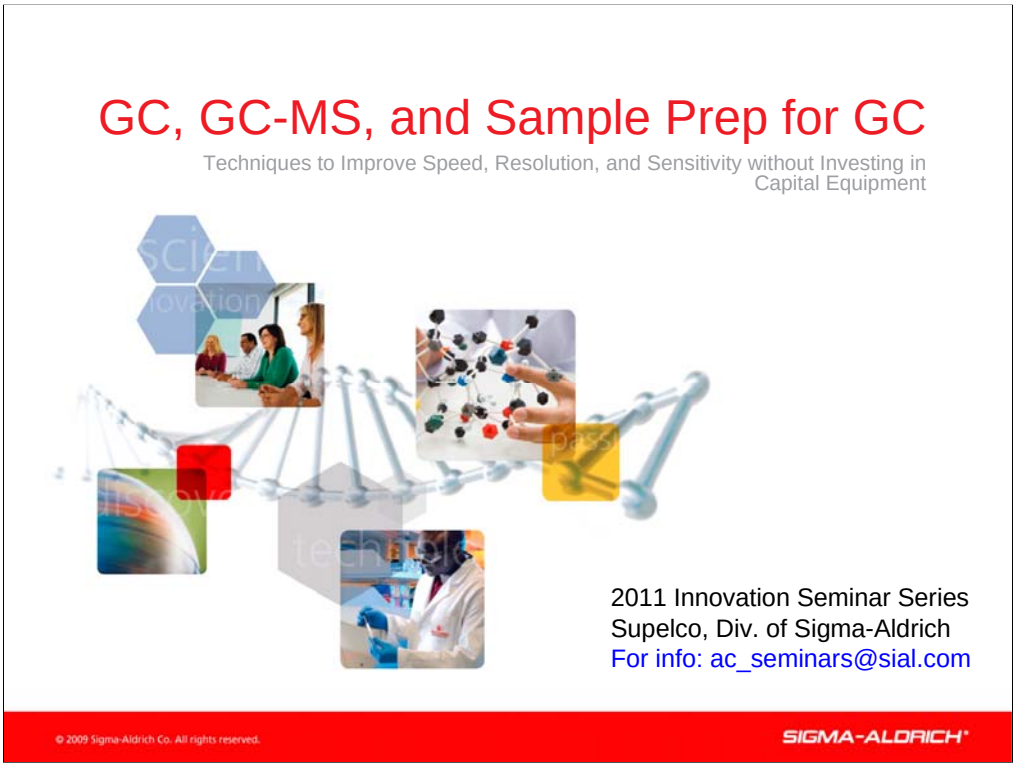
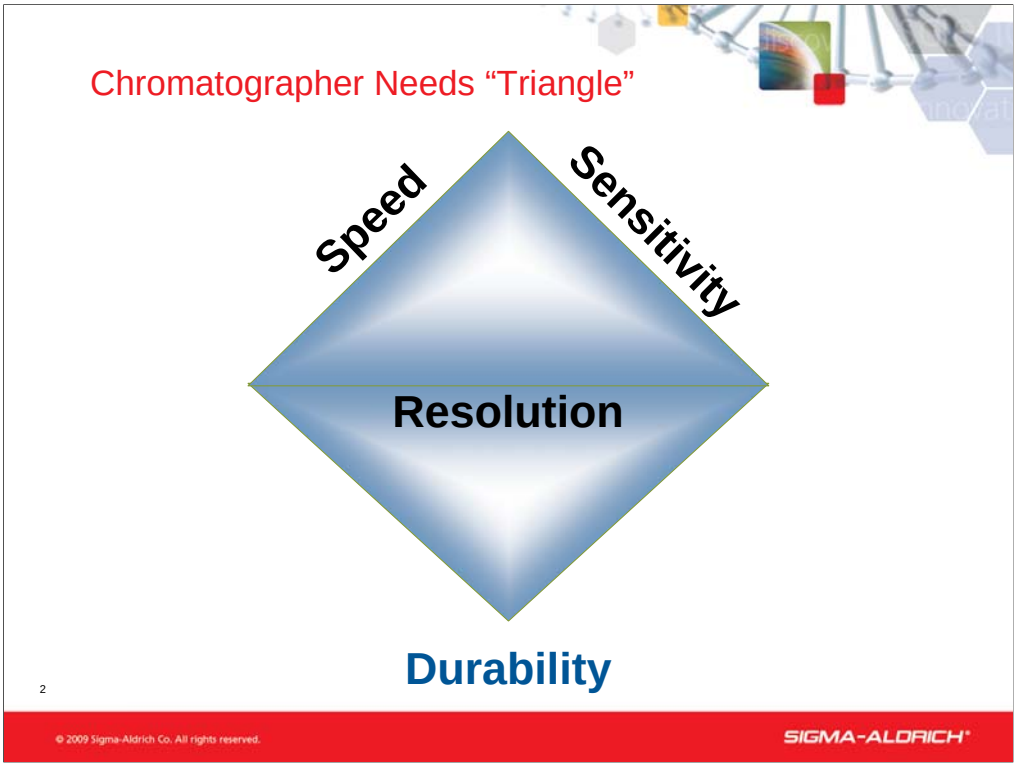


Abstract

Analysts interested in GC, GC-MS, and/or sample prep techniques that employ a GC finish will find this talk valuable. Intended to be instructional, this talk describes the scientific principles behind today's innovative technologies. Topics include ionic liquids, which show the promise of revolutionizing capillary GC because they offer the benefits of polarity/selectivity with higher maximum operating temperatures, ways to increasing throughput using Fast GC, solid phase microextraction (SPME), unique carbon adsorbents, QuEChERS, Ag-ion SPE for FAMEs, and others. Applications will be presented that demonstrate these innovations in pharma, food, forensics, environmental, bioanalysis, and other areas using interesting case studies geared toward the registrants' areas of interest.

GC may be considered a mature technique, but it is still widely used and GC analysts still desire to do things better and faster. Supelco was one of the first companies to offer capillary GC columns and we have continued to innovate with novel phase chemistries and column configurations. This presentation "GC, GC-MS, and Sample Prep for GC" will deal with three innovative technologies that use GC analysis, and how they can be implemented by any GC analyst on conventional instruments. Like the HPLC talk, our aim is to describe why we developed them and the underlying principles behind the products -- why they work. We will put them in the context of the fundamental chromatography equations, so hopefully you will come away knowing not only that they work, but why they work, and how they can be applied to your particular analytical challenge. After the main talk, we have some case studies to choose from that we can talk about.



This is what we call the Chromatographers Triangle.
Speed is how fast the separation occurs and is obviously important for lab throughput. For increasing speed & throughput we usually think of increasing flow rate, but this increases pressure and decreases efficiency, or using shorter columns, but this also reduces efficiency, so we:

- Need columns that deliver the necessary plates, but fast
- Need sample prep processes that eliminate steps

Sensitivity is how low in abundance can you detect your analyte. Sensitivity – we usually think of injecting more, but samples may be limited, columns overload and lose efficiency, or there are too many interferences in the sample. So we:

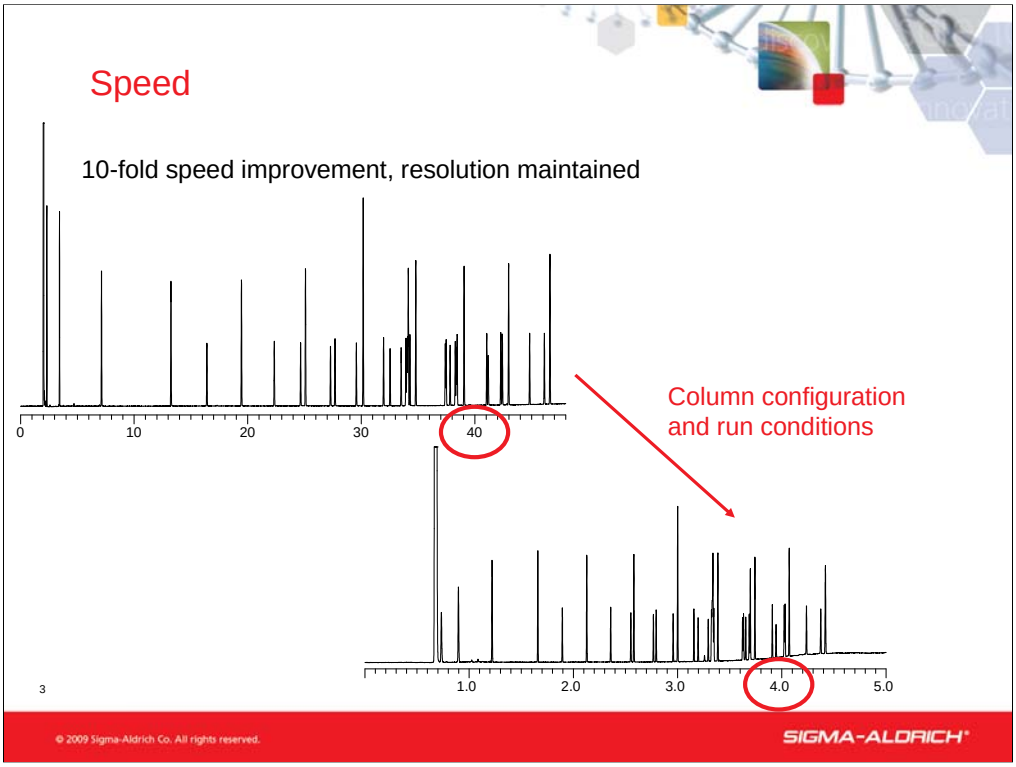
- Need columns that supply peaks with high s/n ratio
- Need sample prep processes that remove interferences

Resolution is being able to see one component in the presence of other components; to be able to pull apart the peaks. We usually think of increasing efficiency to increase resolution, which is good, but also consider selectivity:

- Need columns with different bonded phase chemistries
- Need sample prep devices with controlled surface chemistry

And because no separation is interesting if it can’t be repeated, we want our analysis to be durable; columns should be durable, stable, reproducible, and last a long time. So we really have a Chromatography “Square.”

You’ll hear it said that this shape represents compromise – if you optimize any of “sides,” you will adversely affect the others. So we’ve been working on changing that paradigm; how you move toward having it all. Next, let’s see what is possible.

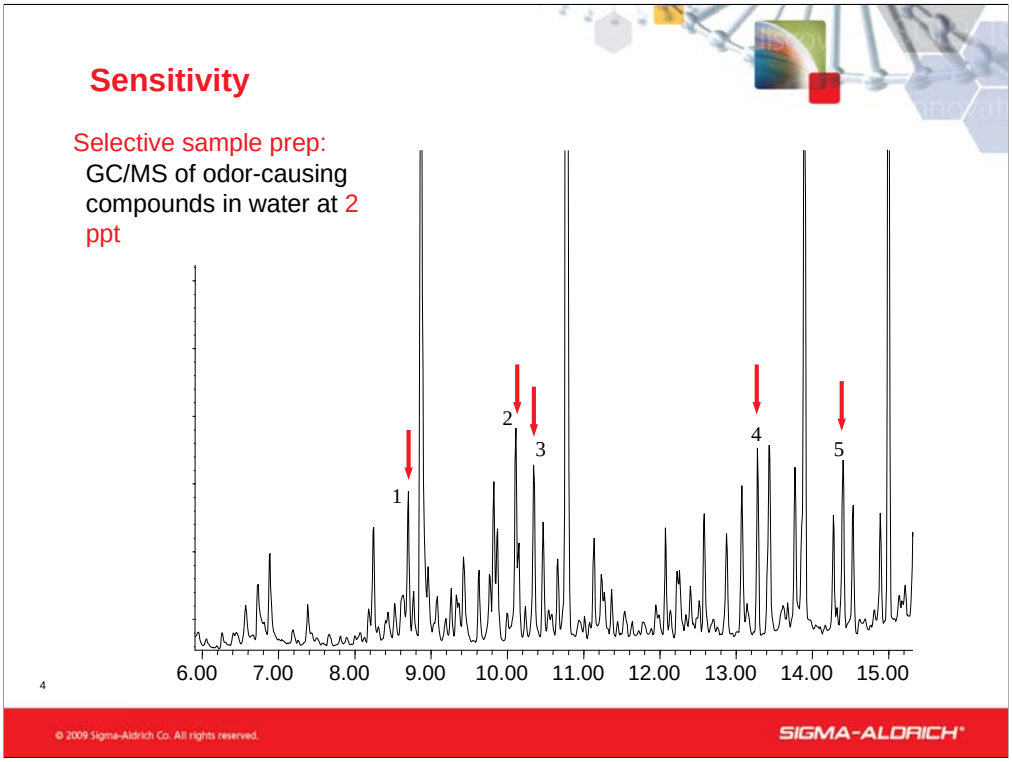


An example of the increase in speed that is possible is shown here. We took a >40 minute GC run, and on the same instrument, we are able to dramatically reduce the run time, here to around 4 minutes, a 10-fold increase in speed. Note that this did not come at the expense of the resolution. We used column dimensions and run conditions for Fast GC to accomplish this, which we'll describe later.

Supelco 37-Component FAME (fatty acid methyl ester) mix

Top: Conventional GC
30 m x 0.25 mm I.D., 0.25 µm film
He, 1 mL/min.

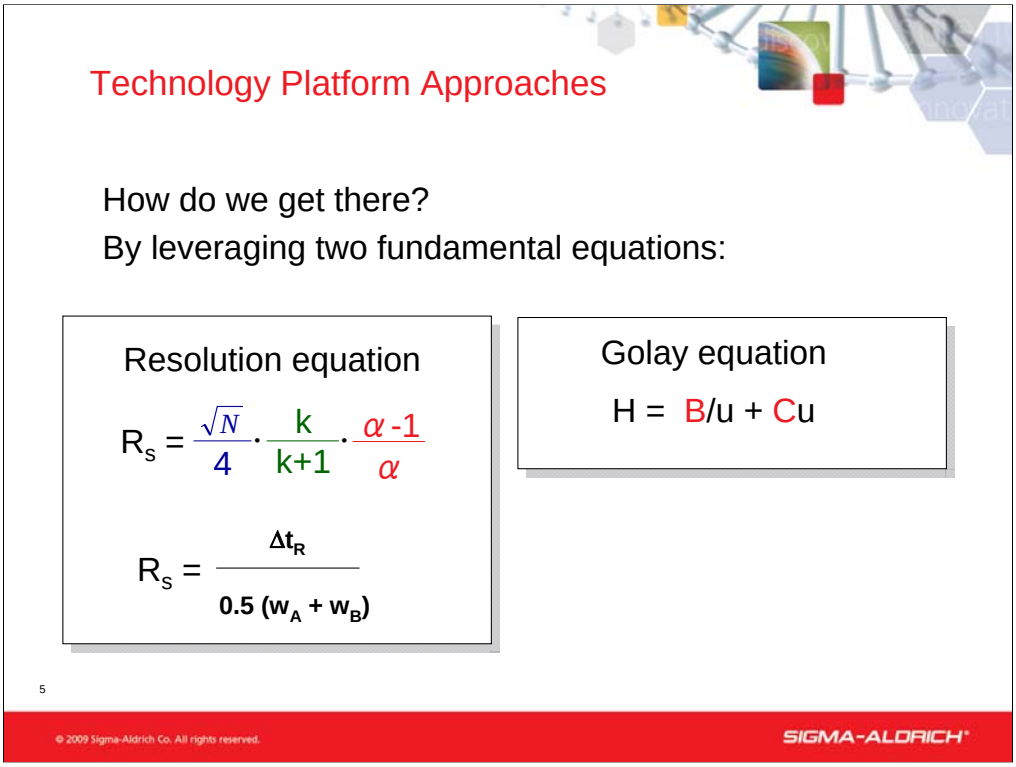
Bottom: Fast GC
15 m x 0.10 mm I.D., 0.10 µm film
H_2, 50 cm/sec.



