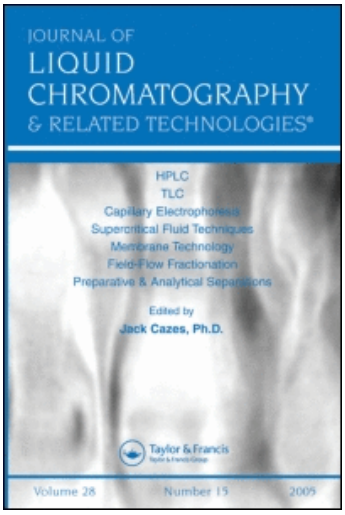

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Liquid Chromatography, Past, Present, and Future

R. P. W. Scott[a]
[a] Georgetown University, Washington, D.C.

To cite this Article Scott, R. P. W.(1987) 'Liquid Chromatography, Past, Present, and Future', Journal of Liquid Chromatography & Related Technologies, 10: 8, 1547 — 1567
To link to this Article: DOI: 10.1080/01483918708066787
URL: http://dx.doi.org/10.1080/01483918708066787

JOURNAL OF LIQUID CHROMATOGRAPHY, 10(8&9), 1547-1567 (1987)

LIQUID CHROMATOGRAPHY, PAST, PRESENT, AND FUTURE

R. P. W. Scott
Georgetown University
Washington, D.C.

In this special issue of the Journal that commemorates the first symposium on liquid chromatography (LC) held forty years ago, it is perhaps, appropriate to review the progress that has been made, from its inception in the late eighteen nineties and early nineteen hundreds, to the present day. It might also be useful to look into the future a little and try to anticipate the changes that may take place in the next decade and those areas where new developments are likely to occur. Chromatography is unique in the history of analytical techniques. On the one hand the development of LC was *painfully slow* and *arduous*, particularly in the early years, whereas, in comparison, the rate of development of gas chromatography (GC) was almost *meteroic*. It took *only* nine years, between, 1951 and 1960, for GC to evolve from a novel method of separation, used only in a few fortunate laboratories to a fully fledged analytical technique. In contrast, the level of development of LC in 1960 lagged very far behind that of GC despite the fact that LC had been discovered half a century earlier. Today the performance of the liquid chromatograph is equivalent to that of the gas chromatograph and the technique, perhaps, is even more versatile. To achieve this, however, far

more research and development effort was required, which was spread over many more years than was necessary for the development of GC. Overlapping the development of GC and LC was the sister technique of LC, thin layer chromatography (TLC). The performance of TLC relative to LC leaves much to be desired but due to its simplicity and the low cost of the necessary equipment, it is still a very popular technique and is extensively used throughout industry and in many universities.

Today the technique of LC can be applied to a very wide range of sample types the only limitation being that their components are soluble in a suitable solvent that can be employed as the mobile phase. In contrast, GC can *only* be used for the separation of samples that are *volatile* and even then, many mixtures which involve volatile solutes that were originally separated by GC are now analysed by LC. It is interesting to note that, in the future, the most exiting areas of application for LC appear to be in the field of biotechnology where, in fact, the technique of chromatography first began.

Early Days

The first scientist to recognize chromatography as an efficient method of separation was the Russian botanist Tswett who employed a primitive form of liquid solid chromatography to separate and isolate various plant pigments. The colored bands he produced on the adsorbent bed evoked the term chromatography for this type of separation. Although color has little to do with modern chromatography the name has persisted and despite its irrelevance is still used for all separation techniques that employ a mobile and a stationary phase. Chromatography was actually discovered by Tswett in the late eighteen nineties but it was not until 1910 that he published a book (1) describing his chromatographic methods for the separation of chromophylls. Unfortunately the work of Tswett was not developed to any significant extent, partly due to the original paper being in Russian and partly due to the condemnation of the method by Willstatter and Stoll (2) in 1913. These scientists repeated Tswett's experiments without heeding his warning *not* to use to "aggressive" adsorbents as these would cause decomposition of the

chlorophylls. Willstatter's experiments failed and their published results and conclusions impeded the recognition of chromatography as a useful separation technique for nearly 20 years.

The next significant development was reported by Kuhn et al (3) in 1931 who used the technique in the manner recommended by Tswett to separate lutein and xanthine. Kuhn and his co-workers also employed the same procedure to separate α and β carotene (4,5) and thus, they were the first to demonstrate that LC could be employed for preparative separations (6). Subsequent to 1931 progress in LC was slow and somewhat desultory and this was largely due to a lack of essential instrumentation.

