation HPLC

1970

1970, Hupe & Busch formed

1973, Acquisition Hupe & Busch

37 Employees, 7 MDM Revenues

1980

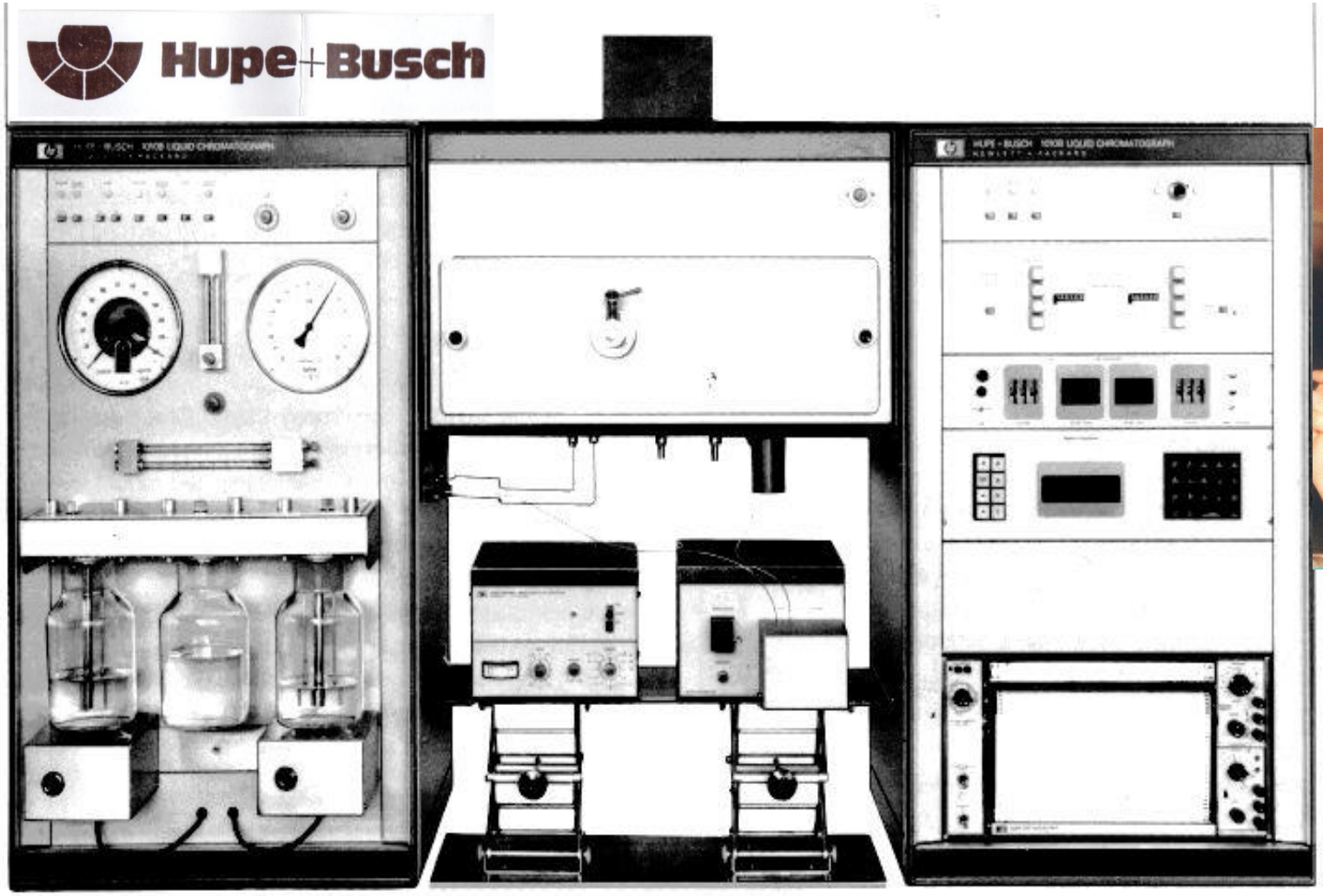
1978, Waldbronn Analytical Division

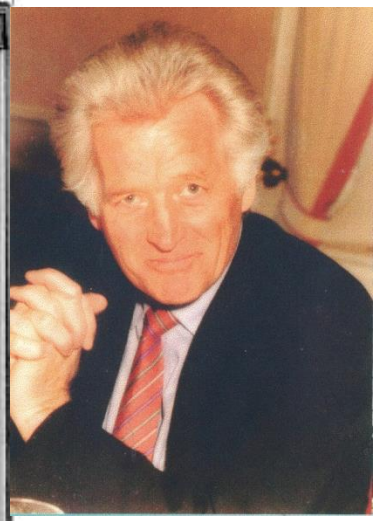
F&M Gas Chromatography

1965, Avondale Division GC

Chem. Anal. EU/D

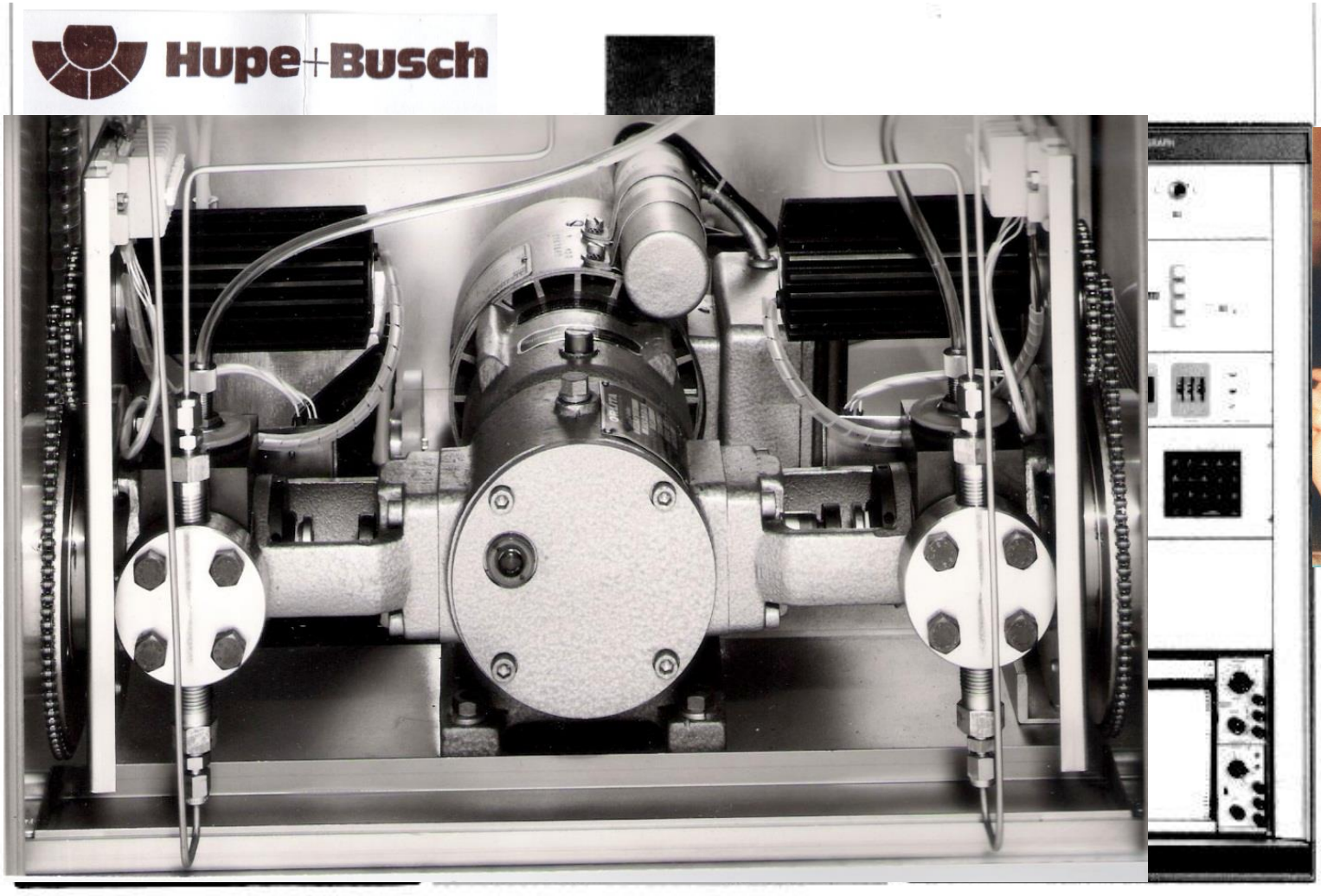
UFC 1000: “Objekt der Begierde”