We can address sensitivity with both columns (by maximizing efficiency), sample prep device, and instrument settings. It is no surprise how effectively removing sample matrix can improve sensitivity. We'll talk about both column and sample prep approaches. In this slide, four odor causing agents are extracted along with trichloroanisole as the internal standard. Note that detection level of 2 ppt was easily obtained.

A quadrupole mass spectrometer was used to generate this slide. It was critical that the detection was done in the single ion mode. Full scan would not detect the analytes. Even with selected ions, the background is still high due to the very low detection threshold. When a single ion is extracted for quantification of an analyte, the background is greatly reduced.

Sample: 25mL water containing odors at 2 ppt 25% NaCl, 40mL vial
SPME fiber: 2cm DVB/Carboxen™/PDMS
Extraction: heated headspace, 30 min, 65° C, rapid stirring
Desorption: 3 min, 250° C, splitter closed
Column: Equity-5, 30m x 0.25mm x 0.25µm film
Oven: 60° C (1 min) to 250° C at 8° C/min
Det.: mass spectrometer, quadrupole, single ion mode
1. 2-Isopropyl-3-methoxypyrazine (IPMP)
2. 2-Isobutyl-3-methoxypyrazine (IBMP)
3. 2- Methylisoborneol (MIB)
4. 2,4,6-Trichloroanisole (I.S. 8ppt)
5. (±) Geosmin



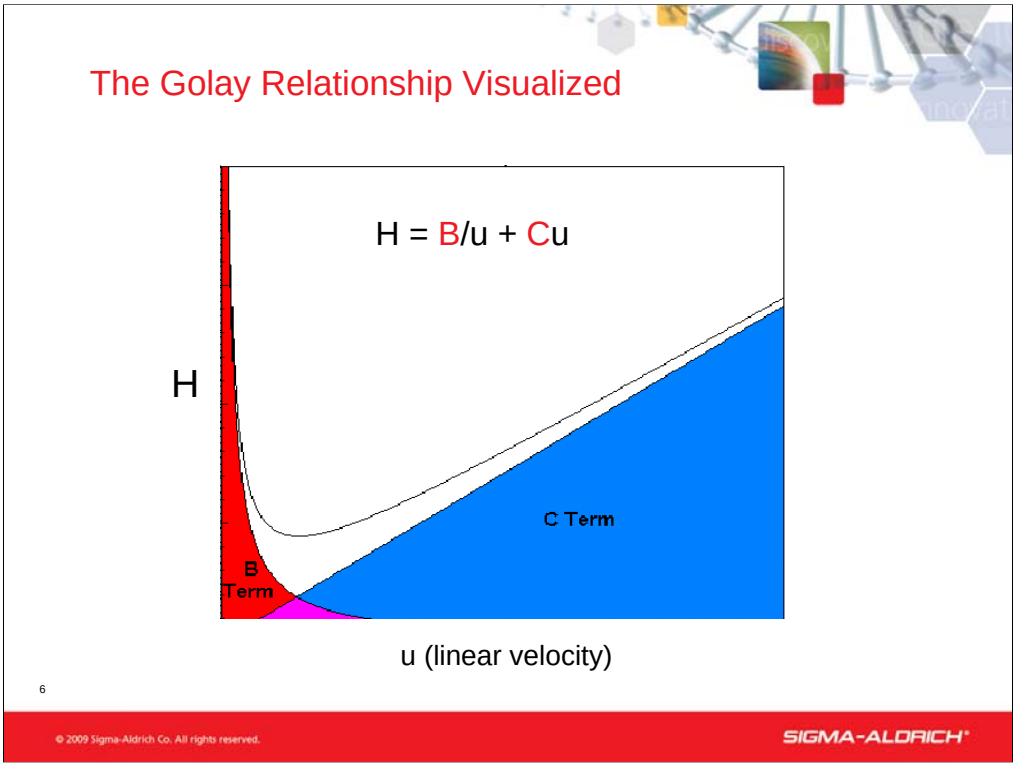
But how do we get to these dramatic improvements? What development paths do we take? Well, like the HPLC discussion, it all boils down to a fundamental understanding of what controls speed, resolution, sensitivity, (and durability) in chromatography. Although a full dissertation on these equations is a college-level course, we can distill it down for the purposes of this discussion into a basic understanding of two equations:
• the Resolution equation (left)
• the Golay equation (right)

On the left are the two most commonly-encountered forms of the resolution equation. We've shown it in these two forms so you can easily see what contributes to chromatographic resolution: efficiency (N, and w), retention (k and Δt_R), and selectivity (α). The terms are related, of course.

The Golay equation on the right is the basic equation describing the dispersion that takes place in an open tubular column. It is the van Deemter equation minus the A-term, which doesn't apply to open tubes. (This is the main reason why capillary GC is so much more efficient than packed GC and HPLC.) H is height equivalent of a theoretical plate, and low H is good. Each of the terms, B and C, have to be minimized to get low H and high efficiency values.

Definition of terms:
N = column efficiency in theoretical plates, $N = 5.54(t_R/w_{0.5})^2$ where $w_{0.5}$ is the peak width at half-height
k = retention or capacity factor, $k = (t_R - t_0)/t_0$
α (alpha) = selectivity, $\alpha = k_2/k_1$
Δt_R is also called differential migration. $\Delta t_R = t_B - t_A$
w_A, w_B = peak widths at baseline. w is also called band dispersion
u = linear velocity, which equals L/t_0 where L is column length. u is usually reported in cm/sec or mm/sec. Average linear velocity is estimated using the retention time, t_0, of an unretained peak and the formula $u = L/t_0$. The flow rate, F, and the estimated (or calculated) void volume, V_0, of the column can also be used to arrive at velocity using the formula $u = LF/V_0$.

Note: Various levels of GC training, from beginner to advanced, are available from Supelco. Please inquire.



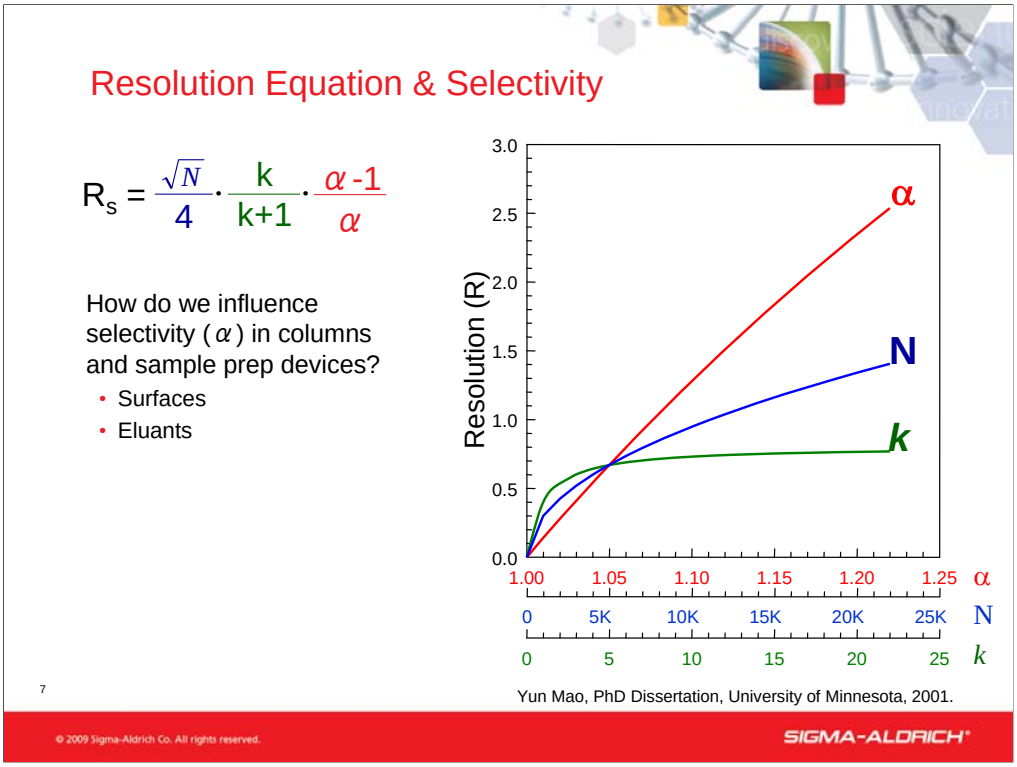
A classic Golay (van Deemter minus the A-term) curve shown here is a plot of column efficiency vs. linear velocity (u). H = L/N. H is plotted instead of N because it is independent of column length to allow easy column performance comparison.

The B-term is *longitudinal (axial) diffusion* that causes bands to spread up and down the column, which is a function of solute size (diffusion coefficient, D) and how long the band stays on the column, which is a function of flow rate and retention factor, k.

The C-term is *resistance to mass transfer (rate of mass transfer)* into the phase (particles do not apply in capillary GC). The C-term is very dependent on flow rate and film thickness, and is also dependent on solute diffusion coefficient in a manner that is opposite to that of the B-term. Small molecules and fast diffusion lower the C-term but increase the B-term and *vice versa* for large molecules. Temperature also has an important (opposing) impact on these two terms. For a given solute, thinner films decrease slope of the C-term by decreasing the diffusion path into and out of the particle.

Several points are worth noting:
1. We want the lowest possible value H because H is inversely proportional to N.
2. We also want to be able to work at high flow rates to get fast separations.
3. B-term: This red portion shows the importance of keeping flow rates high, because diffusion really takes over at low flow rates.
4. C-term: The flatter we can make the curve, the faster we can run separations and maintain efficiency. In capillary GC, the main contributor to the C-term is the phase thickness.

So – what can be done to minimize the B- and C-terms?
1. Reduce column I.D.
2. Reduce mass transfer into and out of the stationary phase
3. Optimize flow rate

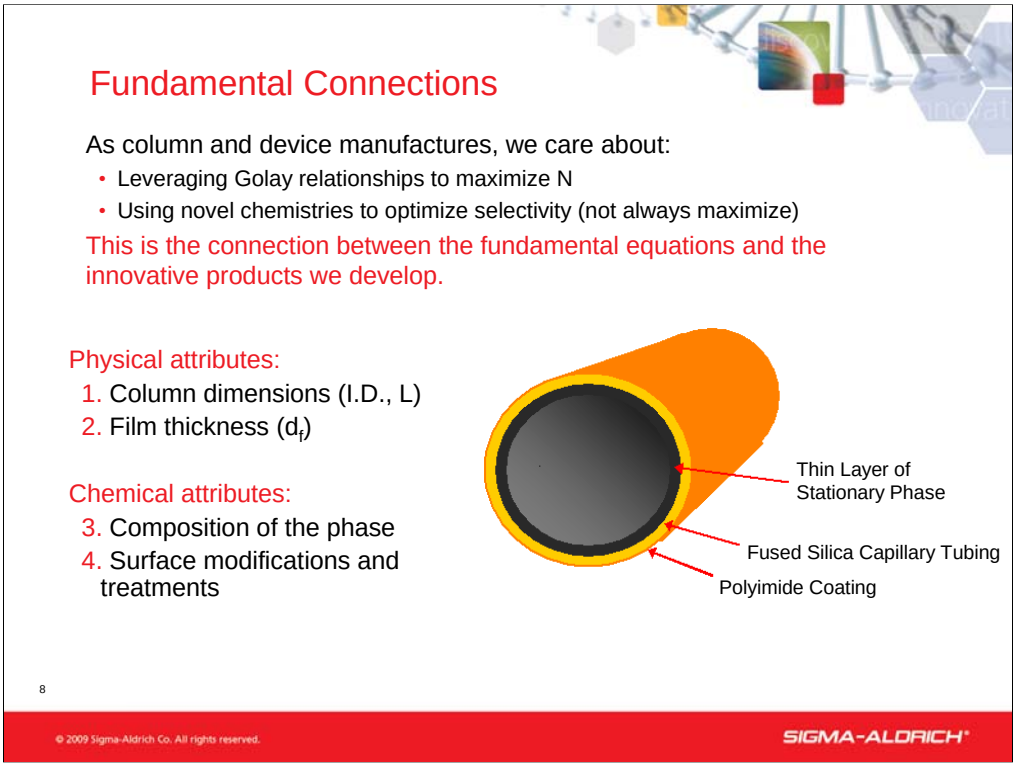


Here's the resolution equation again. It applies in GC just like we discussed in the HPLC section. We just discussed the N term, but you can see that retention (k) and selectivity (α) can have profound influence.

Those terms are defined by these equations. In GC, you have the phase to play with to adjust chromatographic selectivity. Sample prep devices that employ liquid eluants also have the power to alter retention and selectivity using pH, ionic strength, etc.

This graph shows the influence of the three terms on resolution. When plotted, the relative effects of the three terms can be seen. Of the three parameters, selectivity has the greatest effect on resolution and its effect does not LIMIT as does the effect of efficiency and retention. The graph was generated by starting with N = 5,000, k = 5, alpha = 1.05. Fixing two variables, then changing the other generated the three lines. At very low k, increasing k has a large effect on resolution, but above k = 2, it has little or no effect. At very low column efficiencies, increasing N increases R_s dramatically. Selectivity in GC can be changed by changing the stationary phase and possibly the film thickness.

Definition of terms:
Capacity factor, $k = (t_R - t_0)/t_0$
Selectivity, $\alpha = k_2/k_1$
$N = 5.54(t_R/w_{0.5})^2$ where $w_{0.5}$ is the peak width at half-height



So, as column and device manufactures, we care about:

Leveraging Golay relationships to maximize N

Using novel chemistries to optimized (not always maximize) α

This is the connection between the fundamental equations and the innovative products we develop. And the parameters we as manufacturers have to "play" with are:

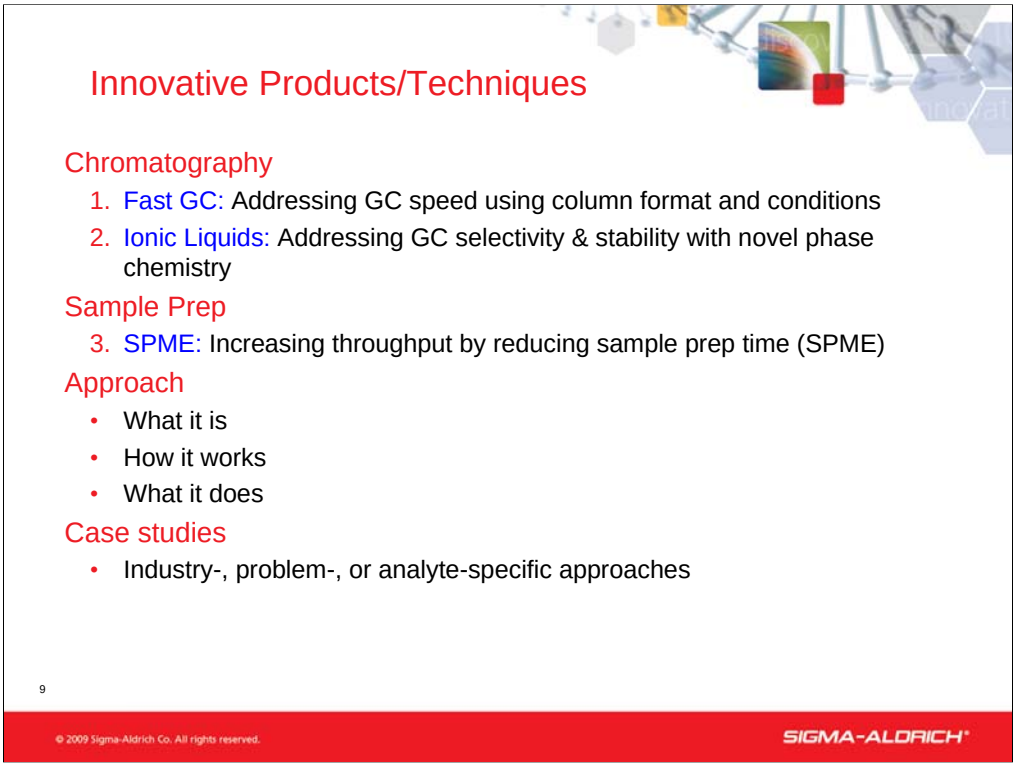
Column dimensions:
- I.D.
- Length
- Thickness of the phase film

Chemical attributes:
- Composition of the phase
- Surface modifications and treatments

The products we'll talk about today have been developed around one or several of these approaches.

