Early LC-MS Developments in the Laboratory of Professor McLafferty at Cornell University

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A brief account is given on LC-MS research conducted in the laboratory of Professor Fred W. McLafferty at Cornell University during the 70's. This pioneer work ended into a commercial interface, the Direct Liquid Introduction (DLI) that was later commercialised by mass spectrometer manufacturers, before being superseded by other systems.

Retrospection to the early days of LC-MS has been the subject of several review papers and dedicated sessions at scientific meetings. For example, early Instrumentation for LC–MS was the topic of the 21st James L. Waters Annual Symposium held during the 2010 Pittcon's conference, and the review by Pullen [1] was another account of the triumphs and failures of LC-MS developments during its pioneer period. Apart from evoking nostalgia, recalling the early steps of a technique has an epistemological significance that can be valuable to future generations. This collection of reminiscent celebrations could also be because many of the actors that were active during these early days have now reached retirement or emeritus status, not to mention those who have left us (Malcolm Dole, 1903-1990; Evans Horning 1916-1993; John Bennett Fenn, 1917-2010). Although they are not frequently mentioned, a few chromatographers also actively participated in early development of LC-MS, giving sound advices to their mass spectrometry colleagues, and credit should be given to Roland Frei (1936-1989), J.F.K. Huber, (1925-2000) and Csaba Horváth (1930 –2004).

This text will not review the early LC-MS interfaces, a well-documented subject [2] [3] [4] [5] to only quote a few of them. Suffice to say that overcoming the volatility barrier, as now routinely done for molecules that have no measurable vapour pressure at atmospheric pressure, is comparable to steps that made it possible to overcome the sound barrier. In both cases, the conquest was achieved after designing new equipments, in a significant rupture with previous methods to attack the problem [6]. Among the many historical reviews that have been published, the interesting account of early LC-MS work published by Thomas Covey et al. [7] is noteworthy. Covey also was a major pioneer of LC-MS achievements, and using a sort of historical map, he brilliantly summarised the mixed influences of research work in Europe and in America, that sometimes converged, but also sometimes diverged and vanished away. This map is another example that a single drawing may more capture the reader attention than a long text.

Motivation to add a few comments to the article by Pullen was because I have been fortunate enough to be in the right place, at the right time. As a witness, I can provide the reader with first hand information, in particular on the LC-MS work developed in Professor Fred McLafferty laboratory at Cornell University (Ithaca, NY, USA) during the 1973-1981 periods. In addition, one figure that I had drawn lacks a correct reference in Pullen's text, but also in other sources from the literature, as just citing a reference, without checking its full content, is prone to propagate citation errors. A few minor details also call for additional comments.

A Personal Perspective of the DLI Origin and Development

I joined Professor McLafferty's group in March 1973, after receiving a PhD on organic geochemistry from Strasbourg University (France) under Professor Guy Ourisson (1926-2006). This period had been the occasion of a very fruitful collaboration with the University of Bristol (UK), and with the Organic Geochemistry Unit directed by Professor Geoffrey Eglinton , that resulted in the first recognition of ubiquitous polycyclic triterpenoid compounds in geological samples [8] [9]. Nearly forty years later, this large family of molecules of bacterial origin is still widely used as chemical markers for environmental and geochemical studies. For my PhD work, I had acquired some hand-on practice on gas chromatography, using lengthy and tedious procedures for making wall-coated open-tubular glass capillary columns, but I had only been a passive mass spectrometry user. The postdoctoral opportunity to join Professor McLafferty laboratory oriented my subsequent academic career.

Arriving in March 1973 in the Baker Chemistry Laboratory building at Cornell University, Pr McLafferty offered me the choice between two possible postdoctoral subjects: either gas-phase ion structure characterisation using a MS-MS technique recently introduced in his laboratory, or the development of an LC-MS interface, which finally was my choice. Years before, while he was Director of Dow's Eastern Research Lab in Framingham, Massachusetts, Pr McLafferty and Roland S. Gohlke had developed one of the first GC-MS instruments [10] [11]. As HPLC was rapidly progressing at the beginning of the 70's, Professor McLafferty believed that LC-MS coupling was a new frontier to be explored.

