Charged Aerosol Detection 101

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Outline

• Introduction to charged aerosol detection (CAD)
• How charged aerosol technology works
• Comparison of CAD to ELSD
• CAD product evolution
• Example applications
• Summary
Introduction to Charged Aerosol Detection (CAD)

- Used to quantitate any non-volatile and many semi-volatile analytes with LC
- Provides consistent analyte response independent of chemical structure and molecule size
- Neither a chromophore, nor the ability to ionize, is required for detection.
- Dynamic range up to four orders of magnitude from a single injection (sub-ng to µg quantities on column)
- Mass sensitive detection – CAD provides relative quantification without the need for reference standards
- Compatible with gradient conditions for HPLC, UHPLC, and micro LC

Comparison of charged aerosol detection to UV and MS
Charged Aerosol Detection – How It Works

Flow path through a Thermo Scientific™ Dionex™ Corona™ Veo™ charged aerosol detector

1. Inlet from column
2. FocusJet™ concentric nebulizer
3. Gas inlet
4. Micro drain pump
5. Evaporation tube
6. Corona charger
7. Mixing chamber
8. Ion trap
9. Electrometer
10. Gas exhaust
Charged Aerosol Detection – How It Works

Flow path through a Thermo Scientific™ Dionex™ Corona™ Veo™ charged aerosol detector

Signal is proportional to the analyte quantity
Particle Charging for Charged Aerosol Detection

- Particle size proportional to mass of analyte
- Charge per particle proportional to particle size
- Charged particles are measured, not gas phase ions as in MS (Independent of analyte net charge).

→ CAD response depends on initial mass concentration of analyte in droplets formed but is independent of its chemical properties.

→ Nearly uniform detector response

However, sample needs to be non-volatile or at least only semi-volatile.
ELSD vs. CAD

Evaporative light scattering detector (ELSD)

- Detection chamber
- Heated nebulizer
- Siphon

Measures the **optical reflection** of solute particles after the sample has been passed through a nebulizer.

Charged aerosol detector

- Nebulizer
- Light source
- Evaporating chamber
- Drain
- Electrometer
- Corona charger
- Evaporation tube

Measures **charged particles** by an electrometer generating a signal that is proportional to particle size (Mass of analyte) after nebulization.
Detector Response Characteristics

- For Rayleigh scattering: \( b = 2 \)
- For Mie scattering: \( b = \frac{1}{3} \)
- For Refraction and reflection scattering: \( b = \frac{2}{3} \)

**ELSD**

Nonvolatiles - Decreasing slope with increasing mass \( (b \sim \frac{2}{3}) \)

**CAD**

* ELSD exhibits a narrower linear calibration range than CAD.*
Detector Response Characteristics

Apramycin and impurities

CAD
- 50 mg/mL
- 20 mg/mL

ELSD
- 50 mg/mL
- 20 mg/mL

ELSD response disappears
Detector Response Characteristics

Apramycin and impurities

- **CAD**
  - 50 mg/mL
  - 20 mg/mL

- **ELSD**
  - 50 mg/mL
  - 20 mg/mL

**ELSD response disappears**
Over short ranges, both ELSD and charged aerosol detector can offer linear response.  

All aerosol-based detectors exhibit a non-linear response over large concentration ranges.  

Several calibration curve approaches are available:  

Selections –

<table>
<thead>
<tr>
<th><strong>ELSD</strong></th>
<th><strong>CAD</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sigmoidal response behavior</td>
<td>Parabolic response behavior</td>
</tr>
<tr>
<td>Log-log</td>
<td>Log-log</td>
</tr>
<tr>
<td>Point to point</td>
<td>Quadratic</td>
</tr>
<tr>
<td></td>
<td>Power function</td>
</tr>
</tbody>
</table>