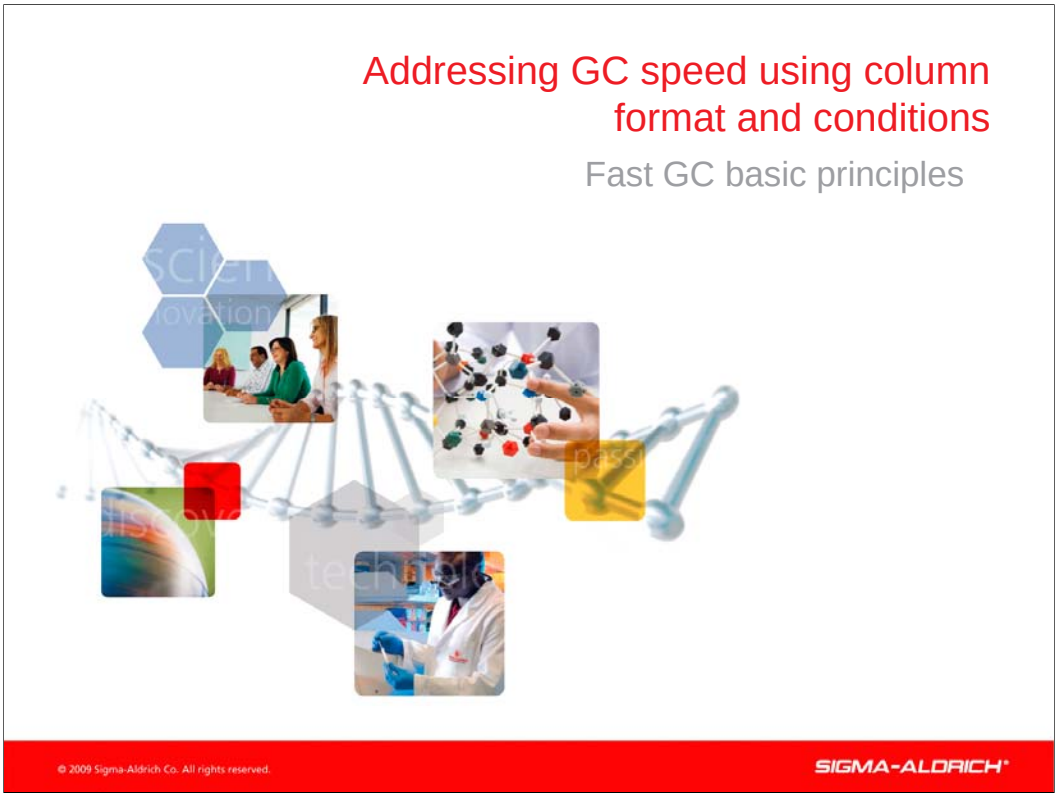
The figure shows the anatomy of a modern GC capillary column. There are other types, including packed columns, but this type comprises ~97% of the market. It has basically 3 parts:

1. It starts with a fused silica tube, called capillary tubing in this figure. There are different types of treatments done to the inner surface to make it more inert, or make the phase layer adhere better.
2. The tube is then coated with a polymer, usually polyimide, to make it flexible without breaking
3. Then the stationary phase is coated onto the inner surface.



These are the three innovative technology areas we will talk about today: What they are, how they work (fundamental equations), and what they can do. Like we did in the HPLC talk.

Will these products have applicability to your particular industry or analytical/sample prep situation? Hopefully! But at the end we have a long list of applications that are geared to specific industries. I've chosen some to talk about, but if you have any specific interests, we can discuss them too.

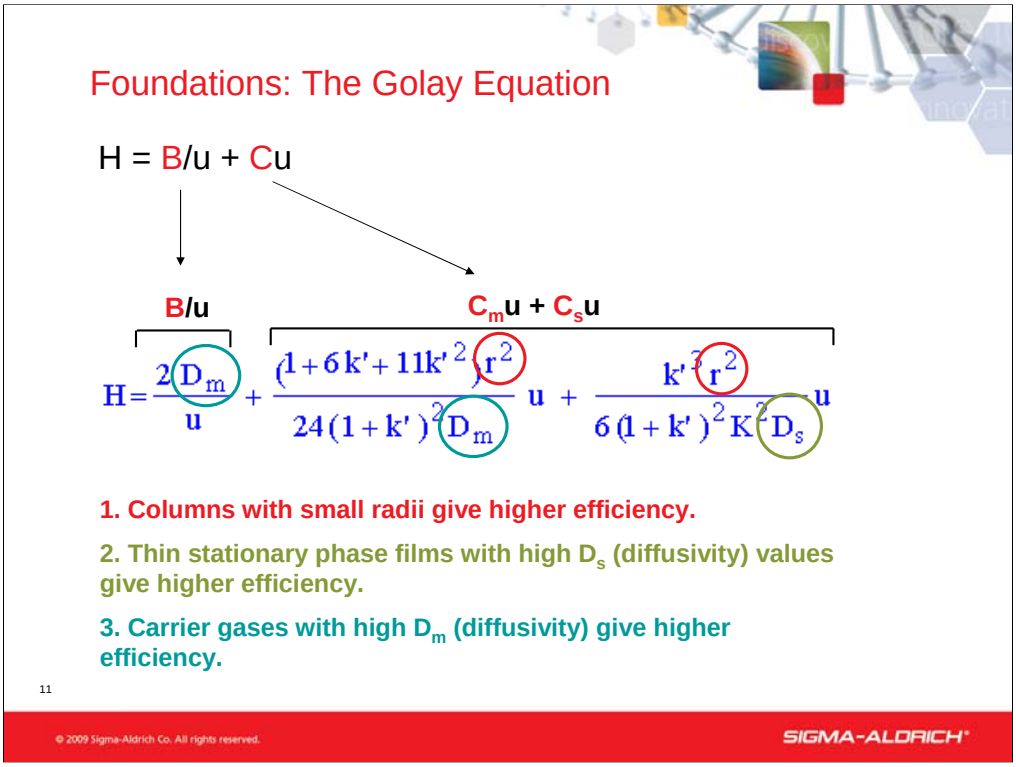


Analytical chemists using GC are continually striving to reduce analysis times, because shorter analysis times increase sample throughput, which translates to the completion of more (sometimes billable) samples per shift. However, any decrease in analysis time must not diminish the resolution necessary to adequately resolve peaks of interest, and identify specific elution patterns. Applying the Principles of Fast GC to any application can achieve both of these objectives. Now, Fast GC isn't a recent innovation. It has been around for nearly 20 years. But it is our experience that the majority of GC users are not taking advantage of Fast GC, maybe they don't know where to start to convert a method. So we hope to at least touch on what parameters we adjust, and why, based on the Golay relationship.

Why should you consider implementing Fast GC into your lab? Time and money! Fast GC yields faster analysis times than conventional GC, often three to ten times faster, offering higher throughput and allowing more samples to be analyzed per shift. The main benefits to a laboratory are that:
• Costs can be decreased if fewer analysts and/or instruments are needed
• Revenue can be increased if more samples are analyzed
• Method development can occur faster

Fast GC can be applied to any application with no sacrifice in quality. Lastly, Fast GC typically does not require any additional capital equipment. We'll walk through the principles behind Fast GC and how you can take advantages of the speed advantages.

We have a full seminar just on Fast GC. So please let us know if you are interested in learning more.



To fully understand Fast GC, it is necessary to take a closer look at the Golay relationship we touched on earlier. This slide shows an exploded version of the Golay equation so you can see the individual contributing factors. The variables are defined below. We didn't discuss this to this level of detail in the HPLC talk, but we will here because Fast GC leverages the r, D_m, and D_s terms.

Again, H = HETP (height equivalent of a theoretical plate) Because N = L/H, the smaller the H value, the higher the column efficiency (N). Changes to any of the terms in the Golay equation that decrease H will increase column efficiency.

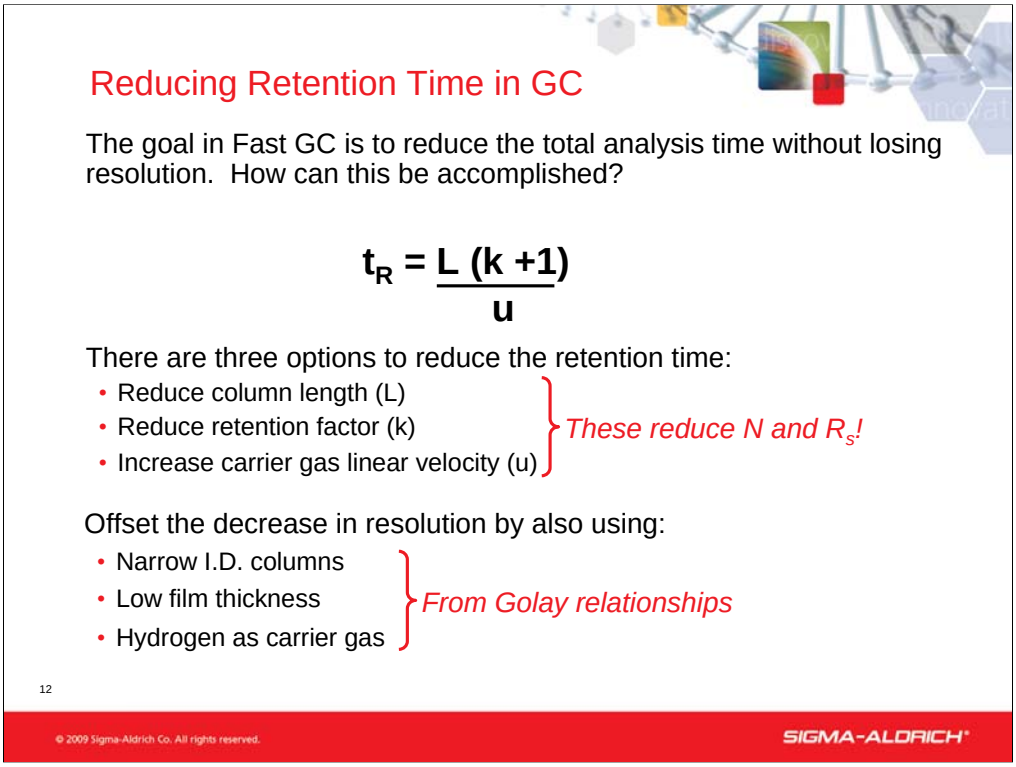
Let's take a deeper look at the B- and C-terms. They have in common u, the linear velocity of the mobile phase (carrier gas). The **B-term (longitudinal diffusion)** has D_m which is the diffusivity of the analyte in the mobile phase. The **C-term (resistance to mass transfer)** has two parts: C_m is resistance to mass transfer in the mobile phase, and C_s is resistance to mass transfer in the stationary phase. The variables in the C-terms are:

- k' (or k) which is the retention factor of the analyte
- D_s which is the diffusivity of the analyte in the stationary phase
- D_m which is the diffusivity of the analyte in the mobile phase (also shows up in the B-term)
- K which is the distribution coefficient of the analyte between the two phases
- r which is the radius of the column

Three points important to this discussion are:

1. Columns with small radii (r) give higher efficiency.
2. Thin stationary phase films with high D_s (diffusivity) values give higher efficiency.
3. Carrier gases with high D_m (diffusivity) give higher efficiency.

Let's see how we can use these variables in Fast GC.



The goal of Fast GC is to reduce the run time, obviously. How to do this?
This equation defines retention time in GC: $t_R = L (k + 1)/u$
Where L is column length, k is capacity factor, and u is linear velocity.

We see that we can decrease t_R by:
1) Decreasing column length (L)
2) Reducing capacity factor (k), by changing the phase, or increasing temperature, for example.
3) Increasing flow rate (u)

This is intuitive, but doing any of these will also reduce efficiency and resolution. So they all have to be balanced to get the throughput improvements without sacrificing quality of the analysis. So, we offset the decrease in resolution by also using:
1) Narrow I.D. columns
2) Low film thickness
3) Hydrogen as carrier gas (note: Nitrogen provides the best efficiency as a carrier gas but the optimal working u window is too small and the optimum u is vey low, so long analysis times result. We use helium and, preferably, hydrogen for speed.)

Before we look at how-to perform Fast GC, let's take a step back and look at why it works through a short theoretical discussion. The underlying Principles of Fast GC are pretty simple.

Analysis times can be decreased by using:
- Short columns
- Fast oven temperature ramp rates
- High carrier gas linear velocities

Fast GC also employs faster temperature ramp rates.

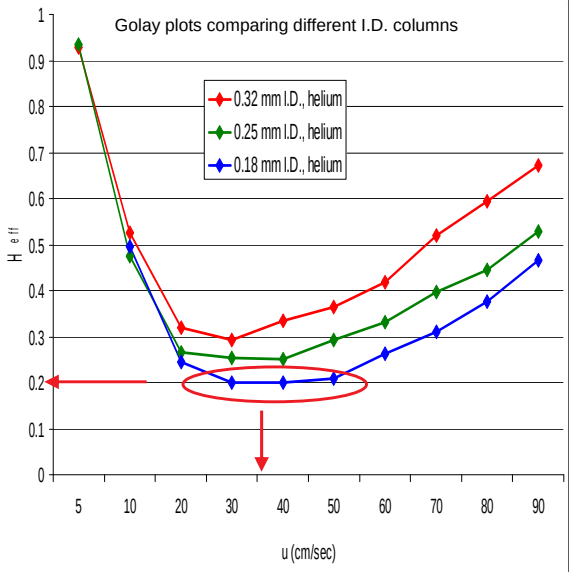
The loss in resolution caused by the above steps can be offset by using:
- Narrow I.D. columns
- Low film thickness
- Hydrogen carrier gas

The more Principles that are applied, the greater the benefit!

Fast GC: Why Use Narrow I.D. Columns?

Narrow I.D. columns provide:

- **Lower H**
  - increases efficiency leading to increased resolution
- **Higher u_{opt}**
  - can use a faster flow rates than with larger I.D. columns
- **Flatter Golay relationship**
  - can be run at flow rates above u_{opt} without a significant increase in H



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SIGMA-ALDRICH

Let's take a moment and look at some data relating to column I.D. and carrier gas. First is column I.D.

Shown here are Golay plots of three different I.D. columns (0.18, 0.25, and 0.32 mm). The x-axis shows linear velocity (u), and the Y-axis shows effective plate height (H_{eff}). The phrase optimal linear velocity (u_{opt}) is used to define the linear velocity value when the Golay plot is at its lowest. As we have already discussed, lower plate height (H) values result in higher resolution, and that higher linear velocity (u) values result in shorter analysis times. From a Fast GC point of view, we want to choose a column I.D. whose Golay plot reaches low and to the right.

