The ten most important advances or themes in separation science in the last 60 years (LCGC ChromSoc 60th Anniversary Magazine Survey)

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Which two of the following ten important advances of themes in separation science in the last 60 years do you feel have had the greatest impact?

1. Comprehensive multidimensional chromatography (LC and GC)
2. Metal pure spherical silica
3. Dual piston LC reciprocating pumps and gradient elution
4. Interfaces for hyphenation of LC and GC with MS detection
5. Enantioselective separations
6. Sub 2-micron particles and UHPLC pumping systems
7. Capillary GC columns
8. Reproducibly packed 1 mm i.d. LC columns and microflow pumping systems
9. Solid core particles (both early and more recent variants)
10. DNA sequencing using electrophoresis
11. Other

1. Comprehensive multidimensional chromatography (CMDC)

The development of chromatography initially utilised single columns, but there is an inherent limitation with this approach, in that the separation is driven by the selectivity of the column, and since this is the same throughout the column it will only separate certain types of compounds. Improving the efficiency of the separation will aid the separation, but ultimately the lack of selectivity will limit the ability of the column to resolve components. Using multiple columns by taking very small aliquots of eluant from the first column, refocussing the analytes and then effectively injecting this onto a second column which utilises a different form of chemistry greatly increases the resolving power, with the optimal peak capacity

So why then is so important, and although this approach does provide better selectivity do chromatographers actually need this level of resolution? To answer this question it is necessary to understand the nature of the samples that chromatographers analyse. The major use of separation science has been to monitor the separation of pharmaceutical compounds, whether that be within a biological specimen, within the environment or within a production environment. Initially the compounds that were predominantly analysed were small molecules, and the range of compounds that needed to be separated was limited, typically tens of compounds. However, the pharmaceutical companies have developed ever more complex drugs, with the latest genre of drugs being based on a protein structure. Consequently, the number of compounds that is require to be separated has increased from something that is very manageable with one mode of selectivity where 20 – 30 compounds are being investigated, to very complex samples where tens of thousands of components exist. It is simply not feasible to separate such complex mixtures with one separation mode, multiple modes are required. The use of comprehensive multidimensional chromatography opens up this world of very complex separations that are simply not achievable using any other approach.

The technology has been developed by the manufactures, initially for GC with a variety of innovative solutions being provided for the focussing of the eluting analytes from the first column onto the

second column. The most successful approach to focussing the eluting analytes from the first column before they are effectively injected onto the second column is to use a thermal modulation technique. In this approach part of the first is heated and cooled, a variety of solutions have been manufactured to ensure that this happens, including spray techniques and also use of a rotating heater.

One aspect that initial users of this technology found challenging was the interpretation of the data, since invariably, although there are two columns, only one detector was being used. The development of the appropriate software to allow the representation of the output from a comprehensive GCxGC chromatograph has also resulted in a better understanding of the requirements of the columns that are employed. Better orthogonality results in a better separation and scientists are developing the ideas on what is more orthoganol based on the interpretation of the data that is produced.

The developments of comprehensive 2D HPLC have not been so good, and there is still some way before manufacturers have developed a fully working solution, however some of the manufacturers have embraced this challenge and have been developing the hardware and the software to allow this to happen. Currently the approaches rely on the use of valve technology to store the samples either on trap columns or in sample loops, and both of these solutions are not as effective as the one developed for the GCxGC chromatograph. There is still much to do in developing the technology here, but many of the great separation scientists are looking at developing the technology that will eventually become common place with analytical laboratories. So although not quite a finished product in HPLC, the use in GC and the substantial potential that this technology offers for HPLC mean that this technology has gained a position in our list.

2. Metal pure spherical silica

At the heart of HPLC is the stationary phase, and without a doubt the most popular stationary phase has been based on a silica substrate. A range of other materials have been utilised, but silica is by far the most popular and the most widely used of the available substrates. The use of silica as a stationary phase arises due to its general compatibility with liquid chromatography. When developing an ideal stationary phase it is necessary to have a substrate that;

- Can be easily bonded to, allowing the development of a range of different stationary phases
- That has a degree of mechanical stability
- That has limited chemical reactivity
- That can be easily modified to allow for a range of active stationary phases.
- Has a large surface area to allow for retention of analytes
- Is financially viable

Silica matches these criteria and the development of chromatography has very much been dependent on the development of this material.

