THE HISTORY OF CHROMATOGRAPHY

My Mentors, Colleagues, and Friends in Separation Science and Technology, Part I

When I reflect on my career in chromatography, what stands out for me are the great people I met along the way — people who became my mentors, colleagues, and ultimately, my friends. In many ways, my story is their story.

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It is amazing, when viewing your life backwards like a movie, to discover how your ideas, thoughts, and actions were affected by a number of people who have become your mentors, colleagues, and ultimately, your friends. The story you will read is not a special one, but it documents, from a personal retrospective, part of the history of the development of separation science and technology that is now part of our daily life.

My Mentors Who Helped Me Enter the Field of Liquid Phase Separations at the Right Time

Istvan Halasz (1922–1988): Istvan Halasz was a young scientist when he left Hungary after the 1956 revolution. He became a docent at the Institute of Physical Chemistry at the University of Frankfurt am Main [**Author: Is this the way we should refer to the university? Or should itd be called "Goethe University at Frankfurt am Main"?**]. I visited Istvan in Frankfurt around 1970 and was impressed by his outstanding knowledge of gas chromatography (GC) and column liquid chromatography (LC), particularly his practical skills in designing and constructing LC equipment. We quickly agreed upon a close collaboration, and he helped my coworkers construct and build their first LC equipment, using three-head membrane pumps (Orlita, Giessen, Germany), a septum injection device similar to those used in GC, a column, and a UV detector. Istvan was interested in our know-how regarding the surface modification of silicas. He hired Ernst Brust as a diploma student who studied the binding of Si-N-R groups on silica in my group.

Two years later, Istvan received a professorship in applied physical chemistry at the University of Saarbrücken, Germany. He had a large group of Ph.D.s who performed fundamental studies in column design and column packings in LC. During this period, he recommended me as a visiting professor to Barry Karger at Northeastern University (Boston, Massachusetts). I spent six months with Barry during 1973, a period that I consider today to be one of the turning points of my scientific life.

During those six months, I was able to personally meet all the tycoons in chromatography along the east coast of the United States, which gave me a clear view on the current status of the field, and at the same time, strong motivation for the future focus of my research activities. Heinz Engelhardt, who also was a visitor at Barry's laboratory, joined Istvan's group and stayed at Saarbrücken until his retirement in 2001. Istvan passed away in 1988.

Josef Frans Karl Huber (1925–2000): I first met J.F.K. Huber at a congress of the German Chemical Society at Karlsruhe in 1972, where we reported on the use of porous spherical silicas as column packing in LC in the session on high-pressure technology.

J.F.K. was one of the leading scientists in GC and was the head of the separations laboratory at the University of Amsterdam. Two of his famous collaborators were Johan Kraak and Hans Poppe. They were heading the group when J.F.K. left Amsterdam and accepted a professorship at the University of Vienna.

J.F.K. Huber, Vienna, 1997.

At that time, the world of high performance liquid chromatography (HPLC) was heavily debating how high-performance columns should be made. The group around Istvan preferred the so-called *porous layer beads* (PLBs) as packings. They were composed of a 30–40 µm thick solid glass core and a porous shell composed of silica particles with a thickness of approximately 1 µm. One could perform highly efficient and fast separations on columns that were dry-packed with these porous layer beads. However, the column capacity (column loadability) was rather low compared to those packed with fully porous particles. A number of commercial products became available, such as Corasil I and II (Waters, Milford, Massachusetts), Zipax (Du Pont de Nemours, Wilmington, Delaware), and Perisorb A (Merck, Darmstadt, Germany).

On the other side of the debate was the group around J.F.K. Huber, which was propagating the use of microparticulate silica packings with average diameters of 5–10 µm. At that time, irregularly shaped silica particles were available that were obtained by grinding, milling, and sizing larger silica xerogel lumps. Johan Kraak was the first in J.F.K.'s laboratory to dry-pack stainless steel columns with microparticulate silicas. It took several years to synthesize spherical silicas, size them by the elutriation technology in narrow size fractions, and pack them in stainless steel columns by the slurry technique. It took nearly another decade for a column manufacturer to produce reproducible, stable, and validated HPLC columns that enabled their wide application in the pharmaceutical and chemical industry.