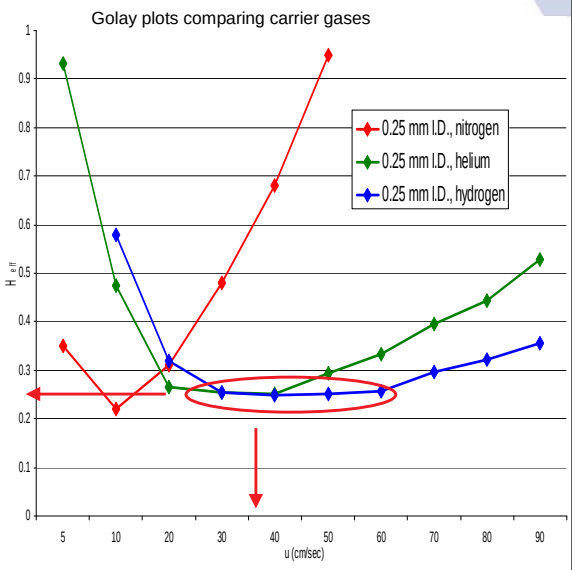
For Fast GC, a narrow I.D. column is the best choice, because:
- It has a lower effective plate height (H_{eff}) than larger I.D. columns, which increases efficiency and leads to increased resolution
- It has a higher optimal linear velocity (u_{opt}) than larger I.D. columns, which allows faster analysis
- It has a flatter Golay relationship than larger I.D. columns, which allows the use of a liner velocity (u) greater than optimal (u_{opt}) without a significant increase in effective plate height (H_{eff})

Note: Data for a 0.10 mm I.D. column with helium carrier gas could not be obtained due to high backpressure.

Fast GC - Why Use Hydrogen?

Hydrogen exhibits:

- **Low H_{eff}**
  - good efficiency and resolution
- **Higher u_{opt}**
  - can use a faster flow rates than with other carrier gas choices
- **Flatter Golay relationship**
  - can be run at flow rates above u_{opt} without a significant increase in H



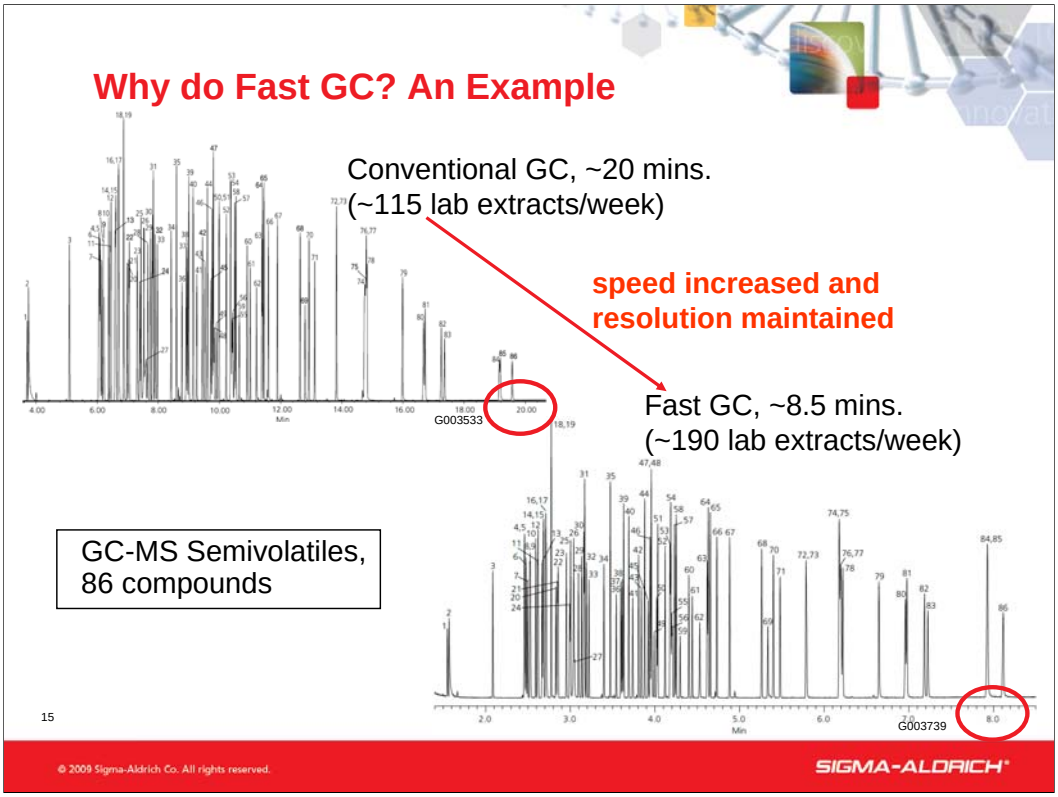
14

SIGMA-ALDRICH

Now let's look at carrier gas. Shown are Golay plots of the common GC carrier gases nitrogen, helium, and hydrogen on the same 0.25 mm I.D. column. As stated on the previous slide, a Golay plot that reaches low and to the right is desirable from a Fast GC point of view.

For Fast GC, hydrogen is the best choice, because:
- It has a low effective plate height (H_{eff}), resulting in good efficiency and resolution
- It has a higher optimal linear velocity (u_{opt}) than other carrier gases, which allows faster analysis
- It has a flatter Golay relationship than other carrier gases, which allows the use of a liner velocity (u) greater than optimal (u_{opt}) without a significant increase in effective plate height (H_{eff})

Note: Although nitrogen provides the best efficiency as a carrier gas, the optimal working u window is very narrow, and the optimum u is very low, so long analysis times result. We use helium and, preferably, hydrogen for speed.



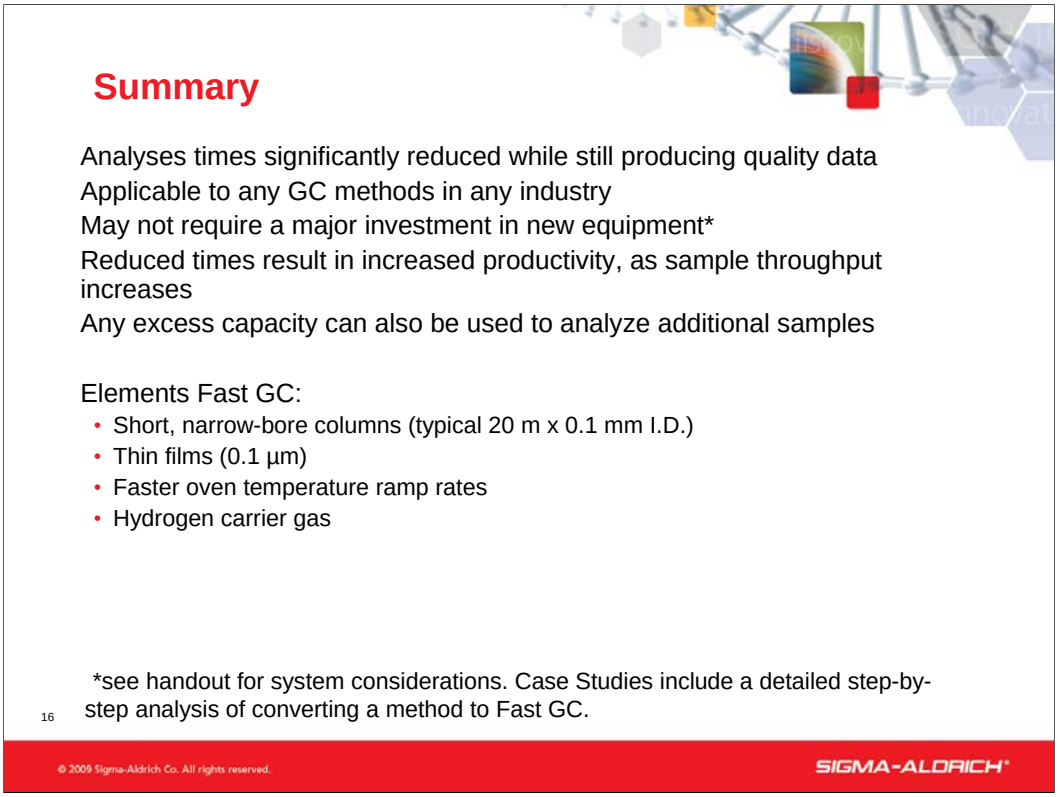
Here is an example of why Fast GC should be considered. Both of these chromatograms are the GC-MS analysis of semivolatiles, an application routinely performed in environmental laboratories. This method requires the GC-MS to be 'tuned' and calibrated prior to the analysis of any lab extracts (blanks, QA samples, and billable samples), and that all lab extracts must be injected within 12 hours of when the 'tune' solution was injected. The shorter the run time, the more lab extracts that can be run within the 'tune' window.

The top chromatogram was obtained using conventional GC. Assuming a single 'tune' window is set-up per day, each instrument can analyze ~115 lab extracts per week after taking into account the cool down period between runs.

The bottom chromatogram was obtained after applying the Principles of Fast GC. Assuming a single 'tune' window is set-up per day, each instrument can now analyze ~190 lab extracts per week after taking into account the cool down period between runs. This increase of 75 lab extracts per week does not require any increase in staff or equipment. Additionally, the quality of the analysis is not diminished!

Conditions (top chromatogram):
- column: SLB-5ms, 30 m x 0.25 mm I.D., 0.25 µm (28471-U)
- oven: 40 ° C (2 min.), 22 ° C/min. to 240 ° C, 10 ° C/min. to 330 ° C (1 min.)
- inj.: 250 ° C
- MSD interface: 330 ° C
- scan range: m/z 40-450
- carrier gas: helium, 1.0 mL/min (11 min.), 10 mL/min2 to 1.5 mL/min. (hold remainder of run)
- injection: 0.5 µL, splitless (0.50 min.)
- liner: 2 mm I.D., straight
- sample: 80 component semivolatile standard at 50 ppm plus 6 internal standards (at 40 ppm) in methylene chloride

Conditions (bottom chromatogram):
- column: SLB-5ms, 20 m x 0.18 mm I.D., 0.18 µm (28564-U)
- oven: 40 ° C (0.7 min.), 55 ° C/min. to 240 ° C, 28 ° C/min. to 330 ° C (2 min.)
- inj.: 250 ° C
- MSD interface: 330 ° C
- scan range: m/z 40-450
- carrier gas: helium, 40 cm/sec, constant
- injection: 0.5 µL, 10:1 split
- liner: 2 mm I.D., fast FocusLiner™ inlet liner with taper (2879501-U)
- sample: 80 component semivolatile standard at 50 ppm plus 6 internal standards (at 40 ppm) in methylene chloride



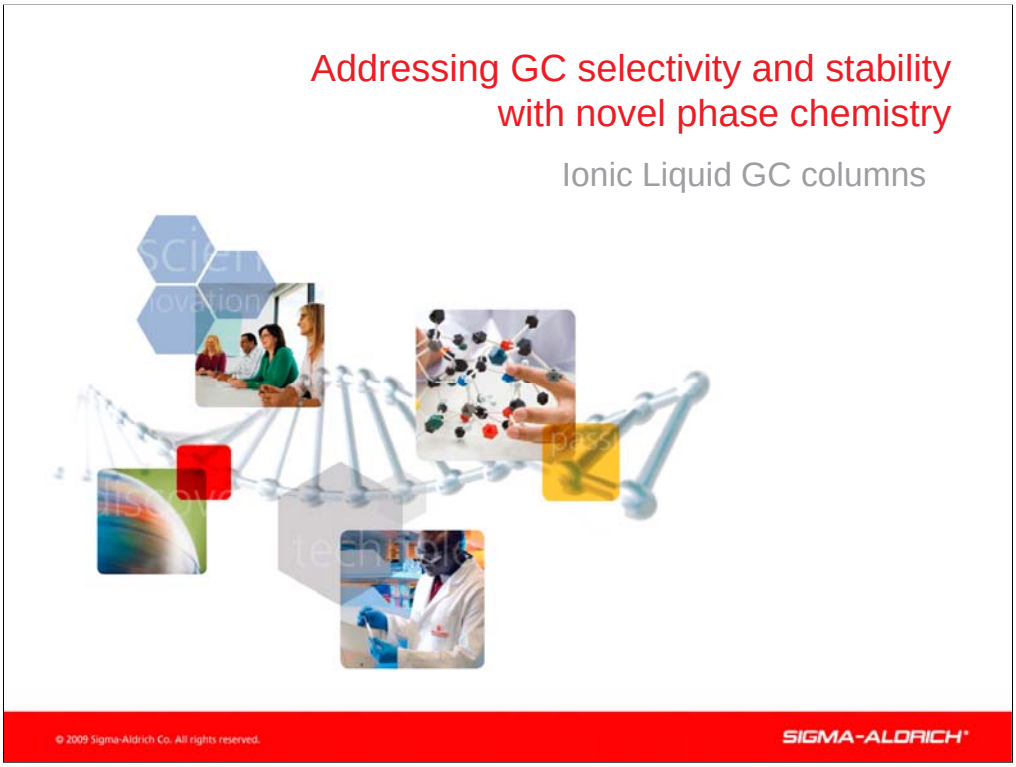
Fast GC can be applied to any application in any industry, any may not require a major investment in new equipment. By applying the techniques of Fast GC such as using shorter, narrower bore columns, faster oven temperature ramp rates, and hydrogen carrier gas, analyses times can be significantly reduced while still producing quality data. These reduced times can result in increased productivity, as sample throughput increases. Any excess capacity can also be used to analyze additional samples, resulting in increased revenue. We have a full hour seminar on Fast GC if you are interested in learning more about how to implement it.

About temperature ramp rates:
Fast oven temperature ramp rates are essential to decreasing analysis time. It is important to know the ramp rate abilities of your GC for the temperature ranges in which you will be operating. Programming a ramp rate that is faster than your GC can handle may result in variations in retention time. Many newer GC instruments have faster ramp rate abilities due to decreased oven volume or 240V power connections. On older GCs, decreasing the internal oven volume through the use of an insert is an inexpensive and simple way to increase ramping ability.

About instruments for Fast GC:
The instrumentation being used may prohibit applying all of the principles of Fast GC. An example of this is when working with GC-MS. Some older MS instrumentation may not work properly with hydrogen as a carrier gas. To find out whether your MS is compatible with hydrogen carrier gas, check your instrument manufacturer.

About detectors:
Because Fast GC produces rapid and narrow peaks, the detector must be able to obtain sufficient data points per peak to ensure proper peak quantitation. Most new detectors are able to work with Fast GC.

About MS carrier gas requirements:
Some older MS instrumentation may not work properly with hydrogen as the carrier gas. Consult your instrument manufacturer.



