CHROMCOM

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ChromSoc
The Chromatographic Society - founded 1956



In memory of John Knox

$$h = Av^{\frac{1}{3}} + \frac{B}{v} + Cv$$

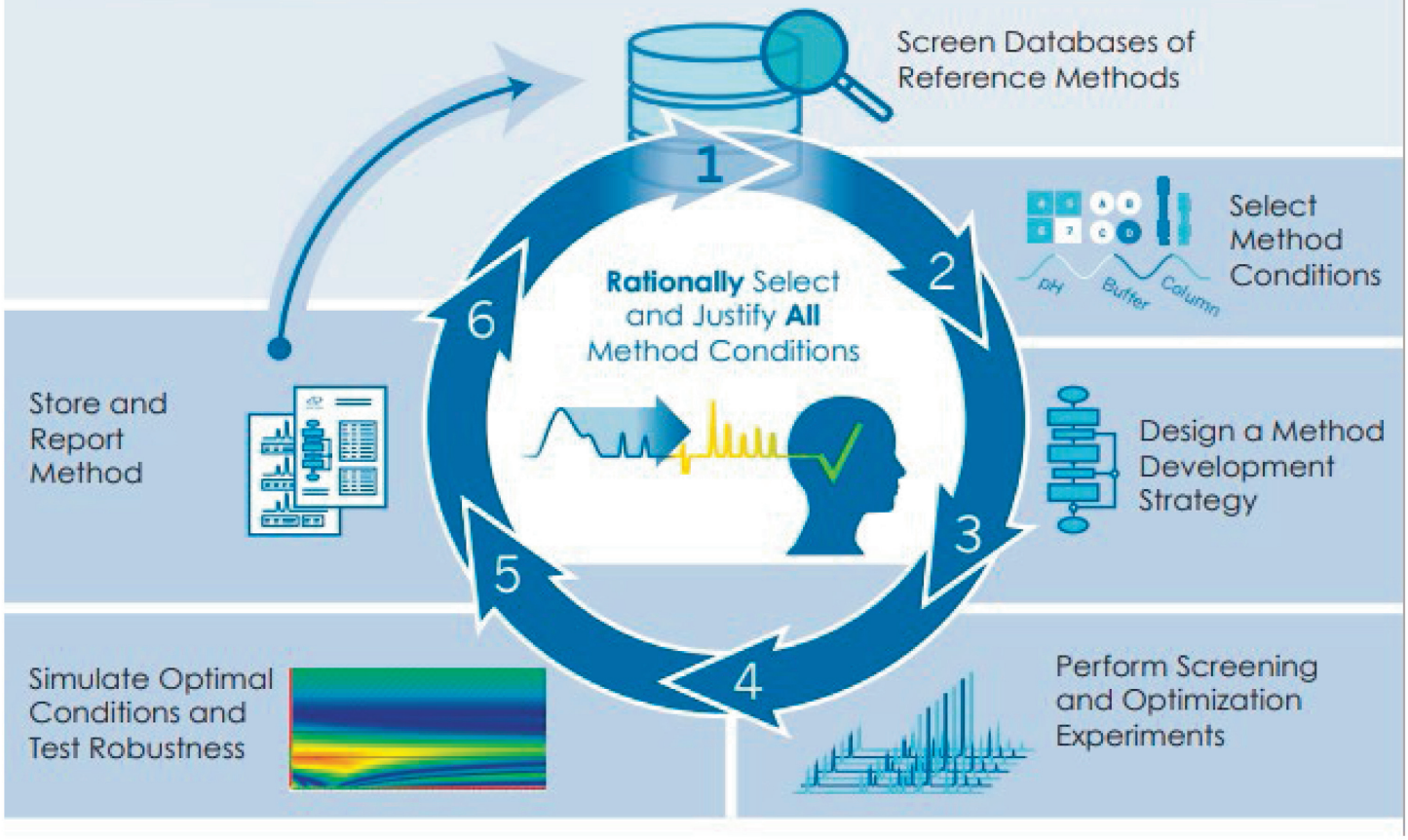
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EDITOR SAYS

Welcome to the sixth edition of ChromCom, a newsletter dedicated to supporting the work of the Chromatographic Society and chromatographers. The ChromCom focusses on fundamental aspects of separation science, which we try to foster in our members through trouble shooting articles as well as primers on the basic theory on which chromatography is developed. In addition, we publish primary data to demonstrate the ongoing state of the art in the field of separations. To support our members' development, we publish chromatographic news and conference reports which highlight major events in both the British and international chromatographic communities.

I regret the delay in the publishing of this issue, COVID19 has taken a toll on all facets of life for all our committee and members. However, we remain committed to providing inciteful articles and updates to our members. Especially considering the dramatic reorganisation in working conditions that most of us are adapting to.

In this edition we pay tribute to Professor John Knox who unfortunately passed in 2018, aged 90,



and look back on the impact that has had on the world of chromatography. Articles include the applications of temperature on LC separations and the use of concentration vs mass sensitive detectors. Our research article focuses on the Hypercarb stationary phase which was developed by John.

Due to the large delay during COVID-19 we will be publishing a follow-up article shortly after to update members on the meetings the ChromSoc has arranged in the past year.

Daniel

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PRESIDENT SAYS...

Firstly, I would like to send a warm welcome to all our members for the sixth edition of ChromCom. Since its inception we have seen a wide variety of topics discussed, but we have very rarely focussed on the work of one individual. This edition will be different and will reflect the impact of the great **Professor John Knox** who sadly passed away at the end of 2018.

His contributions to the understanding of the dispersion processes within a chromatographic column was pivotal. Additionally, he was also responsible for the development of a purely carbonaceous LC stationary phase. Not only was his scientific contribution of the highest order, it is very evident from the numerous kind words that he was a much-loved character -which is a testament to his quiet undemanding personality.

The committee has been busy, as always, during 2019. Unfortunately, with the onset of COVID-19, the events of 2020 has been deferred until next year. A very successful meeting in May 2019 was jointly organised with the RSC North East Analytical Division at the Stadium of light with the focus on **Separation Science: Driving Pharmaceutical Development and Manufacture**, principally organised by **Dr John Lough**. Highlights of the meeting included a presentation on the application of SFC in routine pharmaceutical analysis delivered by **Francesca Hollis Kirk**, from Pfizer and an excellent presentation on the implementation of PAT delivered by **Irene Areri** from GSK. It is always a pleasure to see new, young speakers featuring at our meetings.

The Reid sub-committee organised another engaging **Reid International Bioforum** meeting in September, looking at a range of activities associated with bioanalysis. The Reid continues to grow in stature, but it still holds true to the ethos that has been developed over many decades. The level of delegate engagement is immense, both within the lecture hall but also at the social activities as well. I am not too sure if the karaoke will continue to feature in future Reid meetings! However, I am confident that the organising committee will continue to engage and attract a range of very gifted bioanalytical scientists to deliver updates on their work. Of particular note was a fascinating presentation by Reid Committee member **Dr Richard Houghton**, titled '*Working with the MHRA when it all goes horribly wrong – a case study*'. It truly was a journey through the worst nightmares of a GLP study.

The final meeting of 2019 was organised by **Dr Chris Bevan** and focused on the **Recent Advances in Hyphenated Separations** which took place at the Syngenta site in October. This was Chris's last meeting, and I would like to take this opportunity to thank Chris and **Alan Handley**, both former presidents of the Society for their continued support and guidance. Their advice and direction over many years has put the Society in a very strong position and I am eternally grateful to both of them for their many words of wisdom, as I found my feet in this role. I wish them every success for the future and hope that they will not be a stranger to the Society going forward.

As well as the meetings, the website has seen a gradual upgrade which will make things easier for members of the society to find out about our activities. I encourage all members to try out the site and give feedback on how we can make it better. The website contains information of how to apply for bursaries. I encourage all ChromSoc members to advertise the bursaries and summer studentships, for student membership at only £10 a year, it is an incredible opportunity to attend national and international separation science events or be sponsored for work over the summer period.

The website also features previous medal winners as well as being the best way to get updated on our meetings schedule in 2021. We thank **Karen Rome** for her dedication in this role and welcome **Dan Carrier** as her able successor.

The Society has also been busy with bursaries, with one delegate being awarded the **John Dolphin** to attend HPLC 2019 in Milan and two Grass Roots IV delegates being awarded student bursaries.

Finally, the next instalment of the Grass Roots series of training events was completed at the start of October. **GRIV** was very tiring for the trainers **Tony Taylor**, **Ken Cook**, **Paul Ferguson** and myself and delegates, however the enjoyment had by all was palpable. The theme for the course was an *Introduction to biopharmaceutical analysis*. There were many highlights both from the lectures, but also from the walking and entertainment in the evenings. I am continuously amazed how a group of disparate separation scientists can gel so quickly over such a relatively short period of time, and how the relationships developed during the training continue well after the events. It is also evident that these courses have a positive impact on the delegates who are increasingly attending other ChromSoc events and even presenting.

And so, to this edition of ChromCom, dedicated to the great Professor Knox. This edition starts with a eulogy to Professor Knox from **Peter Myers** a close friend and colleague of the great man and an authority in HPLC. The back to basics discusses a particular interest of Professor Knox's, that of high temperature liquid chromatography. It is much underutilised and misunderstood, and this back to basics looks to address some of these misconceptions. The troubleshooting article looks at the difference between mass and concentration dependent detectors and the importance of understanding the impact that flow can have on sensitivity. Finally, we wrap this edition up with an article from **Dr John Lough** on just one of the diverse range of applications to which the highly versatile Hypercarb stationary phase may be used. The phase was developed by Professor Knox and subsequently commercialised.

The last 17 months have been a busy time for the Society. I am continuously impressed with the levels of enthusiasm that the organising committee members bring to the meetings. This is only matched by the enthusiasm of the delegates and vendors that attend these and other ChromSoc meetings. Even during the COVID-19 pandemic, which has resulted in very uncertain times for many people, in terms of health, job security and in the way in which we live our lives, it is heartening to see the continued level of enthusiasm for chromatography. To feed that enthusiasm we will be releasing a supplementary meetings review edition before the end of the year to highlight our past meetings and give you a taste of what you can expect. For all separation scientists I wish you well in these most troubled of times and look forward to seeing you when normality returns.