The initial forms of silica where irregular particles, with a large degree of heterogeneity between particles and indeed in the surface chemistry of the silica. The development of the Stöber process (Stöber *et. al. Controlled growth of monodisperse silica spheres in the micron size range*, *J. Coll. Int.* Sci. **1968**, 26 (1), 62–69) resulted in the formation of spherical particles of a much more uniform size, which greatly increased the efficiency of the columns. The Stöber process is a polymerisation of a silicate monomer that results in the formation of a solgel. A solgel is a colloidal solution (sol) that

acts as the precursor for an integrated network (or gel) of either discrete particles or network polymers.

The Stöber process in itself produces reasonably regular spherical particles, however, the surface area associated with the spherical particles is limited, and it is the ability to produce spherical porous particles that has the most benefit to separation scientists. The surfactant molecules form micelles, small near-spherical balls with a hydrophobic interior and a hydrophilic surface, around which the silica network grows producing particles with surfactant- and solvent-filled channels. This creates a gel substance which can be solidified by heating at an elevated temperature. This process called calcining, also leads to removal of the surfactant and solvent molecules by combustion and/or evaporation, leaving mesopore voids throughout the structure.

As chromatographers became more aware of the effect of the silica substrate in the separation process, so greater effort was applied to the synthesis of the particle. Initial efforts concentrated on the production of a purer silica particle as it was observed that impurity metal ions from the original precursor caused increased secondary interactions with basic compounds. Unfortunately, this was not reproducible and so not only did the high metal concentrations result in more acidic forms of silica which caused peak tailing, but also the variability meant that separation scientists did not know how the columns would perform. The work by Tanaka (N. Tanaka *J. Chromatogr. Sci.* **1989**, 27 (12), 721-728) and other academics has highlighted this issue, and allowed for the production of very high purity silicas, which give less substrate interactions, but more importantly are much more reproducible to manufacture. Thus for liquid chromatography, silica is fundamental and has to be on any shortlist for significant developments in chromatography.

3. Dual piston reciprocating pump and gradient elution

In liquid chromatography the delivery of the mobile phase at a constant rate through the column is essential to avoid peak broadening effects. This was effectively achieved through the use of dual reciprocating pumps. In today's modern laboratories the use of these types of pumps is considered standard and most users will not be aware of the technology that is incorporated into these pumps to ensure that the smooth delivery of the mobile phase is maintained.

The delivery of a constant flow of liquid can be achieved through the use of a syringe pump, however this has the disadvantage that it relies on a single piston drive pushing liquid through the column. The larger the volume of the piston chamber, the less stable will be the flow rate, due to the nature of the electric motor which drives the piston via a screw thread. This can be overcome by having a smaller chamber where the liquid is stored but this creates an issue on the volume of liquid that can then be used for the chromatographic analysis. The solution to this is to use two reciprocating pumps, both of which can have a small displacement volume which allows for the stable and accurate delivery of the mobile phase by switching which pump is delivering the mobile phase. The design of the reciprocating pump is similar for all manufacturers, and uses a piston driven by a rotating cam. Seals alongside the piston ensure that liquid does not leak from the pump. The use of non-return (check) valves ensures that this is feasible, so as the pump is pushing liquid the outlet check valve is in a position such that liquid can flow through it, whereas the inlet check valve will not allow liquid to pass. When the pump switches to the fill phase, the check valves move to their alternative positions. This can be done using small spherical moveable spheres, which are moved either electronically or by the motion of the liquid from the two positions.

There are two designs which manufacturers have developed; the first was to use two pumps that work in parallel, thus one pump will be filling whilst the other pump is delivering the mobile phase to

the column. The other design is to have the liquid flowing through both reciprocating pumps, with the primary pump being used to deliver the flow, and once the piston has reached the end of its stroke, the second pump then delivers mobile phase, whilst the primary pump refills with the check valve between the first and second pump becoming active to stop the mobile phase being forced through the first pump.

Both designs suffer from the change between the flow being driven from the two reciprocating pumps. At this point there is in general a perturbation in the flow rate, and manufacturers have looked to address this in a variety of manners, either by the introduction of a pulse dampener reservoir located after the pump which utilises a large volume to even out the pressure ripple effect. The other approach is to use complicated algorithms to determine when the when the drive cams should be in operation. The latter approach is favoured as it reduces the delay volume of the solvent before it reaches the column.