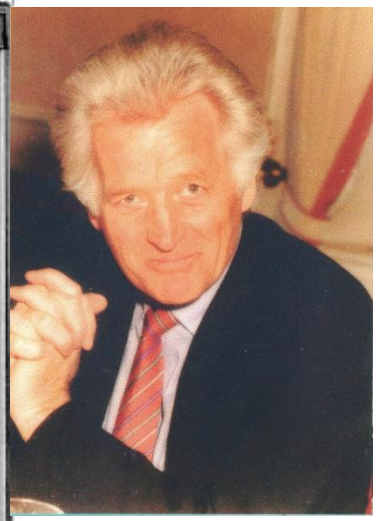




Ann. Rev. 7 Million DM
37 Employees

UFC 1000: “Objekt der Begierde”



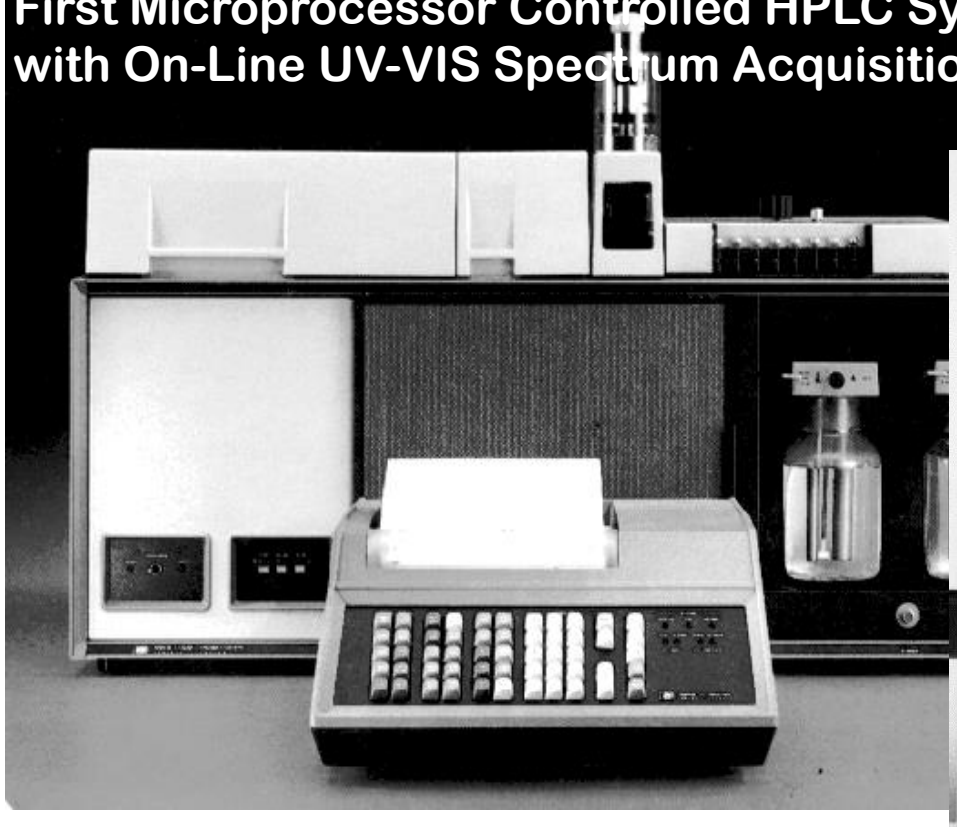


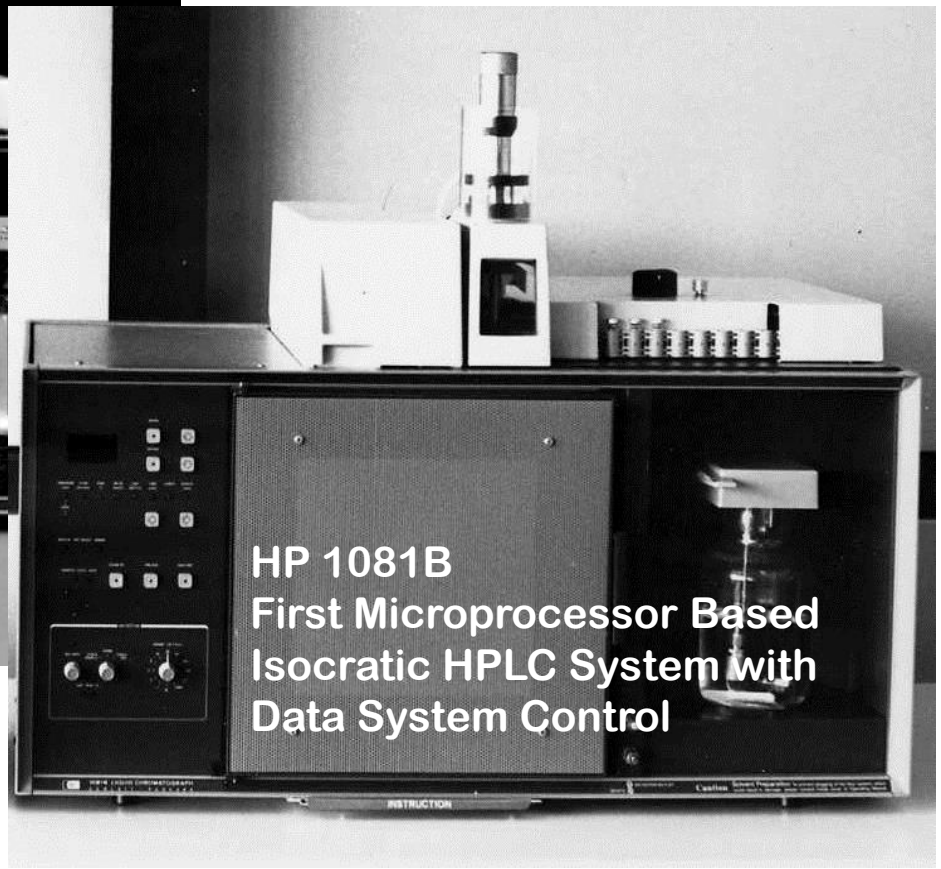
Ann. Rev. 7 Million DM
37 Employees

150 cm, 300 kg

HP1080 Series (1976 -1984)

HP 1084B
First Microprocessor Controlled HPLC System with On-Line UV-VIS Spectrum Acquisition