The most appropriate approach depends upon the data.
## Comparison Review

<table>
<thead>
<tr>
<th>Feature</th>
<th>Evaporative light scattering</th>
<th>Charged aerosol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Response</td>
<td>Sigmoidal</td>
<td>Curvilinear</td>
</tr>
<tr>
<td>Dynamic range</td>
<td>2–3 orders</td>
<td>&gt;4 orders</td>
</tr>
<tr>
<td>LoQ and LoD</td>
<td>LoQ and LoD often higher (Worse) than estimated by SNR</td>
<td>LoQ and LoD often lower (Better) than estimated by SNR</td>
</tr>
<tr>
<td>Sensitivity (LoD)</td>
<td>&gt;10 ng</td>
<td>&lt;1 ng</td>
</tr>
<tr>
<td>Semi-volatility range</td>
<td>Similar</td>
<td>Similar</td>
</tr>
<tr>
<td>Analyte response</td>
<td>Variable - Dependent on compound</td>
<td>Independent of structure</td>
</tr>
<tr>
<td>Flow rate range (0.2 – 2 mL/min)</td>
<td>Possibly several nebulizers</td>
<td>One nebulizer</td>
</tr>
<tr>
<td>Ease of operation</td>
<td>Can be complex</td>
<td>Simple</td>
</tr>
</tbody>
</table>
### Evolution of Charged Aerosol Detectors

<table>
<thead>
<tr>
<th>Year</th>
<th>Model/Description</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>2015</td>
<td>Thermo Scientific™ Vanquish™ charged aerosol detector</td>
<td>Full integration with Thermo Scientific™ Vanquish™ UHPLC platform, slide-in module design, reduced flow path for optimum operation</td>
</tr>
<tr>
<td>2013</td>
<td>Corona Veo RS CAD</td>
<td>Extended micro flow rate range; total redesign with concentric nebulization and optimized spray chamber for enhanced sensitivity, heated evaporation and electronic gas regulation</td>
</tr>
<tr>
<td></td>
<td><strong>Dionex Corp. acquired by Thermo Fisher Scientific Inc.</strong></td>
<td></td>
</tr>
<tr>
<td>2011</td>
<td>Corona ultra RS CAD</td>
<td>Unified with Dionex™ UltiMate™ 3000 UHPLC+ system, added on-board diagnostics / monitoring, automated flow diversion capability and selection of linearization parameters</td>
</tr>
<tr>
<td></td>
<td><strong>ESA Biosciences, Inc. acquired by Dionex Corp.</strong></td>
<td></td>
</tr>
<tr>
<td>2009</td>
<td>Corona ultra CAD</td>
<td>UHPLC compatible, stackable design, enhanced sensitivity, touch-screen user interface with real-time chromatogram display, incorporated precision internal gas regulation system</td>
</tr>
<tr>
<td>2006</td>
<td>Corona Plus CAD</td>
<td>Expanded solvent compatibility with heated nebulization, software drivers for popular CDS systems and external gas conditioning module for improved precision.</td>
</tr>
<tr>
<td>2005</td>
<td>Corona CAD</td>
<td>Introduction of the first commercial charged aerosol detector for HPLC with full control via front panel interface. Designed for near-universal detection on any HPLC system using isocratic or gradient separations</td>
</tr>
</tbody>
</table>
Corona Veo and Vanquish Charged Aerosol Detectors

- Concentric nebulization system improves sensitivity and precision.
- Thermally controlled evaporation scheme widens the scope of applications.
- Corona Veo and Vanquish RS model includes low flow capabilities for micro LC, as well as UHPLC.
- Usability and serviceability have been enhanced.
- CDS drivers available for use with all Thermo Scientific and many other vendor systems.

### Comparison Table

<table>
<thead>
<tr>
<th></th>
<th>Corona Veo</th>
<th>Veo RS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nebulization</td>
<td>FocusJet</td>
<td>FocusJet</td>
</tr>
<tr>
<td>Flow rates</td>
<td>0.2 – 2.0 mL/min</td>
<td>0.01 – 2.0 mL/min</td>
</tr>
<tr>
<td>Data collection</td>
<td>100 Hz</td>
<td>200 Hz</td>
</tr>
<tr>
<td>Evaporation Temp.</td>
<td>35°C or 50°C</td>
<td>RT+5°C – 100°C</td>
</tr>
<tr>
<td>Gas pressure control</td>
<td>manual</td>
<td>electronic</td>
</tr>
</tbody>
</table>