The serious discussions on the pros and cons of porous layer beads versus totally porous particles continued over several years until spherical silica particles took the floor. For me, it is interesting that the same discussions recently began again regarding the pros and cons of columns packed with sub-2-µm porous silica particles and core–shell particles with a 1.6-µm solid core and a 0.5-µm porous shell. Four decades later, we were finally able to approach the limits of HPLC in terms of column performance, speed, and sensitivity!

Josef became my primary mentor in science, and I developed a great admiration of him as a person and scientist until he passed away unexpectedly in 2000. His successor was Wolfgang Lindner, with whom I have a long-standing collaboration and friendship.

Jack J. Kirkland: My first meeting with Jack Kirkland dates back to 1973 when I was visiting the American tycoons in chromatography who were spread along the East Coast from Boston to Washington, D.C., Jack had an excellent background in organic chemistry and GC to resolve complex mixtures. Around 1970, he stepped into modern LC by synthesizing spherical silicas named *Zorbax*, a material that is still on the market. Jack worked at the experimental station of DuPont de Nemours, an impressive place for researchers in polymer chemistry and other areas. Among them was Ralph

Lecturers at a course on HPLC in Uppsala, 1976. From left to right: Klaus K. Unger, Göran Schill, John Knox, and Joseph Huber.

Iler, the renowned senior scientist of silicate chemistry. I had the pleasure to meet Ralph several times while I was visiting Jack. Jack introduced me to Lloyd Snyder, with whom he taught LC courses in the United States for many years, and to Carl Giddings, with whom he developed a sedimentation field flow fractionation (SFFF) instrument at DuPont to separate particles and macromolecules. Jack and I became close friends.

Jack left DuPont in 1992 and opened Rockland Technologies. Rockland was later taken over by Hewlett-Packard, and later spun off as part of Agilent Technologies. [**AUTHOR: I reworded previous sentence. Meaning OK?**] In 2007, Jack retired from Agilent and became vice president of a small start-up company in Wilmington, Delaware, called Advanced Material Technology. AMT produces so-called *Halo* columns, which are packed with core–shell silica particles exhibiting a column performance comparable to columns packed with 2-µm silica particles but generating a much lower column pressure drop.

In my experience, Jack is among the most influential people in HPLC in the United States. He was a real practitioner and had clear concepts and convincing arguments. Jack is now 85 years old.

John H. Knox: John Knox is a physical chemist and the chair of physical chemistry at the University of Edinburgh, Scotland. He was able to install the Wolfson LC unit and developed the spherical silicas marketed under the trade name *Hypersil*. At the end of the 1980s, he joined my group at the Technical University of Darmstadt through a visiting professorship for six months. We both worked on the synthesis of porous carbons. John chose the route of pyrolysis of synthetic polymers, followed by activation and graphitization. Using porous graphitized carbon, we tried to activate special cokes and subjected the intermediates to graphitization. Since that time, we have become close friends. I have visited John several times at Scotland, and we went sailing twice around the West Scottish islands, in 1995 and 2009. John and his wife also have visited me in South Sweden.

John Knox, Jack Kirkland, and Josef Huber became my teachers and supporters in LC for many years.

Göran Schill (1918–1992): But there was a fourth man in the league: Göran Schill from Uppsala University in Sweden. Göran worked on the analysis of pharmaceuticals by LC and invented and propagated, together with John Knox, the mode of ion-pair chromatography, which make it possible to analyze polar ionized analytes by the formation of neutral ion pairs. Ion-pair chromatography developed into a versatile and powerful method that was applied in the pharmaceutical industry at that time. Göran also introduced the validation of analytical methods in industrial laboratories. I got to know Göran through two of his famous former Ph.D. students: Bengt-Arne Persson from AstraZeneca in Mölndal, Sweden, whom I first met in Boston in 1973, and Jurgen Vessman, also from AstraZeneca. There is a third chap in this circle: Karl Gustav Wahlund, who is the chair of analytical chemistry at the Technical University of Lund.