Tony Edge

IN MEMORIAM
JOHN KNOX

PETER MYERS (REPRODUCED WITH PERMISSION)

I first met John in the early 80's through his relationship with Hypersil and my involvement with Phase Separations. Over the years we had many excellent meetings and discussions. Apart from the many tips I picked up on the theory of chromatography John also taught me how to drink whisky. In the early 80's we must have discussed every aspect of silica and I remember clearly how Johns' thoughts moved in the direction of graphitic materials that of course became Hypercarb. In the 90's our discussions moved on to capillary electrochromatography. Again, we talked extensively about the surface of silica but with CEC we discussed and argued about the type of bonding's that should be applied to the silica surface.

John was a regular attendee at the Desty Memorial meetings. He really enjoyed meeting the emerging chromatographers and talking to them in one of his favourite buildings the Royal Institution. John also had a very close association with Denis Desty as John and Denis have consecutive signatures in the Fellows book of the Royal Society.

I have so many memories of John at the Desty lectures. In particular, I remember one very long discussion on the manufacture of monodispersed silica we had that started in the afternoon of a Desty lecture at the Royal Institution and ended in the early hours of the morning at our hotel. I will always remember this as John couldn't work out how I made a particular monodispersed silica, the only thing I knew that John didn't. Needless to say, that by our next meeting he had worked it out.

John received a number of accolades for his work in chromatography. Including receiving the Martin medal from the ChromSoc in 1985.



IMPACT OF TEMPERATURE ON EFFICIENCY AND SELECTIVITY IN HPLC

TONY EDGE, PRESIDENT OF THE CHROMATOGRAPHIC SOCIETY

INTRODUCTION

Temperature is often used in many forms of chromatography as a key variable to impact analyte behaviour [1], in particular, retention of volatile analytes in GC. However, it is seldom considered in LC as a means to modulate selectivity. Why is this, and what would the advantages be of using temperature as a variable within liquid chromatography? I will begin with the perceived disadvantages to try to understand why the use of temperature as a modulating parameter is often considered peripheral by many practitioners of chromatography. There are three major considerations;

- Column stability
- Compound stability
- System hardware compatibility

Column stability is definitely an issue to consider, in general most silica columns will struggle to operate for extended periods of time above 70°C [2]. Above this temperature the stationary phase will be gradually cleaved from the silica surface. This will result in an increase of the silica activity (i.e. generate more silanol groups), so for basic compounds it is possible that there will be an increase in retention times, but more importantly an increase in the tailing factor under conventional low pH conditions. Additionally, selectivity may change through the imposition of an electrostatic retention mechanism on top of the usual hydrophobic interactions. Hybrid phases, ones where an organic molecule is incorporated into the bulk silica structure, are more stable at high temperature, as are other slightly more exotic phases such as carbon [3] or zirconia [4]. However, their potential disadvantages are excessive retention or decreased efficiency.

In terms of compound stability, it is true that as the temperature is increased there is a greater possibility that the compound may degrade [5]. However, this tends to be rare. It has been shown that both temperature and the residence time of the analyte on the column are important considerations [6], alongside the resilience of the chemical structure to thermal degradation effects. Having already discussed some practical issues in terms of column and compound stability, it is also important to be aware that heated solvents will also evaporate or possibly even boil at temperatures in excess of 150oC, unless adequate considerations are taken. This is typically achieved by placing a back-pressure regulator post-detector to reduce the potential for boiling within the column - thus keeping the system pressure above the vapour pressure of most chromatographically relevant solvents. Another consideration is that not all detectors respond well to solvents at elevated temperatures. For example, if the UV detectors flow path is not heated to the same temperature as the column, thermal mismatch [7] may be observed. This can lead to thermal contraction which may negatively impact peak shape or generate additional baseline noise. Consequently, the solvent may need to be cooled before it can go into the detector, but this would not be an issue for MS detectors for example. Many modern column ovens have pre-heater and post column cooling units to negate this. However, there is an associated extra column band broadening with these modifications which leads to a compromise. A final physical parameter to consider is the stability of the connectors at elevated temperatures. PEEK is not stable above 150°C so stainless steel fittings should be used at much elevated temperatures. If much higher temperatures are used, plastic sheaths with the column information on may also melt.

THEORY

EFFECT OF TEMPERATURE ON EFFICIENCY

One of the most obvious areas that temperature affects is the viscosity of the mobile phase. There are many documented relationships between the temperature and the viscosity [8], but perhaps the simplest is the Andrade Equation (Equation 1);

$$\eta_T = \eta_0 e^{b/T} \qquad (1)$$

where

η_T – is the viscosity at a temperature T in Kelvin.

b – is a constant which is dependent on the fluid.

T – is the thermodynamic temperature in Kelvin.

η_0 – is the viscosity at 0 Kelvin (clearly a theoretical value) in poise.

As may be seen from Equation (1), an increase in solution temperature will lead to a decrease in viscosity. As the temperature is raised, the intermolecular forces in a fluid are generally reduced as the transient hydrogen bonds absorb more energy and move closer to the energy threshold required for spontaneous dissociation. This reduces the viscosity of the fluid and in turn increases its compressibility [9] which, for the chromatographer results in a reduction in the pressure within the chromatographic system. How does this pressure vary with temperature? Here we can use information gathered from chemical engineers. If the flow is laminar, the pressure drop can be defined by the Blake-Kozeny Equation (Equation 2).

$$\Delta P = \frac{\phi \eta_T u L}{D_p^2} \qquad (2)$$

where

ΔP – is the pressure drop across the column, Nm^2

φ - is a stationary phase packing term

u – is the velocity of the mobile phase, m/s

D_p – the particle diameter, m

L – the length of the column, m

and all other terms have been defined previously.

By combining Equations (1) and (2) and rearranging, we find the following relationship (equation 3)

$$LnP = Ln\left(\frac{\varphi\eta_0 uL}{D_p^2}\right) + \frac{b}{T} \quad (3)$$

We can now see a direct relationship between pressure and the temperature in packed columns (Equation 3). As the mobile phase temperature increases, the pressure will decrease proportionally. This may be exploited in different ways to improve chromatographic efficiency e.g. decrease the particle size or increase column length, or to reduce analysis times by increasing the mobile phase velocity (assuming the system pressure is below the maximum operating pressure of the LC instrument).

THE EFFECT OF TEMPERATURE ON THE VAN DEEMTER EQUATION

The van Deemter equation (Equation 4) relates solute dispersion on the column to three key parameters, namely, eddy diffusion, longitudinal diffusion and mass transfer - classically known as the A, B and C terms respectively. Both the B and C terms are directly influenced by changes in temperature and subsequently influence theoretical plate height.

$$H = A + \frac{BD_m}{u} + \frac{Cu}{D_m} \quad (4)$$

where

H – is the plate height

A – is the A term or eddy diffusion

B – is the B term or longitudinal diffusion

C – is the C term or resistance to mass transfer

D_m – is the diffusion coefficient for an analyte, m^2/s

and all other terms have been defined previously.

LONGITUDINAL DIFFUSION – THE *B* TERM

So we have discussed some of the macroscopic effects of varying temperature, but how does raising the temperature affect the chromatography? First let's consider band dispersion and return to the van Deemter equation to get a better understanding of how the peak width is affected by the increase in diffusion and lowering of viscosity.

If we consider the three terms within the van Deemter equation it is clear the A term is unaffected by the temperature as it relates to the size and shape of the column packing (and therefore the inter-particle channel spacing within the column). Although there may be some thermal expansion of the silica at elevated temperature, this is negligible as silica is a very good thermal conductor and dissipates heat rapidly.

Longitudinal diffusion can be defined as the propensity of a concentrated solution to diffuse following a concentration gradient - a thermodynamic property of all solutions. Therefore, raising the temperature will increase the degree of Brownian motion which in turn will increase the amount of longitudinal diffusion, as we can see from Equation 5.

$$D_m = 7.4\ x\ 10^{-8}\ \frac{(\alpha M)^{1/2} T}{\eta V^{0.6}} \quad (5)$$

where

α – is the association constant between the solvent and the solute

M – is the molecular weight of the solvent

V – is the molar volume of the solute, m^3

and all other terms have been defined previously.

For small molecule diffusion [10] in water and associated liquids, we can observe that diffusion is a function of the relationship between; temperature and solvent molecular weight (intramolecular bonding effects). Thus, as temperature increases so does diffusion. Additionally, (Equation 1), solution viscosity is inversely proportional to temperature, and in turn is inversely proportional to diffusion rate. Therefore, as temperature is increased solution viscosity decreases, and this leads to an additional increase in the diffusion coefficient. Thus, the B term will have a greater effect at higher mobile phase velocities with increasing temperature (Figure 1). As such, the minima of the van Deemter curve, the point at which there is the least dispersion and consequently the point with the narrowest peak width, will shift to higher flow rates.

MASS TRANSFER – THE C TERM

Mass transfer, or more appropriately the resistance to mass transfer, relates to the equilibrium distribution of a solute between the interaction points on the stationary phase and the mobile phase. As can be seen in Equation 4 the diffusion rate affects the C term in an inverse manner to the B term. In fact, these two terms are acting in opposition to each other (the B term relates to longitudinal diffusion and the C term relates to radial diffusion). So, for the C term, the increase in diffusional rates means there is less resistance to mass transfer since an equilibrium concentration is reached quicker than at lower temperatures.