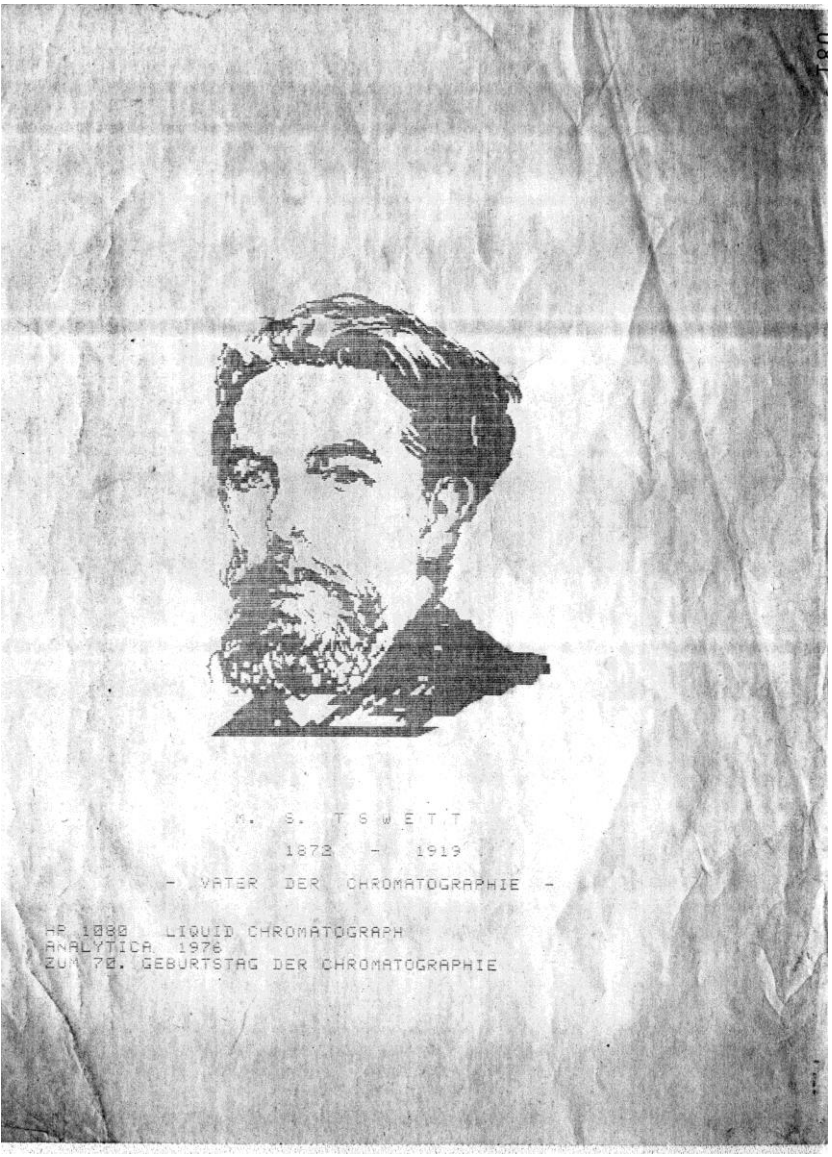




HP 1081B
First Microprocessor Based Isocratic HPLC System with Data System Control

HP 1080 Series – Analytica 1976

First Microprocessor Controlled HPLC



Dedication:

M.S. Tswett
1872 – 1919
Vater der Chromatographie

HP 1080 Liquid Chromatograph
Analytica 1976
Zum 70. Geburtstag der Chromatographie

HP1080 Series - 1978

Variable Wavelength Detection by „reversed optics“

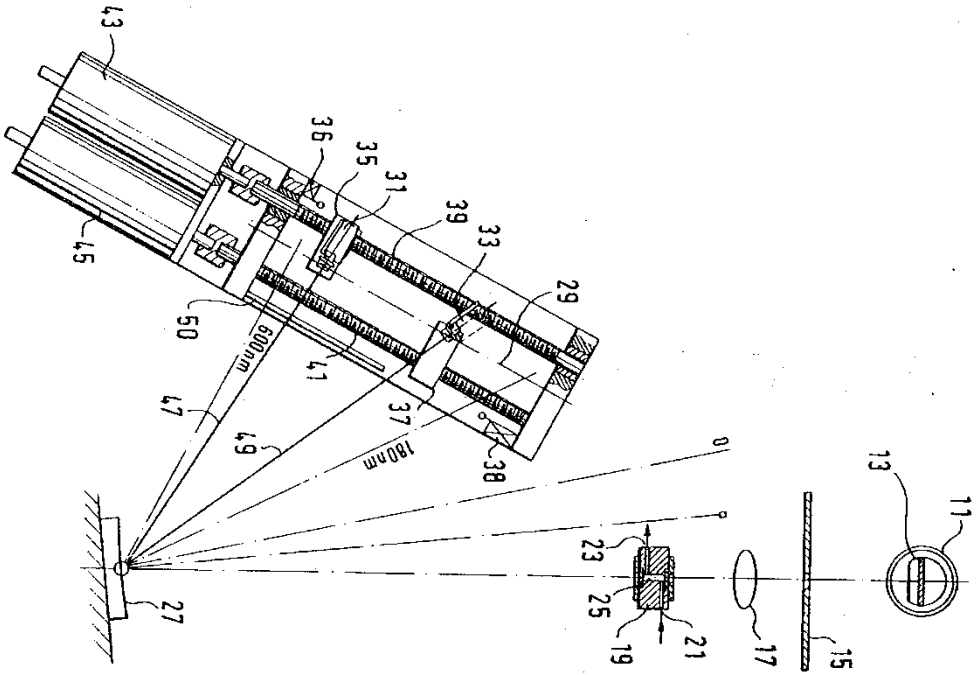


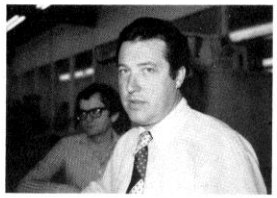
FIG. 1

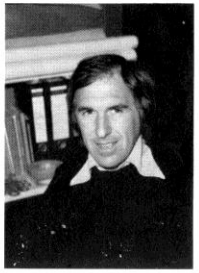
PHOTOMETRIC ABSORPTION DETECTOR

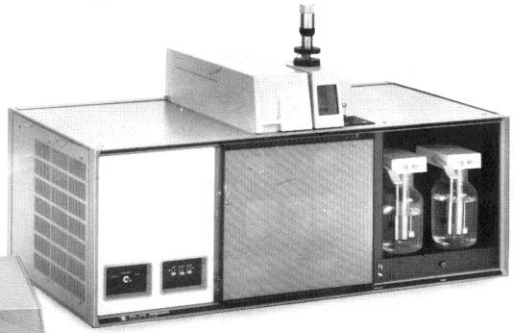
Inventors: **Alfred Maute**, Holzgerlingen; **Konrad Teitz**, Pfinztal-Berghausen; **Klaus Baier**, Karlsruhe; **Hans Heid**, Bammental-Heidelberg, all of Fed. Rep. of Germany