Göran was a nature lover, and he went every year with his wife Brita to the Swedish Lappland. He inspired me to take my first trip to Sarek, in North Sweden, in 1985. From then until 2004, I went hiking every year in that wonderful landscape — alone with a tent, food supply, and my cell phone.

Göran was a great teacher and scientist. It is sad to say that he passed away soon after his retirement in 1990. With John Knox, Josef Huber, and Göran Schill, I started to teach numerous courses on HPLC within Europe. With the help of the Hamilton foundation, we were even able to conduct courses in Russia, Czechoslovakia, Romania, Bulgaria, and other countries in Eastern Europe.

Barry L. Karger: Barry is the director of the famous Barnett Institute of Chemical and Biological Analysis and the James L. Waters Chair in analytical chemistry at Northeastern University in Boston, Massachusetts. He started in GC and later moved into the fields of HPLC, capillary electrophoresis, and mass spectrometry (MS). He performed the basic analytical work in capillary electrophoresis for the separation of DNA and RNA, respectively, which led to success in the genome project.

When Barry began his career at Northeastern University, he had the idea to assemble renowned scientists in the fields of HPLC, GC, and MS. It started with Istvan Halasz, Bengt-Arne Persson, Johan Kraak, Wolfgang Lindner, me, and others. Barry invited me as a visiting professor to Northeastern in

Heavy discussion on an unknown subject. From left to right: Jack J. Kirkland, Klaus K. Unger, N.N. (a chap from Scotland), and John H. Knox.

1973 for six months. During that time I tried to pack 1–3 µm silica particles into HPLC columns and to measure the performance of the columns.

An Experience I Will Never Forget: My Colleagues in Research from Japan

[**Author: In your original heading for this section, you mentioned Nakanashi above but never mentioned him in the text. Do you want to add something about him?**]

My most remarkable entrance into the culture and science of Japan occurred in the early 1980s. Nobuo Tanaka organized one of the first international meetings on HPLC in the mountain area around Kyoto. It was a place located in nature, and far enough from the noisy city to be peaceful and quiet. There were no more than hundred attendees, and we stayed in simple rooms and enjoyed the Japanese baths and cuisine. The meeting took place in early January, and in the middle, it started to snow. I remember that Peter Földi, Eli Grushka, Roland Frei, and Milos Novotny were all there. For the first time, we visited the cultural sites of Nara with all the temples. Kyoto, the old capital of Japan, offered many historical places, among them the wooden temple of the emperor of Japan.

In the following 20 years, I visited Kyoto nearly every year and had the opportunity to notice the social changes in Japan. During my visits, I also got to know the seniors in separation science in Japan as well as the newcomers, including Nobuo Tanaka, Shigeru Terabe, and Takao Tsuda. The others were scientists in the field of adsorption and catalysis, which was and still is an active field of research in Japan. The senior Seiichi Kondo became my friend, as did Kazumi Kaneko of the University of Chiba, Japan, and Aki Matsumoto of the University of Toyohashi, Japan. Early on, I had good contacts with Yoshio Kato from Tosoh Biosciences, formerly Toyo Soda Manufacturing Co. in Yamaguchi, Japan. With Yoshio, I explored the island of Kiushi south of Yamaguchi. Barry Karger and I were invited as guests at the 50th anniversary of Tosoh in Osaka. In 2000, I was awarded a fellowship by the Japan Society for the Promotion of Science. My job was to give approximately 10 lectures at Japanese universities. I began at Chiba University in the vicinity of Tokyo, went south to Toyohashi and Nagoya, spent two weeks at Kyoto, and finally visited Shigeru Terabe at Himeji University. I will never forget this impressive period, which was crowned by a one-week stay at a famous Buddhist monastery located in the surrounding mountains of Kyoto. As I remember, it was spring, and I joined a two-day celebration in which two young men became acknowledged as monks in the monastery.

