Evolution of Three Decades of Separations Research

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Chromatography: the beginning

Figure 2. Separation of products on a Sephadex LH-50 column (2.5 m × 3.0 cm) formed from the condensation of alanyl adenylate in the presence of aqueous (---) and reversed micelles (-- ---). Peaks correspond to alanine (7), low molecular weight peptides (2, 3, and 4), AMP (also 4), high molecular weight polypeptides (1), and surfactants (5 and 6).

Est. M.W. ~ 1.5-10 K

Interaction of nuclear fission fragments with surface molecules. The large energy deposition produces a localized “hot spot,” resulting in volatilization. Results show that for some molecules, such as amino acids, ion-pair formation takes place by proton transfer within a desorbed dimer.

(Macfarlane and Torgerson, 1976)
In: *J. Am. Chem. Soc.* 100 (1978) 4605, we published the 1st high M.W. protein/peptide mass determination (LC-MS).
The first deliberate use of micelles in chromatography (separations)

Advent of the 3-Phase Model and Pseudophase Theory of Separations

\[ \frac{V_s}{V_e - V_m} = \left[ v\left(\frac{K_{mw} - 1}{K_{sw}}\right) \right] \times C_m + \frac{1}{K_{sw}} \]
Over the next 20 years micelle-based separations grew tremendously giving rise to the fields of:

- Micellar HPLC
- Micellar Capillary Electrophoresis (often miss-named micellar electrokinetic chromatography)
- Micelle based extractions
- Micelle based membranes
- And more

This also led to the use deliberate use of surfactants in other areas of analytical importance including atomic absorption & flame emission spectroscopy and HPLC-mass spectrometry.
The 3-phase model & theoretical framework and the pseudophase concept have been used to:

- Explain chiral separations in HPLC, GC and CE
- Calculate binding constants of molecules to micelles, cyclodextrins, proteins, etc.
- Calculate binding constants of volatile compounds to any pseudophase by headspace GC
- Explain some extractions and microextractions
- Explain molecular dynamics in complex microfluidic laminar flow streams
One of the bigger advances was solid phase micro extraction (SPME) in 1990


The 3-phase model & theory is used in “head space SPME” and separations containing macromolecular or colloidal entities.
Our micelle work led to cyclodextrin research beginning in 1979, which contributed to:

- The 1st commercial reversed phase CSP (in 1983) with thanks to Tom Beesley of Advanced Separation Technologies (Astec)!

- The practice and understanding of enantiomeric separations.

- The elucidation of the molecular chiral recognition mechanisms.


- The issuing of new FDA guidelines in 1992 concerning the development of stereoisomeric drugs.

- The development of the most broadly utilized macrocycle in the world (separations, excipients, consumer products, broad variety of scientific studies, etc.). And one of the few molecules to have its own conference!
Fig. 1. Computer projections of inclusion complexes of (A) d-propranolol and (B) l-propranolol in β-cyclodextrin, from x-ray crystallographic data. Dotted lines represent potential hydrogen bonds (distances noted in the text). The configurations shown represent the optimal orientation of each isomer on the basis of the highest degree of hydrogen bonding and complexation.
Used in various formats: 1987-2000
HAPPY CHRISTMAS

Ring in 1988 with Cyclodextrins from

SEASONS GREETINGS

Sterling Organics
BUSINESS DEVELOPMENT GROUP
Hydroxypropyl-β-Cyclodextrin as an Excipient

At one point during the zenith of this work there were ~50 invitations/year to speak and requests for ~400 reviews in one year.
2,6-Di-O-pentyl-3-O-trifluoroacetyl cyclodextrin liquid stationary phases for capillary gas chromatographic separation of enantiomers

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(First received January 24th, 1990; revised manuscript received February 23rd, 1990)