The use of a dual reciprocating pump allows for isocratic flow deliveries, however it does not allow for the mobile phase composition to change during a chromatographic run. For modern chromatographers, where reducing analysis times and analysing a wide range of compounds with differing physiochemical properties, this is important as it can dramatically reduce the analysis times, as it allows compound which may have substantially different isocratic elution times to be chromatographed in a single run by varying the elutropic strength of the mobile phase.

Two approaches are employed to allow the generation of a gradient. One approach requires coupling two dual reciprocating pumps together and mixing the eluants from both to allow different compositions of mobile phase to be generated. By having the pumps delivering different flow rates at different times during the chromatographic run will allow for the production of a gradient. The other approach uses a single dual reciprocating pump and alters the mobile phase composition by the use of a proportioning valve prior to the pump, switching source of the mobile phase dependant on the requirements of the user.

In HPLC the use of gradients and dual reciprocating pumps is common place, and it is a testament to the development by the manufacturers that this technology is deemed to be commonplace and as a consequence of this; gradient and dual reciprocating pumps have earned a slot on our list.

4. Interfaces for hyphenation of LC and GC with MS

Within analytical chemistry there are two major techniques that dominate the industry. One is chromatography and the other is mass spectrometry. Both techniques individually are very powerful, but the combination of chromatography and mass spectrometry offers the analytical scientists the ability to quantify and identify in a single analysis. The coupling of these two techniques has seen some substantial challenges primarily due to the mechanisms by which both operate. Chromatography is performed at atmospheric conditions, or certainly the pressure at which the eluant flows from the end of the column is at atmospheric pressure, whereas with mass spectrometry, the pressure is very low, typically 10^9 lower than the atmospheric pressure observed at the outlet of the chromatography system. Another factor is that in chromatography the compounds of interest are substantially diluted by the mobile phase, which would interfere with the analysis in mass spectrometry and so it has to be removed before the analyte can be interrogated by the mass spectrometer.

Mass spectrometry is a technique that allows for the separation of a series of charged species based on their mass to charge ratio. As has already been stated it requires very low pressure to operate effectively and ideally it is suited to analysing one compound at any point in time. It is an incredibly

powerful analytical technique as it allows for some structural determination of the analyte and it can also be effectively tuned to a single compound, meaning that it can be highly selective but for a broad range of compounds. It is limited in that the compound does have to be ionisable, however many of the compounds that are analysed using chromatographic techniques are ionisable and so this would suggest a good combination.

The first successful coupling of mass spectrometry to chromatography was achieved when a gas chromatograph was coupled to a MS. This is relatively easy to achieve due to the difference in the physical properties of the carrier gas and most of the analytes. Helium is substantially lighter than the components that are being analysed and so it is relatively easy to isolate in the mass spectrometer. The use of vacuum pumps removes the gaseous eluant from the GC, and since it is a gas it is relatively easy to remove. An electron impact (or a chemical ionisation) ionisation source at the exit of the GC column ensures that the analyte molecules are charged allowing for the mass spectrometer to differentiate based on the mass to charge ratio of the eluting components.

The use of a mass spectrometer as the detector in gas chromatography was developed during the 1950s (A.T. James & A.J. Martin, *The Biochemical Journal* **1952** 50 (5), 679–90). These comparatively sensitive devices were originally limited to laboratory settings, although portable GC-MS's are now available and routine used in a variety of industries including environmental, and homeland security. The development of affordable and miniaturized computers has helped in the simplification of the use of this instrument, as well as allowed great improvements in the amount of time it takes to analyse a sample.

The combination of liquid chromatography and mass spectrometry was not so smooth, since it is much harder to remove a liquid mobile phase than it is a carrier gas. At very low pressures the mobile phase will evaporate converting to a gas, with a very large increase in volume. This volume is substantially greater than the volume that is observed with the carrier gas used in GC. There were several approaches that were initially developed, including;

- Moving belt and particle beam interface which relied on the selective vaporisation of the elution solvent.
- Direct liquid introduction and continuous flow fast atom bombardment rely on reducing the flow of the liquid to the interface either through the use of flow splitters, or by using capillary columns to about 5 μL/min.