In the late thirties and early forties Martin and Synge introduced liqiud-liquid chromatography by supporting the stationary phase, in this case water, on silica in a packed bed and used it to separate some acetyl amino acids. They published their work in 1941 (7) and, in their paper, recommended the replacement of the liquid mobile phase with a suitable gas. The use of a gas they considered to be advantageous as the transfer between the two phases would be faster and thus provide more efficient separations. Thus, the concept of *gas chromatography* was born but little notice was taken of the suggestion and it was left to Martin himself and A. T. James to bring the concept to practical reality some years later. In the same paper in 1941 Martin and Synge put forward the first general theory of elution chromatography, namely, the Plate Theory. The theory they devised at that time was the exponential form of the plate concept which had limited use, but nevertheless constituted the first successful attempt to derive an explicit equation for the elution of a solute from a chromatographic column. Unfortunately, there was no Rate Theory available at this time and so there was no way of optimizing column design to provide high efficiences other than by an arbitrary experimental approach which, in practice, led to very limited improvement in column performance. It was necessary to wait for the development of gas chromatography before a useful Rate Theory was to be developed.

The greatest obstacle to the development of LC in the early days was the lack of a sensitive on-line detector. The elution curve for each of the solutes was monitored by collecting a large number of fractions of the column eluent and subsequently analysing each fraction by such techniques as colorimetry and titration. The concentration of a solute in each fraction was then plotted against the fraction number, producing a form of chromatographic *histogram*. This procedure was both time consuming and tedious and was only effective for well resolved mixtures. Moreover, the chromatographic histogram gave a very poor representation of the form of the elution curve and was ineffective in aiding column technology. It was not until 1942, when Tiselius and Claesson (8) developed the first form of refractive index detector, that the true form of the elution curve could be traced. From an accurate recording of the elution curve, the factors that affect the band dispersion would, in due course, begin to be identified and, as a consequence, the chromatographic performance could be improved. For a period of time, interest was aroused in the development of LC on-line detectors and, in 1951, Martin and Randall (9) described the first electrical coductivity detector. In the same year, however, James and Martin published their epic paper describing the first gas chromatograph (10). They separated a series of fatty acids using a titration procedure as a detector employing a microburette. The microburette was eventually automated providing a very effective on-line detector with an integral response (11).

The invention of GC seemed to sound the death knell for LC development. For almost a decade there was little progress and LC became the Cinderella of the separation techniques. The spark of interest in LC was kept alive by such stalwarts as E. Lederer, M. Lederer, S. Moore, L. R. Snyder, E. Stahl and W. H. Stein.

The Barren Years

In contrast to LC, the technique of GC developed at an amazing rate. In retrospect, it is difficult to identify the cause of this spectacular growth. Gas chromatography seemed to attract the attention of scientists of widely

differing skills and disciplines. Physicists, chemists, engineers and mathematicians contributed to the development of GC, all with almost limitless enthusiasm, diligence and effort. This symbiosis was highly productive. In the nineteen fifties, there was a camaraderie between the workers in the field that was unique in the history of analytical instrument development and which, unfortunately, has not been so apparent since. The development of GC may not appear germane to this discussion but some of the advances will be discussed because much of the work provoked similar research to take place during the renaissance of LC, which would not occur until a decade later.

The first symposium on GC was held in London England in 1956 and was entitled "Vapor Phase Chromatography" the name originally given to the technique by James and Martin. A number of existing detectors were discussed, the Gas Density Balance by Munday and Primavesi(12), the Katharometer by Davies and Johnson (13) and two new detectors were described, the Flame Thermocouple Detector by Scott (14) and Wirth (15) and the .β ray detector by Boer (16). The approach to detector design and the method of defining detector performance described in this symposium on GC would in due course be emulated in LC. Probably the *most significant contribution* to the meeting was the paper by Keulemans and Kwantes (17) that gave experimental support to the Rate Theory that had been proposed previously by Van Deemter *et al* (18). The Rate Theory was the long awaited, essential piece to the chromatography puzzle that would provide an understanding of the processes that caused band dispersion in the column. This understanding would initially lead to improved GC columns but above all, it would eventually show how efficient liquid chromatography columns could be made.