ABSTRACT

A series of liquid cyclodextrin derivatives, 2,6-di-O-pentyl-3-O-trifluoroacetyl α-, β- and γ-cyclodextrins (DP-TFA α-, β- and γ-CD), have been used as highly selective chiral stationary phases for capillary gas chromatography. More than 150 pairs of enantiomers were resolved; 120 on DP-TFA-γ-CD, which is the first reported γ-cyclodextrin stationary phase that is more widely useful than the β-cyclodextrin analogue. The enantiomers resolved include chiral alcohols, diols, polyols, amines, amino alcohols, halohydrocarbons, lactones, α-halocarboxylic acid esters, carbohydrates, epoxides, nicotine compounds, pyrans, furans and so on. Identical α values were observed for diol, amine and γ-halocarboxylic acid ester homologues, respectively. The relationship between the unusual selectivity behavior and separation mechanism is discussed.
This column (ChiralDEX G-TA) (and a close analogue) have been sent on at least two space missions:

1. The ESA’s Rosetta Mission to reach Comet 67P and to detect & quantitate organic molecules.*

2. NASA’s Mars Mission (Curiosity Lab’s analytical unit, a.k.a. Sample Analysis at Mars (SAM)).**

** Analytical Chemistry 85 (2013) 8024-8030.
If synthetic, it will be racemic and have a distinct diastereomeric ratio characteristic of the synthetic process.
Enantiomeric composition of the DMAA in synthetic standards and the supplements by enantioselective GC (ChiralDEX GTA)

Banned by the FDA in 2012
Then in 1994 we discovered that the macrocyclic glycopeptides (antibiotics) were exceptional chiral selectors.

They rapidly became the method of choice for the separation of chiral natural and unnatural amino acids, carboxylic acids and a variety of polar drugs and other unusual chiral molecules.
Macrocyclic Glycopeptides

(A) Structure of the Cu-vancomycin complex. (B) Molecular model of the Cu-vancomycin complex. The copper atom is color-coded orange, the carboxylic acid is red and the amino groups are green. The disaccharide unit and hydroxyl groups are colored cyan (light blue), the amide nitrogens coordinated with copper are purple and the coordinating oxygen and the amido linkages of the agly disaccharide unit are depicted in

CHIRALITY 8:590-595 (1996)
With the Petrich group at Iowa State University

Figure 1. Structures of hypericin enantiomers.

Figure 2. Chromatogram obtained using 100% MeOH, 1% TEAA (vol/vol), 0.4 mL/min on Chirobiotic TAG column for separation of racemic hypericin.
EMAC = Extended Metal Atom Chains

- Synthesized by F.A. Cotton group
- Use as molecular wires
- Enantioseparation of tri-metal complexes never reported

\[
\begin{align*}
X-M-M-M-M-X \\
\text{X=Y=Cl}^-
\end{align*}
\]

\[
\begin{align*}
\text{2-dipyridylamine (dpa)}
\end{align*}
\]

M=Ni, Cr, Co, or Cu

Chirality of EMACs

End-on view of Ni(dpa)$_4$Cl$_2$

P-helix (plus or clockwise rotation)

M-helix (minus or counterclockwise rotation)

Nickel EMAC
Chirobiotic V

Optimized Separation Conditions:
90/10 ACN/MeOH with 0.4% w/v NH₄NO₃ and 0.2% w/v NH₄TFA
Flow rate 0.4 mL/min

Specific Rotation of Nickel Complex

\[ [\alpha] = \frac{\alpha}{bc} \]

- \([\alpha]\) = specific rotation
- \(\alpha\) = optical rotation
- \(b\) = cell pathlength
- \(c\) = concentration

<table>
<thead>
<tr>
<th>Peak</th>
<th>([\alpha])</th>
<th>(\alpha)</th>
<th>(c)</th>
</tr>
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<tbody>
<tr>
<td>Peak 1</td>
<td>-5000(\pm)192°</td>
<td>-0.0156°</td>
<td>3.12 (\times) 10^{-6} g/cc</td>
</tr>
<tr>
<td>Peak 2</td>
<td>+5205(\pm)182°</td>
<td>+0.0228°</td>
<td>4.38 (\times) 10^{-6} g/cc</td>
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</table>