Hewlett-Packard Culture
Innovation, Teamwork, Contribution

Dieter Höhn, GM

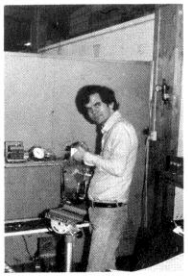






Dr. Alfred Maute

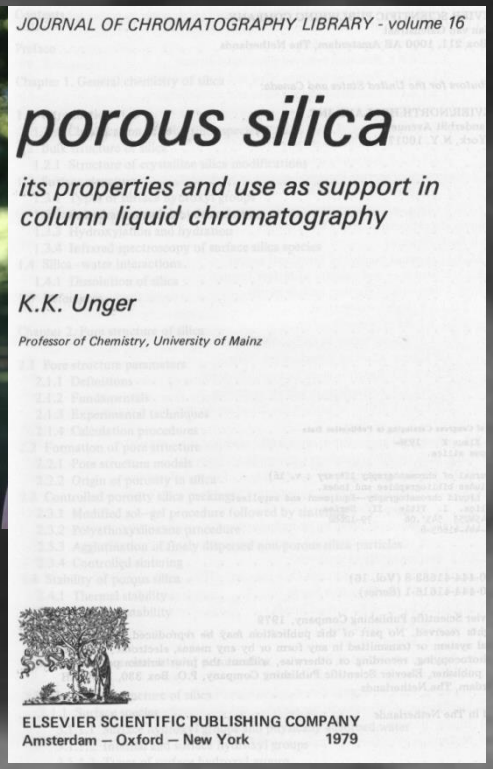
Dieter Lindenau



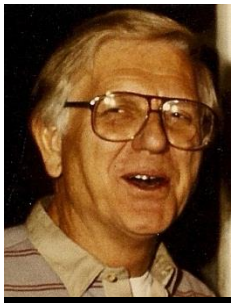
Dr. Bob Brownlee

The 70ties – Stationary Phases

The Master Of Porous Silica



The 70ties – Stationary Phases



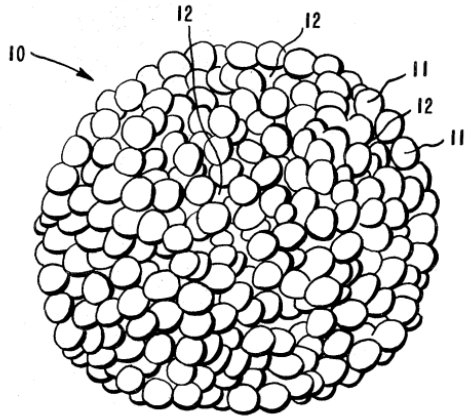
Jack Kirkland

COMPLETELY POROUS MICROSPHERES FOR CHROMATOGRAPHIC USES

Inventor: **Joseph J. Kirkland,** Wilmington, Del.

Assignee: **E. I. du Pont de Nemours and Company,** Wilmington, Del.

Filed: **Apr. 7, 1972**



US Patent 3,782,075

Istvan Halasz

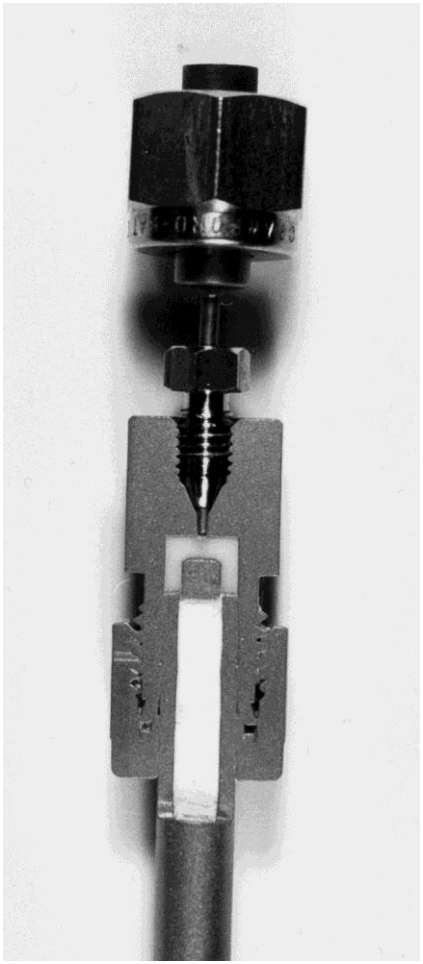
PREPARATION AND PROPERTIES OF REVERSED PHASES*

KARL KARCH, IMRICH SEBESTIAN and ISTVÁN HALÁSZ

Angewandte Physikalische Chemie, Universität Saarbrücken, Saarbrücken (G.F.R.)

J.Chrom., 122, 3, (1976)

The 70ties – Column Technology



4.6 mm i.d.
10 – 30 cm length
5 – 10 µm particles, irregular, spherical
reversed phase C18
5000 – 20000 plates

Many Separation Modes (besides Liquid Liquid Chromatography)

- Reversed Phase Chromatography
- Ion Exchange
- Size Exclusion Chromatography
- Ion-Pair (Soap) Chromatography

Small Molecule Separations not well suited for GC

- Amino Acids
- Carbohydrates
- Polar/Ionisable Pharmaceuticals

OPTIMIZATION IN LIQUID CHROMATOGRAPHY

Georges Guiochon

Laboratoire de Chimie Analytique Physique
École Polytechnique
Palaiseau, France

I. Introduction 1
II. Resolution and Efficiency 2
III. Analysis Time, Flowrate, and Pressure 6
IV. Flow Velocity and Column Efficiency 7
V. What to Optimize in Liquid Chromatography 15
VI. Equations Used for Optimization 17
VII. Ways to Achieve 5000 Plates in 5 Minutes 19

High-Performance Liquid Chromatography,
Advances & Perspectives,
Ed. Cs. Horvath,
Vol.2, page 1, (1980)

The 80ties

2nd Generation HPLC Systems

Bio-chromatography

HP1090 – Project Goal

Objective

To design an instrument which would be able to meet a least the demands governed by a 100x2.1 mm column packed with 5 µm particles to generate 5000 plates and concomittantly satisfy the general demands to quality

“The Iron Rule of Chromatography”

Criterion for peak fidelity:

Accept maximally 10% loss in peak resolution due to external zone broadening for a peak with k’= 1.

$$R / R_{max} \geq 0.9$$

$$R = \frac{V_{R,j} + V_{R,i}}{\sigma_{v,j} + \sigma_{v,i}}$$

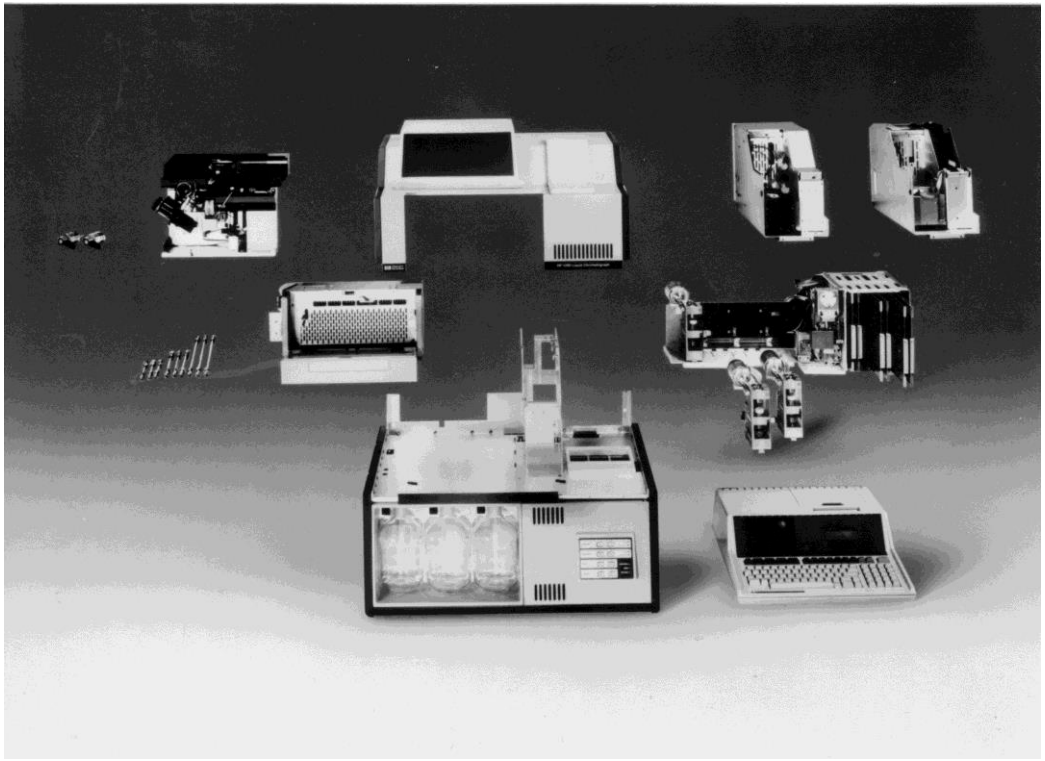
$$0.9 \geq \sigma_{v,col} / \sigma_{v,tot} \rightarrow \sigma_{v,ext} \leq 0.5\sigma_{v,col}$$