The Road Map to Biopolymer Separations, and the ISPPP Team

In 1981, I had the privilege to take part in the first international symposium on HPLC of proteins and peptides, which

Three of the five chairmen of the ISPPP: From left to right: Jan-Christer Janson, Milton T.W. Hearn, and Klaus K. Unger.

Meeting at Riquewihr, D'Alsace, prior to a conference held at Baden-Baden, 1983. From left to right: Karin Kirkland, Jack Kirkland, Johan Kraak, Milton Hearn, Bengt-Arne Persson, and Göran Schill.

convened in Washington, D.C. It was organized by Lena Chow from Varian. The idea was to open a new venue of conferences for the separation of biopolymers, while the international HPLC meetings dealt mainly with low molecular weight compounds. It was a great success in terms of scientific caliber and number of attendees. Almost one year later, the second International Symposium on HPLC of Proteins, Peptides, and Polynucleotides (ISPPP) took place in Baltimore, Maryland.

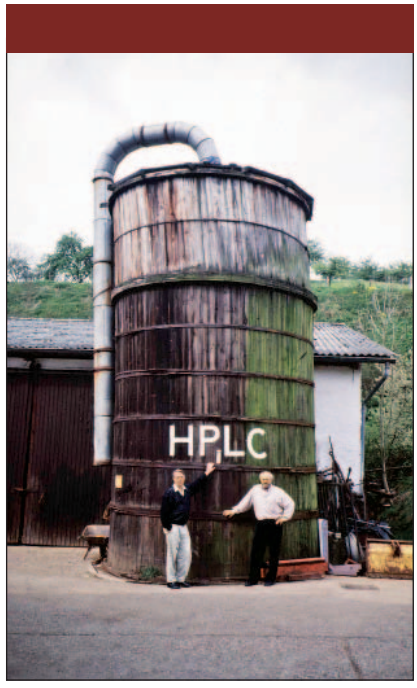
The chairmen of the first meeting were Milton T.W. Hearn, Fred Regnier, and T. **[Author: Tim?]** Wehr. At the second meeting, two more chairmen were added: Jan-Christer Janson from Pharmacia (Uppsala, Sweden) and me. We suggested bringing the meeting to Europe with Pharmacia as the sponsor. Indeed, the third ISPPP meeting took place in Monaco. When the ISPPP moved over to Europe and Pharmacia became a major sponsor, the organization of the meeting was handed over to Bengt Österlund of Pharmacia, who had a background as a biochemist. Bengt managed both ISPPP meetings, the one in the United States and the one in Europe, until Pharmacia stepped out. The rest of the story one can read in a special report entitled: *ISPPP – The successful route to biopolymer separations.* **[Author: can you provide a reference?]**

My Lifelong Relationship with Chromatography Down Under

My first encounter with Milton T.W. Hearn dates back to the early 1980s. Milton is a renowned biochemist in the department of biochemistry of Monash University in Melbourne, Australia. His special fields are peptide syntheses and purification, protein chemistry and analysis, and downstream processing. He is the editor of a number of books on these subjects.

Milton became highly interested in our work on the development of novel stationary phases for peptide and protein separations, and we decided on a close collaboration in early 1980. Birger Anspach was the first Ph.D. student to spend several years in Milton's group, before he returned to the National Institute for Biotechnology (GBF, Braunschweig, Germany). The next was Juergen Wirth, who joined Milton for several years evaluating thoria-based materials for biopolymer separations. Juergen has now settled in Australia, where he is now a manager at SGE Analytical Science in Melbourne, a company that specializes in the manufacture of fused silica capillaries.

Our closest collaboration was in the field of capillary electrochromatography (CEC), where Milton helped us to select appropriate hormonal peptides for separation. Marion Huber and Karin Walhagen, two of my Ph.D. students, were involved in this subject. Last but not least, I sent Tom Hennessy to Milton's laboratory to investigate miniaturized



A silo at a farm at Hochstädten, Germany — one of the world's largest prep columns? From left to right: Johan Kraak and Klaus K. Unger.

hybrid techniques for peptide profiling. Tom stayed two years with Milton, then came back and completed his Ph.D. exam at the University of Mainz. He became an application chemist in the field of proteomics at Agilent in Australia, and is now located in Singapore.