These approaches were not so successful and it was not until the Nobel laureate John Fenn (M. Yamashita & J.B. Fenn, *J. Phys. Chem*., **1984**, 88, 4451) developed electrospray ionisation. Electrospray and heated electrospray technologies are based on applying a charge to the mobile phase as it enters an atmospheric chamber prior to being moved into the mass spectrometer. The charge will remain with molecules that are able to accept the charge. The use of a nebuliser gas aids desolvation (removal of the mobile phase), as does the use of a heated drying gas, typically nitrogen.

The subsequent commercialisation of this process has seen a dramatic increase in the use of HPLC-MS, with very few laboratories not having access to this technology. Today LC-MS is used to test a variety of different types of samples, including forensics, pharmaceuticals, environmental, petrochemical, food and clinical. It is seen as one of the major analytical technologies for a laboratory to have, and with the introduction of cheaper and easier to use instrumentation being released this technology is becoming accessible to a whole new generation of analytical scientists and no longer the preserve of a specialised few.

The coupling of the two great analytical technologies developed in the last century, mass spectrometry and chromatography has seen a quantum change in the types of analysis that are being performed, and as a consequence rightly has a place on our list.

5. Enantioselective separations

On a molecular level, within organic chemistry there are many different arrangements of the atoms that can result, and molecules that are nominally the same in terms of their constituent atoms are referred to as isomers. In many cases isomeric compounds will have different physical and chemical properties, which mean that from the separation scientist they do not present too great a challenge to separate. However, there is a subset of isomers which relates to compounds that are structurally very similar and only differ because they are mirror images of each other, these are referred to as enantiomeric compounds. Enantiomers have the same chemical properties and so present a much greater challenge for a separation scientist, as the separation has to be based on a spatial recognition process and not just chemistry. Within biology, nature often uses just one form of an enantiomer, amino acids are classic examples, where only one of the chiral forms of the molecules exist in Nature. The pharmaceutical industry has also discovered that having an enantiomerically pure substance can have substantial benefits in terms of efficacy and also in terms of safety, the often cited and unfortunate case is that of the drug, thalidomide, where only one form of the racemic mixture was efficacious and safe to use. In 2005, approximately two thirds of all drugs manufactured were chiral.

In order to ensure the purity of a racemic mixture it is necessary to perform a chiral separation. There are three approaches that can be employed;

- Modify the original molecules to form diastereomers
- Use a chiral additive in the mobile phase and separate the resulting diastereomers with a non-chiral column
- Use a chiral stationary phase (CSP)

There are a range of chiral stationary phases that are available and these have historically been classified into 5 categories by I. Wainer *TrAC* **1987**, (5), 6.

- Type I – the solute forms a complex with the CSP through 3 distinctive points of attractive interactions such as hydrogen bonding, dipole stacking and pi-pi bonding. The Pirkle type phases are good examples of this type of phase.
- Type II – the primary mechanism is the formation of a solute-CSP complex, through attractive interactios, but where inclusion complexes also lay an important role. The wide range of derivatised polysaccharide phases are examples of this type of category.
- Type III - Cavity phases in which the solute forms an inclusion complex with the CSP, example phases include modified cyclodextrins, crown ethers
- Type IV – the solute forms a metal diastereomeric complex. This approach is also known as chiral ligand exchange chromatography.
- Type V - Protein phases. The exact mechanism is not well understood, but since naturally occurring proteins are made from amino acids which can have chirality associated with them, it is suggested that this may be a driving factor in the separation.

It was suggested that the mechanism of chiral separations was based on three distinct points of interactions which would allow for a discriminative effect of the stationary phase. These models were first developed by Easson and Stedman as early as 1933 (L. H. Easson, E. Stedman *Biochem. J.*

1933, 27, 1257), but this work has been developed further and has also been incorporated into molecular modelling studies, (K. B. Lipkowitz B. Baker & R. Zegarra *J. Comp. Chem.* **1989**, 10 (5), 718-732).

Without the understanding and the application of the stationary phases, many of the drugs developed up to 2010 could not have been used safely, and so the importance of chiral separations can never be underestimated.