During the preliminary discussions to delineate my work, Professor McLafferty gave me a copy of the paper by Malcom Dole et al. [12] which had already attracted a wide interest in the mass spectrometry community, although in 1973 no other laboratory had attempted to duplicate the experiments. This was to be done, after 1975, by John B. Fenn at Yale University, and ended in the development of electrospray ionisation (ESI) [13]. Another direction was also considered, but finally not followed: Bill McFadden at Finnigan Corporation had already started to conceive a transport interface coupled to a quadrupole mass spectrometer, although first public results were published in 1976 [14]. All mass spectrometers in Cornell laboratory were magnetic sector instruments, but designing a laboratory-built moving belt interface was assumed to be feasible. Such an approach was beyond my technical skills, and far too complex to be completed during a time-limited postdoctoral stay. The decision was to continue the preliminary experiments achieved in Cornell by Michael A. Baldwin, who was a visiting scientist from the group of Allan Maccoll (1914-1999), at University College London. In addition, because of his strong background, Michael Baldwin was a well-trained mass spectrometry expert, and could be my guide, for the period of my stay. Michael Baldwin was a very patient and efficient mentor, and I learned a lot about mass spectrometry from his wise advices. The MS instrument for this project was a huge double focusing high-resolution mass spectrometer, Hitachi HRMS2, with a 10 kV accelerating source voltage.

Figure 1. Picture of the Hitachi PerkinElmer, model HMR-2 high resolution mass spectrometer, used for LC-MS interfacing development at Cornell University (by courtesy of Professor Fred McLafferty, 2012).

Chemical ionisation (CI) mass spectrometry, introduced by Field and Munson at the end of the 60's [15] was extensively investigated during the following years. In addition to the traditional CI gases including methane, isobutane, or ammonia, organic chemists had started to use wider panoply of more exotic reactant molecules, in particular vaporised organic liquid solvents. CI solvent vapours and flash vaporised samples were simultaneously introduced into a MS, although via separated lines. During this period, it was also demonstrated that sample volatility was significantly increased by disposing the sample on an inert surface, next directly introduced and rapidly heated within the CI source block: the so-called "direct chemical ionisation" effect, as exampled by Buehler et al. [16] for a series of underivatised peptides. Baldwin and McLafferty merged these different concepts into a new experiment in which liquid solutions of different underivatised oligopeptides were introduced into small glass ampoules. Each vial was partly sealed by glass blowing down to a few micron aperture, and was next placed at the end of the solid probe inlet of an AEI model MS902 double focusing mass spectrometer.

Protonated molecular ions for each oligopeptide could be recorded for a few minutes, until the solution was completely sucked dry by the vacuum [17]. In a subsequent paper, submitted after the previous one, but in fact published a few months before [18], Baldwin and Mc Lafferty suggested that the method could be a means to achieve an on-line coupling of liquid chromatography with mass spectrometry. Since both reference [17] and [18] contain no figures, I have reproduced a schematic representation of the experimental setup used for this initial work *(Figure 2)*. As proof of concept of this approach, I designed a continuous liquid introduction system.

James (Jim) L. Waters provided the Baker Chemistry Laboratory with a M-6000 HPLC pump, a gradient controller and a UV detector. He had set up a company in the basement of his parent's house in Framingham, Massachusetts, before moving to new quarters in the same city, where he established Waters Associates Company in 1958, i.e. not far from Dow's Eastern Research Laboratory where Fred McLafferty was active at this time. In the spring of 1973, I was introduced to Jim Waters, who offered me a training course in modern analytical liquid chromatography in Framingham in September.

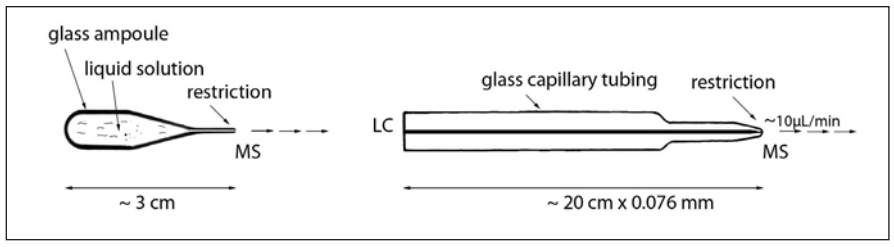

Figure 2. Left: schematic representation of the partly sealed ampoule used by M. Baldwin et al. [17] [18] to introduce liquid solutions directly within a MS ion source. Right : modified glass tube used by Arpino et al. [19] as a link between a HPLC system and a MS ion source.