The impetus provided by the 1956 symposium accelerated the activity in GC even further and brought many more scientists into the field. The next two years progress culminated in the 1958 Symposium on Gas Chromatography. The more rational term *Gas Chromatography* was used to replace the original and somewhat inappropriate term *Vapor Phase Chromatography*. This symposium was the climax of GC development and

although there were many symposia on GC yet to come, none would have the same novel and exciting technical content of this one. The theory of GC was extended by Littlewood (18) and Bohemen and Purnell (19) and the capillary column, which was eventually to revolutionize GC, was introduced by Golay (20). The theory Golay introduced would have important ramifications for LC, not only with respect to columns, but also to extra column connecting systems. The application of Golay's results to LC, unfortunately, would not take place for another decade. In the 1958 symposium, McWilliams and Dewar (21) described the flame ionization detector which was to become the work horse detector for all future gas chromatographs, and Grant (22) described the emissivity detector. Long, high pressure (200p.s.i.), high efficiency packed columns were described by Scott(23) and temperature programming was introduced by Harrison (24), another technique that would be incorporated in the design of all future gas chromatographs. Many new applications of GC were also reported and the five hundred participants left the meeting confident that GC was now a firmly established analytical technique. Despite the extraordinary advances that were taking place in GC there was still relatively little progress in LC and this lethargy persisted throughout the whole of the fifties.

In 1960, the third of the triad of symposia that contained the majority of the essential developments of GC was held in Edinburgh. This meeting, although highly successful, lacked the verve of its predecessor. The papers presented were largely extensions and confirmation of the ideas put forward in the previous two symposia, together with many new applications. Although, as the future would show, there would be further developments in the technique, the impression at the end of the meeting was that the development of analytical GC was more or less complete. This impression may have been the catalyst that initiated the renaissance of LC in the middle and late sixties. There would be many more symposia on GC but they would deal, for the most part, with applications, relatively minor modifications and the extrapolation of the work already reported in the first triad of symposia. As the years passed, it became increasingly

difficult to tell whether the GC symposia were organized merely for the sake of having symposia or for the proper purpose of helping to develop the technique further and impart knowledge. Nevertheless, the completion of the 1960 symposium heralded the beginning of the renaissance of LC that would take place in the sixties and continue through the middle seventies.

The Renaissance

The factors that initiated the renaissance of LC, even today, are obscure and not easily identifiable. Perhaps, despite the glamour that surrounded GC, the recognition that it could only separate volatile materials played a part. There were certainly many more nonvolatile substances of interest than there were that were volatile, particularly in the fields of biochemistry and general biotechnology. In addition, there were many other separation problems remaining that could not be solved by GC. It was extremely difficult to pack efficient LC columns at that time and the possibility of applying the new found understanding of dispersion in packed beds derived from GC theory to LC columns was also an attractive challenge. Similarly, experience gained in the development of GC detectors might be used to design new LC detectors and, indeed, there was a dearth of effective LC detectors in the early sixties. Whatever the cause, interest in LC development started in the early sixties and increased rapidly until the mid seventies. During this period many of the scientists involved with the development of GC feeling, perhaps, that there was little challenge left in the new technique, turned their attention to LC as an exciting new area in which to work. In fact, many of the scientists responsible for the advances made in LC during the renaissance and after were originally pioneers in the development of GC.

The greatest impediment to the development of LC, as already stated, was the lack of a sensitive, linear detector. It was not suprising, therefore, that much of the early work of the renaissance concentrated on the development of detectors. Vanderheuvel and Sipos (25) extended the work on the refractive index detector of Tiselius *et al* (8), Claxton (26)

introduced the heat of adsorption detector and James *et al* (27) described the first transport detector, improved later by Scott and Lawrence (28). The transport principal was eventually employed by Scott *et al* as an LC/MS interface(29). In 1966 Horvath and Lipsky (30) described the first small volume UV detector which in 1968 was improved by Kirkland (31) and eventually became the work horse of LC in much the same way that the flame ionization detector was of GC.

During the sixties and seventies, many scientists contributed to the renaissance of LC and, in an article such as this, it is is impossible to mention everyone. It must be sufficient to cite those who, in the authors opinion, made a particular impact on the future of LC during those times .The extensive work of L. R. Snyder during this period, on liquid-solid adsorption deserves special reference and his book", Principals of Adsorption Chromatography " (32) became the authorative text of that time.

Up to about 1969, the only stationary phase that could be used effectively in LC, was silica gel. Then, in that year, Halasz and Sebastian (33) introduced the first of the bonded Phases. The bonded phase of Halasz and Sebastian contained silicon-oxygen-carbon linkages between the silica matrix and the bonded organic moiety and consequently was somewhat unstable. Nevertheless, their new concept was probably *one of the most important in the evolution of LC.* Today over ninety percent of all LC analyses are carried out on a bonded phase. In the following year Kirkland (34) described an alternative synthesis involving the use of chlorosilanes which linked the organic moiety to the silica by the more stable carbon-silicon bond. These types of bonded phases are the most reliable and easy to use stationary phases available today.