6. Sub 2-micron particles and UHPLC pumping systems

In the late 1990's and early 2000's, chromatographers were focused on approaches to improve resolution through increases in LC particle efficiency. The most obvious way to increase efficiency (without dramatically increasing analysis time through the use of longer columns) was to reduce particle size. Some companies such as Micra Scientific produced solid particles of approx. 1.5 micron in the early 1990's, but commercially, these were not very successful because the back pressure generated by these particles was extremely high, sample loading was limited and LC instrumentation with suitably low extra column dispersion to fully utilise these wasn't available at that time.

Around the beginning of the millennium, Agilent launched the first commercial fully-porous sub 2-micron (1.8) particle for liquid chromatography called their 'rapid resolution' line, but lacked the instrumental hardware to exploit these fully.

In 1997, Jim Jorgenson's group at the University of Carolina had published a seminal paper on ultrahigh pressure liquid chromatography (J.E. McNair *et. al. Anal. Chem.*, **1997**, *69* (6), 983–989). Waters Corporation, realizing the potential of this technology worked with Jorgenson's group to commercialise a 1000 bar (~15000 psi) pumping system which would allow the use of sub-2 micron particles at their optimal linear velocity. This instrument was released in 2004 and trademarked as 'UPLC' – ultra performance liquid chromatography. Other companies quickly followed suit including Jasco (with their X-LC) and Agilent (via their 1200 SL series of instruments which exploited higher temperatures to allow sub 2- micron particles to be used on these 600 bar systems). Nowadays, every major LC instrument company has at least one LC system capable of pumping liquids at 1000 bar or greater. These systems are fully optimised for these particles with extremely small extra column band broadening credentials to ensure best performance is obtained from the particles.

The coupling of sub-2 micron particles and 1000 bar pumping systems created a revolution in chromatography. Increases in chromatographic efficiency and the ability to dramatically shorten analysis times (and also reduce solvent usage) has played an important role in the development of chromatography. Undoubtedly, analysts working with complex mixtures such as biological fluids have benefitted most from these technologies, but every laboratory and industry utilising liquid chromatography will have been impacted by these developments and fully deserves to be on our list.

7. Capillary GC columns

Gas chromatography is considered a mature technique having been established in routine use long before liquid chromatography. Modern GC was invented in 1952 by Nobel winner Martin and James (A.T. James & A.J.P. Martin, *Biochem. J.* **1952**, *50*, 679). Griffin and George Ltd in the UK were considered to have manufactured the first commercial GC system in 1954, and several companies,

including Perkin Elmer, Fisher/Gulf, Barber Coleman, Podbelniak and Pye Unicam, produced similar commercial instruments over the next two years.

Chromatographic columns at that time were made of stainless steel, 1 to 5 meters in length and 1 to 5 mm internal diameter and packed with a variety of derivatised or coated particle types. Although these columns had a high sample capacity and were cheap, the resolution of packed columns was and still is limited by their length and the pressure drop resulting from gas flow resistance. Martin in 1956 proposed the poor efficiency of the columns could be overcome by the use of long wall-derivitised capillary columns and these were subsequently developed by M.J.E. Golay in 1957. In 1959, future Martin Medal winner Ray Scott demonstrated the effective use of nylon capillary columns. He coated these with squalane and dinonyl phthalate and could be coated onto extremely long lengths demonstrating very high peak efficiencies for petroleum hydrocarbons (R.P.W Scott *Nature* **1959**, *183*, 1753 – 1754),

Also in 1959, Dennis Desty from British Petroleum (and also a founder of The Chromatographic Society) developed a glass drawing machine to make coiled glass capillary columns. Glass could be made more inert than stainless steel, but it was not easy to manufacture. Glass capillary's also had a number of physical issues. The glass was not stable in very high and low temperatures, was active towards highly polar analytes leading to poor recovery and peaks shapes and the columns were difficult to coat with an even film of stationary phase. However, over the next 20 years capillary columns became firmly established with many stationary phases being developed by a plethora of vendors.

In 1979, the invention of the flexible quartz capillary tubes by Dandeneau and Zerenner of Hewlett Packard (now Agilent) was an evolutionary step to solve of these issues, including many issues regarding the physical shape and connectivity of the columns. Some initial teething problems were experienced in coating these columns, but these were soon overcome and today, most stationary phases can be coated as thin films on the walls of quartz capillaries (R.D. Dandeneau & E.H Zerenner, *J. Sep. Sci*., **1979**, *2(6)*, 351-356). Today, the majority of all capillary columns are thin film high-quality fused silica.