Making a continuous introduction system was achieved by means of a glass tube, partially closed at one extremity, and connected to the Waters HPLC system at the other end *(Figure 2)*. The glass tube was modified to fit into the modified solid probe inlet of the Hitachi HRMS2 mass spectrometer, removing excess glass by dissolution into a HF solution. The first real "HPLC peak" was observed in the fall of 1973, following the injection of benzanthraquinone, showing perfect similitude between the UV trace, from the online installed UV detector, and the total ion current trace from the mass spectrometer. Other results and the experimental set up were presented at the 22nd ASMS meeting, in Philadelphia in May 1974 [19], and were published the same year in the inaugural issue of Biomedical Mass Spectrometry (now called Biological Mass Spectrometry) [20]. The illustration shown as *Figure 1* in the article of Pullen [1] was not published in ref [18], as quoted in the figure legend, but it was an illustration in reference [20]. To draw this cut-away figure, I had bought on my own expenses sheets of tracing paper, china ink pens, and a technical drawing book [21], from the Cornell Campus Store. In spring 1974, as the end of my postdoctoral stay was approaching, Bobby Dawkins joined me to continue the LC-MS work after my departure in September 1974. Published results were illustrated by a perspective representation of the same experimental set up (Figure 2) [22].

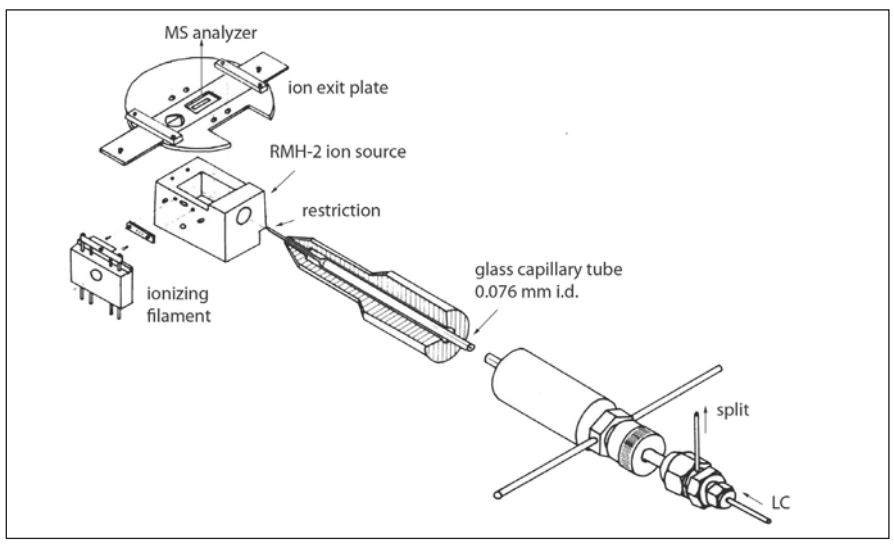

Figure 3. Inlet probe and MS source block for continuous introduction of solutions (adapted with permission from ref. 19)

Ruedi Knutti and Rengachari Venkataraghavan designed the computerised data acquisition and processing system for the LC-MS experiments [23]. Direct liquid introduction method into a chemical ionisation mass spectrometer was also the subject of a patent granted in 1975 [24], that was licensed to Hewlett-Packard who used diaphragms instead of restricted capillary tubes, as flow restrictors. In the literature, the interface was referred to as 'direct liquid introduction' or DLI. The complete LC-MS-computer system was applied to sequencing some oligopeptide standard mixtures [25] [26].

Further modification to the Cornell LC-MS system was based on rapid vaporisation of the liquid droplets by means of laser shots to a solution spray [27] [28], in a similar fashion as Marvin Vestal was experimenting in Houston, on a modified quadrupole mass spectrometer [29]. Laser vaporisation did not led to significant improvement to the Cornell experimental set-up, while Marvin Vestal observed that the laser beam produced better results when directed to the liquid vaporiser, rather than to the liquid spray [30].