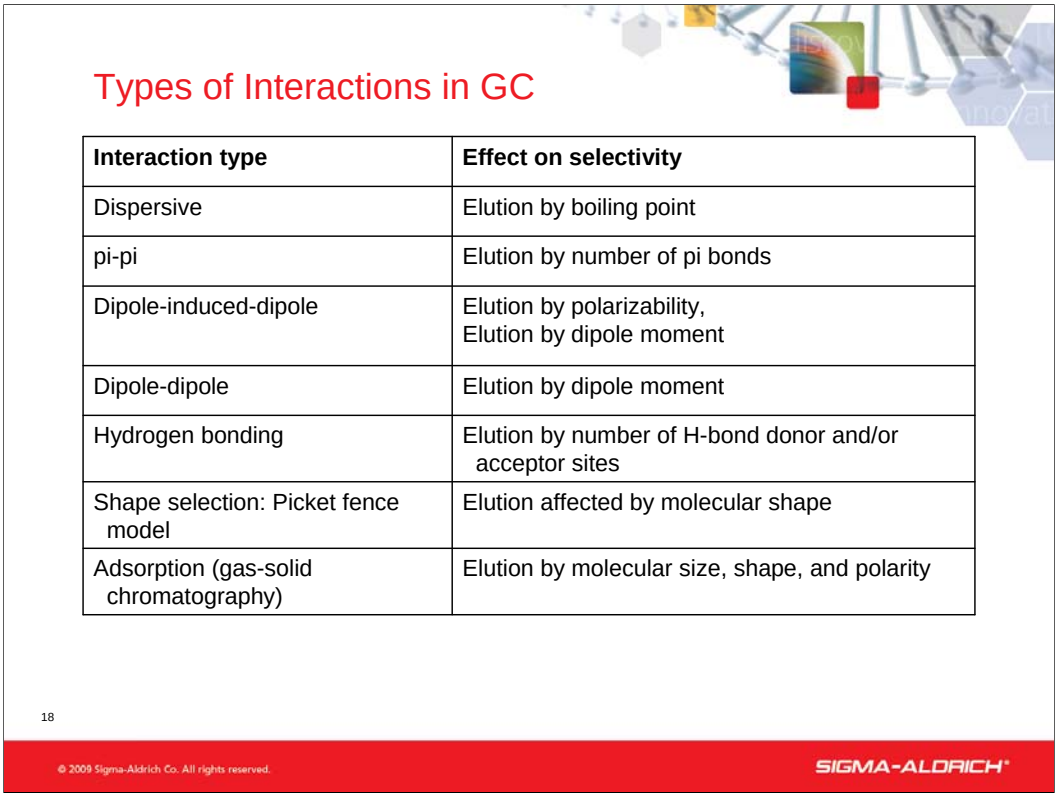
This next topic, ionic liquids for GC, are truly new and innovative. There haven't been that many true innovations in the GC field for many years. It was considered a mature field. But application of ionic liquids for GC phases has shifted that paradigm. In 2005, Prof. Daniel W. Armstrong (University of Texas at Arlington) showed that dicationic and polycationic ionic liquids could successfully be used as viable GC stationary phases. They also do not require any instrument modifications to take advantage of them. Supelco is in the process of commercializing columns that use the IL chemistry.

Whereas Fast GC leverages the Golay equation, ionic liquids let us leverage the selectivity and retetion terms in the Resolution equation. And we remember that alpha is the most power term in that equation.

What are ionic liquids?

Ionic liquids are a class of nonmolecular ionic solvents with low melting points. These liquids are a unique combination of cations and anions and can provide a variety of different selectivities when used as stationary phases in capillary gas chromatography. There are numerous combinations of cations and anions possible, so tailoring the material to a specific application or function is a potential benefit of these materials.

(These materials are patent pending.)



Types of Interactions in GC

Interaction type	Effect on selectivity
Dispersive	Elution by boiling point
pi-pi	Elution by number of pi bonds
Dipole-induced-dipole	Elution by polarizability, Elution by dipole moment
Dipole-dipole	Elution by dipole moment
Hydrogen bonding	Elution by number of H-bond donor and/or acceptor sites
Shape selection: Picket fence model	Elution affected by molecular shape
Adsorption (gas-solid chromatography)	Elution by molecular size, shape, and polarity

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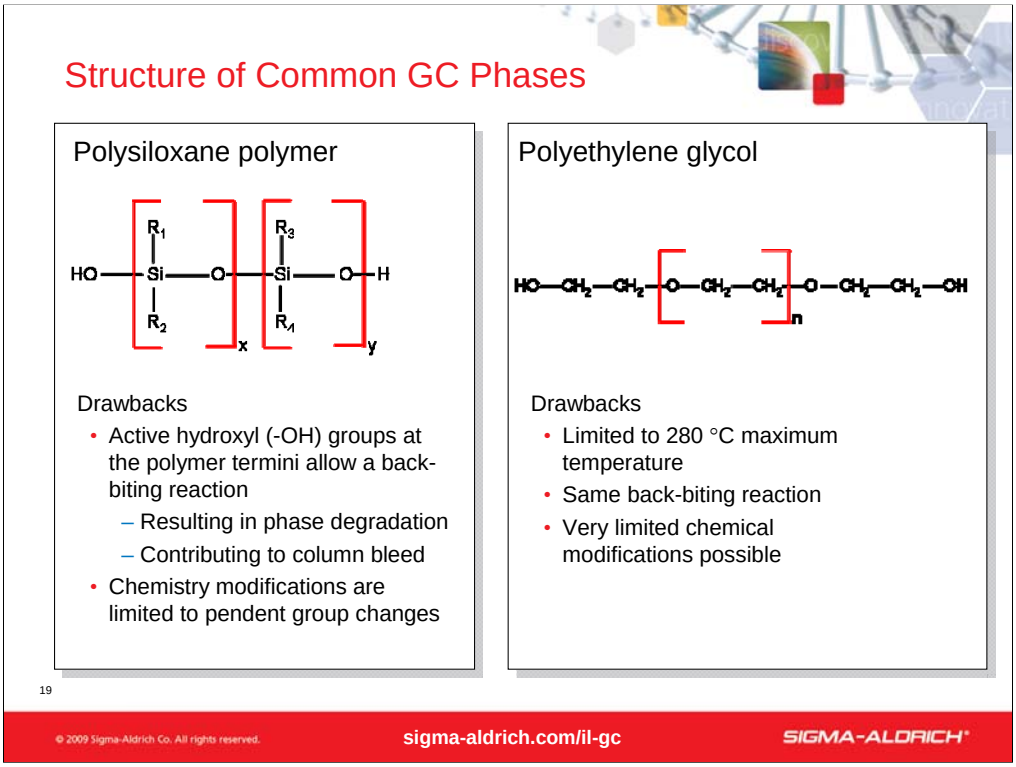
Just as a quick review. When we think of GC, we think of elution by boiling point. While this is true, boiling point is not the only separation criteria. The chemical nature of the GC phase has a strong influence on retention and selectivity. This table outlines the major types of interactions in GC, and their effect on selectivity. So, we can leverage stationary phases in GC just as we do in LC to pull apart closely-eluting compounds, reverse elution order, increase or decrease retention, whatever the aim of the separation. This is where ionic liquids come in.

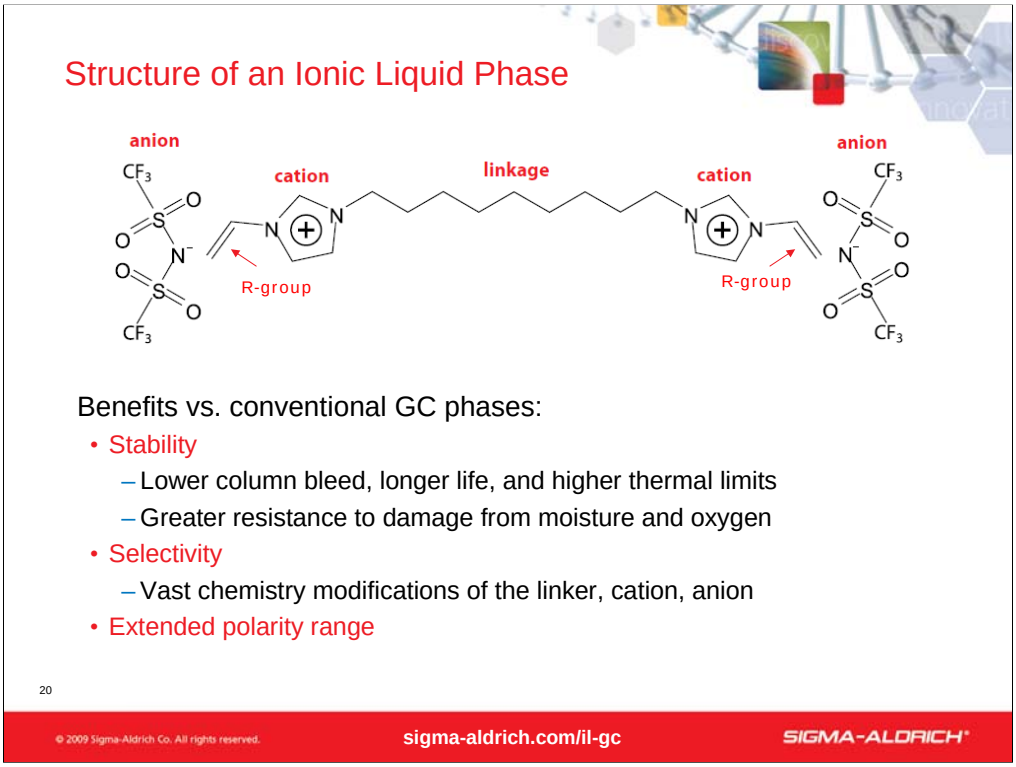
For further reading:
Dr. Matthew Klees, Basis of Interactions in Gas Chromatography – Part 3: Stationary Phase Selectivity, Glass Half Full? GC Solutions, Issue 10: December 2010, www.sepscience.com



Two of the most common GC phases are shown here. The polysiloxane polymers and polyethylene glycols. These have widespread utility, but have some drawbacks:
• In both, active hydroxyl (-OH) groups at the polymer termini allow a back-biting reaction that result in phase degradation that contributes to column bleed.
• They also give us few sites to modify to alter phase selectivity.
• A major limitation of the PEG phases is their thermal limit of around 280˚ C.

Polysiloxane legend:
R = methyl, phenyl, fluoropropyl, and/or cyanopropyl (listed from least polar to most polar).
x,y = percentage in the overall polymer composition.

PEG legend:
n = number of monomer repetitions to make the overall polymer.
Carbowax 20M (commonly used to make these) has a MW of 20,000.



This is an example of an ionic liquid GC phase (called SLB-IL100). You can see by the long chemical name why we give them the shorthand "ILXX!" They basically comprise four parts:

1) The cation (polycationic). The phase here contains two imidazolium is shown here, so it is a dication.
2) The anion counterion. nTF_2^- is shown here.
3) The linker – here is shown C9, but it can be nearly any number.
4) The functional groups (R-groups) on the cation. The vinyl is shown here.

The composition of these four parts affects the performance of the IL.

So what benefits do they offer?

Offer greater stability compared to polysiloxane polymers and polyethylene glycols

- Lower column bleed, longer life, and higher thermal limits
- More resistant to damage from moisture/oxygen

Vast chemistry modifications are possible to make columns with unique selectivity

- Dicationic (*shown*) or polycationic
- Limitless choices for cations, linkages, and anions
- Pendent groups can be added to cations and/or linkages

But perhaps the most interesting aspect of ILs is the potential to make very polar phases with high thermal stability.

Note: We can manufacture ionic liquid GC columns that incorporate any number of desirable properties, features, and benefits. For example, they can be engineered with identical selectivities as traditional, often flawed, non-ionic liquid columns...

but with lower column bleed, longer life, less susceptibility to damage from moisture/oxygen, and/or higher operating temperatures

can be engineered with high thermal stability...

decreasing analysis times

allowing additional higher boiling compounds to be analyzed

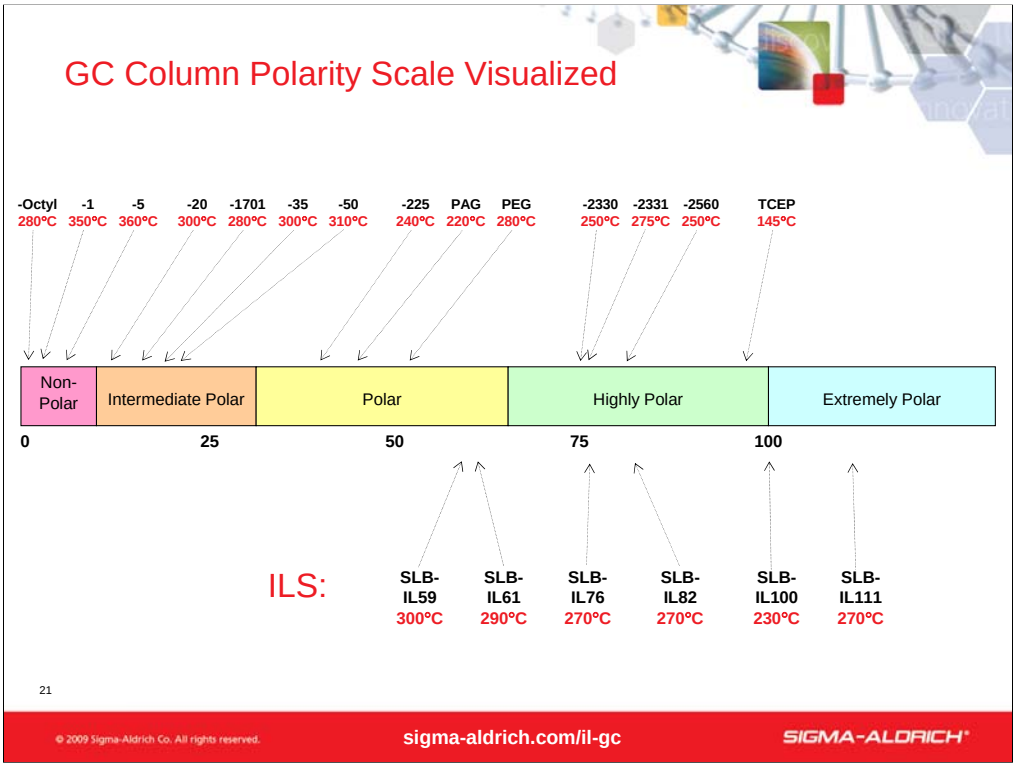
can be engineered with completely unique selectivities compared to any/all traditional non-ionic liquid columns...

producing good peak shape and resolution for compounds of varying functionality

expanding the polarity range upward

should play a significant role in multidimensional separations...

due to their engineered orthogonality and high thermal stability

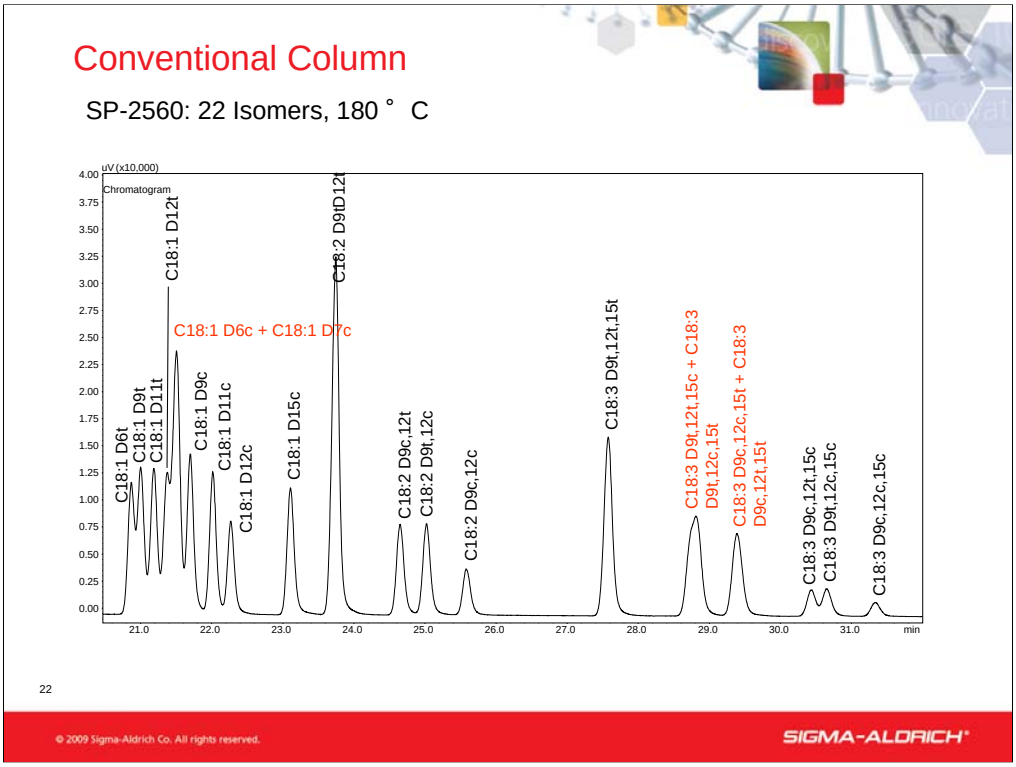


Here is a visual representation of the relative position of the current ionic liquid phases (below scale) and conventional GC phases (above scale) along a polarity scale continuum. We used the McReynolds constants* to represent polarity. We found this approach to be a simple way to describe the relationship of ionic liquid columns to each other and also to non-ionic liquid columns. Also reported in red text is the maximum temperature of the phase. Note several important points:

1. Some ILS offer identical selectivity to conventional phases – but with improvements. For example, SLB-IL100 has selectivity similar to TCEP*, but its maximum temperature is much higher (230 vs. 145˚ C). Other ILS may have lower bleed, longer life, or be more resistant to damage from oxygen or moisture.
2. Some ILS offer totally unique selectivity. For example the SLB-111 has no equivalent, and the SLB-IL59 and 61 fill “gaps” in the conventional phase range. They are orthogonal to the conventional phases.
3. High temperature operation allows the use of temperature to alter selectivity. This is not possible with non-polar phases because temperature masks the dispersive interactions.