However, although different methods of solute retention were beginning to be exploited, and a number of sensitive and linear LC detectors were becoming available, there had been little improvement in column performance particularly in column efficiency. The average column, 25cm long, exhibited efficiences of only 200 to 300 theoretical plates. The first step forward was made by Horvath and Lipsky in 1967 (35)

with the introduction of their *pellicular* packings .These authors coated an ion exchange resin as a thin film onto the surface of tiny glass beads. This thin coating, as predicted by the Van Deemter equation, would reduce the resistance to mass transfer in the column and thus produce higher efficiencies. At the same time the relatively large beads they employed (*ca* 80μ) gave a high permeability to the bed and thus permitted longer columns to be used. This type of packing produced between 1000 and 2000 theoretical plates from a column one meter long. Although not very impressive when compared with the efficiencies of modern columns, it was a great step forward in 1967. It is ironic that, in the light of present knowledge, the pellicular columns of Lipsky and Horvath could probably have provided efficiencies of about 20,000 theoretical plates if the chromatographic system had been designed to have adequately low *extra column dispersion*. A column with *20,000 theoretical* plates would, indeed, have been *mind boggling* in 1967.

The Van Deemter equation predicted that a reduction in particle size should also decrease dispersion and increase column efficiency and, furthermore, this would also apply to LC columns. However, packed beds of very small particles have a high flow impedance and, consequently, would require high column inlet pressures to function satisfactorily. J. F. K. Huber and J. J. Kirkland were two of the first workers in the field to emphasize the need for high pressures if high column efficiencies were to be attained, a concept to which not every body agreed at first. Their insistance on the advantages of high pressure was, in due course, completely justified, but before this could happen a method of packing small particles into a column had to be developed. This was achieved by a slurry packing procedure developed by both Kirkland himself(36) and independently by Majors (37). So was born the term HPLC, *high pressure liquid chromatography*. Today, the interpretation of the term HPLC is more often taken to mean *high performance liquid chromatography* or sometimes more cynically *high priced liquid chromatography* but the original term referred to *pressure* and the concept pioneered by Huber and Kirkland. Thus, by the early seventies the

renaissance had produced a number of effective detectors, high pressure solvent systems, microparticulate columns and bonded phases. LC was beginning to match the performance of GC and, furthermore, successfully tackle separation problems that were impossible for GC.

The renaissance period ended in about the mid to late seventies with the onset of the association of LC with the computer, the mass spectrometer (LC/MS) and the introduction of microbore columns. The association of the liquid chromatograph with the computer, however, was more of a natural extension of the computer capabilities than an advance in LC. Not so, the association of the liquid chromatograph with the mass spectrometer. The improvement in column efficiency and phase selectivity had resulted in the separation of a number of hitherto, unresolvable mixtures disclosing an unexpected complexity of many natural substances and many new and unknown compounds. The need for the association of the liquid chromatograph with some procedure for solute identification immediately became apparent. The first tandem system to be investigated was LC/MS. It was quickly found that interfacing a liquid system to a mass spectrometer was far more difficult than that of a gas system as in GC/MS. The first successful LC/MS system was described by McLafferty *et al* (38) who employed the direct inlet system using the vaporized solvent as the chemical ionization agent. Chemical ionization spectra, however, are not so useful for the structural identification of unknown solutes as electron impact spectra. In an attempt to solve this problem Scott *et al* (29) utilized the transport system as an interface between a liquid chromatograph and a quadrupole mass spectrometer to provide electron impact spectra of the eluted solutes .Finally, near the end of the renaissance period, small bore columns were introduced by Scott and Kucera (39) who demonstrated that three quarters of a million theoretical plates could be achieved in LC and that more than a million plates was practically possible. The renaissance period ended in about the mid seventies and was indeed an exciting time for scientists developing LC. The advances in instrumentation, together with the basic understanding of the processes

involved, laid a solid foundation for the exploitation of the technique that would occur in the next decade, '*the golden years*'.

The Golden Years

The 'golden years' of LC extended from the mid seventies until the present day, although, in the last year or so, the gilt may have become a little tarnished. It has been a period of consolidation and exploitation both technically and commercially; a decade characterized by the introduction of sophisticated and elaborate instrumentation sometimes with a versatility that was often more bemusing than useful. The 'golden years' produced such devices as the computer controlled pump, the computer cotrolled solvent programmer providing complex gradients with up to four solvents and the diode array detector. The diode array detector proved to be an extremely useful detector and was developed, during the renaissance, by Talmi (40) and later extended by Grushka *et al* (41). The detector monitors the column eluent continuously over a range of different wavelengths. Consequently, as the complete spectra of any solute can be regenerated from the computer memory after development is complete, the detector is extremely valuable for solute identification and the determination of peak purity.