\(\lambda\) = 589 nm, \(b\) = 1 dm, samples in CH\(_2\)Cl\(_2\)
Cyclofructans (CFs)

- Cyclic oligosaccharide
- Inuline, fructosyltransferase
- Beta-(2-1) linked D-fructofuranose
- 6,7,8 fructofuranose units
- Crown ether skeleton
- UV transparent
- Solubility: >1.2g/ml
- No health hazardous
- The 3-D structure shown here is incorrect
Native vs. Derivatized CF6

Native CF6
Acetonitrile/methanol/AA/TEA 90/10/0.3/0.2

CF6
3,5-dimethylphenyl carbamate
Acetonitrile/methanol/AA/TEA 75/25/0.3/0.2
Separation of Primary Amines

Column: IPCF; 60ACN/40MEOH/0.3AA/0.2TEA
Macrocycles in Separations Today

1. >95% of all GC chiral separations are done on cyclodextrin derivative stationary phases.

2. >90% of all CE chiral separations are done with cyclodextrin-based chiral selectors.

3. CDs are the dominant water-based NMR chiral shift reagents.

4. CDs are used for only about 5-10% of LC chiral separations.

5. Macrocyclic glycopeptides have superseded crown ethers for the separation of chiral amino acids and many other compounds. They are the dominant macrocycle used for chiral LC separations.

6. Cyclofructans are the newest and least developed chiral macrocycle
Figure 3. Capillary electropherogram showing the separation of three bacteria and baker’s yeast (S. cerevisiae) where small amounts of 600,000 MW PEO are added to the running buffer. Note the relatively short migration times and the high efficiency. The arrow denotes the migration time of the EOF marker mesityl oxide. See the Methods section for other experimental conditions.
Simultaneous Separation of *B. infantis*, *L. acidophilus*, and *S. cerevisiae* and Determination of Their Viabilities

Channel A

Channel B

Live (**green** fluorescence curve)

Dead (**red** fluorescence curve)
Developed into a Rapid Test for Microbial Contamination

Published in the U.S. Pharmacopeia, 2012
Ionic Liquids in Separations and Mass Spectrometry


GC commercialization with thanks to Supelco and esp. Len Sidisky.
Examples of More Analytically Useful ILs

Synthesized in Our Work

IL 82

IL 59

IL 94

IL 126

IL Blend
Why the Renaissance in GC?

1) Miniaturization
2) Advent and Commercialization of Effective, Efficient Comprehensive GCxGC
3) Advent of the 1\textsuperscript{st} New Class of Stationary Phase in 40 Years: Ionic Liquids
4) Availability of Statistical Methods to Efficiently Obtain and Treat Data
5) Growing Necessity to Analyze Complex Samples (Metabolomics, Nutritional, Petro, Environmental, Etc.)
Figure 6. GC x GC separation of diesel fuel on the (a) IL x HP-5 column combination, (b) the DB-Wax x HP-5 column combination, and (c) the HP-50+ x HP-5 column combination. Both the IL x HP-5 and DB-Wax x HP-5 configurations generated distinct chromatographic regions for the saturated hydrocarbons, monoaromatics, and diaromatics. The HP-50+ x HP-5 configuration had nearly complete separation of the saturated hydrocarbons from the aromatics, but no clear separation of the aromatics into monoaromatic and diaromatic regions.

Water detection by GC

1. Water cannot degrade the stationary phase.

2. Water must produce a reasonably sharp, efficient peak.

3. Water must be well separated from all solvents (matrices) and an internal standard.

4. Analysis times should be short.

5. Results must be highly accurate and reproducible.

6. Effective for any concentration from <1 ppm to >99%.
Chromatograms illustrating the separation of water from organic solvents

Chromatograms A and C are isothermal separations. Chromatogram B is for the same sample as in A, however a temperature gradient was used to decrease the analysis time and further “sharpen” the water peak. This enhanced the sensitivity and precision of the method.