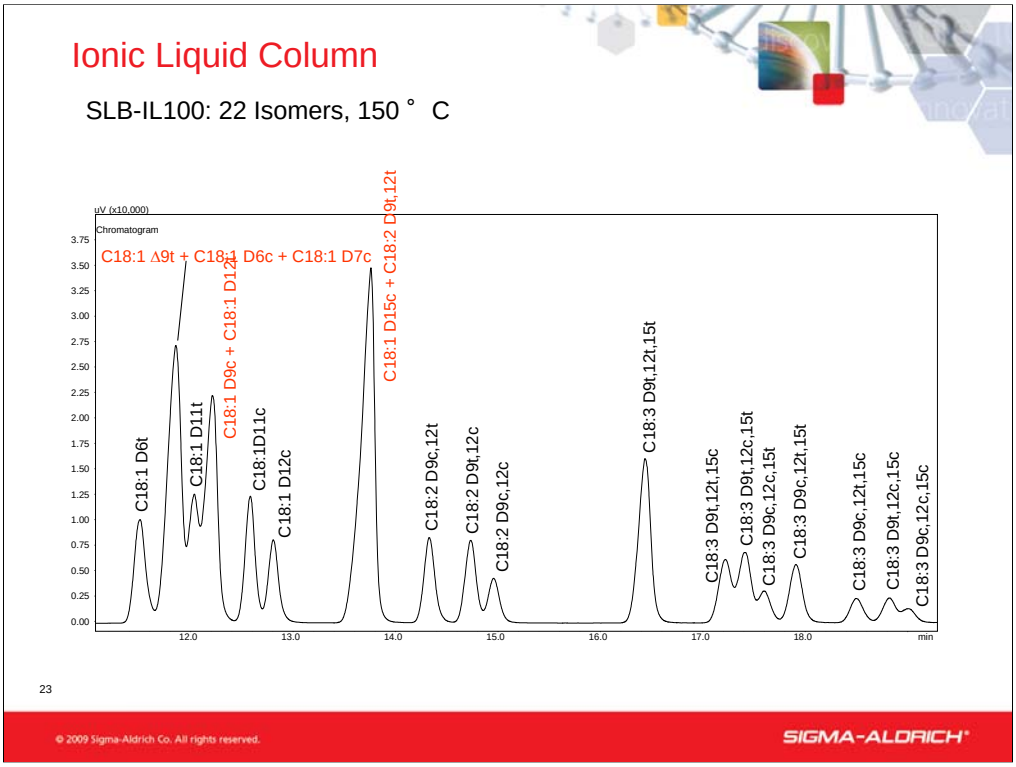
* McReynolds constants are a way of characterizing GC phases based on the relative retention of a set of test probes.

* TCEP is tris-cyanoethoxypropane, the most polar conventional GC phase available.



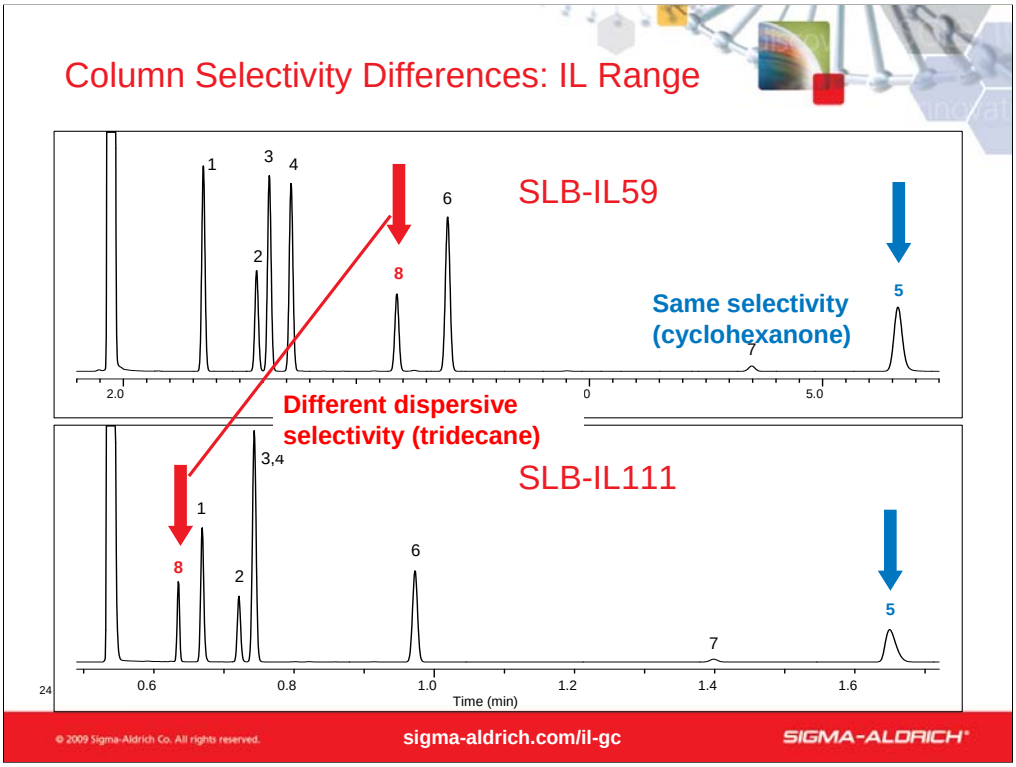
This is the SP-2560 column which was designed for positional geometric isomers, C18:1 fames, but also 18:2 and 18:3. There should be 8 18:3 isomers but only 6 are shown. Note the 100 m column length.

Column: SP-2560, 100 m x 0.25 mm I.D. 0.20mm film; Inj. vol.: 1µL; Sample: C18:1 isomer mix + Linolenic acid methyl ester isomer mix (Supelco Cat. No. 47792) + Linoleic acid methyl ester mix, cis/trans (Supelco Cat. No. 47791) in dichloromethane. Split ratio: 1:20 (220 ° C); Temp. progr.: Isothermal at 180° C; Press. progr.: 161.6 kPa at linear velocity constant; Carrier gas: H2; u: 25.0 cm/s; Detector: FID (220 ° C) H2: 50 mL/min., Air: 400 mL/min, Make-up: 50 mL/min kPa (N2); Sampling rate: 80 msec; Filter Time Constant: 200 msec



The different selectivity of SLB-IL100 allows it to resolve all 8 C18:3 isomers, what the SP-2560 column cannot do. It offers such greater selectivity that we can use a much shorter column (30 m).

Column: SLB-IL100 30 m x 0.25 mm I.D., 0.20mm film; Inj. vol.: 2 µL; Sample: C18:1 Isomer mix + Linolenic acid methyl ester isomer mix (Supelco Cat. No. 47792) + Linoleic acid methyl ester mix, cis/trans (Supelco Cat. No. 47791) in dichloromethane. Split ratio: 1:20 (240 ° C); Temp. progr.: Isothermal at 150° C; Press. progr.: 52.8 kPa at linear velocity constant; Carrier gas: H2; u: 30.0 cm/s; Detector: FID (240 ° C) H2: 50 mL/min, Air: 400 mL/min, Make-up: 50 mL/min kPa (N2); Sampling Rate: 80 msec; Filter Time Constant: 200 msec



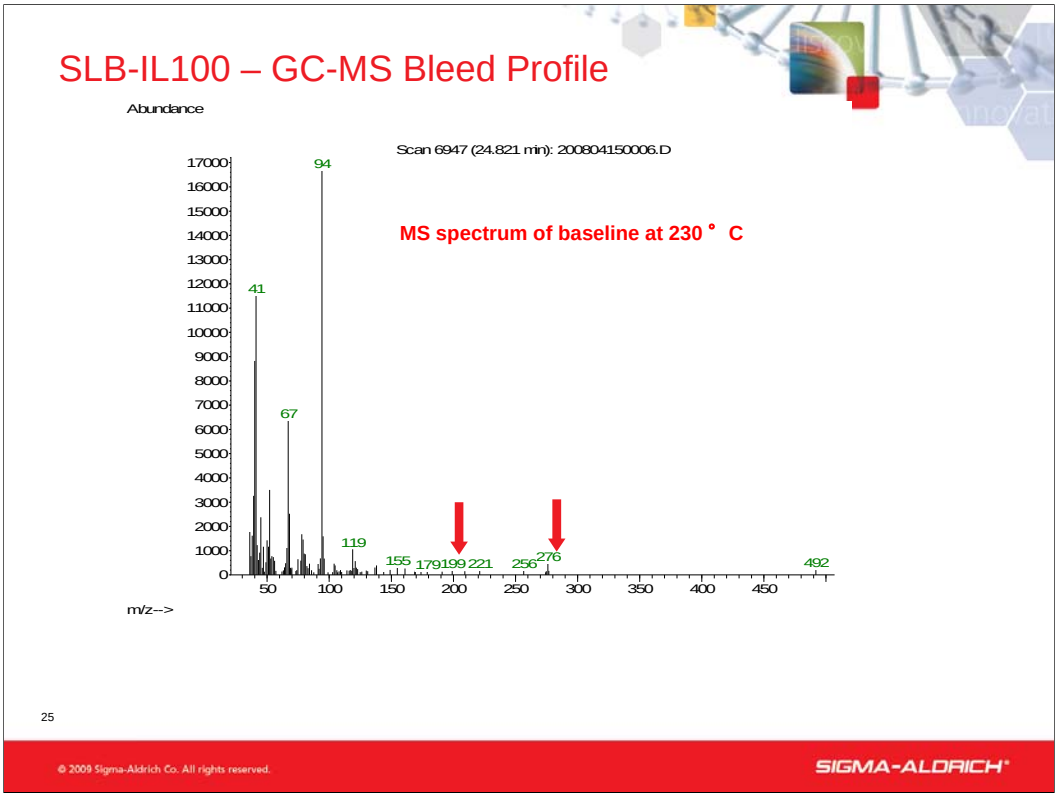
We've chosen the current IL phases at the far left and far right ends of the polarity scale to demonstrate selectivity differences. The aromatic compounds (xylene, toluene, ethylbenzene, trimethyl- and tetramethyl benzene, 1,2,3,4,6,7) interact by pi-pi interactions and we can see that between the two phases there is little selectivity difference. Same with the cyclohexanone (5) that likely has dipole-induced-dipole. But – when we look at tridecane (8) which only has the dispersive interactions, there is great differences.

So when we evaluate these ionic liquid phases, we use temperature to tune in and tune out types of interactions (e.g. dispersive goes away at high temperature), and phase chemistry to add types of interactions.

Selectivity chromatograms of 0.25 mm I.D. ionic liquid columns are all shown with an isothermal oven temperature of 110 ° C and helium carrier gas to allow direct comparison of different phases.
column: SLB-IL59, 30 m x 0.25 mm I.D., 0.20 µm (28891-U)
oven: 110 ° C
inj.: 250 ° C
det.: FID, 250 ° C
carrier gas: helium, 26 cm/sec
injection: 1.0 µL, 100:1 split
sample: each analyte at various concentrations in isooctane,

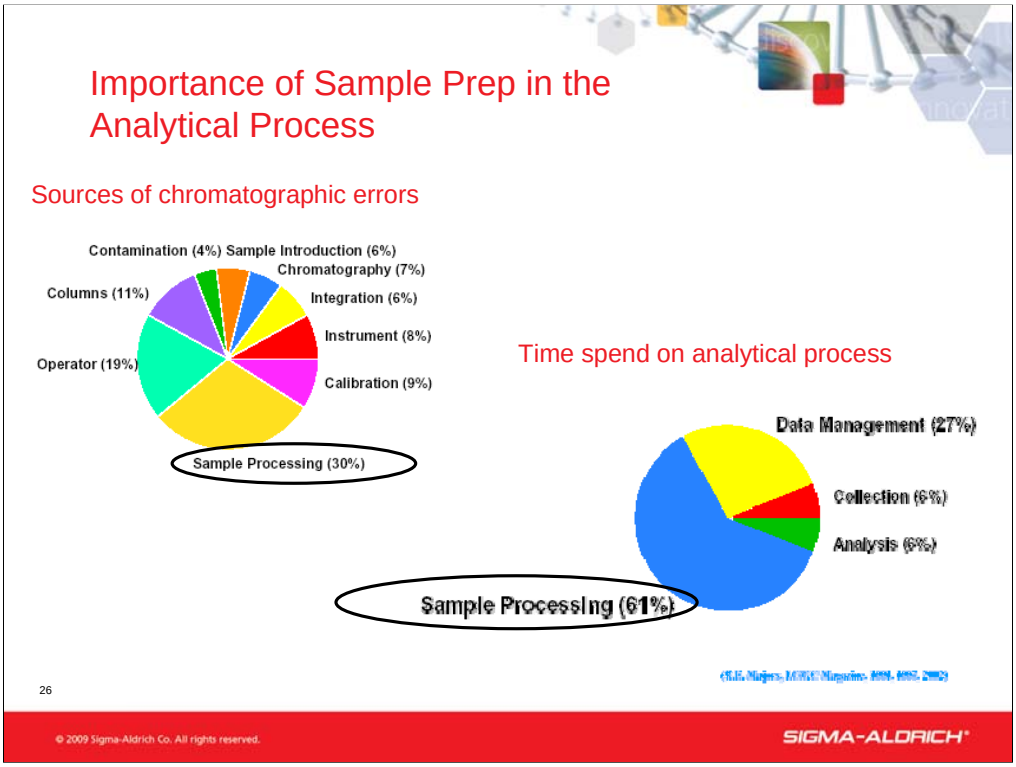
column: SLB-IL111, 15 m x 0.10 mm I.D., 0.08 µm (28925-U)
oven: 90 ° C
inj.: 250 ° C
det.: FID, 250 ° C
carrier gas: hydrogen, 50 cm/sec
injection: 0.5 µL, 300:1 split
sample: each analyte at various concentrations in isooctane,

Analytes listed in order of increasing boiling point:
1. Toluene
2. Ethylbenzene
3. p-Xylene
4. Isopropylbenzene (Cumene)
5. Cyclohexanone
6. 1,2,4-Trimethylbenzene
7. 1,2,4,5-Tetramethylbenzene
8. n-Tridecane (C13)



As mentioned the ionic liquid phases are low bleed. This figure is the MS spectrum at 230° C on the SLB-IL100 phase. It exhibits simpler (smaller) m/z bleed fragments (compared to predominant m/z 207 and m/z 281 typically formed by polysiloxane polymers) resulting in easier mass spectral identifications due to less interference.