Thus, the laser was just an expensive heater, later replaced by an electrical device, and this was the start of the development of the Thermospray interface.

The LC-MS work in Professor Fred McLafferty laboratory was conducted during a relatively short period, less than 10 years, but was sufficient to associate Professor McLafferty's name to the long list of mass spectrometry innovations, after GC-MS [10], and before MS-MS [31] [32], electron capture dissociation [33], top down proteomics [34], to name only a few of them. The 18 months spent in Cornell laboratory was the chance of my life, as this tremendous experience definitely oriented my research career in the subsequent years, and I deeply acknowledge Pr McLafferty for his kind guidance and support.

Making a Long Story Short

To conclude on some non-classical LC-MS illustrations, I indeed drew the 'fish and bird' cartoon represented in *Figure 7* in Pullen's article [1], but not in 1974, as written in the legend, which also lacks an appropriated citation to the paper in which it was published [35]. The cartoon was drawn later, after my return to France, while working in the analytical chemistry laboratory of Professor Georges Guiochon at the Ecole Polytechnique (Palaiseau, France). In October 1979, a user meeting of the French quadrupole instrument manufacturer Ribermag was organised at Antwerp University. Eddy Esmans in his lecture compared the difficulty of coupling LC to MS to that to marry a bird and a fish, and this prompted me to translate this bright comparison into a drawing illustrating a paper published the next year in TRAC [35].

I also summarised my opinion on another early LC-MS interface, using a drawing. I did not attempted to build a moving-belt LC-MS interface in Cornell, but I observed its development [2], as the device was commercially available during the 70's from both Finnigan (now Thermo Fisher), and Vacuum Generator (VG, now Waters). Professor Dai Games in Cardiff was an ardent supporter of the moving belt, and we often attended same meetings, and same LC-MS sessions. During the 1982 LC-MS meeting in Montreux (Switzerland), and while we were joining a dinner organised by Roland Frei, I gave to Dai Games a drawing that I had specially prepared for him, illustrating the sort of moving-belt that never fails to work. In the subsequent years, Dai Games frequently showed the drawing during his presentations, until this LC-MS interface rested in peace, along other early LC-MS interfaces, including the DLI.

Figure 4. Pr McLafferty, on receiving the Lavoisier Medal from Professor Armand Lattes, discerned on September 2004 by the French Chemical Society.

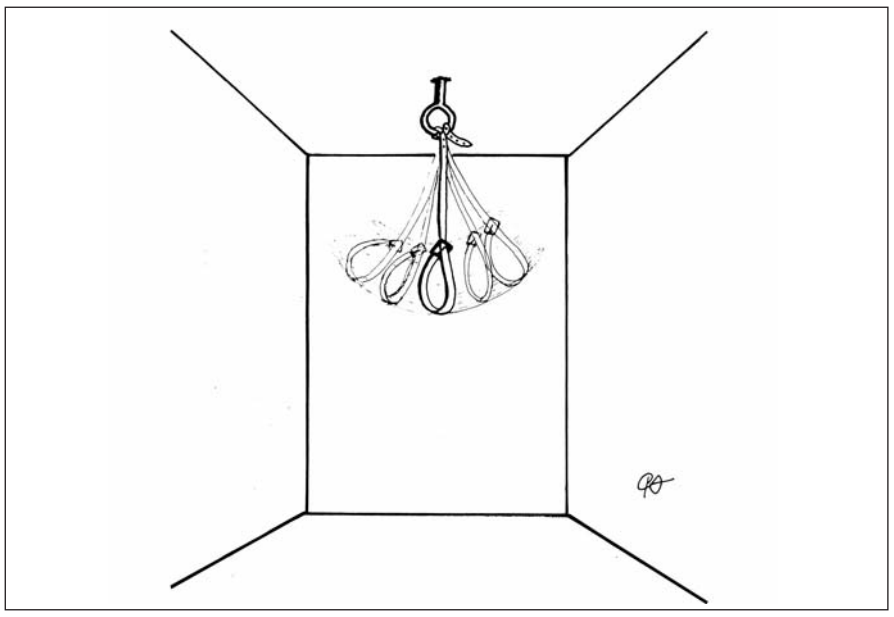

Figure 5. The moving belt: a dead end

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