$$\sigma^2_{v,tot} = \sigma^2_{v,ext} + \sigma^2_{v,col}$$

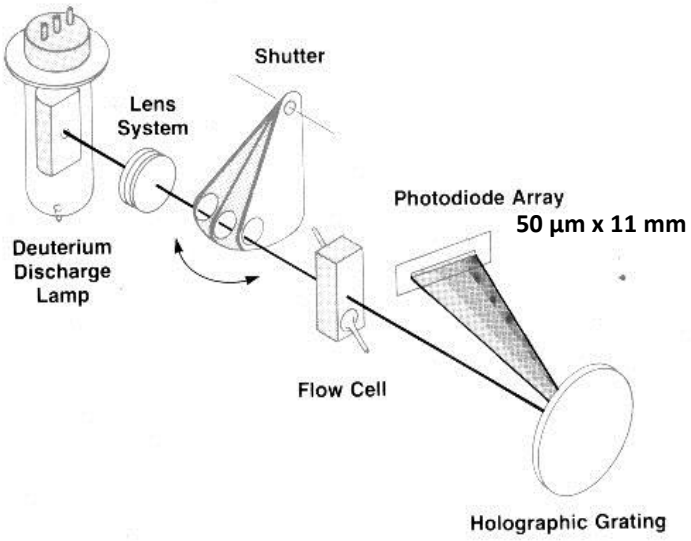
Design Center for HP1090:
Column, 100x2.1 mm, 5 µm particles
➔Low Dispersion Liquid Chromatography

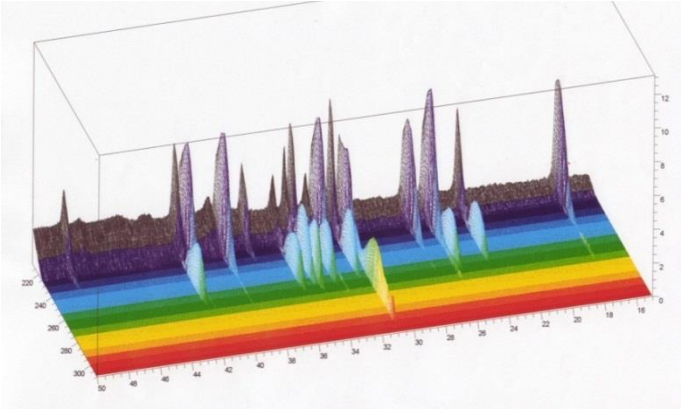
$$\rightarrow \sigma_{v,ext} \leq 3.5\mu L$$

“Integrated Modularity” – HP1090

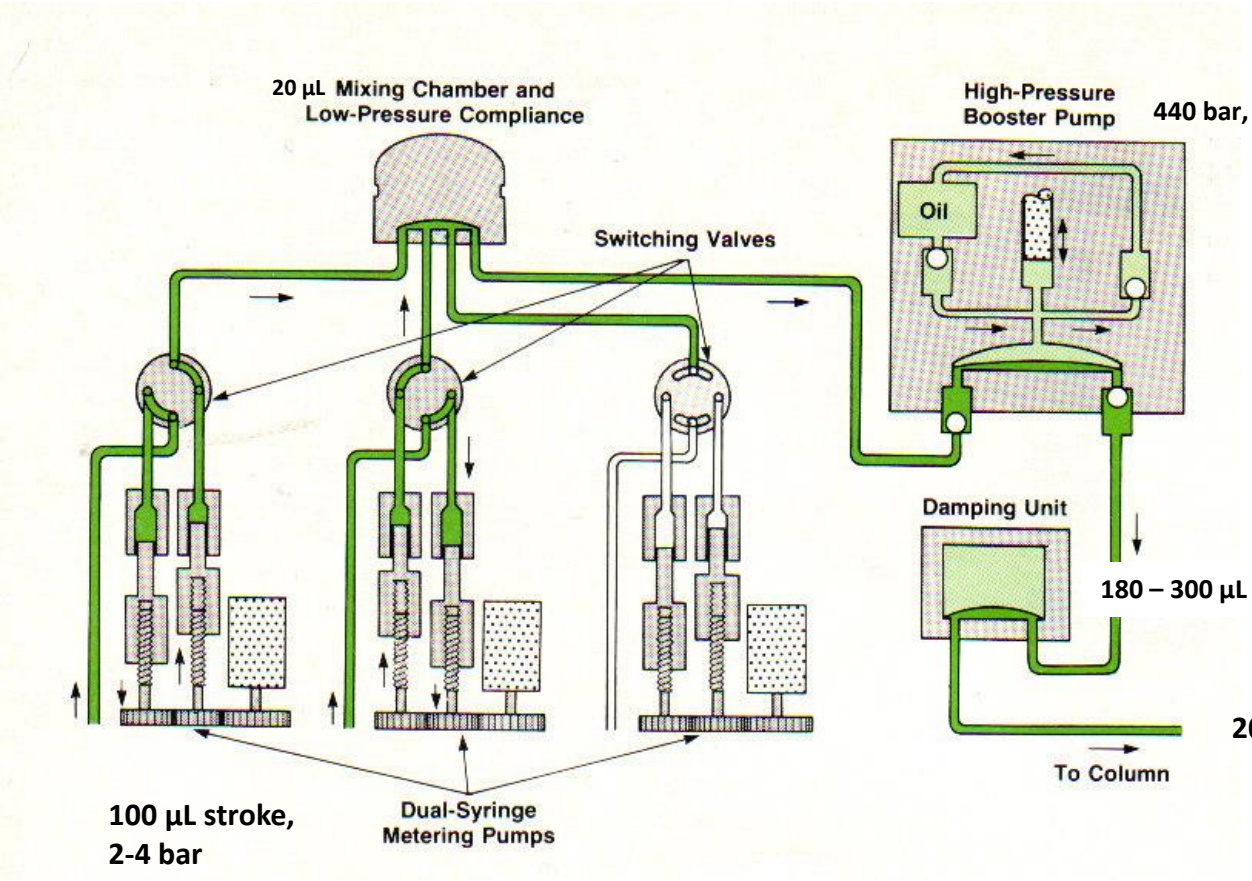


Diode Array Detector Reversed Optics





High Dynamic Range Solvent Delivery



A Great Machine – HP 1090 Series

When it finally worked, it worked GREAT !!