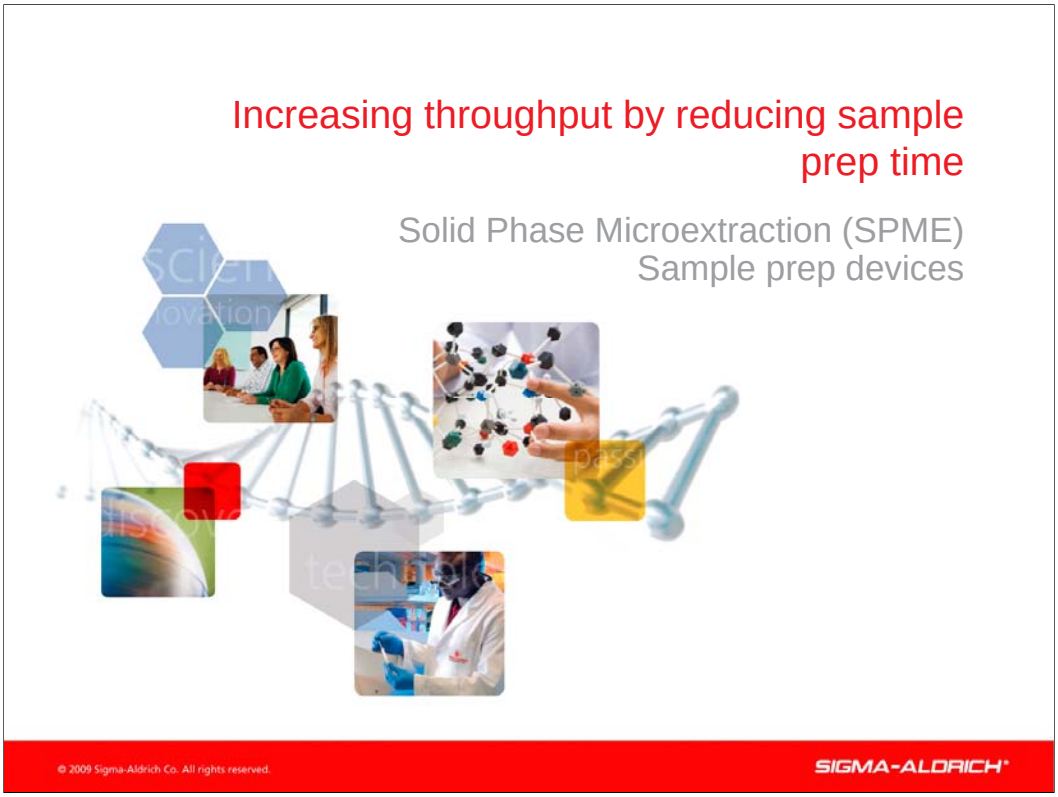
column: SLB-IL100, 30 m x 0.25 mm I.D., 0.20 µm (28884-U)
oven: 60 ° C (1 min.), 8 ° C/min. to 230 ° C (5 min.)
inj.: 250 ° C
MSD interface: 220 ° C
scan range: m/z = 35-500
carrier gas: helium, 1.5 mL/min. constant
injection: 1 µL, splitless (1.0 min.)
liner: 4 mm I.D., single taper



The previous two innovations we talked about, Fast GC and Ionic Liquids, address the analysis, but what about sample prep? From both time and sources of error standpoints, sample prep can have major negative impact. So we look at ways to reduce both error and bottlenecks related to sample pre.

The left pie chart is a breakdown of the sources of “getting the wrong answer” when using chromatographic techniques. As you can see, the largest source of error is in sample processing.

On the right, if the time spent to analyze a sample is broken down, we can see that the vast majority is spent on sample processing; further emphasizing the importance of this aspect of analytical analysis.



The innovative sample prep device that uses a GC finish we'll talk about is SPME (solid phase microextraction). It has been called "solventless sample prep" also "sample prep made easy"… but it does a lot more as we'll see. It generally uses a bind-elute strategy, and the typical aim is to concentrate analytes. We talked about SPME briefly in the HPLC talk, but we'll go into a little more detail here.

Solid Phase Microextraction (SPME)

- Holder assemblies (manual, autosampler, robots)
- Coated fibers (adsorbent and absorbent)
- Janusz Pawliszyn, Univ. of Waterloo; unique and proprietary to Supelco
- Economical enrichment technique mainly for trace analysis
- Initially for GC analysis, now new fibers for LC

Features:
- Very limited or no use of solvents
- All types of samples & matrixes
- Direct immersion or headspace
- Designs for manual, autosamplers and robots

Benefits:
- Economical
- Highly consistent, quantifiable results
- Portable (field use) and reusable
- Reduces lab animal sacrifice

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Solid phase microextraction, a simple, effective adsorption/ desorption technique, eliminates the need for solvents or complicated apparatus for concentrating volatile or nonvolatile compounds in liquid samples or headspace. SPME is compatible with analyte separation/detection by gas chromatography or HPLC, and provides linear results for wide concentrations of analytes. By controlling the polarity and thickness of the coating on the fiber, maintaining consistent sampling time, and adjusting several other extraction parameters, an analyst can ensure highly consistent, quantifiable results from low concentrations of analytes.

SPME is a solvent-free extraction technique that can be used on a variety of samples and matrixes
It is an alternative to head space GC and solid phase extraction (SPE) techniques
The SPME technique involves two processes:

Sample extraction

Analyte desorption

SPME is mainly interfaced with GC (gas chromatography), but an HPLC interface is also available
New bioanalytical fibers have recently been introduced.

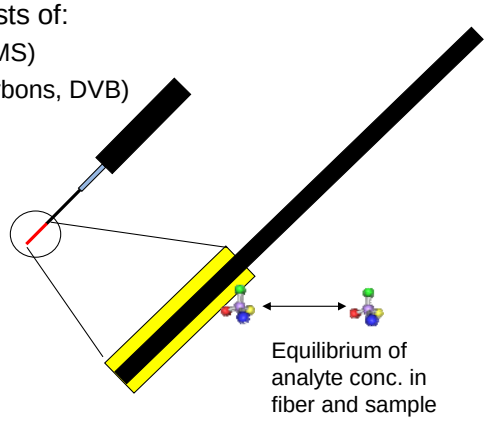
SPME Fiber Coating: The Business End

Not an exhaustive extraction technique

An equilibrium is set up between analytes dissolved in the sample (solution or gas phase) and in the liquid coating on the fiber.

The fiber coating consists of:

- Matrix/binder (e.g. PDMS)
- Particles (e.g. C18, carbons, DVB)



Enlargement of the SPME fiber coating

Equilibrium of analyte conc. in fiber and sample

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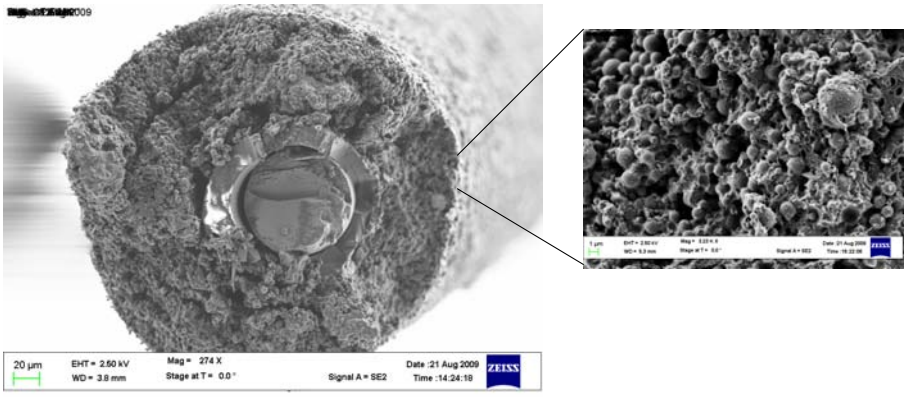
The heart of the SPME device is the fiber, which does the actual extracting. During the extraction process, an equilibrium is established between the analytes (whether in solution or gas phase) and the coating on the fiber. There are a variety of fiber coatings available, and they consist of a matrix, usually GC-type phases such as PDMS, and particles such as C18-silica, Carboxen and DVB.

PDMS-DVB Fiber SEM

Cross section of the PDMS-DVB fiber. The center is a fused silica core, surrounded by a Stableflex core. The 3-5µm DVB particles are suspended in PDMS and layered over the cores.



30 Photomicrograph of SPME fiber provided by Prof. Dan Armstrong, U. Texas Arlington

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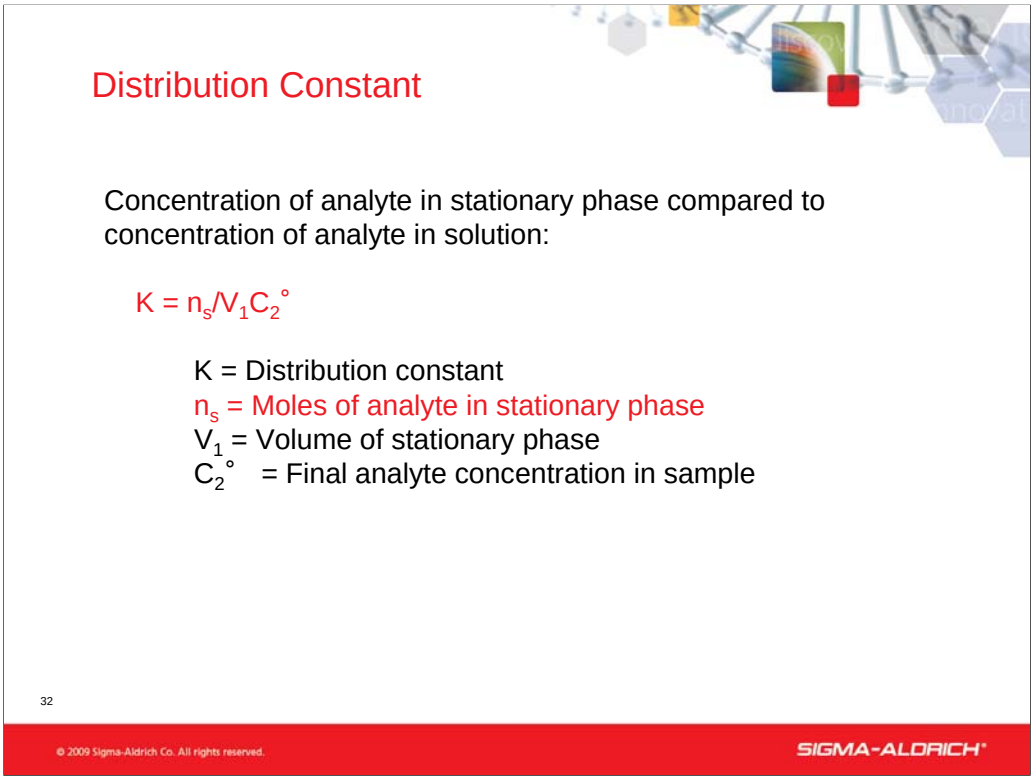
Left: SEM (275x mag.) of cross-section of SPME fiber.
Right: 3000X magnification of the Carboxen PDMS coating. The 3-5µm Carboxen particles are suspended in PDMS.

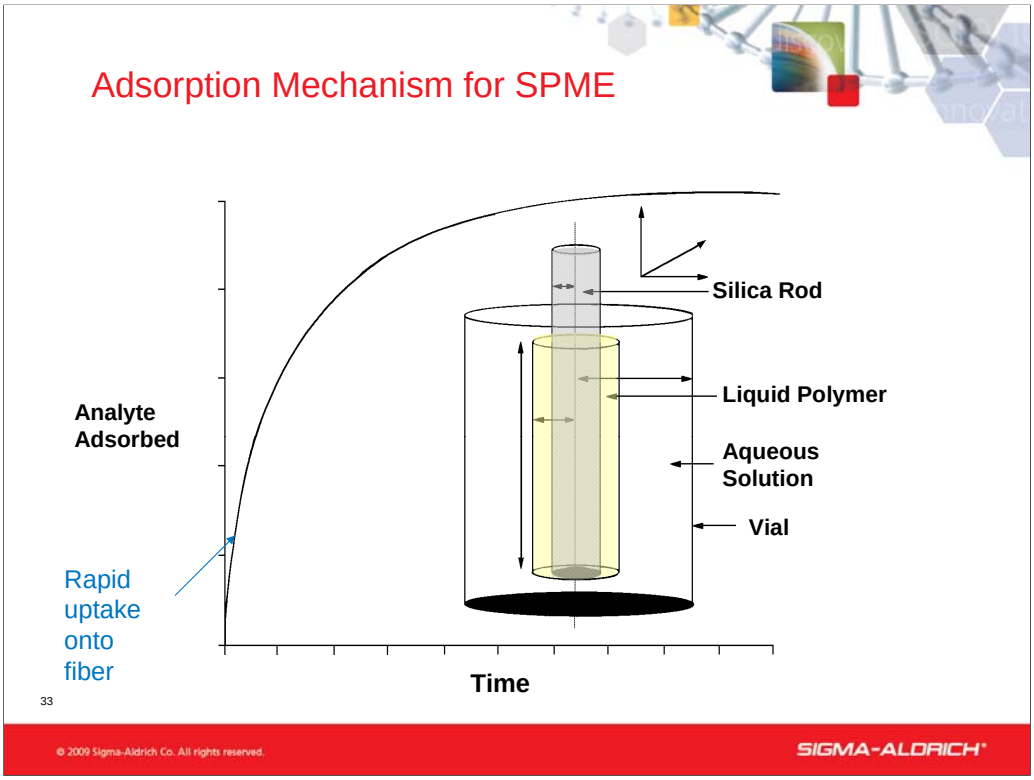
Inside – fused silica core
Middle layer – Stableflex
Outside layer – Coating (here, PDMS-DVB, the DVB are particles). The thickness of the coating dictates sample capacity.

The SPME Concept

Animated process of sample extraction by SPME.



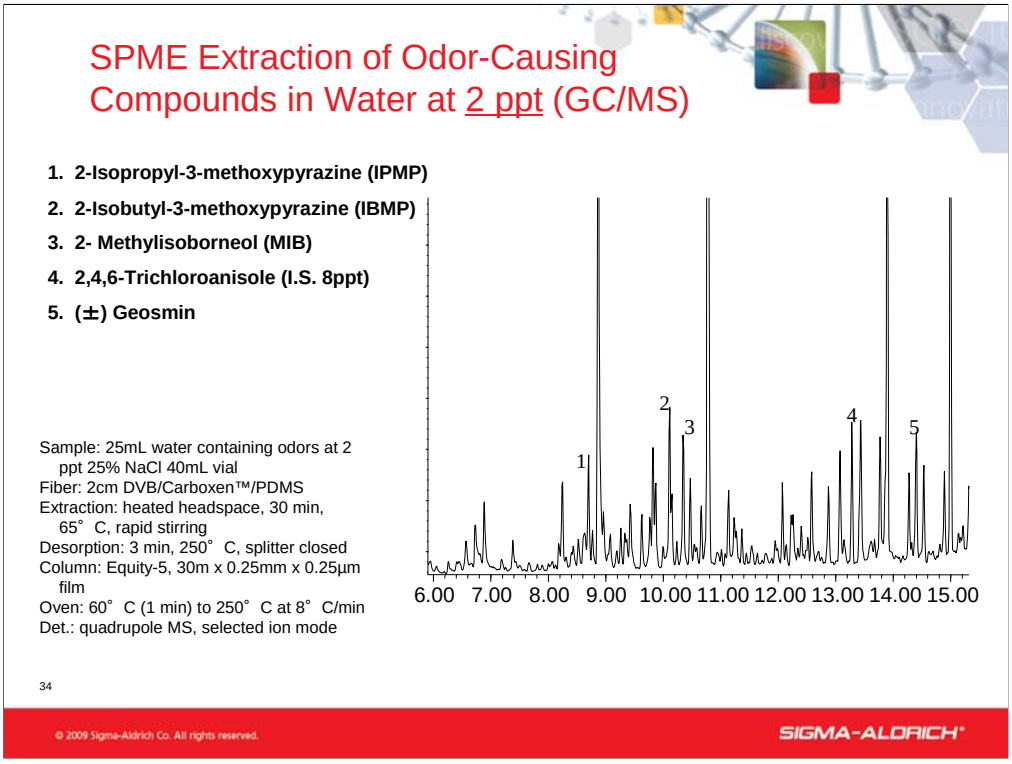
SPME, unlike SPE, is not an exhaustive extraction technique. Rather, extraction is governed by distribution constants. This equation describes the relationship between analytes on the extraction device (fiber) and in the sample. The distribution constant is essentially the affinity of the analytes to the phase coating compared to their affinity to the matrix and is based upon Fick's laws of diffusion. The distribution constant (K) can be altered by fiber polarity and thickness and chemical adjustments to the matrix. The higher the K value, the stronger the affinity of the analyte for the stationary phase on the fiber. So to maximize n_s (moles of analyte in the stationary phase), we need to select a fiber and extraction conditions that optimize the K value.