### Vanquish CAD

- FocusJet

### Corona Veo RS

- Coastal N2 flow
- Capillary inlet

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**Vanquish CAD model**

<table>
<thead>
<tr>
<th>Vanquish CAD model</th>
<th>Flow rate range (ml/min)</th>
<th>Data rate (Hz)</th>
<th>Evap temp (°C)</th>
<th>Positioning</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horizon</td>
<td>0.01 – 2.0 (Microflow)</td>
<td>2 – 200</td>
<td>Settable from ambient +5–100</td>
<td>Ideal for R&amp;D and methods development Labs</td>
</tr>
<tr>
<td>Flex</td>
<td>0.2 – 2.0</td>
<td>2 – 100</td>
<td>Selectable 35, 50 or 70</td>
<td>Suitable for routine analysis in QC/QA Labs</td>
</tr>
</tbody>
</table>
Pharma and Biopharma Application Areas

- Drug composition
  - Impurity testing
- Formulation
  - Counterions
  - Surfactants / Excipients
- Degradation / Stability testing
- Characterization
  - Glycan analysis
  - Adjuvant analysis
- Excipient raw material analysis and lot-to-lot variability
Pharma and Biopharma Application Areas

- Drug composition
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- Characterization
  - Glycan analysis
  - Adjuvant analysis
- Excipient raw material analysis and lot-to-lot variability

- Cleaning validation
- Mass balance
- Extractables / Leachables
- PEGylation and antibody-drug conjugates
- siRNA lipid delivery vehicles
- QbD
- MIST (Metabolites in safety testing)

Visit the charged aerosol detection website and the free Thermo Scientific AppsLab library of analytical applications, to see more examples of HPLC-CAD solutions:
www.thermofisher.com/cad
www.thermofisher.com/appslab
Analysis of Gentamicin standard
(200 μg/mL)

Column: Thermo Scientific™ Acclaim™ RSLC
PolarAdvantage II, 2.2 μm, 2.1 × 100 mm

Mobile phase
A: 0.025:95:5 HFBA:water:acetonitrile
B: 0.3:95:5 TFA:water:acetonitrile

Gradient:
0 to 1.5min,1 to 10%B
1.5 to 7min,10 to 100% B
7 to 10min,100% B
4 min. pre-injection equilibration

Flow rate: 0.45 mL/min
Inj. volume: 1 μL
Detector: Corona ultra RS, 15 °C, 60 Hz
Orthogonal and Complimentary Detection with DAD and CAD

Metoprolol and impurities A, M and N

Isocratic HILIC chromatographic method using both UV and charged aerosol detection
Formulation – Counterions

Drug counterions

Instrumentation: Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLC system
Column: Acclaim Trinity P2, 3 μm, 3 × 50 mm
Col. temp: 30 ºC
Flow rate: 0.6 mL/min
Inj. volume: 2 µL
Mobile phase A: Water
Mobile phase B: 100 mM ammonium formate, pH 3.65
Gradient:

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>-8.0</th>
<th>0.0</th>
<th>1.0</th>
<th>11</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>%A</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>%B</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Charged aerosol: Corona Veo RS; 55 ºC, 5 Hz, 2 s, PF 1.5
Sample: 20 to 100 ng/µL each in deionized water

Peaks
1. Phosphate
2. Sodium
3. Potassium
4. Chloride
5. Malate
6. Bromide
7.Nitrate
8. Citrate
9. Fumarate
10. Sulfate
11. Magnesium
12. Calcium

Anions, cations, organic and inorganic ions simultaneously
Drug counterions

Instrumentation: Ultimate 3000 RSLC system

Column: Acclaim Trinity P2, 3 μm, 3 x 50 mm

Sulfonated nano-polymer bead

Anion-exchange /HILIC functionality in inner pore area

Sulfonated nano-polymer beads attached to surface

Nano-polymer/Silica hybrid particle

Peaks
1. Phosphate
2. Sodium
3. Potassium
4. Chloride
5. Malate
6. Bromide
7. Nitrate
8. Citrate
9. Fumarate
10. Sulfate
11. Magnesium
12. Calcium