The introduction of very sophisticated computer data aquisition and processing systems was also a feature of the 'golden years'. Clever software that compensated for poor column resolution including peak skimming, peak deconvolution and other procedures ensured that the maximum information was obtained from each chromatogram. However, it is extremely doubtful whether any amount of brilliant mathmatical algorithms will ever be a satisfactory substitute for good chromatography. Similarly, the presentation of a chromatogram in "glorious technicolor" does nothing whatsoever to improve the accuracy or precision of the analysis. However, the prettier the toy the more fun to play with. Originally there was one chromatography symposium held every two years and there was sufficient research and development being carried out in the field to ensure that each had an exciting program filled

with papers describing original work. During the last decade there has been rapid increase in the number of symposia and now there can be as many as three or four international symposia in one year. Unfortunately, the amount of research and development has not increased to anything like the same extent, resulting in much repetition between the programs of the different symposia and the inclusion of work of limited interest or inferior standard.

The 'golden years' also contained much basic research on the mechanism of solute retention. Among the many contributors to this area of knowledge were C. G. Horvath, J. F. K. Huber, B. Karger, R. J. Laub, C. H. Lochmuller, J. H. Purnell, R. P. W. Scott and L. R. Snyder. These chromatographers worked assiduously to try to elucidate the nature of the molecular interactions that controlled the distribution of a solute between two phases and, consequently, its retention. There were a number of milestones passed during this period. Purnell and Laub(42) showed that the distribution coefficient or retention of a solute on a GC column, carrying a binary mixture as a stationary phase, was linearly related to the volume fraction of either component. Consequently, if the retention volume of a solute was known for each pure component of the stationary phase then the retention could be predicted for any binary mixture of the two phases. There was one caveat; there must be no association between the two stationary phases. Scott *et al* (43) showed a similar relationship for liquid-liquid distribution systems including bonded phases. Thus, the retention of a solute in LC could also be calculated for a binary solvent mixture from the volume fraction of the mixture and the retention of the solute on each of the pure solvents. There was again a caveat; any modification of the stationary phase, that could arise from the presence of the solvent, must be complete and there was no association between the solvent components. These authors also demonstrated that a methanol-water mixture, the mobile phase frequently used in reverse phase systems, was in fact a ternery system consisting of 'free water', 'free methanol' and 'associated methanol-water'. This misunderstanding had confused the interpretation of retention data from reverse phase systems

for some time. The exact nature of the surface of a bonded phase has always been somewhat enigmatical and, to some extent, still is. Karger, Lochmuller and Gilpin, among others, have examined the surface of bonded and reverse phases in many and varied ways. Lochmuller(44) was the first to demonstrate the interaction of the hydrocarbon moieties, with themselves in mobile phases of high water content and this was confirmed from thermodynamic measurements made by Gilpin(45). Although great strides have been made in the elucidation of the mechanism of solute retention, much remains to be done and future study will provide much challenge and interest for chromatographers and physical chemists in the future.

During the last decade, column theory and column technology has also advanced considerably. The major contributors in this area have been J. J. Van Deemter, J. C. Giddings, J. F. K. Huber and J. A. R. J Hullsman, G. J Kenedy and J. H. Knox, and Cs. Horvath and H. J. Lin. In particular the paper by J. H. Knox and M. Saleen (46), published in 1969 during the renaissance, laid down much of the foundations of modern column technology. However, their ideas and predictions had to wait until the late seventies and early eighties before they were confirmed. Today column theory is well advanced and it is possible to design high performance columns that cannot be constructed employing present day column packing technology. It can be calculated, for example, that for certain separations, particles of 2 micron or less are necessary for optimum performance (47). Unfortunately, to date, particles with diameters less than 2 microns can not be manufactured and even if they became available there is no known method to pack them. An example of the progress that has been made in column design is given in Figure 1, which shows the separation of a five component mixture containing p-xylene,anisole,nitrobenzene,acetophenone and dipropyl phthalate in less than 3.5 seconds (47). It was carried out on a column 2.5 cm. long, 2.6 mm in diameter packed with silica gel, particle diameter 3 micron, and a mobile phase of 2.2%w/w methyl acetate in n-pentane.