Dieter Höhn
Vice President HP

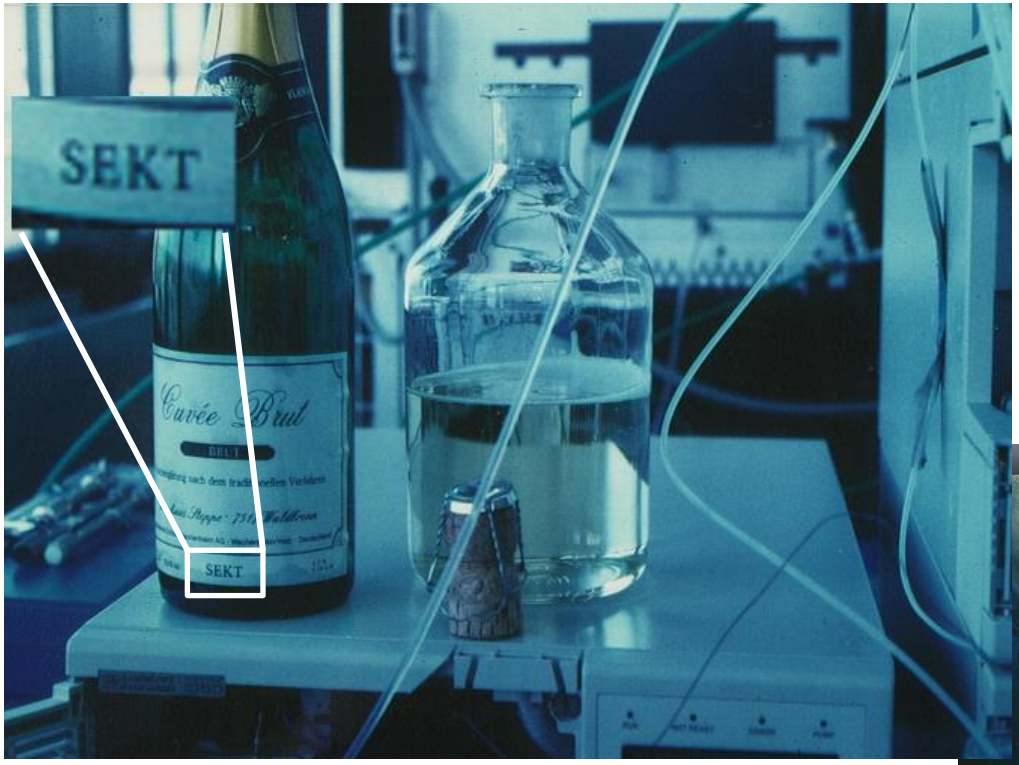
HP1050 Series -1988

Modular HPLC System

Multiple Detection Options

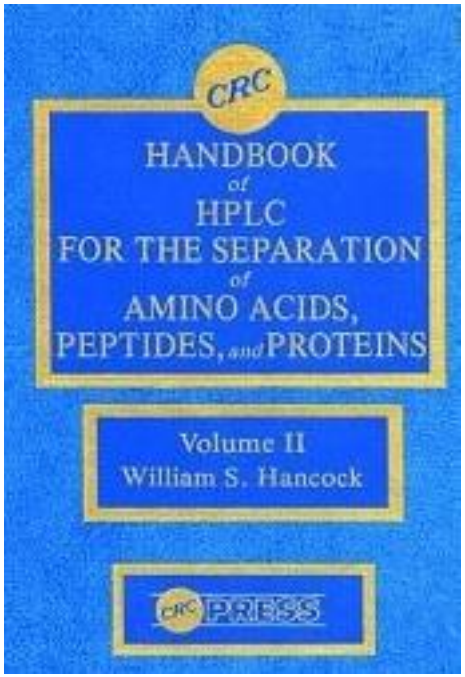
Electronic Data Storage

HP 1050 Series



Pumping “bubbles”!!

Biochromatography

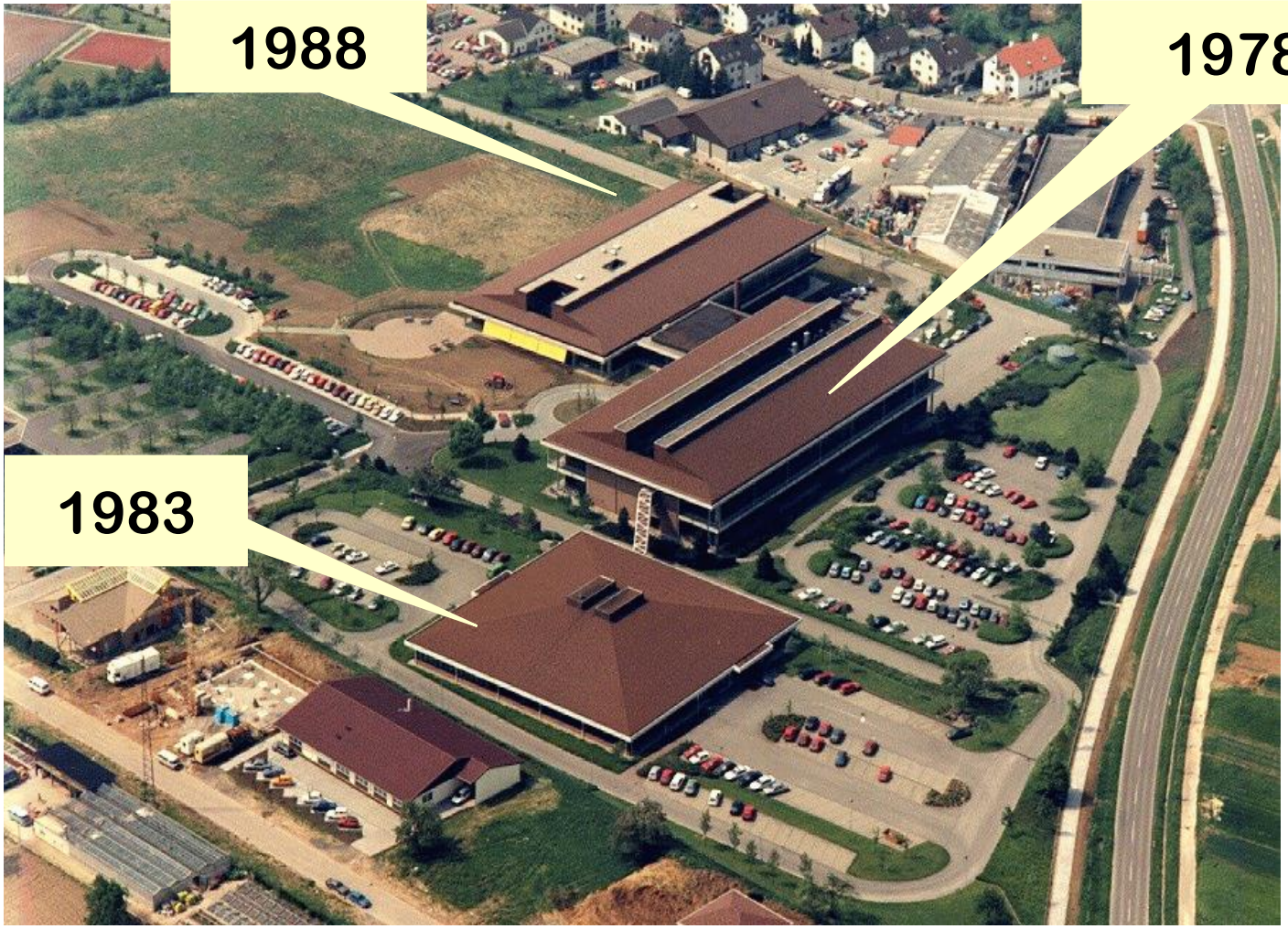


The Biocompatibility Issue:

Inertness ➔ no interaction between protein and system
Corrosion resistance ➔ no interaction between solvent and system

Non-metal HPLC systems.