Capillary GC columns were probably the most significant advance in the history of GC and have seen many iterations and developments to arrive at the columns we use today. If this capillary format had not been developed, we simply would not be able to analyse the complex samples we find in the petrochemical, environmental monitoring or fragrance industries to name a few and is highly worthy of being on this shortlist.

8. Reproducibly packed 1 mm i.d. LC columns and microflow pumping systems

It was Professor Pat Sandra (University of Ghent) at our 'Advances in Microcolumn Separations' meeting in London earlier this year that suggested that 1 mm columns should be on this list of most important. Reduced diameter LC columns have been available for many years, and much of the early work in understanding chromatography was performed on for example 1 mm internal diameter columns. The reasons for this are more likely to be practical in so much as less material is required to pack a small i.d. column than a larger one, but much research has gone into their development.

With the advent of biological sciences, the need for LC techniques which provide high resolution and high sensitivity while requiring only nanolitre sample volumes was very important. Additionally, mobile phase use is significantly reduced in capillary formats and offers the direct coupling to many detection techniques. Historically microcolumns were claimed to provide poorer performance than larger internal diameter columns which was considered to be attributable to three factors, (i) limited experience in packing small i.d. columns, (ii) unsuitable instrumentation for achieving best performance with these columns and (iii) significant wall effects (M. Novotny *Anal. Chem.* **1981**, *53 (12)*, 1294A-1308A). Even with these limitations, chromatographic column efficiencies of 10^5-10^6 could still being generated, but only on very long (14 m!), 1 mm i.d. columns (R.P.W Scott & P. Kucera *J. Chromatogr.* **1979**, *186*, 475-481).

Over recent time, great strides have been made in the packing of microbore columns. Many aspects of packing have been researched and optimized such as packing bomb volume, slurry density, packing solvent (e.g. J.P.C. Vissers *et. al. Anal. Chem*. **1995**, *67*, 2103-2109). Obviously commercial organisations have also focused great resource on addressing these challenges. As a result of the commercial sensitivity of many these approaches, although there have been great strides in improving the packing performance of narrow bore columns, the approach taken is difficult to determine. However, in brief summary, there are several physical properties of the solvent and the stationary phase that need to be considered, including;

- Charge/zeta potential of the solvent and stationary phase
- Compressibility of the substrate particle, with larger pore materials this is very prominent
- Viscosity of the fluid being used to pack, as well as different solvents the use of temperature can be interesting here
- The different solvents that can be used during different parts of the packing processes (initial transfer of the particles to the column hardware, a bed compression solvent, decompression solvent, etc.)
- Sonication and vibrational effects (frequency and energy and location of vibrating source)

Manufacturers guard the packing process very carefully, and even within these organisations there are very few people who have access to the 'secret' recipes that are developed to ensure optimum performance. These recipes will consider not only the packing process but also the stability of the particle to undergo the pressures observed during packing. In general the pressures that are used in packing the columns are more extreme than those that separation scientists would employ and so manufacturers have looked at developing stronger particles, either through the use of hybrid silicas, or by careful synthesis of the pore structure.

Alongside this, there has been major progress in the development of microflow LC instrumentation. Historically, instrumentation aligned with 1 mm or smaller i.d. capillary columns were essentially standard HPLC's with an external capillary flow splitter and occasionally a modified injector. The use of this configuration of instrument dramatically increases system volume which contributes significantly to extra column band broadening (and therefore resolution) and gradient delay. Today's microflow instruments are largely based on UHPLC technology and the accurate pumping systems they now possess can deliver precise gradients at very low flow rates.

Biological sciences are progressing towards a time when the column efficiencies generated by sub. 1 mm i.d. columns only will be sufficient to analyse these complex samples and provide the insight required to progress this branch of science. Without the pioneering work already undertaken, this would simply not be possible.

9 Solid core particles (both early and more recent variants)

Solid core particles (also known as pellicular, superficially porous or fused core particles) were first developed as polystyrene-benzyl-dimethylammonium derivatised 50- micron particle diameter solid glass bead ion-exchange materials in 1967 for the separation of phosphate nucleotides by Horvath and co-workers (C.G. Horvath *et. al. Anal. Chem.* **1967**, *39 (12)*, 1422-1428). As readers will be aware, by reducing the diffusional path length of the analyte in the particle, mass transfer is enhanced and reduced plate heights are observed. The state of the art in chromatography for nucleotides at that time was the use of column chromatography and analysis times of 20 hours or greater were not unheard of. In Horvath's work, this was reduced to ca. 90 minutes with a number of additional benefits such as the non-shrinking or swelling of the particles which also improved performance compared to the more commonly used polymeric particle methods.