Chromatogram A: 1ml injection, 50°C, analysis time: 9 min, Internal Standard: acetone (0.4%)

Chromatogram B: 1 ml injection; 50°C (hold 2min), ramp 10dpm to 80°C; analysis time: 6min, Internal Standard: acetone (0.4%).

Chromatogram C: 0.2 ml injection; 110°C, analysis time: 8min, Internal Standard: acetone (0.2%).

Rapid Analysis of Ethanol and Water in Commercial Products Using Ionic Liquid Capillary Gas Chromatography with Thermal Conductivity Detection and/or Barrier Discharge Ionization Detection

Quantitation of Water in Solids (i.e., APIs)

- **Weight loss by drying**
  - Detects mass of all volatile solvents
  - Nonselective
- **Karl Fischer**
  - Labor intensive and not a high throughput method
  - Needs careful control of solvents
  - Need to control ambient moisture
  - Side-reactions can affect results
Headspace Gas Chromatography (HSGC)

• A common method in pharmaceutical products to analyze residual solvents

• Increases sensitivity compared to direct injection

• Nonvolatile portions that can damage the column are not injected, cleaner injections

• Economical
FAP ILs: the anion is all important

1-hexyl-3-methylimidazolium trifluorotris(pentylfluoroethyl) phosphate (HMIM FAP)

1-butyl-3-methylimidazolium trifluorotris(pentylfluoroethyl) phosphate (BMIM FAP)

1-ethyl-3-methylimidazolium trifluorotris(pentylfluoroethyl) phosphate (EMIM FAP)
Trifluorotris(pentylfluoroethyl) Phosphate (FAP) Anion

- Low content of residual water
- Low water uptake
- Hydrolytic stability
- High thermal stability
- Low viscosity

*Chemistry Today 2004, 22, 42-43.*
<table>
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<th>Sample</th>
<th>Percent Water</th>
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<tr>
<td></td>
<td>HSGC</td>
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<tr>
<td>Ascorbic acid</td>
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<tr>
<td>Bupivacaine</td>
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<td>b</td>
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<td>Carnitine</td>
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<td>Cholecalciferol</td>
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<tr>
<td>Ephedrine</td>
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<td>Ethnylestradiol</td>
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<tr>
<td>Homocysteine</td>
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<td>Ibuprofen</td>
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<td>Promethazine</td>
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<td>Propranolol</td>
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<tr>
<td>Rifampicin</td>
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<tr>
<td>Scopolamine</td>
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<tr>
<td>Sodium tartrate dibasic dihydrate</td>
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<td>15.6</td>
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A New Approach for Ultra-Sensitive Anion Analysis a.k.a.:

PIESI

For

Paired Ion Electrospray Ionization
Detection of Anions

- Three different ways to detect ions
  1) Anion SIM
  2) Cationic complex SIM
  3) Use MS/MS (SRM)
    - Trap m/z of complex
    - Excite this m/z to break complex
    - Monitor m/z of deprotonated cation (m/z 289)
Chromatographic separation and detection of nineteen acidic pesticides in positive ion mode LC-ESI-MS.
### Improvement

<table>
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<th>Absolute LOD (pg)</th>
<th>References</th>
<th>Improvement</th>
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<td>PB</td>
<td>1000 - 40000</td>
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<td>APCI-NI, SIM</td>
<td>2500 - 50000</td>
<td>[2]</td>
<td>4200×</td>
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<td>[3]</td>
<td>830×</td>
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<td>APCI-NI, SIM</td>
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<td>[4]</td>
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<tr>
<td>ESI-NI, SIM</td>
<td>150 - 1600</td>
<td>[6]</td>
<td>250×</td>
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<tr>
<td><strong>ESI-PI, SRM</strong></td>
<td><strong>0.6 - 19</strong></td>
<td>Present method</td>
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‡ The LOD range reported is based on LODs of 19 pesticides obtained using dication C₆(bpyr)₂ in the SRM positive ion mode.

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