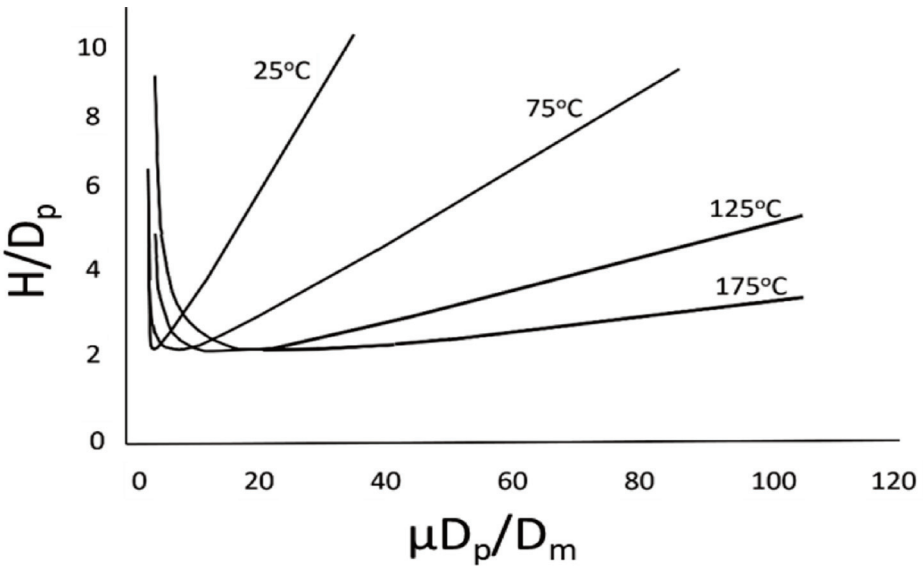


Figure 1. *Effect of temperature on the shape of the van Deemter plot. Increasing temperature leads to the optimal efficiency (curve minima, y axis) being retained for increasing linear velocities (x axis). μ is the linear flow velocity.*

Overall (Figure 1), although the minimum theoretical plate height does not change, the linear velocity at which this height is reached increases, and the C term (which defines the shape of the curve at higher flow velocities) has less of an effect on the dispersion process so the curve becomes flatter at higher temperatures, it is worth noting the Knox equation expanded on the terms above by introducing so-called 'reduced terms' to normalise for stationary phase particle size and analyte diffusion coefficient.

EFFECT OF TEMPERATURE ON CHEMICAL SELECTIVITY

To understand how the analyte interacts with the stationary phase we need to understand the two forms of binding energy that exist and their relationship. To form a chemical bond there is a mixture of entropic and enthalpic forces involved - with the overall binding energy being given by Equation 6:

$$\Delta G = T\Delta S - \Delta H \qquad (6)$$

where

ΔG - Gibbs free energy
binding or energy of reaction)

ΔS – entropy

ΔH – enthalpy

and all other terms have been defined previously.

This can be rearranged to give the following expression (Equation 7) which relates retention factor (k) to system entropy and enthalpy;

$$Lnk = \left(\frac{-\Delta H}{RT}\right) + \left(\frac{\Delta S}{R}\right) + ln\,\Phi \quad (7)$$

where

k - is the retention factor of a compound

R – is the Boltzmann constant

Φ – is the phase ratio

and all other terms have been defined previously.

It appears only the enthalpy term is sensitive to changes in temperature. Strictly speaking however, entropy is also affected by temperature but the effect over the temperature ranges under which chromatographic separations are performed is minimal. The effect of temperature on a chromatographic separation is often visualised using the van't Hoff relationship between k and T (Equation 8):

$$lnk = a + \frac{b}{T} \qquad (8)$$

where,

$a = \Delta S/R + \ln \Phi$

$b = -\Delta H/R$

and all other terms have been defined previously.

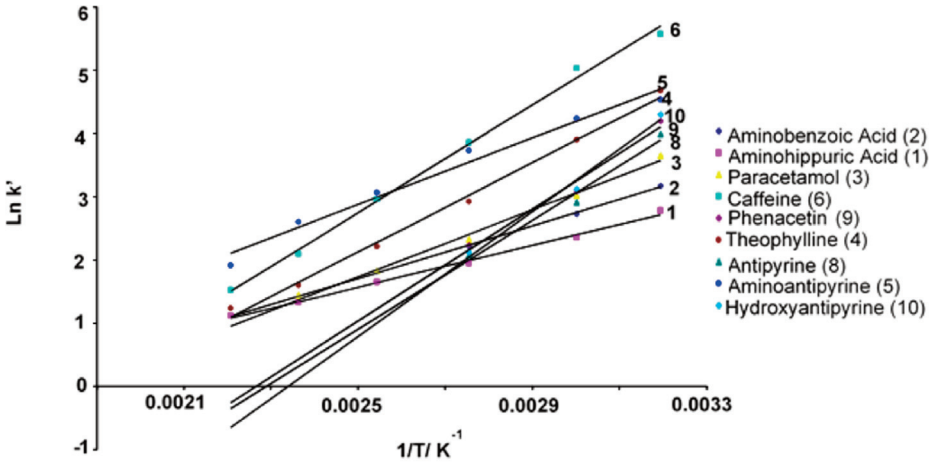


Figure 2. *Effect of temperature (x axis) on the retention (y axis) of a variety of structurally different compounds. Elution order changes with increasing temperature.*

Experimentally (Figure 2) it can be seen that altering the temperature certainly impacts analyte retention time. However, It is important to note that the gradient slope generated in relation to the change in temperature is analyte specific and, very importantly, can lead to changes in separation selectivity/elution order.

CONCLUSIONS

While it is common practise in LC method development to optimise the elution and selectivity of analytes through mobile phase pH, %organic solvent, or through stationary phase chemistry, temperature is often an afterthought. While it is obvious there are several factors which must be taken into account when fine tuning column temperature in HPLC, the benefits of doing so can be considerable. In some cases, merely changing the temperature a few degrees may yield baseline separation or improve peak-pair selectivity. Considering how facile temperature optimisation can be in comparison to other factors it would be wise to bring temperature to the forefront of method optimisation.

ACKNOWLEDGEMENTS

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TROUBLESHOOTING

CONCENTRATION SENSITIVE VS MASS SENSITIVE DETECTORS

TONY EDGE
President of the Chromatographic Society

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Honorary Secretary of the Chromatographic Society

DANIEL MESTON
Editor in chief of ChromCom

INTRODUCTION

The concept of mass or concentration sensitive detection in LC can be a tricky concept to understand. We therefore thought it would be useful in this article to clarify the operating principles and differences behind these two modes of detection.

We begin by describing the critical difference between a concentration sensitive and a mass sensitive detector. The easiest way to think about it is that a concentration sensitive detector is volume dependent and detector response will therefore decrease with increasing flow rate in a fixed volume flow cell (Table 1).

Table 1. *Effect of flow rate on a concentration sensitive detector*

Flow rate / mL min^{-1}	Area
0.2	1166.1
0.6	388.7
0.8	291.5
1.0	233.2
1.2	194.3
1.4	166.6
2.0	116.6

If this data is plotted and a curve fitted through the data we start to see something quite interesting (Figure 1).

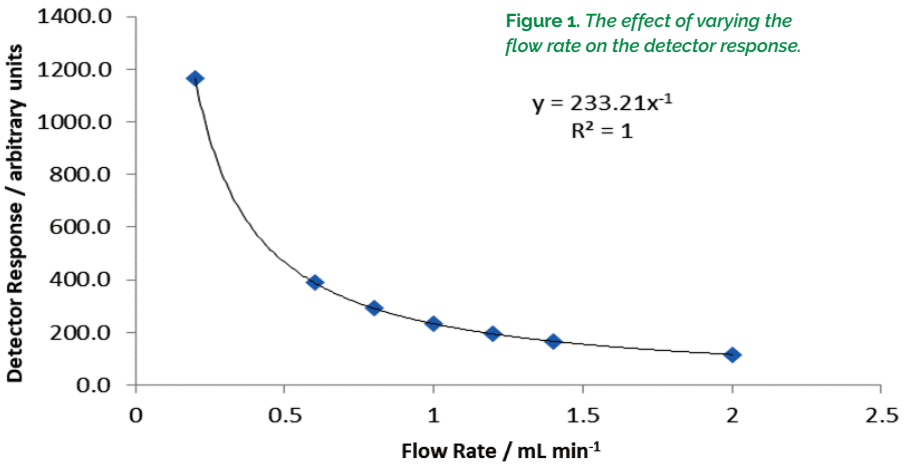


Figure 1. *The effect of varying the flow rate on the detector response.*

This is an issue that newcomers to chromatography often struggle with when first using UV detection or another concentration sensitive detector. So what is happening and why is the response decreasing with increasing flow rate?

First, we have to make some sensible assumptions before we can get behind the mathematics of this effect. The first assumption is that the same mass of analyte is injected on all occasions. We can then consider what happens with the absorption characteristics of the detector, noting that the same principle is applicable to other concentration sensitive detectors such as fluorescence or refractive index detectors. When we use a concentration sensitive detector to monitor the response, then the detector will generate a specific peak area dependent on the concentration within the detector at that point in time. If we have a system with zero column or instrumental band broadening we can assume we would generate a perfectly rectangular peak. As we reduce the flow rate, the concentration of the square peak would remain the same but the signal per unit time would increase. Therefore, as the flow rate is reduced the peak area that is measured will increase (Figure 2).

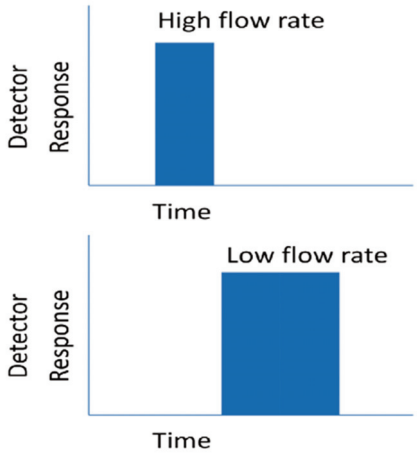


Figure 2. *Effect of different flow rates on a square wave chromatogram.*

It is important to understand what the relationship is between the flow rate and the peak area. For this a little mathematics is required (Equation 1).

$$A_i = \frac{k_i m_i}{Q} \qquad (1)$$

where

A_i – is the area response from the detector to compound i

k_i – is the detector response factor (sensitivity of detector to compound i)

m_i – mass of compound i injected

Q – flow rate

Thus, increasing the flow rate will result in a decrease in the response from the detector. This is not the case for mass sensitive detectors such as evaporative light scattering detectors (ELSD) or charged aerosol detectors (CAD). A concentration dependant detector is indirectly measuring the number of particles present per unit volume, while a mass sensitive detector is directly measuring the number of particles per unit time.

In general, mass sensitive detectors tend to be destructive detectors, and are often affected by the elution solvent parameters, specifically the degree of "interference" that comes from coeluting compounds. Consequently, these detectors will give a finite signal dependent on the total number of interactions that occur with the molecule of interest and the detector. An easy way to determine if a detector is mass sensitive or concentration sensitive is to stop the flow when the compound of interest is eluting, with a concentration sensitive detector there is no change in the detector response, but for a mass sensitive detector the response will drop to zero very quickly as the compound being analysed is effectively destroyed.