Anions, cations, organic and inorganic ions simultaneously
Adderall® (Shire Pharmaceuticals) and counterions

**Instrumentation:** UltiMate 3000 RSLC system
**Column:** Acclaim Trinity P2, 3 μm, 3 × 50 mm
**Col. temp:** 30 ºC
**Flow rate:** 0.6 mL/min
**Inj. volume:** 5 μL
**Mobile phases:**
- A: Acetonitrile
- B: Water
- C: 100 mM ammonium formate, pH 3.65

**Gradient:**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>-8.0</td>
<td>35</td>
<td>59</td>
<td>6</td>
</tr>
<tr>
<td>0.0</td>
<td>35</td>
<td>59</td>
<td>6</td>
</tr>
<tr>
<td>0.5</td>
<td>35</td>
<td>59</td>
<td>6</td>
</tr>
<tr>
<td>5.0</td>
<td>35</td>
<td>0</td>
<td>65</td>
</tr>
<tr>
<td>10</td>
<td>20</td>
<td>0</td>
<td>80</td>
</tr>
<tr>
<td>12</td>
<td>20</td>
<td>0</td>
<td>80</td>
</tr>
</tbody>
</table>

**UV detector:** UV diode array; 254 nm, 5 Hz, 0.5 s
**Charged aerosol:** Corona Veo RS; 55 ºC, 5 Hz, 2 s, PF 1.5
**Peaks:**
1. aspartate 24 μg/mL
2. sodium
3. saccharin 24 μg/mL
4. amphetamine 122 μg/mL
5. sulfate 26 μg/mL

**Ref:** AN20870

Complimentary detection by CAD and UV/Vis
Analysis of diclofenac sodium salt (1 mg/mL)

Column: Acclaim Trinity P1, 3 μm, 3.0 × 50 mm
Mobile phase A: 75% Acetonitrile
Mobile phase B: 25% 200 mM Ammonium acetate pH 4
Flow rate: 0.8 mL/min
Inj. volume: 5 μL
Col. temp: 30 °C
Detector: Corona Veo RS CAD
Evap. temp: 60 °C

Charged aerosol even detects chloride impurity.
Stability – Forced Degradation

Follow forced degradation of Amikacin sulfate

Corona ultra RS CAD

Follow forced degradation of Amikacin sulfate
Glycoprotein Characterization – Released Glycan Analysis

**Bovine fetuin**

N-linked glycans

- Monosialylated
- Disialylated
- Trisialylated
- Tetrasialylated
- Pentasialylated

**Mucin**

O-linked glycans

**Detection of released glycans – No labeling required**

**System:** Thermo Scientific™ Vanquish™ UHPLC system

**Column:** Thermo Scientific™ GlycanPac™ AXR-1, 1.9 μm, 2.1 × 150 mm

**Mobile phase A:** Deionized water

**Mobile phase B:** 1.00 mM Ammonium formate, pH 4.4

**Gradient:** 4 % B to 39% B in 35 min

**Flow rate:** 0.4 mL/min

**Inj. volume:** 2 μL

**Col. temp.:** 30°C

**Detector:** Vanquish Charged Aerosol Detector H

50°C, PF 1.0, 10 Hz, 5s
Food and Beverage Application Areas

• Simple carbohydrates
• Lipids
  • Profiling methods
  • Targeted methods
• Artificial sweeteners
Food and Beverage – Simple Carbohydrates

Analysis of simple sugars

Add 20 mL of 85% ACN to 1 gram juice, mix & spin

Isocratic HILIC separation
Corona Ultra RS CAD

Simplified sample preparation “Dilute-and-shoot” method
**Global lipids – Algal oils**

**Column:** Thermo Scientific™ Accucore™ C18, 2.6 μm, 3.0x150 mm

**Mobile phases:**
- A: Methanol:water:acetic acid (600:400:4)
- B: Tetrahydrofuran:acetonitrile (50:950)
- C: Acetone:acetonitrile (900:100)