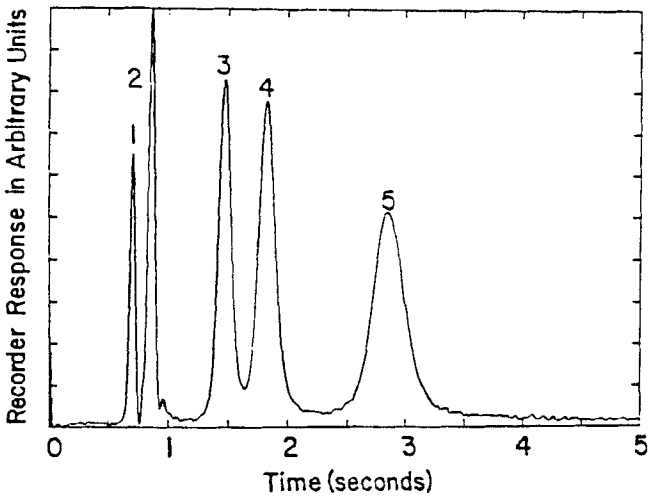

Figure 1
The Separation of a Five Component Mixture in 3.5 Seconds

This type of separation is, in general, too fast for normal LC analysis but illustrates the advanced level of development of column technology that has been achieved during the 'golden years'.

The advances in column technology, and the consequent separation of highly complex mixtures that often contained many compounds of unknown structure, provoked further active development of tandem chromatograph-spectrometer systems. A spray system for use with the transport LC/MS interface was developed by Hayes *et al* (48) that increased the amount of sample that entered the mass spectrometer and, consequently, its sensitivity. Fan *et al* (49) introduced another transport system that could produce either secondary ion mass spectra (SIMS) or laser desorption mass spectra (LDMS), both being extremely useful in producing spectra from very high molecular weight solutes.

Many attempts were made to link the liquid chromatograph with the nuclear magnetic resonance spectrometer (NMR) with varying degrees of success. The relative lack of sensitivity of the NMR instrument was a

serious drawback to this tandem system. Nevertheless, good progress has been made, the most successful being the system of Laude and Wilkens (50) who actually inserted a small bore column into the NMR magnet very close to the sample tube. This was achieved without seriously disturbing the homegenuity of the magnetic field. In this manner, increased sensitivity and good resolution was obtained and the spectra of column eluents could be monitored during chromatographic development. More sensitivity could be obtained, if required, by stopping the flow and capturing the solute peak in the NMR cell and accumulating a number of spectra by the Fourier Transform procedure

Some work has been carried out to improve the LC/IR combination, but the infra red spectrometer also has relatively poor sensitivity. The lack of sensitivity, coupled with the fact that the most useful LC solvents are opaque in the IR has rendered LC/IR the least successful tandem system. Nevertheless tandem systems, where a separation instrument is coupled to an indentification instrument, is becoming more and more desirable as the performance of LC continues to improve.

LC is now a well established, extremely important, analytical technique which, despite the recent heavy 'oversell' of capillary columns, has completely eclipsed GC in both versatility and popularity. In fact, a large number of analyses, previously carried out by GC, are now carried out by LC. It is likely to become the *primary analytical technique* in *environmental* work and is already the *chosen separation technique* in the field of *biotechnology*. However, LC is not the 'Philosophers Stone' of the analyst; there are still many separation problems it *cannot* solve and,from that point, we can ask, "How will LC change in the future?"

LC in the Future

Liquid chromatography is, today, in the same state of of maturity as GC was at the beginning of the sixties. Unless a completely novel concept is introduced (and this could happen at any time) future changes that are predictable, are likely to take the form of improvements and extensions of existing systems and devices. Advances in any analytical technique are

usually (but not always) provoked by *need*. Consequently, in order to look into the future and predict the changes that are likely to take place, it is necessary to identify those areas where contemporary LC is, in some way, inadequate or has shortcomings. One very obvious area, where there is already much research and development being carried out, is the separation of high molecular weight substances, those of prime importance being biological polymers. This will require the development of new phase systems, not only to provide improved selectivity for such compounds, but also acceptably 'gentle' environments so that the biological polymers of interest are not denatured. Many of the substances that exhibit interesting and useful biological activity are extremely labile and, therefore, need special mobile phases to maintain stability. Suitable stationary phases may require new packing techniques to be developed whereas, passive mobile phases may need novel or modified detecting devices. As more products evolve from biotechnological research and development, the new interest in *large scale* LC may rapidly grow. Chromatographic systems of a scale normally associated with chemical plant could become necessary, with columns three or four feet in diameter and twenty or thirty feet long, handling charges involving tens or maybe hundreds of kilos of material. Such a chromatographic plant would demand entirely new approaches to pump design, gradient systems, detectors, automatic controllers and even solvent recovery processes. An exciting area, indeed, for research and development.