This then raises an interesting point about coupling a mass spectrometer (MS) to a HPLC system. This is clearly a destructive detector and yet it can be classified as a concentration sensitive detector. How is this possible? articles by Gallagher [1], and also Klink [2] are recommended reading. In both articles it becomes clear that at higher flow rates an MS becomes a concentration sensitive detector, and that it is only mass sensitive at lower flow rates, since at higher flow rates not all of the analyte goes into the MS. These articles the following experimentally derived data is presented and demonstrates the advantages of running at lower flow rates (Figure 3).

Historically, LC-MS has been subject to a unique physical disadvantage which is the fundamental incompatibility of reversed-phase LC (RPLC) with MS. This is the necessity of RPLC to have uncharged compounds in predominantly aqueous mobile phases for good chromatographic performance versus the MS generating gaseous ions.

An interface is required to move analytes from a liquid to gaseous state and the main challenge of this interface is the complete vaporisation of mobile phase. It has been shown [3] that above a certain threshold of solvent volume per unit time, residual mobile phase attenuates ionisation in the source and leads to lower peak areas for compounds.

This effect is of course flow rate dependant (as higher flow rates will introduce more mobile phase into the source per unit time), as well as column i.d. dependant (as the greater the volume of the column the more mobile phase is introduced to the ionisation source).

Therefore, one can make the assumption that with mass sensitive detectors the degree of sample dilution has a major impact on the effective mass sensitivity of the detector.

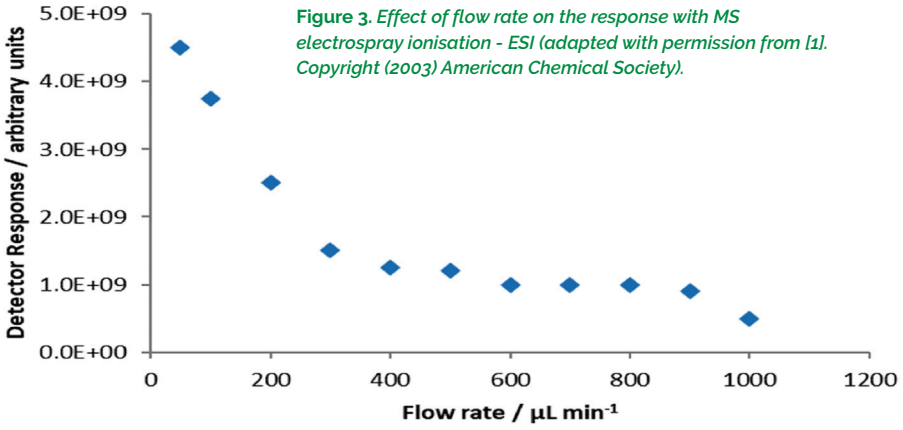


Figure 3. *Effect of flow rate on the response with MS electrospray ionisation - ESI (adapted with permission from [1]. Copyright (2003) American Chemical Society).*

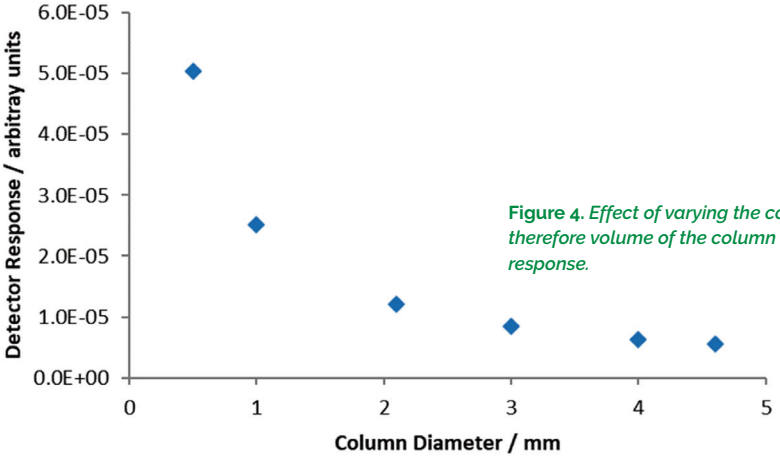


Figure 4. *Effect of varying the column diameter and therefore volume of the column on the detector response.*

It should also be noted that as smaller i.d. columns are used there is inherently an increase in the sensitivity, which arises from the following equation (Equation 2).

$$Response = \frac{mN^{1/2}}{(2\pi)^{1/2}V_0(1+k)} \quad (2)$$

Where;

M is the response is dependent on the absolute mass of sample loaded onto the column

N is the system efficiency,

k is the retention factor and

V_0 is the column dead volume which is clearly related to the column diameter.

It should be noted that by keeping the mass loaded onto the column constant whilst reducing the column i.d. will lead to an increase in detector response and potentially column overloading (Figure 4), which will in turn affect the chromatographic peak efficiency and quantification.

CONCLUSION

In conclusion, we can make a statement about concentration vs mass sensitive detection methods which underpin the main difference simply put if we can completely remove the mobile phase we approach mass sensitivity. However, it is important to be aware of both the effect of the column i.d. and also the effect that the flow rate can have in terms of the sensitivity of an assay. It is also important to be aware that the injection volume should be scaled in accordance with the column diameter to avoid overloading the column and saturating the detector, which will result in poor peak shape. Finally, it should also be noted that dead volume of the chromatographic system can also effect the peak shape, and consequently the sensitivity. We have shown that flow rate can have a profound effect on analyte peak shape and assay result. Therefore, care should be taken that the optimisation of the chromatographic parameters is made in conjunction with the optimisation of the detector settings.

ACKNOWLEGEMENTS

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MEDALS & BURSARIES



WINNER OF THE 2020 CHROMATOGRAPHIC SOCIETY MARTIN MEDAL

PROFESSOR GERT DESMET

Each September The Chromatographic Society executive awards committee convenes to discuss the award of their Jubilee and Martin medals for the following year. The Martin Medal is named after Professor A.J.P. Martin who together with Richard Synge received the Noble Prize for Chemistry in 1952 for their seminal work on partition chromatography. The 'Martin Medal' is the highest honour the Society confers. Indeed, it is recognized by the international chromatography community as being one of highest accolades that can be bestowed upon a chromatographer and is awarded to scientists who have made outstanding contributions to the advancement of separation science.

The nominations for the Martin Medal category for 2020 were extremely strong and included several highly prominent separation scientists. The Chromatographic Society is now pleased to announce that Professor Gert Desmet from the Vrije Universiteit Brussel (VUB), will be awarded the Martin Medal for 2020. The award has been made in recognition of his outstanding contributions to the advancement of fundamental chromatographic theory.

Professor Desmet is a full professor in chemical engineering, heading the Department of Chemical Engineering at the Vrije Universiteit Brussel (VUB), Brussels, Belgium. He is an author of over 300 peer reviewed scientific papers, 11 patent applications and promotor of 35 PhD students. During his career Professor Desmet received numerous scientific awards, among others the Nanotech-Montreux Award (1999), most innovative scientist awarded at the HTC conference (2004), Desty Memorial award (2006), Emerging Leader in Chromatography awarded by LCGC magazine (2008), the Agilent Technologies Award from the Agilent Technologies foundation (2008), The Chromatographic Society Silver Jubilee Medal (2009), JFK Huber Lecture Award from the Austrian Society for Analytical Chemistry (2017), Istvan Halasz Medal award from the Hungarian Society for Separation Sciences (HSSS) (2019) and American Chemical Society Award for Chromatography (2019). He is currently an Associate Editor of "Analytical Chemistry", a journal of the American Chemical Society. Apart from his outstanding contribution to the advancement of fundamental chromatographic theory, development of chromatographic process simulation approaches Prof Desmet's recent research work led to the development of micro-pillar array columns and other miniaturized separation techniques.

Professor Desmet is well known in the academic community and his engagement either directly or through the work of his students has had a substantial impact on the development of chromatographic science. He is often seen at the major scientific events and his approachable manner and ability to communicate with the next generation of scientists at all levels is something that the awards committee responded to well.



WINNER OF THE 2020 CHROMATOGRAPHIC SOCIETY JUBILEE MEDAL

PROFESSOR DEIRDRE CABOOTER

To mark its 25th anniversary in 1982, The Chromatographic Society created the Silver Jubilee Medal. The purpose of this award is to recognise up-and-coming separation scientists, those who have made major use of separation science in their own field or to scientists who have made important contributions to a particular area of separation science.

This year, The Chromatographic Society is pleased to announce the award of the Silver Jubilee Medal to Professor Deirdre Cabooter from the University of Leuven, in recognition of her contributions to advancement of fundamental understanding of band broadening in liquid chromatography.

Professor Cabooter, obtained her MSc degree in Bio-engineering from the Free University of Brussels (VUB), Belgium (2005) and a PhD in Chemical Engineering at the VUB (2009). She is currently an Associate Professor at Faculty of Pharmacy, University of Leuven, Belgium. Professor Cabooter is active on a broad range of topics in separation science with particular emphasis on an in-depth understanding of the phenomena contributing to band broadening in liquid chromatography. She has worked on innovative approaches to improve and streamline the process of method development in liquid chromatography by developing novel hardware and currently, her research team also develops software strategies based on artificial intelligence to streamline this process in an algorithmic way. Professor Cabooter authored in excess of 85 peer reviewed articles and is an editor of Journal of Chromatography A. She received an Emerging Leader in Chromatography Award LCGC magazine (2017). Since 2012 she has been a member of the organizing and scientific committee of the International Symposium on Hyphenated Techniques in Chromatography (HTC).

Both Chromatographic Society award winners have chosen to receive their medals at the 2020 ISC symposium in Budapest, Hungary. Unfortunately, due to the global Covid-19 pandemic an alternative arrangement is sought and will be announced in a due course.

Please join me in congratulating Professors Desmet and Cabooter on receiving these prestigious awards.