**Gradient:**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Flow Rate (mL/min)</th>
<th>%A</th>
<th>%B</th>
<th>%C</th>
</tr>
</thead>
<tbody>
<tr>
<td>-10.0</td>
<td>1.00</td>
<td>90</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>-0.1</td>
<td>1.00</td>
<td>90</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>0.0</td>
<td>0.25</td>
<td>90</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>20.0</td>
<td>0.50</td>
<td>15</td>
<td>85</td>
<td>0</td>
</tr>
<tr>
<td>35.0</td>
<td>0.50</td>
<td>2</td>
<td>78</td>
<td>20</td>
</tr>
<tr>
<td>60.0</td>
<td>0.50</td>
<td>2</td>
<td>3</td>
<td>95</td>
</tr>
<tr>
<td>65.0</td>
<td>0.50</td>
<td>90</td>
<td>10</td>
<td>0</td>
</tr>
</tbody>
</table>

**Flow rate:** 1.0 mL/min

**Inj. volume:** 2 μL

**Col. temp:** 40 °C

**Detector:** Corona Veo RS CAD

**Evap. temp:** 40 °C

**Complex sample – Minimal sample prep**
Lipids – Targeted Methods

Column: Thermo Scientific™ Hypersil™ ODS C18 5 µm, 4.6 × 250 mm, 2 in series, plus 2 in series for gradient compensation

Mobile phase A: 2-propanol:hexane 1:1 (v/v)
Mobile phase B: Acetonitrile

Gradient:

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Flow rate (mL/min)</th>
<th>%A</th>
<th>%B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>1</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>80</td>
<td>1</td>
<td>90</td>
<td>10</td>
</tr>
</tbody>
</table>

Inverse gradient: Yes
Flow rate: 1.0 mL/min
Inj. volume: 4 µL
Col. temp: 30 °C
Detector: Corona CAD 100 pA; 35 psi, low filter

Sample: B, TG standard GLC#435 and D) GLC#437, dissolved in hexane


**Complex real samples – minimal sample prep**
Food Additives: Steviol Glycosides (Sweetener)

Column: Acclaim Trinity P1, 3 µm, 2.1 × 150 mm
Mobile phase: 88:12 (v/v) Acetonitrile:10 mM ammonium formate, pH 3.1
Flow rate: 0.8 mL/min
Inj. volume: 2 µL
Col. temp: 30 ºC
Detection: Dionex Corona Veo RS CAD
Settings: 2 Hz, 5 second filter, PF 1.0, evap. temp 35 ºC

CAD exhibits more uniform response.
Artificial Sweeteners: Steviol Glycosides

Column: Acclaim Trinity P1, 3 µm, 2.1 x 150 mm
Mobile phase: 88:12 (v/v) Acetonitrile:10 mM ammonium formate, pH 3.1
Flow rate: 0.8 mL/min
Inj. volume: 2 µL
Col. temp: 30 °C
Detection: Dionex Corona Veo RS CAD
Settings: 2 Hz, 5 second filter, PF 1.0, evap. temp 35 °C

ELSD

CAD

CAD exhibits more uniform response.
Summary

• Charged aerosol detection delivers accurate and precise quantification of lipids, carbohydrates, surfactants, amines and counterions that UV/Vis absorbance cannot detect.

• For analytes with chromophores, charged aerosol detection provides uniform response independent of the extinction coefficient.

• Charged aerosol detection provides a good estimate of the amount of unknown impurities and degradation products.

• Charged aerosol detection is superior to ELSD in terms of sensitivity, dynamic range, response uniformity, precision and ease of use.

• More information on charged aerosol detection can be found at www.thermofisher.com/cad

• Bibliography of charged aerosol detector applications can be downloaded from http://analyteguru.com/resources/charged-aerosol-detection-list-of-published-articles/
Thank You Very Much for Your Attention!

Questions?

Do you have additional questions or do you want to talk to an expert from Thermo Fisher Scientific?

Please send an E-Mail to analyze.eu@thermofisher.com and we will get back to you.