Probably one of the greatest deficiencies in contemporary LC is a lack of an adequate theory that describes the mechanism of solute retention. This deficiency has rendered the prediction of the optimum phase system for the resolution of a given mixture virtually impossible or, at best, very approximate. There are a number of arbitrary equations that purport to optimise solvent systems to provide maximum resolution. They are, however, either satisfactory for only very simple mixtures or attempt to explain a separation that has already been optimized rather than predict the optimum conditions in the first place. Furthermore, existing equations usually employ capacity ratio (k') data as the retention

variable. Unfortunately the exact measurement of k' values is a subject of considerable disagreement and uncertainty at this time and, consequently, any theories based on experimental measurements of k' values, must be suspect. This uncertainty arises from the disagreement that is rife as to the best method of measuring the dead volume of a column to which the calculation of k' is extremely sensitive.

The development of a satisfactory theory to predict solute retention is difficult and it is exascerbated by the fact that the interaction of the solute with the two phases is not the sole factor that controls retention. Firstly, the mobile phase also interacts with the stationary phase (thus modifying the interactive properties of the stationary phase with respect to the solute) and, secondly, where the mobile phase is a mixture, the individual components interact with each other in competition with the solute. There is obviously a great opportunity for chromatographers and physical chemists to provide a real service to LC in the immediate future. It must be emphasized, however, that arbitrary curve fitting of experimental data to convenient equations must be abandoned. Experimental data must be tested against equations that can be derived for model systems that are feasible on sound physical chemical reasoning. Such an approach will allow the physical nature of the molecular interactions taking place in the chromatographic distribution system to be understood and, ultimately, the prediction of solute retention possible. Such work may, perhaps, not be within the 'realm' of the general chromatographer but more pertinent to the the activities of the physical chemist. Hopefully, active work in this area will be continued by such physical chemists as Laub, Lochmuller, Martire and Purnell and, perhaps, the challenge of this rather difficult study will incite others to become involved. Understanding the nature of solute-solvent interactions in general distribution systems will, in fact, have a much wider importance than the mere prediction of solute retention in LC.

This historical synopsis cannot be closed without some mention of the likely changes in LC instrumentation that will occur in the future. The market for both GC and LC instruments has changed, and again it is GC that

is pointing the way. The departure from the conventional attitude to instrument purchasing was probably evoked by the recession that occurred in the early 'eighties'. Capital funds were severely restricted during this period and the chromatographer found that it was not possible to buy the 'all singing, all dancing ' chromatograph that was the usual choice in the past. Funds were only available to buy instruments that would carry out the required analysis in an efficient manner, and nothing more. In fact, this sensible approach to the purchase of an instrument was well overdue in view of the relative maturity of the technique. After a fairly short period of time it was found that the new rationale was both technically satisfactory and economically attractive. It became clear that the extreme versatility of the expensive instrument was rarely used and was for most work, in the average laboratory, unnecessary. It was soon realized that low cost need not mean poor performance or unreliability; in fact the converse applied. Thus the chromatograph market changed and it was apparent that the era of expensive instrument exclusivity was passing. Nevertheless, the birth of the reliable, efficient low cost instrument was, to say the least, difficult and protracted. The three major pioneers of low cost instrumentation in the USA have been certain Japanese companies, Gow Mac and Hewlet Packard. Together, they invaded the GC instrument market to such an extent that the market share of the GC product groups of all the other major US instrument companies was jeopardised. Shares began to fall, 'cut-backs and 'lay-offs' began to take place throughout the industry usually under the euphamistic terms of 'streamlining, rationalization and reorganization for 'future growth'! The instrument industry was in trouble-it could not supply the instruments that the customers wanted. Now that the instrument market has become fairly large, it is fair to compare the disparity between the complexity of the modern car and that of the liquid chromatograph in the light of their approximately equivalent costs. As a result, the customer is beginning to expect more for the money or alternatively to pay less. The epitome of the reliable low cost gas chromatograph is probably the HP model 5890, and as a result,this particular instrument has proved

extremely popular. Nevertheless, progress towards low cost instruments is still slow despite the market pressures. Some companies, are finding the transition to low cost instrumentation difficult as it is often contrary to their established product concept. However, life is not always easy , progress is inexorable, as Tennyson said in his epic poem "The old order changeth yielding place to new....." and instrument companies must ultimately move with the market needs if they are to survive. It follows that the low cost chromatograph will, without doubt, be an important feature of future LC instrument development.

There still remain many exciting areas to be explored, and it is likely that the liquid chromatograph of the year 2027 will be as unrecognizable to us today as contemporary instruments would be to those who attended that LC symposium forty years ago. The inspiration of M.S.Tswett that has motivated so many of us for so long still endures and will continue to sustain the study and development of chromatography for many years to come.

References

1/ M.S.Tswett,Khromphylii Rastitelnom i Zhivotnom Mire ,Izd. Karbasnikov,Warsaw,191

2/ R.Willstatter and A. Stoll, Utersuchungenuber Chlorophy, Springer,Berlin 1913.