Milton eventually left the department of biochemistry and became the director of the Institute of Green Chemistry at Monash University.

My Dutch Connections

My first encounter with the group from the University of Amsterdam came through Josef Huber, who was the head of the analytical department. When Josef left for Vienna, Hans Poppe took over his responsibilities until his retirement a few years ago. Johan **[author: Kraak?]** (1940–2004) was the most practical and experienced research fellow of the group, a description that also could be applied to Hans. We developed a very close collaboration with the Amsterdam group in the field of nonporous silica beads and in CEC. We both were active in education and trainee of students. Johan, Hans, and later Wim Kok, organized the International Summer School on HPLC at Amsterdam, which I joined as a lecturer. In return, Johan attended my HPLC courses organized by the German Chemical Society (GDCh). I went to Amsterdam with my group several times, the highlight of which always was a sailing tour with Hans's old sailing boat in the Issel Sea. Hans even sailed this boat from Amsterdam to Hamburg when I was chairman of the international HPLC meeting in 1993. Hans retired **around 2004 [Author: edit ok? I moved this up from after the following paragraph; originaly it said "soon after" referring, I believe to 2004 when Johan died]**. He bought a new sailing boat and seems to be happy.

Johan retired around 2000, at the same time I left the University of Mainz. He married Thuy, a Vietnamese researcher, and moved to Southern France where his brother lived. In 2004, he was diagnosed with lung cancer. It was too late for a treatment, and he passed away in December 2004.

At the same time that I was connected with the University of Amsterdam, I became acquainted with Roland Frei (1936–1989) from Basel, who chaired the analytical department of the Free University of Amsterdam. With Roland, I had a long intensive collaboration in the field of sample cleanup. It is sad to say that Roland passed away shortly after celebrating his 50th birthday. His successor was Udo Brinkman, and after Brinkman's retirement, Hubertus (Huby) Irth, whom I got to know later along with Henk Lingeman, his co-researcher.

In 1991, I received an invitation from G.J. (Ad) de Jong of Solvay Duphar, near Amsterdam. Ad was involved in the purification of pharmaceuticals by preparative LC. Soon after, he was named the chair of pharmaceutical analysis at the University of Groningen, Netherlands. Around 2000, he accepted a position at the Utrecht University, Netherlands, in the same field. His vacant chair at Groningen was converted into a chair for biopolymer separations. Rainer Bischoff from AstraZeneca R&D (Lund, Sweden) applied for and received the position. Rainer is very successful and happy at Groningen.

When Ad was at Utrecht, the Netherlands Proteomics Centre was established and the chemistry faculty was looking for a specialist in liquid separation techniques. They offered a prestigious chair, the W.C. Donders chair, for which they asked me to apply. After a lengthy debate, I received the honorary professorship in 2004. I very much enjoyed my stay in Utrecht, which is a vivid, beautiful, and old Dutch city. I got to know the department of Albert Heck and others, and gave a series of lectures and courses in separation science. On top, I invited Ad's group to my place at Illstorp, Österlen, for a week in the summer of 2006.

My Swedish Link

[Author: In your original heading for this section, you mentioned B. Österlund, but then you never mentioned him in the text. Did you want to add something about him?]

When I visited Boston in 1973, I met Bengt-Arne Persson from AstraZeneca R&D (Mölndal, Sweden) and his young family. Bengt was a member of an even bigger family: He was a Ph.D. student of Göran Schill at Uppsala University in Sweden. Göran's former Ph.D. students could be found at nearly every pharmaceutical company in Sweden. One also met them in many pharmacies throughout the country. Through Bengt-Arne, I became acquainted with Göran. Karl Gustav **[Wahlund?]** became a professor at the department of analytical chemistry at the Technical University of Lund dealing with field flow fractionation after he had joined Carl Giddings post doctorate.