JOHN DOLPHIN BURSARY REPORT - CHRISTINA VANHINSBERGH

UNIVERSITY OF SHEFFIELD, CHROMSOC MEMBER

My name is Christina and I am a PhD student at the University of Sheffield, UK. I work within the Chemical and Biological Engineering department of the University within Professor Mark Dickman's research group. I am undertaking a CASE industrial PhD and work in partnership with GlaxoSmithKline. My PhD research focuses on optimising two-dimensional HPLC methods for analysis of oligonucleotide therapeutics and their manufacturing impurities. Oligonucleotide therapeutics are nucleic acid pharmaceuticals that interrupt gene expression by preventing translation of mRNA into protein. They work in a range of different ways; either utilising proteins such as the RNA induced silencing complex or other enzymes to facilitate the degradation of mRNA. Some also adopt 3D conformations to bind to ligands- similarly to antibodies, or stimulate the immune system.

I applied for the John Dolphin Fellowship funding to attend HPLC 2019 in Milan and present my PhD research. This year HPLC 2019 had three multidimensional chromatography sessions, highlighting the growing interest in this field. My presentation would engage the wider audience to explain the applications of 2D-LC to analysis of oligonucleotides. I also wanted to widen my network of multidimensional chromatographers and find out about other interesting advances in liquid chromatography. I was excited to attend two courses directed at students and early career researchers, and to catch up with those researchers already within my network.

Although I was a little nervous to speak in front of many highly respected chromatographers, presenting at the conference would give me valuable experience for the future. I also feel that it is somewhat of a baptism for PhD students to orally present at larger meetings rather than submitting posters, as it shows how far you have progressed with your research and professional development.

I arrived at the conference and attended two short courses. In total there were 16 courses to choose from, my choices being "Two-dimensional Liquid Chromatography: A tutorial focusing on best practices" (hosted by Dwight Stoll, Matthias Pursch and Stephan Buckenmaier) and "Drug Quality Fundamentals: Quality control of small molecules" (hosted by Michael Dong). The 2D-LC workshop was useful to me as I learnt a little more about modulation techniques which gave me many new ideas about how it could be implemented in my own workflows. The drug quality fundamentals workshop introduced the drug development process and attributes of small molecule drugs that are appropriate to monitor during and after manufacture. It was useful to learn more about various methods of analytical development and the importance of method validation and QC with quality by design strategies in place.



The opening ceremony saw various medals and awards given - starting with the Georges Guiochon Faculty Fellowship presented to Professor Ryan Kelly. The Uwe D Neue award in separation science, sponsored by Waters was presented to Dr Christopher Welch. The JFK Huber Lecture Award was presented by the Austrian Society of Analytical Chemistry to Dr Fabrice G. Gritti. The Chromatographic Society Martin Medal was given to Professor Robert Kennedy and the Jubilee Medal to Professor Frederic Lynen. After this, the art of Leonardo Da Vinci was discussed by Martin Kemp alongside operatic musical performances, such as Ave Maria by Schubert.

That evening I saw many past attendees of the ChromSoc's Grass Roots training events. Having been to two so far, I have met many people from academia and industry who have attended. We caught up on each others' lives and progress over drinks in central Milan, which was a great start to the conference.

I would like to discuss a few topics I found interesting at the conference. The first being the miniaturisation of liquid chromatography using chip technology, 3D printing or miniaturised systems. Could miniaturisation be the way forward for PAT or continuous analytical monitoring? It seems it is beneficial for high throughput requirements and some of the technology for chip-based columns can even negate dispersive effects.

Columns such as the µPAC™ seem out of this world but have potential to improve chromatographic efficiency by micro-pillar array design. Another technology that caught my eye, was the integration of organoids (miniature organs) and biomolecules in liquid chromatography to analyse metabolism of drugs and biological reactions. Miniaturisation of a

biological system in this way assists with sensitivity issues and widens the capability of biological monitoring practices. Lastly, the machines are here to stay! By that I am of course referring to the impressive use of machine learning to aid compound identification and build databases for *in silico* suspect screening.

My presentation was in the third multi-dimensional chromatography session. I felt it went well and gave me the experience I needed to progress professionally. I was able to discuss my work with others involved in oligonucleotide therapeutic analysis after the session. I hopefully was also able to give some context about oligonucleotide therapeutics to people unfamiliar with them. I presented using the traditional format, however, there were two alternative, engaging sessions during the conference – Separation Science Slam and HPLC Tube.

The Separation Science Slam involved 7 contestants creatively presenting their research to the audience. The winner – Nàndor Lambert from the University of Pécs, Hungary- rapped his research on insulating LC columns in polyurethane foam. As he rapped to music and presented slides on screen, the audience clapped along with a cheery demeanour. HPLC Tube was a mirror of the infamous website, where presenters introduced short videos to disseminate their work. The wining video was an old silent movie mimic by Dr Simone Diamartino's group at the University of Edinburgh, discussing present LC technology.

The poster topics were wide ranging from sample preparation to SFC to forensic and food analysis. A couple of posters specifically caught my eye. Samantha Bowerbank was presenting a poster entitled "How safe is your garden". Sam works at Northumbria University and was presenting an LC-MS method for the analysis of alkaloid compounds found in poisonous plants. I also liked a poster by Gregory Jones and Jun Shi of Alnylam entitled "Design and application of a scalable automated platform using multi-dimensional chromatography for impurity characterization of an RNAi Therapeutic". The poster highlighted that analytical timescales were heavily reduced with the approach and was stimulating for me as it presented an application very relatable to my research.

During the conference, my aim was to try and talk to as many people as I could to widen my network. Whilst networking, I met Arif Butt from Select Science who presents science related topics via televised programmes at www.thescientistschannel.com. I think something like Arif's channel would benefit many individuals at all stages of their career, for example, when learning a new technique.

I also approached Professor Susan Olesik of the Ohio State University to discuss her presentation on using Enhanced Fluidity LC-MS. We didn't speak for long but I hope to connect with her via LinkedIn to discuss other applications of EF-LC-MS. In addition I spoke with Dr Giacomo Russo about the alternative applications of a biomimetic 2D-LC platform. Giacomo presented just before me in "Multidimensional Chromatography 3" and gave an inspiring presentation on comprehensive 2D-LC - assessing intestinal absorption of pharmaceutically relevant compounds.

More networking went on at the Gala Dinner at Milan University. The grounds we ate in are in the Renaissance Palace of Ca' Granda and are beautiful! Around my table were ChromSoc members, PhD students, ChromSoc's president Tony Edge, Jenny Bain from Waters and Lee Noyes and colleagues from the Analytical Scientist Magazine. We ate delicious beef followed by a desert of Tiramisu. In time the dinner closed with dancing and laughing by all.

In summary, the conference was a great way to meet other professionals and more senior chromatographic researchers. I was able to re-connect with those already within my network and discuss my own work with oligonucleotide analysts. I presented my PhD research to a range of individuals, some who know a lot about multidimensional chromatography and some who know a lot about oligonucleotide therapeutics. I have learnt about new techniques, technologies and approaches to chromatographic analysis that has opened my eyes to what could be done in the world of oligonucleotide therapeutic analysis. I would like to conclude with a note of thanks to Tony Edge (president of the Chromatographic Society). I was attending the conference alone and felt that his constant words of support, company and scientific discussions made me more confident to network and present.



COMMITTEE MEMBER SPOTLIGHT

ROMAN SZUCS

In this edition of the committee spotlight, we introduce the Vice-President of the ChromSoc, **ROMAN SZUCS**.

The route to my current role as the Vice-President of The Chromatographic Society took me through multiple countries, companies and even political regimes, but one thing never changed: my keen interest and curiosity in "how things work."

My early interest in chemistry, as it happens in many cases was initiated by an inspirational chemistry teacher at my high school, which focussed on chemical technology. When I came 4th at the National Chemical Olympics games (a much better achievement than my sporting endeavours) in my final year, the future was decided and I went to study analytical chemistry at what is now known as the Slovak Technical University, Faculty of Chemical Technology, in Bratislava. I did my Masters degree in a brand-new trace analysis laboratory equipped with the latest instrumentation for atomic spectroscopy (ICP-OES, AAS) and related sample prep techniques.

After finishing at the University, I was lucky enough to join Professor Pat Sandra at the University of Ghent in Belgium to study for a PhD. Although I abandoned my first analytical love, atomic spectroscopy, I instantly fell in love with electro-kinetic separation techniques, which were at that time in their infancy. That and the application area in the field of hop and beer bitter acids led to a very happy four years spent in Belgium. Pat was not only a fantastic scientist, but also an extremely supportive, encouraging and motivational PhD supervisor who provided seemingly limitless resources for his students. This was the time when I started to attend the ISCC series of symposia in Riva del Garda and where I started to discover the big world of separation science with its huge number of scientists.

My next stop was only a short distance away, this time the Unilever Research laboratory in Vlaardingen, The Netherlands. This is where I continued to apply my knowledge of electro-kinetic techniques to natural product research but also expanded into field polymer characterisation using size exclusion/gel permeation chromatography with multiple detection techniques.

After two years of Postdoc research at Unilever I was ready to settle and find a proper job. At Pfizer I found an exceptionally supportive environment where I managed to combine my developing career of analytical scientist with keeping one foot firmly in academic research. This was achieved through multiple academic collaborations. The one that I am particularly proud of was a multi-year collaboration with two Universities (Ghent and Hobart) named the Pfizer Analytical Research Centre (PARC). This collaboration created a large number of PhD and post-doc opportunities for students from around the world, many of whom are now prominent members of the Chromatographic community working in academia as well as in industry, some of them for Pfizer but also many with our competitors. Nearly a hundred peer reviewed publications and countless number of poster and oral presentations generated during this collaboration is testimony to the hard work and innovative thinking of two academic leaders that I've had the enormous pleasure to work with, Profs Pat Sandra and Paul Haddad. The end of the PARC collaboration led to a new scientific endeavour, driven by my keen interest in everything computational. The holy grail of separation science, i.e. prediction of retention times from chemical structure was a trophy worth shooting for. Led by me, Pfizer entered into a new collaboration with Prof Haddad and the University of Tasmania during which we developed a significant number of computational approaches to predict the retention times for ion chromatography, HILIC as well as RP-LC. Three PhD students and 12 peer reviewed papers later, this approach is now finding its way into routine practice in method development activities within Pfizer.