Seeing the obvious benefits of this technology, Dr J. Jack Kirkland, then of DuPont commercialised the use of solid-core materials under the brand name Zipax in 1968 which were 20 to ~40 micron in diameter. The technology developed incrementally over the years – principally in phase types, but had largely been superseded by sub 10 micron fully porous silica particles (which Kirkland was also at the development forefront). However, in 1992, Kirkland resurrected the particles through the development of 7 micron solid-core wide pore material at Rockland Technologies (now part of Agilent) offering significantly enhanced performance over any other solid-core material up to that time. However, it is possibly the more recent incarnations of this type of material that younger chromatographers are more familiar with. In 2006, Kirkland, now working at his new company Advanced Materials Technology developed the 2.7 micron superficially porous particle which offered UHPLC like performance, but due to the larger particle size (and therefore lower backpressure) could be used on regular 400 bar HPLC instruments. This approach rapidly became very popular, particularly in laboratories who couldn't afford the transition to expensive UHPLC instruments (although best performance is still found when using these particles with UHPLC systems). While the reasons for enhanced performance are not completely understood, it is likely that all terms in the van Deemter equation are improved, and there are possibly additional parameters which have not been fully defined. Many other column manufactures have now followed suit, some offering high pH stable variants of this technology with wide application over small, medium (peptides, oligonucelotides) and large (mAbs, proteins).

Undoubtedly, the next developments in LC particle technology will be based on solid-core technology. Historically, the technology has been pivotal in the development of large biomolecule separations, but will undoubtedly be at the forefront of many additional developments in the future.

10 DNA sequencing using electrophoresis

DNA sequencing is the process of determining the order of nucleotides within a DNA molecule. Knowledge of DNA sequences has become indispensable for basic biological research, and in numerous applied fields such as medical diagnosis, biotechnology, forensic biology and virology. The advent of rapid sequencing methods has greatly accelerated the sequencing of complete DNA sequences, or genomes of numerous types and species of life, including the human genome and other complete DNA sequences of many animal, plant, and microbial species.

The first DNA sequences were obtained in the early 1970s by academic researchers using laborious methods based on two-dimensional chromatography. Following the development of fluorescence-based sequencing methods with a DNA sequencer, DNA sequencing has become easier and orders of magnitude faster.

The first automated DNA sequencer, the AB370A co-invented by Professor Lloyd Smith (who was then post-docing at CalTech with Dr Leroy Hood – see L.M. Smith *et. al. Nucleic Acids Research* **1985,** 13, 2399-2412 and L.M. Smith *et. al. Nature* **1986**, 321, 674-679), was introduced by Applied Biosystems in 1987 (whom Smith was also a consultant for). This first generation of DNA sequencers employing Sanger sequencing were automated electrophoresis systems that detect the migration of labelled DNA fragments. This was performed with fluorescent dideoxy nucleotides and polyacrylamide gel sandwiched between glass plates which also incorporated the thermocycling amplification of the DNA fragments. The AB370A was able to sequence 96 samples simultaneously, 500 kilobases per day, and read lengths up to 600 bases. The next major advance was the release in 1995 of the AB310 which utilised a linear polymer in a capillary in place of the slab gel for DNA strand separation by electrophoresis. These techniques formed the base for the completion of the human genome project in 2001.

Since then, several new methods for DNA sequencing (NGS – next generation sequencing) were developed in the mid to late 1990s and have been implemented in numerous commercial DNA sequencers since that time.

The advent of genome sequencing has undoubtedly provided the keys to understanding biology at a molecular level which will unlock many important developments in medical treatments in the future.

11 Other

The above list is not exhaustive and after much debate, we still needed to leave many major developments out of our top ten. Other topics discussed included axial compression columns in preparative chromatography, the GC FID or ECD detectors, chemically bonded LC stationary phases, gradient elution LC pumps amongst many more. Please let us know if you think other chromatographic developments should have been on the list!