The extraction mechanism for SPME is based upon Fick's laws of diffusion which govern the transport of mass through diffusive means.* In the case of SPME, the analytes will partition into the fiber coating from the matrix, whether solid or gas. The amount of analyte that migrates into the coating is dependent upon the affinity of the analyte for the phase coating compared to the affinity for the matrix. There is typically a rapid uptake period. After a given amount of time the analytes will reach an equilibrium where there is no change in the concentration between the matrix and the fiber coating. The time that it takes to reach equilibrium is dependent upon the fiber thickness and analyte size.

To emphasize again, SPME is not an exhaustive technique and extraction is governed by distribution constants, so the amount of analytes that migrates into the coating on the fiber will be dependent on an analyte's affinity for the coating as compared to the sample matrix. After a given amount of time, the amount of analyte that is adsorbed by the coating versus what is in the sample will not change, or it will reach equilibrium. During the method development process, a study is often conducted to determine this time. Being sure that the extraction time is at equilibrium is especially important in quantitative determinations.

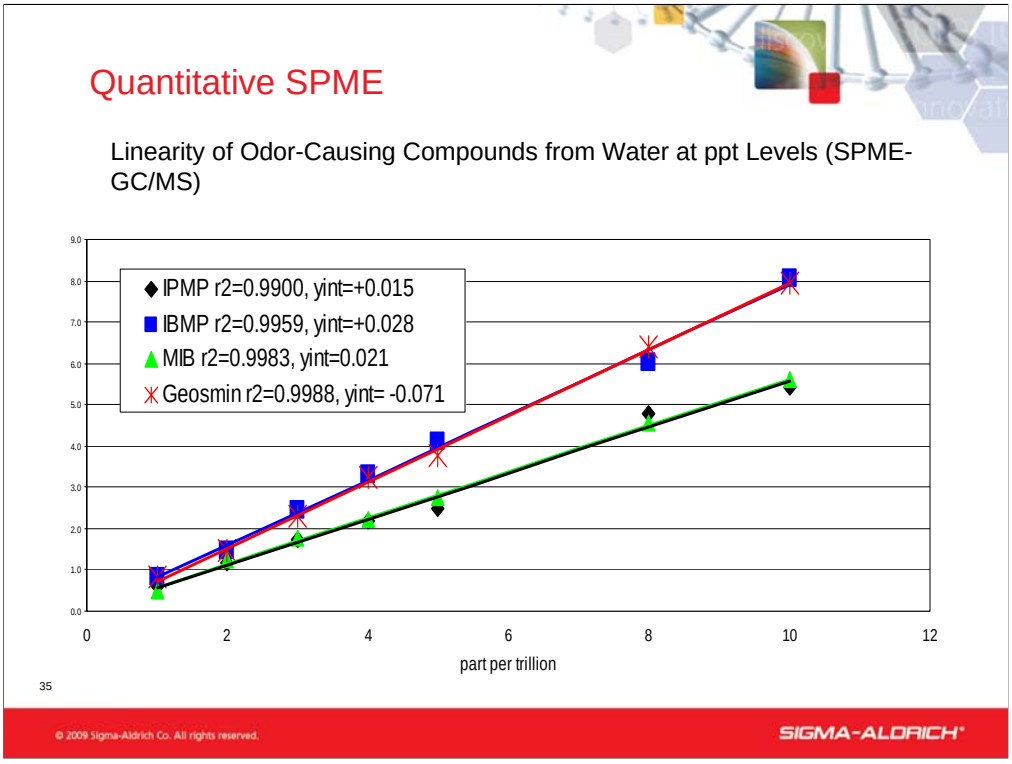
*Fick's First Law postulates that the flux goes from regions of high concentration to regions of low concentration, with a magnitude that is proportional to the concentration gradient (spatial derivative). In one (spatial) dimension.
*Fick's Second Law predicts how diffusion causes the concentration field to change with time.



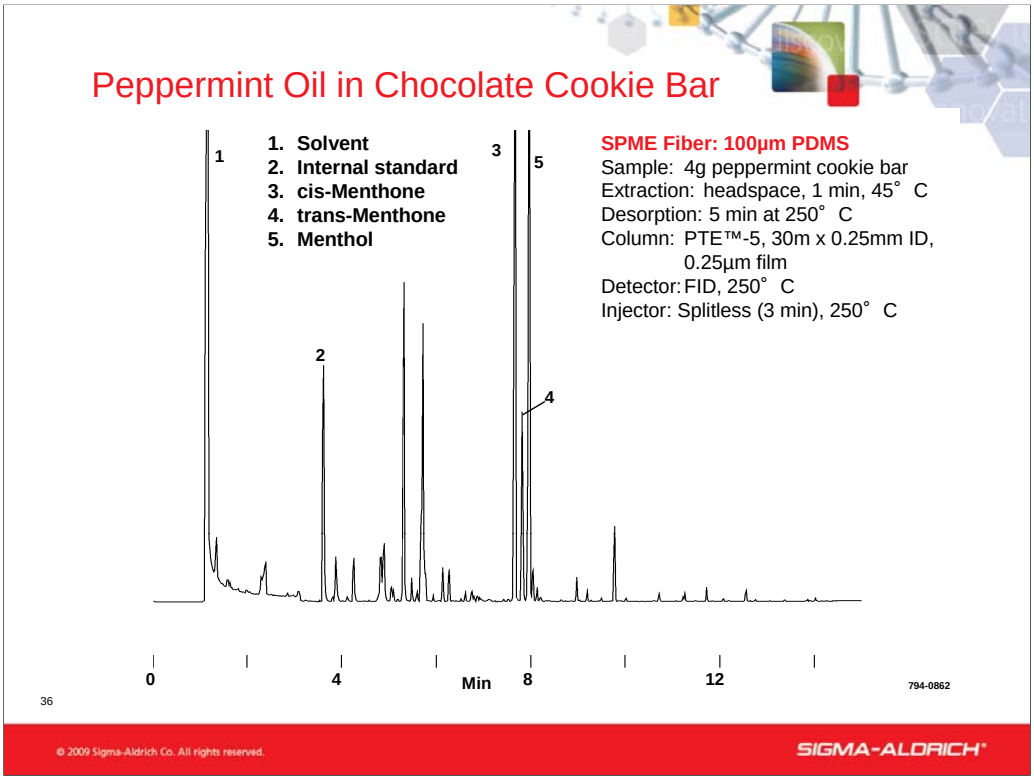
SPME is sensitive and quantitative, as the next two slides show. In this slide, four odor causing agents are extracted along with trichloroanisole (TCA) as the internal standard. This is an AWWA* method. These are microbe-produced compounds that give water a musty, moldy smell. A quadrupole mass spectrometer was used to generate this slide. It was critical that the detection was done in the single ion mode. Full scan would not detect the analytes. Even with selected ions, the background is still high due to the very low detection threshold. When a single ion is extracted for quantification of an analyte, the background is greatly reduced.
Interesting to note that TCA, the IS here, is also a compound analyzed in corks to assess quality. It is also an SPME method.

Sample: 25mL water containing odors at 2 ppt 25% NaCl 40mL vial
Fiber: 2cm DVB/Carboxen™/PDMS
Extraction: heated headspace, 30 min, 65° C, rapid stirring
Desorption: 3 min, 250° C, splitter closed
Column: Equity-5, 30m x 0.25mm x 0.25µm film
Oven: 60° C (1 min) to 250° C at 8° C/min
Det.: mass spectrometer, quadrupole, single ion mode

*AWWA – American Water Works Assoc.

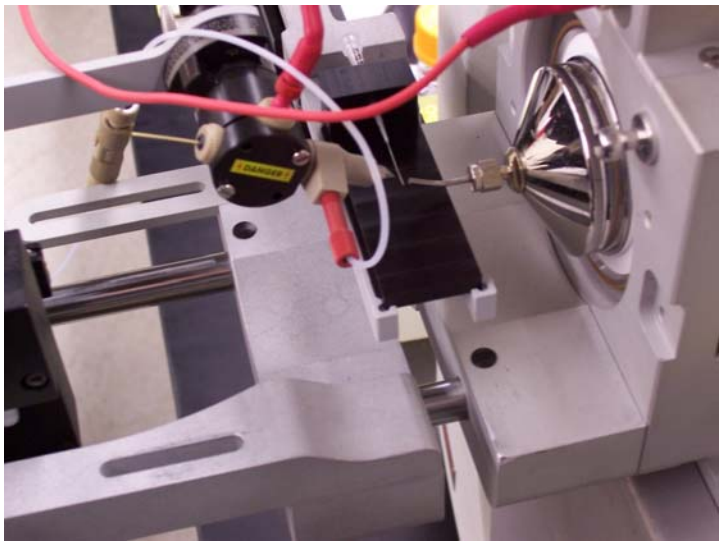


The slide shows that the extraction procedure for the 4 odor agents is linear and reproducible. The regression coefficient was 0.99 and better for all of the analytes. Also the y intercept was quite low, under 0.07ppt. This indicates that there were very few interfering peaks.



SPME is used for solid samples as well as liquids, as this slide shows. A food manufacturer came to us to help with a quality problem in their chocolate mint cookie. The mint flavor was off, and they wanted to look at the menthol profile. SPME was used to monitor the amount of mint in the bar during the process. Here we see the SPME headspace extraction of mint flavors (menthol compounds) from the ground-up cookie. Even though menthol has a high boiling point, it is easily extracted by SPME at 45° C. According to their expertise, this profile was not as it should have been. (Analysis note: The PTE-5 column is 5% diphenyl, 95% dimethyl polysiloxane. Today, we would recommend using the SLB-5ms capillary GC column for this application.)

SPME fiber Holder with Automated DESI-1D Source



Courtesy of Joseph Kennedy of Prosolia

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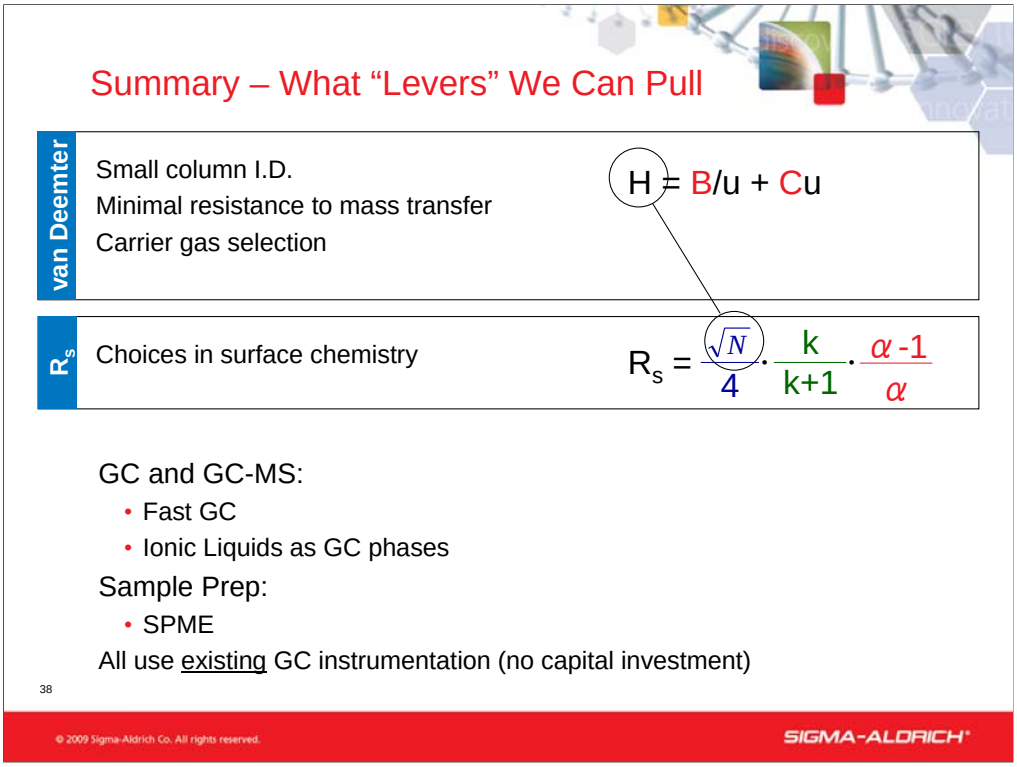
SPME is finding uses outside traditional headspace or direct immersion with GC analysis. For example, something very new is the SPME-DESI interface. DESI stands for direct electro spray ionization and is a simplistic way of introducing samples into an MS system. Since electro spray ionization is accomplished at ambient atmosphere, it is easy to desorb SPME fibers using this method.
Prosolia developed an interface that uses an nebulizer to ionize and release analytes from adsorbents. SPME fibers are ideal for this application. By using DESI the offline desorption step for removing analytes from the fibers is eliminated. Instead, the mobilized spray removes the analytes and they are directly introduced either into a high resolution MS or a system with MS/MS capability. This is a major time reducing step.



So summarizing, we've shown that we can leverage the two fundamental GC equations, Golay (top) and Resolution (bottom), to give us speed, resolution, and sensitivity. The two equations are related in the N (efficiency) term.

We know now that for the best chromatography for GC we need to minimize the B and C-terms of the Golay:
•Operate at the u optimum
•Use small column I.D.
•Minimize resistance to mass transfer by using thin films
•Use carrier gases with high diffusivity

Leveraging the selectivity term of the Resolution equation:
• Have choices in chemistry of phases or other surfaces. Innovative sample prep devices typically employ some novel surface functionality.
• Be able to operate across a wide temperature range. (Ionic Liquids permit high temperature operation.)

The innovative GC and sample prep products we've talked about here to improve speed, resolution, and sensitivity are:

• Fast GC – reduces B and C terms of the Golay by using narrow column I.D., H_2 carrier gas, and thin films.
• Ionic liquids – provides novel selectivity, especially for polar compounds, low bleed even at very high temperatures.
• SPME – for solventless sample prep, well entrenched for GC analysis, but now expanding into LC, DESI, and other areas.

These products can be used on any instrument. (Although there are some instrument considerations for Fast GC.)

Much literature and more detailed talks are available, please ask.

The background is the view of “Happy Valley.” You can see Penn State, and the Supelco facility in Bellefonte is just to the right, outside of this photo.

I would like in closing to acknowledge some of the research teams with whom we collaborate, as well as our internal R&D groups.