Towards the winter of 2018 I accepted a part-time position (60%) at Pfizer focusing on the implementation of computational approaches to chromatographic method development. I am spending the rest of my time as a senior lecturer at the Comenius University, Faculty of Natural Sciences, Department of Analytical Chemistry in my home town Bratislava, Slovakia where I teach various bits and pieces of analytical chemistry while I am trying to establish a new research group focussing on advancing computational approaches applied to various fields of separation science. Although this arrangement is hugely demanding especially during the winter months when traveling is nothing but painful, teaching gives me a great deal of personal satisfaction while I have the opportunity to continue my research activities in a field that combines my natural curiosity in finding out how things work with my desire to predict the future.

HYPERCARB™: UNIQUE RETENTIVITY

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ABSTRACT

Porous graphitic carbon HPLC packing material developed by John Knox's research group and commercialised as Hypercarb™ has been available on the market for over thirty years. However, the unique properties of this phase still prove to be fundamentally novel and provide solutions to difficult separation applications. Of these properties, its lack of an elutropic series and hence ability to provide good retention not just in reversed-phase HPLC but even with non-aqueous solvents was put to good effect in this work.

Herein it was demonstrated, the resolution of permethrin isomers, the isolation of coal tar extract constituents, the separation of a homologous series of garlic oil constituents, the clean-up of plant extract fractions and the provision of orthogonal selectivity for the separation of flurbiprofen and its related substances.

Keywords: Hypercarb™, porous graphitic carbon, retentivity, selectivity, elutropic series

INTRODUCTION

It is a view held almost universally that John Knox, along with the likes of Martin and Synge, was one of the most influential British separation scientists not just of his time but all time. To borrow a phrase from one of his contemporaries, he was the doyen of UK chromatographers. While he was renowned as a world leader in chromatography theory, he brought much more than this to the field of separation science [1]. One of his more interesting avocations was his use of porous graphitic carbon as a chromatography stationary phase. It had the inherent advantages over silica that it was more stable, e.g. over a wider pH range, and did not contain acidic silanol groups that can cause peak tailing with basic analytes. However, time would tell that there was much more to it than just these two properties.

Even before it was commercialised, as Hypercarb™, Knox announced at HPLC 1985 in Edinburgh that it did not have an elutropic series of solvents. In the subsequent publication there was more emphasis on its unique selectivity [2]. This and other interesting properties were subsequently highlighted by other authors. Poor correlation of log *k* and log P values on Hypercarb™ at pH 14 for a series of organic bases demonstrated that the retention mechanism in operation was orthogonal to the hydrophobic partition mechanism in reversed-phase HPLC [3]. By this unique mechanism it was shown to be able to retain very polar compounds [4]. It also proved to be an effective support material when chiral mobile phase additives were being used to bring about enantiomer separations [5-7]. Over the years many similar reports based on the unusual properties of Hypercarb™ have continued to be published.

Knox's work on this material began in earnest in the late 1970's, continued through the 1980's until late 1988 when it was ready for release onto the market as Hypercarb™, produced by Knox's commercial partners Shandon Scientific Ltd. The company name morphed over the years (Shandon Hypersil, Hypersil) and although Shandon still exists as a separate entity the HPLC products were subsumed into ThermoFisher Scientific.

As alluded to earlier, just over thirty years on from its launch, Hypercarb™ is still proving to be an intriguing material and at the University of Sunderland it continues to be a valuable resource in PhD and undergraduate projects. Recently, interest in the material was revived by work on pharmaceutical formulations (manuscript in preparation) in which the need to use a highly retentive stationary phase was required for on-column focussing [8] of early eluting related substances. On-column focussing increases both the peak capacity of the separation and the sensitivity, with the sharper peaks having improved signal-to-noise and thereby being easier to detect and integrate.

In brief, Hypercarb™ proved to be so retentive that non-aqueous mobile phases were required to elute the analyte and in turn this proved to be the key to the eventual LC conditions used being adaptable for use with a wider range of drug formulations. This was the prompt to revisit older work to reflect on just how often this very high degree of retention had been a feature of Hypercarb™ application work at the University.

MATERIALS AND METHODS

INSTRUMENTATION

Three LC systems were used; a modular isocratic system based on (a) Shimadzu hardware (Kyoto, Japan) i.e. an LC-6A pump and SPD-6AV detector with data collection using a Dionex (PeakNet v4.3) data system. (b) an Agilent 1200 series chromatographic system (Agilent Technologies, Waldbronn, Germany) consisting of G1361A pumps, a G2260A autosampler, G1315 diode array detector using Agilent ChemStation software, and (c) an Agilent 1290 Infinity (Agilent Technologies, Waldbronn, Germany) system consisting of a G4220A 1290 binary pump, G4226A 1290 autosampler, G1316C Infinity TCC (Thermostatted Column Compartment) and a G4212A Infinity DAD detector. Data was collected using Agilent ChemStation software.

MATERIALS

The Hypercarb™ (100 x 4.6 mm i.d.) columns used were either gifts from the University of Edinburgh (*cis*-permethrin work) or were purchased from ThermoFisher Scientific (Runcorn, Cheshire, UK) or equivalent predecessor companies. The column used for work on flurbiprofen contained 3 µm particles. Permethrin technical (Mitchell Cotts Chemicals) was supplied by Robert Young & Co. Ltd. (Glasgow, UK) for a project involving 'pour-on' and shampoo insecticide formulations for use on sheep and goats. Ecoguard® garlic oil was a gift from Ecospray (Suffolk, UK).

Coal tar was a gift from Stiefel Laboratories Ltd (Maidenhead, Berkshire, UK). Flurbiprofen and structurally-related compounds were supplied by Aesica Pharmaceuticals (Cramlington, Northumberland, UK). Solvents were purchased from Sigma Aldrich (Poole, Dorset, UK) and Fisher Scientific (Loughborough, Leicestershire, UK).

METHODS

Method details are given in figure legends. Unless otherwise stated, analytes were dissolved in mobile phase at a concentration of ~0.2 mg mL^{-1}.

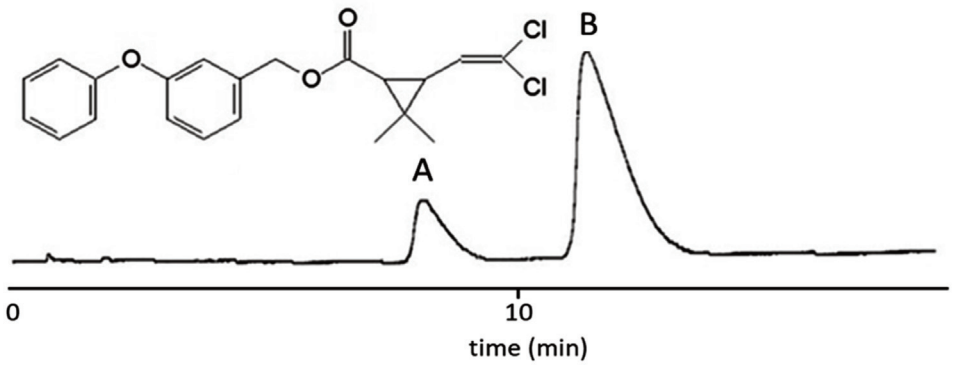
Gradient conditions for Figure. 5.

Time (min)	%A	%B
0	80	20
2	80	20
3	85	15
5	85	15
6	80	20

RESULTS AND DISCUSSION

The first encounter with the need to use non-aqueous solvents to elute an analyte from Hypercarb™ came in the development of an assay to determine the content of the insecticide, *cis*-permethrin, in a sheep dip formulation. For the reversed-phase LC conditions on alkyl- bonded phases developed, the separation of the cis- and trans- isomers of permethrin was just baseline (R_S < 1.3). Hence it was logical to employ Hypercarb™ as it was already known at that time to have useful stereoselective properties [8]. As anticipated, when using Hypercarb™, there was a clean separation (R_S = 2.0) of the two isomers (Figure. 1) albeit with poor peak symmetry on the pre-manufacturing batch of porous graphitic carbon used.

Figure 1. *Resolution of minor trans-permethrin isomer (A) from the major, and active, component cis-permethrin isomer (B) in Permethrin Technical on Hypercarb™ (150 x 2.1 mm i.d.) with mobile phase methanol – tetrahydrofuran (80:20, v/v) with flow rate 0.6 mL min-1 and UV detection at 254 nm.*



However, it had been necessary to use non-aqueous solvents in order to elute the compound off the column. Because of this, and the symmetry issue, the use of Hypercarb™ for the determination of the active insecticide in the water-based sheep dip formulation was discounted. In hindsight, with the benefit of knowledge of recent work on the analysis of creams and ointments, this might not have been the best move. After a short time in regular use the developed reversed-phase LC method proved to be a little troublesome. The resolution of the isomers just held up but over time the baseline began to show a peculiar undulation, fortunately not serious enough to significantly compromise quantitation. Had Hypercarb™ been used with the non-aqueous mobile phase the relative retention between surfactant and analyte would have been different with surfactant eluting as a discrete band either before the analyte or after it without building up on the column.