Growth in the Eighties

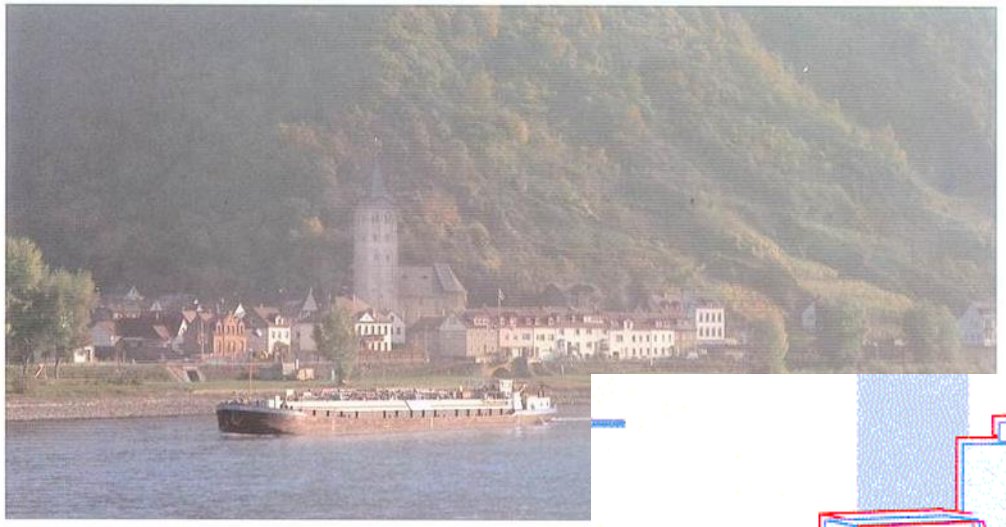


The 90ties

Routine Pharma
Solutions
Hyphenated Methods esp. LC-MS
Proteomics

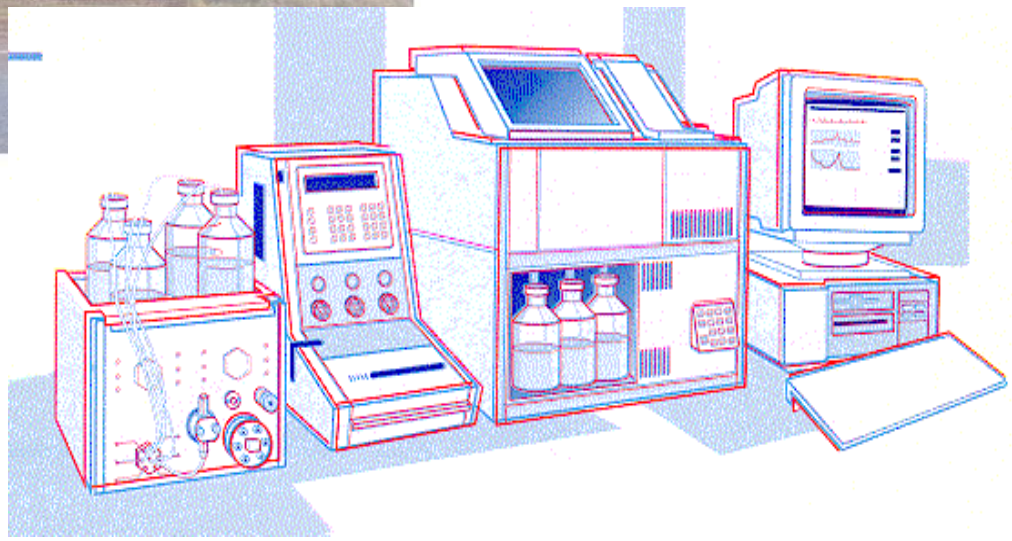
Hewlett-Packard Values, Community - Rhine Basin Program - 1989

A European program for a better quality of life



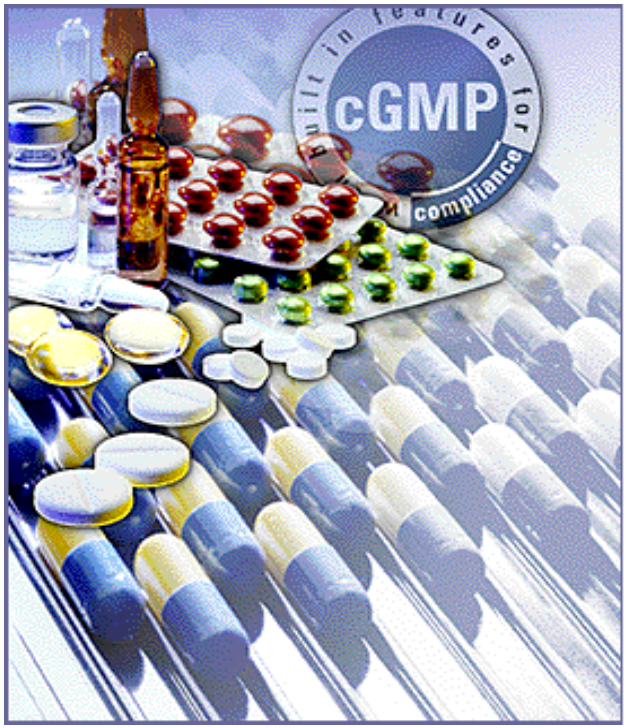
The Rhine River

A vital ecological system, cultural lifestream for millions of Europeans



HP 1100 Series - 1995

HPLC Becomes Routine in Pharma



- **Accuracy and precision of quantitation**
- **Reproducibility (day-to-day, system-to-system)**
- **Sensitivity (impurity detection)**
- **Standardization of methods (Pharmacopoeia)**
- **System and method validation**
- **Robustness of methods**
- **Ease-of-use**
- **Cost-of-ownership**

The New Millennium

HP Measurement Business becomes Agilent Technologies

HPLC Rediscovered → UHPLC

Miniaturization → HPLC-Chip

The Key Issue in HPLC

How to achieve a particular plate number N_{req} , say 50,000 plates, in the shortest time possible at **given maximal pressure** available ?

According to:

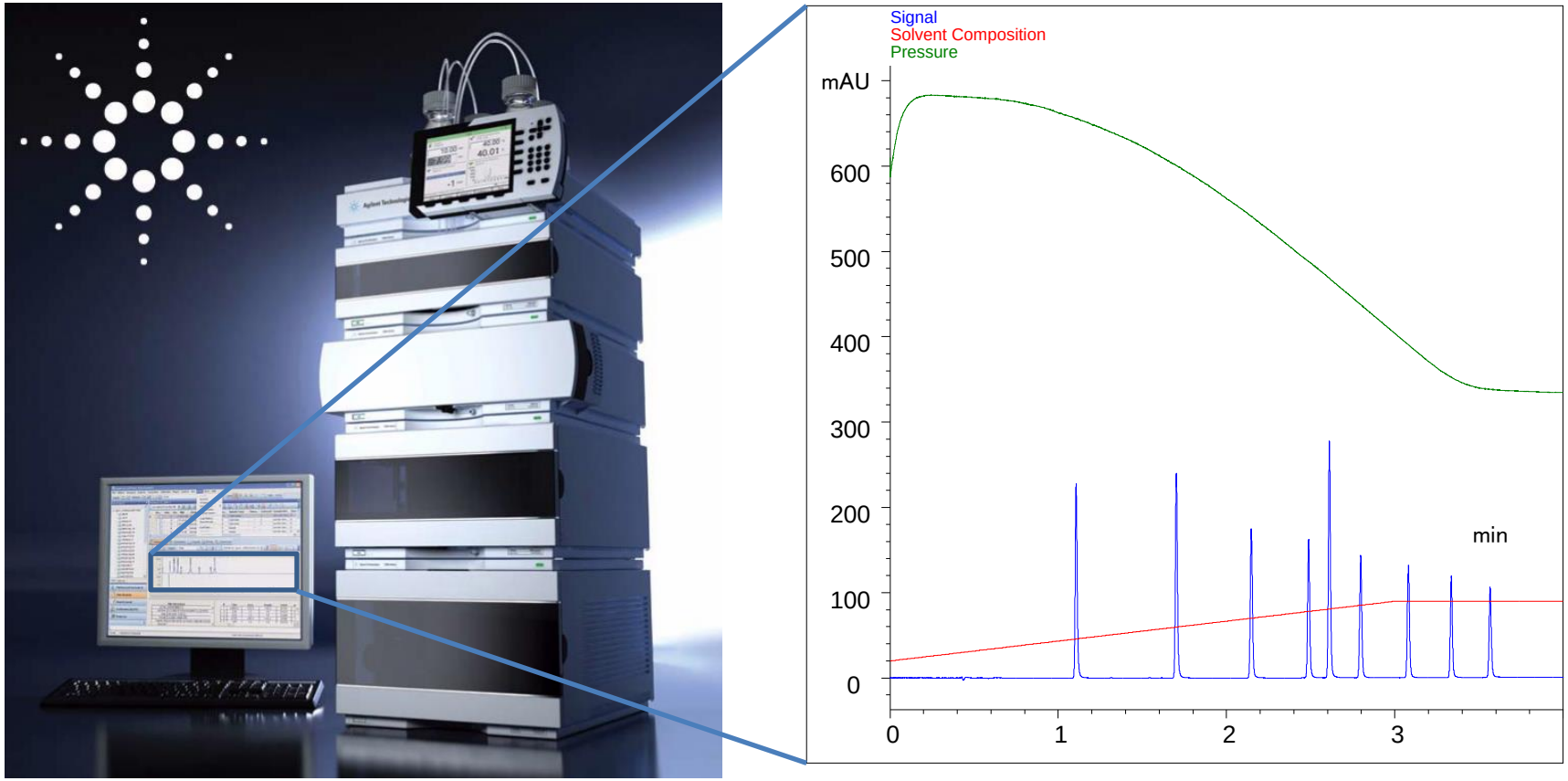
J.H. Knox and M. Saleem. J. Chromatogr. Sci., 7, (1969), p. 614
G. Guiochon, Anal. Chem., 52, 2002 (1980)
H. Poppe, J. Chrom. A., 778, (1997) 3-21

According to:

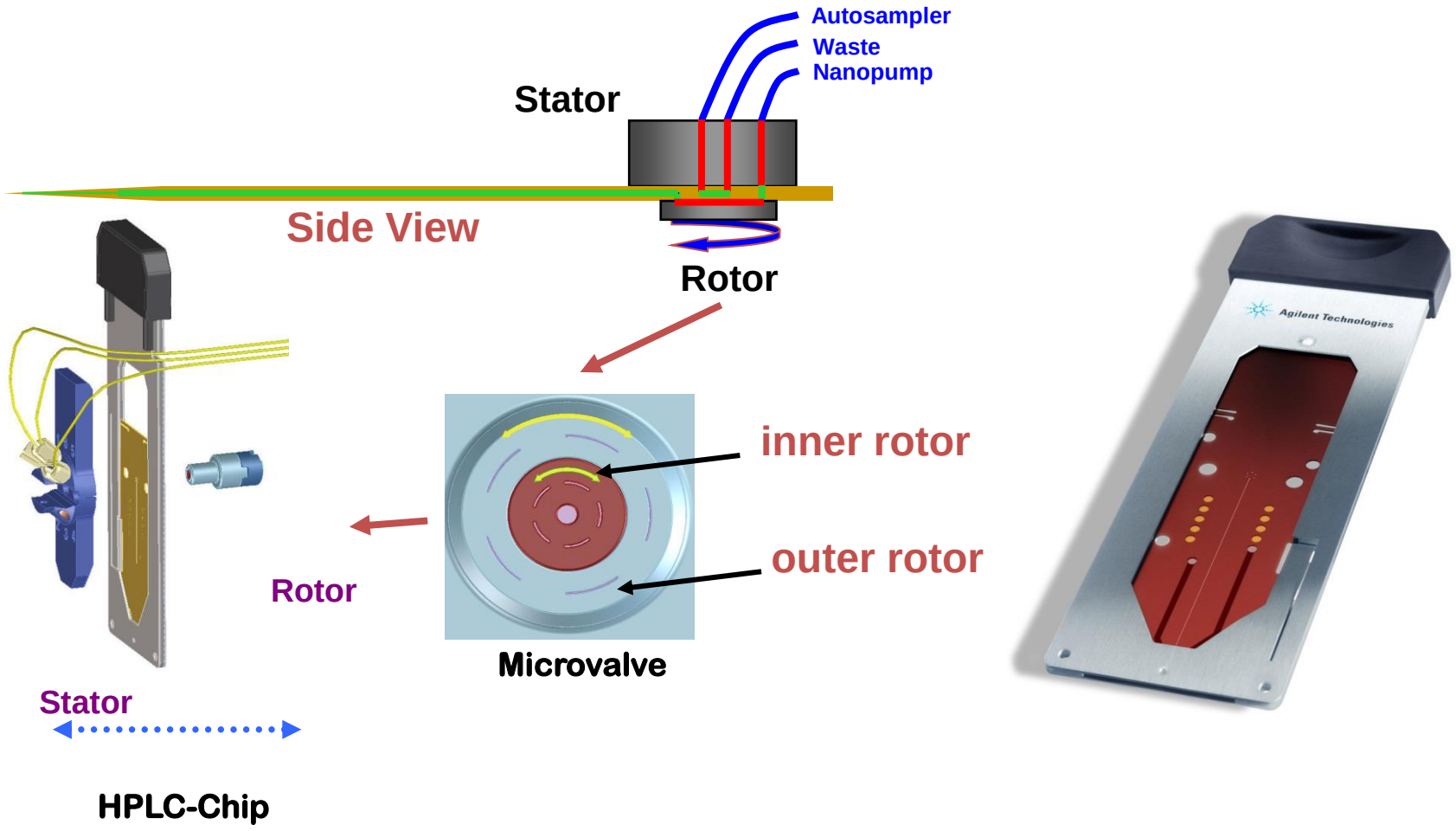
J.H. Knox and M. Saleem. J. Chromatogr. Sci., 7, (1969), p. 614

200 bar			
N_r	t_0	d_p	L
1000	0.2 s	0.5 μm	1.1 mm
10000	20 s	1.7 μm	35 mm
100000	2000 s	5 μm	1100 mm
1000000	2.3 days	17 μm	35 m
400 bar			
1000	0.1 s	0.39 μm	0.78 mm
10000	10 s	1.22 μm	24.4 mm
100000	1000 s	3.87 μm	774 mm
1000000	1.16 days	12.25 μm	24.5 m
1000 bar			
1000	0.04 s	0.25 μm	0.5 mm
10000	4 s	0.77 μm	15.5 mm
100000	400 s	2.45 μm	490 mm
1000000	0.46 days	7.75 μm	15.5 m

HPLC - 100+ Years after M.S. Tswett



HPLC-Miniaturization



Agilent Technologies Waldbronn - 2013

Acknowledgements

- Prof. K.K. Unger, Seeheim, Germany
- Prof. K-P. Hupe, Baden-Baden Germany
- Dr. Günter Nill, Waldbronn, Germany
- Dieter Höhn, USA
- Colleagues @ Agilent Technologies, Waldbronn

Perspectives on UHPLC

- Is 1200 bar enough?
  - Theory predicts lessening gains with further pressure increase
  - Engineering effort for reliability will grow exponentially
  - Frictional heat problem mandate very narrow i.d. columns
- Address time/skill/reliability issues
  - Intelligent automation
  - Autonomous systems
  - Constant pressure HPLC