I got to know the Biomedical Center at Uppsala University with Jerker Porath, Stellan Hjerten, Jan-Christer Janson, and others. After Pharmacia became a sponsor of the ISPPP, I often visited Pharmacia and LKB, where Peter Földi worked on amino acid analysis. Later, LKB would be acquired by Pharmacia AB. Pharmacia would then be bought by Kabi, and finally Kabi disappeared after being taking over by GE Healthcare.

A notable change in my relationship to Sweden occurred at the beginning of 1990, when I received a prestigious

Humboldt professorship in 1992 for one year at the University of Lund. Gillis Johansson was my host who worked in the field of electrochemistry. I was unable to stay away from Mainz for a full year (my large group at Mainz would not allow such a long leave). Thus, I split my duties into several years and went to Lund in between the teaching periods of the semesters. In 1991, I bought a beautiful cottage in Illstrop, Österlen, which is approximately 80 km away from Lund. I went by car to Lund and back every day and enjoyed the landscape.

It was a vivid period in the department. Gillis was close to his retirement and young researchers such as Lo Gorton and Ake Jönsson became the leading folks. For me, Lund was a fantastic experience, because of its biochemical departments, Klaus Mosbach, Ideon Science Park as a place for start-up companies, and the surrounding pharmaceutical companies (Astrazeneca, Ferring, Pharmacia, etc).

I got to know nearly all of them and started joint projects. With Anne-Marie **[Olsson?]** from Ferring AB (Malmö, Sweden), we managed to receive a fellowship for Karin Walhagen, enabling her to come to Mainz to perform a Ph.D. on the CEC analysis of peptide hormones.

At Lund, I became acquainted with Börje Sellergren, who had completed his Ph.D. with Klaus Mosbach's group on molecularly imprinted polymers (MIPs). Börje had just returned from a post doctoral year in the United States and did not really know what to do. I saw a chance to invite him to Mainz; the chemistry department was strong in the field of assembled monolayers and design of surface chemistries for molecular recognition (Klaus Möhwald, Hellmut Ringsdorff, and others), which matched Börjes's interests. Secondly, I had a docent position available for six years, which gave Börje the option to carry out his **habilitation** [**Author: what does that term mean?**]. Börje accepted my invitation with pleasure. He started to assemble a huge group on several EU projects. He continued research in the field of MIPs. Börje completed his habilitation and received a position at the University of Bochum, Germany, where he is still active. He founded a start-up company at Ideon, in Lund, called MIP Technology, which was recently bought by Biotage.

AstraZeneca was the main focus of our activities in several proteomics and peptidomics projects. Karl-Siegfried Boos and Klinikum Großhadern at the University of München, Germany, helped us to step into the field by tuning the restricted access materials for the sample clean up of biofluids. Knut Wagner, one of my Ph.D. students with excellent engineering skills, built a multidimensional LC unit hyphenated to matrix-assisted laser desorption ionization (MALDI) where we could demonstrate the potential of LC compared to 2D-gel electrophoresis. After Knut finished, Egidijus Machtejevas, one of my most experienced postdoctoral students in this field, stepped in. He was extremely successful in reducing the complexity of the analytical multidimensional LC–MS platform. He installed, in total, five units at AstraZeneca in Lund and in Mölndal for biomarker search. During this period, we worked together with Eric Edholm, Györgi Marko-Varga, and his Ph.D. student Tasso Miliotis. Tasso moved to Mölndal to continue research, and Györgi left AstraZeneca Lund for early retirement. Recently, the whole unit of AstraZeneca Lund, which had 800 employees, was disintegrated.

I still have contacts to Lund, in particular to Peter James at the Institute of Protein Technology. With Peter, we organized proteomics courses at Lund and at the Universidad Complutense in Madrid, Spain (with Concha Gil of the department of microbiology), which were supported by the European Proteomics Association (EUPA).

Conclusions

The report covers mainly the first part of my scientific activities. A second, even more fascinating area started in 1990 until the end of my activities in 2010 — with the impact of liquid chromatography in life sciences, starting with multidimensional LC–MS, proteomics, and related approaches. This will be published in Part II.

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