For the next two instances the use of Hypercarb™ with non-aqueous mobile phases was an obvious choice. The first application was a company-commissioned attempt to use preparative reversed-phase LC to fractionate coal tar extract in the hope that a fraction could be found that was active against psoriasis but was not carcinogenic. An optimum combination of solubility / loading / resolution was not easily attainable. Accordingly, it was decided to use normal phase solvents, such as propan-2-ol / n-hexane, dichloromethane and chloroform, in which the coal tar constituents are much more soluble. Retention and resolution was low on all of the usual normal phase adsorbents tried and so it was decided to use Hypercarb™. Not only was good retention and resolution obtained but also it was possible to use 100% dichloromethane as the mobile phase (Figure. 2). This was highly convenient as it was a facile exercise to collect peaks, evaporate off solvent and then identify compounds by mass spectrometry. The process of collecting of fractions for biological testing was not undertaken as all the compounds identified were known constituents of coal tar that could be purchased. It was deemed that it would be easier to test all the constituents individually and then prepare a blend of the

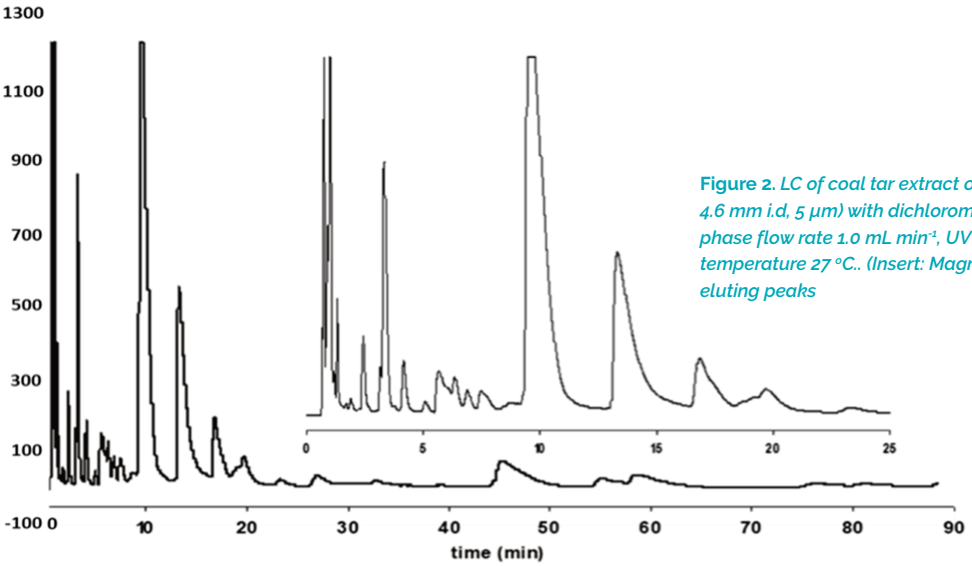


Figure 2. *LC of coal tar extract on Hypercarb™ (100 x 4.6 mm i.d, 5 µm) with dichloromethane as the mobile phase flow rate 1.0 mL min^{-1}, UV detection 280 nm, temperature 27 °C.. (Insert: Magnification of the early eluting peaks*

most active / least carcinogenic compounds. Which carried the risk that the most active compounds would be the polyaromatic hydrocarbons which were carcinogenic.

The second application addressed was the analysis of garlic oil, the use of Hypercarb™ with non-aqueous solvents again helping to solve a problem. LC was being used to assess the composition of the garlic oil for quality control purposes. Using reversed-phase the chromatography was perfectly satisfactory and it was apparent that the oil contained a mixture of related compounds believed to be allylpolysulphides of different numbers of sulphur atoms. However, on collection and reinjection of each analyte, it was apparent that the collected solutions had quickly reverted to giving chromatograms similar to that of the original mixture. This transformation was thought to perhaps be brought about by bimolecular S_N2 nucleophilic displacement reactions. This seemed to be faster in the presence of water. As in the previous examples, thoughts turned to Hypercarb™. The separation of the allylpolysulphides had been very good when using reversed-phase systems. However, good retention and resolution was also observed when using 100% of organic solvents including methanol, acetonitrile, dichloromethane and chloroform. Chloroform and dichloromethane would have been preferable from the point of view of evaporating off solvent to isolate constituents. However, when using methanol there was better resolution of the early eluting peaks (Figure. 3). The collected eluate could be reinjected to still give a single peak for these early eluting peaks. However, on longer term storage at ambient and also on evaporating off solvent under vacuum between 30 – 40 °C, the later eluting peak solutions showed signs of reverting to a pattern similar to the original mixture. This behaviour supported the notion that the oil might well simply be an equilibrium mixture. The garlic oil samples could well be analysed using this Hypercarb™ / organic solvent system given the relative stability of the constituents in chloroform. However due to the rapid structural degradation / reintegration transformations under the influence of external factors it was felt that applying these, or any other, conditions for quality control purposes would only yield a temporary snapshot of the content that would not necessarily have been representative of the product as used.

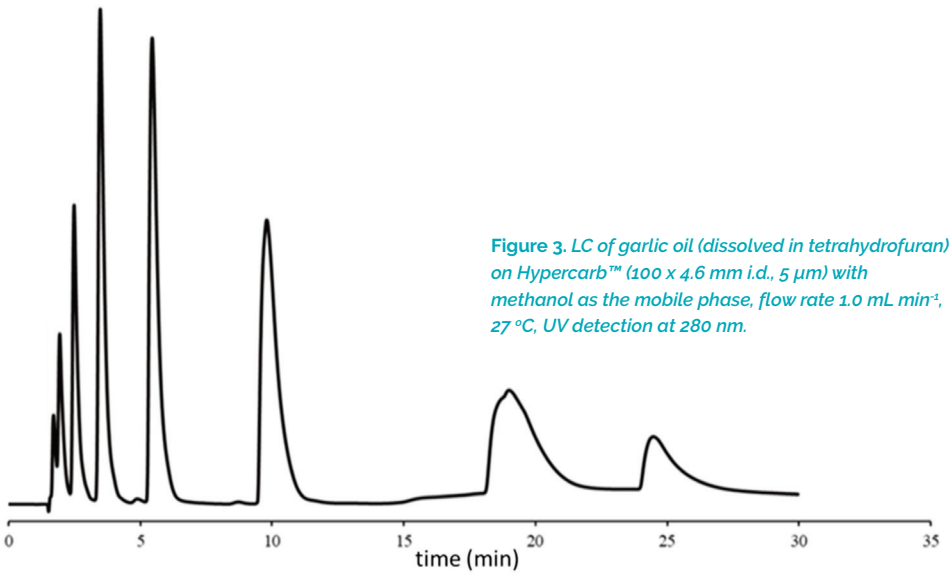


Figure 3. *LC of garlic oil (dissolved in tetrahydrofuran) on Hypercarb™ (100 x 4.6 mm i.d., 5 µm) with methanol as the mobile phase, flow rate 1.0 mL min^{-1}, 27 °C, UV detection at 280 nm.*

Hypercarb™ was also used with non-aqueous solvents in the analysis of the petroleum ether (pet ether) plant extracts. In this case it was the unique selectivity of Hypercarb™ which led to its use and at least, not initially, its high retentivity. In studies of the extracts of *Zanthoxylum ovalifolium* the compound suberosin (Figure. 4a, insert) had been found in one of the active fractions from preparative reversed-phase HPLC. However, the fraction contained other compounds and it was sought to ascertain if the activity did in fact arise from suberosin and not from one of the other compounds present in the preparative LC fraction. While suberosin was a known compound, it was rare and very expensive (£750 per mg) to purchase. Given the previous experiences of good selectivity for closely related compounds and the ease with which collected fractions could be evaporated down, the use of Hypercarb™ with dichloromethane was the first port of call. The resolution of the main peak from a peak which had barely been separated from it in the reversed-phase work e.g. on a cyano-bonded phase was very large (Figure. 4a, peaks A and B). The amount of compound available did not justify the purchase of a preparative column. There was scope for injecting a much higher load (20 µg *v* 5000 µg i.e. 5 mg) onto the analytical-scale Hypercarb™ column (Figure. 4b) and still maintaining resolution. In this way a pure sample (by ^{1}H NMR) was obtained and was also confirmed as suberosin by the ^{1}H NMR. Further, It was shown that the suberosin sample had very similar activity to that of the total original pet ether extract thereby supporting the suggestion that it had been responsible for the activity. It was later shown that the 'clean' sample isolated from Hypercarb™ was similar to an authentic sample which eventually had been purchased.

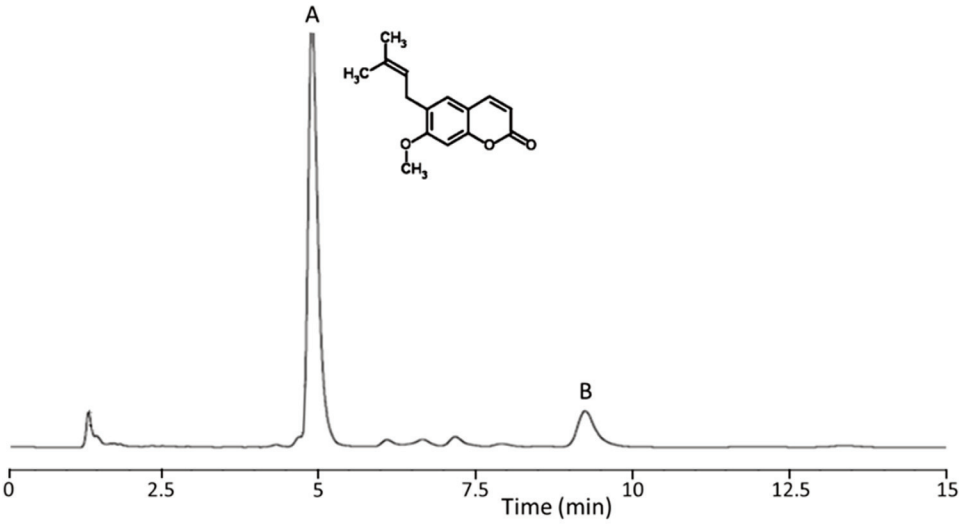


Figure 4a. *LC of Zanthoxylum ovalifolium on Hypercarb™ (100 x 4.6 mm i.d. 5µm) 0.6 mg mL^{-1} in dichloromethane, 20 µL injection volume, flow rate 2.84 ml min^{-1}, UV detection at 254 nm. (A) and (B) were well resolved*

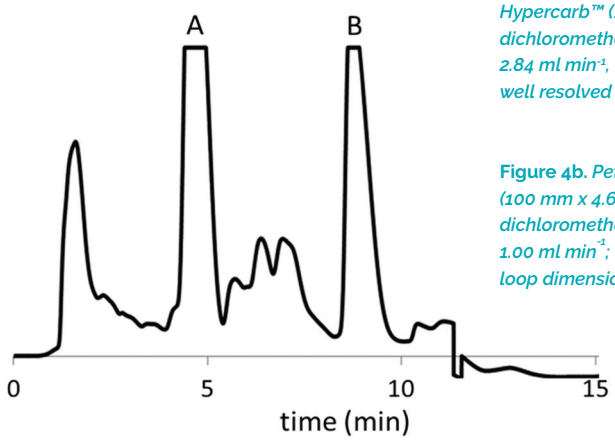


Figure 4b. *Petroleum ether extract on Hypercarb™, (100 mm x 4.6 mm i.d. 5µm). 10.0 mg mL^{-1} in dichloromethane; injection volume, 500 µL; flow rate, 1.00 ml min^{-1}; UV detection at 254 nm. Larger injection loop dimension used.*