3/ R.Kuhn,A.Winterstein and E.Lederer,Hoppe-Seyler's Z. Physiol. Chem.197((1931)141.

4/ R.Kuhn and E. Lederer, Hoppe-Seyler's Z.Physiol. Chem. 200(1931)246

5/ R. Khun and H. Brockmann, Ber.64(1931)1859

6/ R.Kuhn and E. Lederer, Naturwissenschaften,19 (1931) 306

7/ A.J.P.Martin and R.L.M.Synge,Biochem.J.35 (1941)1358

8/ A.Tiseliusand D.Claesson, Arkiv. Kemi Mineral. Geol. 15b (No.18) (1942)

9/ A.J.P.Martin and S.S.Randall, Biochem.J.,49 (1951) 293

10/ A. T. James and A. J. P. Martin, Biochem.J., 48 (1951) vii

11/ A. T. James and A. J. P. Martin Biochem.J. 50 (1952) 679

12/ C.W.Munday and G.R.Primavesi, "Vapor Phase Chromatography 1956" (Ed.D.H. Desty) Butterworths Scientific Publications, London (1957)146
13/ A. J. Davies and J. K. Johnson, *ibid* 185
14/ R. P. W. Scott , *ibid* 131
15/ M. M. Wirth, *ibid* 154
16/ A. I. M. Keulemans *ibid* 15
17/ J. J. Van Deemter, F. J. Zuiderweg and A.Klinkenberg, Chem. Eng. Sci. 5(`956)271
18/ A. B. Littlewood, "Gas Chromatography 1958 ", (Ed. D. H. Desty) Butterworths Scientific Publications, London, (1958)23
19/ J. Bohemen and J. H. Purnell, *ibid*, 6
20/ M. J. E. Golay, *ibid*, 36
21/ I. G. McWilliam and R. A. Dewar, *ibid*, 142
22/ D. W. Grant, *ibid*, 153
23/ R. P. W. Scott, *ibid*, 189
24/ G. F. Harrison,P. Knight,R. P. Kelly and M. T. Heath, *ibid*, 2
25/ F.SA A. Vanderheuvel and J. C. Sipos Anal. Chem. 33(19621)21
26/ G. J. Claxton, J. Chromatog.2(1959)136
27/ A.T.James,J.R.Ravenhill and R.P.W.Scott,"Gas Chromatography 1964" , TheInstitute of Petroleum London, (1965)197
28/ R. P. W. Scott and J. G. Lawrence, J. Chromatog. Sci. 8(1970)65
29/ R. P. W. Scott, C. G. Scott,M. Munroe and J.Hess JR,"The Poisoned Patient : the role of the Laboratory" Elsevier,Holland , 155
30/ C. G. Horvath and S. R. Lipsky, Nature,,London,211(1966)748
31/ J. J. Kirkland, Anal.Chem.,40(1968)391
32/ L. R. Snyder,"Priciples of Adsorption Chromatography", Marcel Dekker,New York, 1967.
33/. I. Halasz and I. Sebastian.Angew Chem.Interat. Ed.,8(1969)453
34/ J. J. Kirkland and J. J. DeStefano,J. Chromato. Sci. 8(1970)309
35/ C.G. Horvath and S. R. Lipsky, Anal. Chem. 34 (1967)1422
36/ J. J. Kirkland, J. Chromatog. Sci.9(1971)206
37/ R. E. Majors, Anal. Chem. 44(1972)1722

38/M. A. Baldwin and F. W. McLafferty,Org.Mass Spec.7(1973)1111
39/ R. P. W. Scott and P. Kucera, J. Chromatog., 125(1976)251
40/ Y. Talmi, 47(1976)20
41/ M.J.Milano, S.Lam and E.Grushka,J. Chromatog.,125(1976)315
42/ R. J. Laub and J. H. Purnell, J. Chromatog.112(1975)35
43/ E. D. Katz,K. Ogan and R. P. W. Scott J.Chromatog. 352(1986)67
44/ C.H.Lochmuller and D.R.Wilder,J.Chromatog.Sci., 17(1979)574
45/ R. K. Gilpin and J. A. Squires, J.chromatog.,Sci.,
46/ J. H. Knox and M. Saleen,J. Chromatog. Sci.,7(1969)614
47/ E.D Katz and R.P.W.Scott, J. Chromatog. 253(1982)159
48/ M. J. Hayes, E. P. Lanksmayer, P. Vouroo and B. L. Karger, Anal. Chem.55(1983)1745
49/ T.P.Fan,E.D.Hardin, and M.L.Vestal,Anal.Chem.56(1984)1870
50/ D. L. Laude and C. L. Wilkens, AnAl. Chem> ,57(1985)1464