The final example of using Hypercarb™ with non-aqueous solvents was different in that it was not a case of using them as an alternative to what would be regarded as reversed-phase conditions but as a continuum from those reversed-phase conditions. Quite simply for some aromatic analytes with more than one benzene ring, when using polar organic solvent – aqueous component mobile phase with Hypercarb™ the analyte will not elute quickly with 100% methanol or even 100% acetonitrile (unless an additive is used to 'deactivate' the Hypercarb™). This proved to be the case in the study of flurbiprofen and its related substances. A historical company method for these compounds had proved to be an interesting vehicle for student method development exercises. Essentially in an isocratic reversed-phase method the total run time was 120 minutes largely because of the late-eluting neutral compound. The keto- compound eluted at ~20 minutes and all the acidic compounds eluted before 12 minutes but the separation of these acids was not robust. The run time issue was simply addressed by applying gradient elution and the robustness issue was addressed by working at a lower mobile phase pH (moving away from acetic acid which had a pK_a very similar to that of the acidic analytes) and using alternative silica-based bonded phases. However, it was thought that with the known orthogonal selectivity of Hypercarb™ and the likelihood that retention would be influenced by the aromatic nucleus rather than the functional group (or lack of it), that it might form the basis of an isocratic method. The anticipated orthogonal selectivity was duly delivered but not really in the way that had been hoped for, e.g. the acidic compound, 2-fluorobiphenyl-4-carboxylic acid now had a similar retention time to the neutral 4-acetyl-2-fluorobiphenyl. However as can be observed (Figure. 5) the need for a gradient was even more pronounced. Both of the afore-mentioned compounds, each containing a carbonyl- group adjacent to the biphenyl- system, had been very strongly retained relative to the propionic acid compounds and that was the reason that it had been necessary to resort to complete the gradient. This was not a problem in itself. Dioxane is still water-miscible and re-equilibration with the starting mobile phase did not prove to be an issue. In this particular case while the latest eluting peak was resolved from the preceding peak the efficiency was lower than would normally be expected. Given that the compound is strongly adsorbed this may be because of slow mass

transfer. However, the overriding message was that Hypercarb™ gave rise to orthogonal selectivity and bridged the gap between reversed-phase and what would normally be regarded as normal-phase.

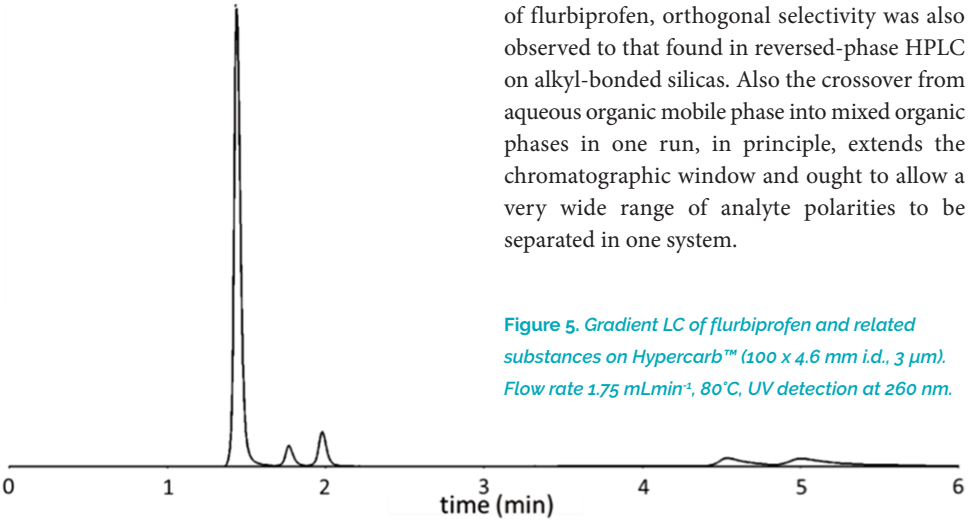


Figure 5. *Gradient LC of flurbiprofen and related substances on Hypercarb™ (100 x 4.6 mm i.d., 3 µm). Flow rate 1.75 mLmin^{-1}, 80°C, UV detection at 260 nm.*

On reflection the very high retention of some of these aryl- compounds should have been even more expected than it was. An attempt at adsorbing naproxen on Hypercarb™ to make a chiral stationary phase had had to be abandoned because the column just kept adsorbing without ever reaching saturation. However, if revisited, such an adsorbed material would offer some potential of being used in reversed-phase as a coated chiral stationary phase without any 'bleed'. Another interesting spin-off of this very high aromatic retentivity might be to use a very short Hypercarb™ column or cartridge after a reversed-phase alkyl-bonded phase to bring about post-column peak focussing.

CONCLUSIONS

In all five instances of Hypercarb™ use described it was clearly necessary or highly convenient to use its good retentivity extending beyond typical aqueous reversed-phase eluents across into non-aqueous typical normal-phase solvents. An affinity was shown for aromatic systems in particular. In three of the cases (coal tar extract, garlic oil, *Zanoxylum ovalifolium* extract) this resulted in conditions that made for facile evaporation of solutions of collected peaks. In two cases (permethrin, *Zanoxylum ovalifolium*) Hypercarb™ still exhibited good stereoselectivity under these non-aqueous conditions. In the case of flurbiprofen, orthogonal selectivity was also observed to that found in reversed-phase HPLC on alkyl-bonded silicas. Also the crossover from aqueous organic mobile phase into mixed organic phases in one run, in principle, extends the chromatographic window and ought to allow a very wide range of analyte polarities to be separated in one system.

It was also noted that LC on Hypercarb™ using solvents such as methanol, dichloromethane and in particular chloroform might render it suitable in miniaturised systems for carry out LC-NMR with deuterated solvents. Also with respect to miniaturised systems, the very high retentivity of Hypercarb™ has been used in-house for project work involving on-column focusing (or peak compression [8]) and now in modern LC systems short, narrow Hypercarb™ columns could be used for the same purpose in order to bring about fast, efficient second dimension chromatography without the need for active solvent modulation valves [9].

It would be ironic if one of the richest veins for future new applications was based on the idiosyncratic lack of an elutropic series which Knox addressed when he first introduced his new material to an international audience. The material's property of giving strong retention with both solvents commonly thought of reversed-phase and those thought of as normal phase may yet lead to it being hailed as the most authentic facilitator of unified chromatography.

ACKNOWLEDGEMENTS

The authors would like to thank Vanessa Tang and James Clarke for the alignment of company codes with chemical names.

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JOHN H. KNOX
AN APPRECIATION

W. JOHN LOUGH

In 1997 it was an honour to write a guest editorial for the journal, Chromatographia, entitled "John H. Knox - 70 Years Old". The emphasis then was that there was much more to his achievements in separation science than just being a global leader in the development of chromatographic theory. It is also worth noting that there was much more to him than his scientific achievements. He was not just revered as a scientist but very well-liked by everyone that knew him. He was a thoroughly good human being. Maybe a shy man, he was nonetheless welcoming and non-judgmental with all, be it his peers, his co-workers, research students, exhibitors at his training courses or staff at the University refectory.

John Knox also had an aura about him but fortunately not one that engendered fear. His research group and team from his Wolfson Unit were in his image – a friendly, good-natured bunch. I was not in the group but worked for John Knox's Wolfson Unit lieutenant, Dick Wall, incidentally a very able and trusted aide to Knox especially when it came to equipment and column hardware design, but was in no way made to feel out of things. It all felt perfectly normal when one Friday lunchtime another great man, Jack Kirkland ("Big Jack") joined the Knox group for a meal at a small diner nestling at the foot of the Pentland Hills just outside Edinburgh.

The Pentland Hills had other connotations. Knox was known for his love of the healthy outdoor life and in particular his strong commitment to sailing at Cramond just to the north of Edinburgh on the Firth of Forth but the Pentland Hills were where he looked to do his running. Every Wednesday lunchtime he would be the prime mover to make sure everyone was up for it. I recall one freezing cold Wednesday in winter doing an 8-mile run when there was six inches of snow lying on the hills. The social side of things was more to the fore just after everyone had helped out on the highly successful RSC (Scottish Region) HPLC training courses staged by Knox and his fellow conspirator, Prof. Tony Fell, then at Heriot-Watt University. As far as I am aware Knox would never make a joke or say anything untoward about anyone. However, he wasn't so unworldly and naïve that he couldn't have a laugh at a joke at someone else's expense. Some saw tragedy when a celebration was ruined by a flooded toilet and smoke billowing out over the restaurant from on open spit. Knox, like a few others, enjoyed a good chuckle. He could also laugh at himself.

After one Christmas lunch Knox came into his research lab (DuPont constant pressure pumps and all) and was totally fascinated when shown a perfectly regular series of set of chromatographic peaks which had purportedly been obtained by injecting a sample of the Bull's Blood recovered from the restaurant. He wasn't at all upset when, after ten minutes of deliberation on the chromatogram, all and sundry collapsed in laughter when the prankster research student admitted to moving injected a polymer standard mixture and not Bull's Blood.

Finally, to get back to John Knox's scientific prowess, while he was known as by some as a humble, self-effacing man, that is not to say he was unaware of just how good he was. At the lectern he wasn't your usual captivating orator but there was definitely a presence. One was left with the feeling that it had been a privilege to an audience due to a deep conversation between the man and his thoughts. He was always totally immersed in the subject of his lecture. He was very much the same with scientific problems and discussions. Once when working in industry I contacted the man himself regarding a compound giving the most peculiar peak shapes. Essentially I wanted advice on how to fix the problem. What I got back was a 2-page letter from a man very intrigued by the issue which contained a series of equations describing what he thought were the various equilibria coming into play to give rise to the peak shape. There was no getting away from what everyone knew: John Knox was very good with equations!



Photo: John Knox (centre) April 1st 2004 at the RSC (Chromatography and Electrophoresis Group) meeting "A Century of Separation Science — Celebrating the Past: Predicting the Future" at the Royal York Hotel Conference Centre at which he" gave an erudite account of the development of high performance liquid chromatography (HPLC) interspersed with details of his own contributions (well into his 70's seen here looking much healthier than the younger Tony Fell (University of Bradford) and the much younger John Lough (University of Sunderland).

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W.J.Lough (1997). "John H. Knox - 70 Years Old". Guest editorial, Chromatographia, 46, 467-469

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