Charged Aerosol Detection: Factors Affecting Uniform Analyte Response

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ABSTRACT

The charged aerosol detector (CAD) uses evaporative aerosol techniques and a downstream measurement process that imparts a size-dependent charge independent of particle composition. Because the downstream measurement provides a uniform analyte response, most factors affecting CAD response uniformity are related to the upstream spray drying process.

CAD response can be influenced by four factors related to the drying process:

1) Mobile phase composition: Changes in organic content of the mobile phase during gradient elution can impact detector response.

2) Analyte volatility: Loss of analyte response due to its evaporation during nebulization and drying processes.

3) Salt formation: The interaction between ionizable analyte and mobile phase additives (i.e., pH modifiers, pH buffers and ion pairing agents). Salt formation can be leveraged to convert analytes that behave as semi-volatiles and volatiles into those that behave more like non-volatiles.

4) Analyte density: This is only a minor influence on analyte response.

We used flow injection analysis to study the CAD response of 58 chemically diverse analytes and observed an approximate volatility limit. For some semivolatiles with ionizable functional groups, volatile eluent additives had a profound effect on response. After correcting for salt formation, the relative standard deviation of CAD response for 36 diverse analytes was 5.8%.

INSTRUMENTATION FOR FLOW INJECTION ANALYSIS

Thermo Scientific™ Vanquish™ Flex Quaternary UHPLC system consisting of:
- System Base Vanquish Flex (P/N VF-S01-A-02)
- Dual Pump Flex (P/N VF-P32-A-01)
- Split Sampler FT (P/N VF-A10-A-02), 25 µL sample loop
- Column Compartment (P/N VH-C10-A-02)
- Corona Veo / Vanquish Flex Charged Aerosol Detector (P/N 5081.0010 / VF-D20-A)

METHOD

Samples were prepared at 0.5 µg/µL in mobile phase A. Specifically, when flow injection was performed without TEA, samples were dissolved in water. When flow injection was performed with TEA, samples were prepared in 0.01% TEA.

Flow Injection Conditions	
Connection from Autosampler to CAD	0.1 x 550 mm Thermo Scientific™ Viper™ Capillary fingertight fittings
Mobile Phase	For flow injection without TEA: A: Water (20 %) B: Acetonitrile (80 %) For flow injection with TEA: A: water with 0.01% TEA, pH ~10.5 (20%) B: acetonitrile with 0.01% TEA (80%)
Flow Rate	0.4 mL min^{-1}
Injection Volume	1 µL
Detector settings	35°C evaporation temperature, 5 Hz data collection rate, 0.5 s filter

CAD UNIFORM RESPONSE

The CAD is a mass-flow sensitive detector (response ∝ mass per unit time) that shows outstanding response uniformity. Response is independent of analyte chemical properties, as shown in Figure 1. The variability of response in Figure 1 is less than 6%. Unlike UV detectors, the CAD can quantify all analytes in a run with a single standard, called a universal calibrant. The comparison between CAD and UV detector uniform response is shown in Figure 2.

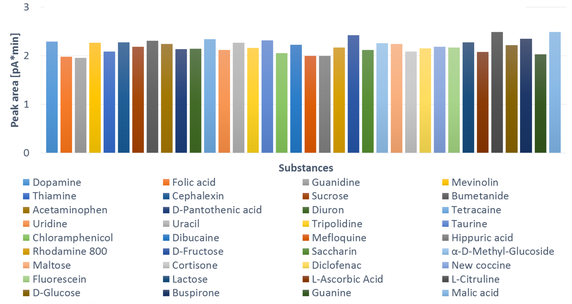


Figure 1. CAD response, corrected for purity of the solid material, for 36 compounds introduced by flow injection at 0.5 µg. Variability of response is less than 6%. Several outliers, not shown, are undergoing further investigation.

MOBILE PHASE COMPOSITION EFFECTS

Changes in organic content of the mobile phase during gradient elution can impact detector response. In order to achieve uniform response with a CAD, a constant composition of mobile phase must reach the detector inlet. This constant composition is accomplished by adding a "make-up" or "inverse" gradient using a second pump. Effects are shown in Fig. 3(A) and (B).

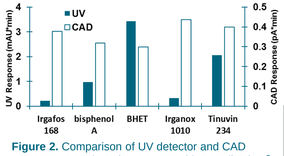


Figure 2. Comparison of UV detector and CAD response to analytes in an extractables application.[3]

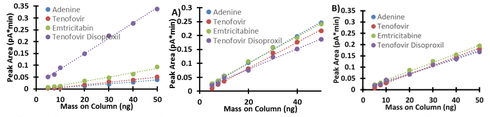


Figure 3. Calibration curves for analytes in tenofovir (A) without inverse gradient, (B) with inverse gradient, and (C) with inverse gradient and correction for one acetate per analyte molecule.

ANALYTE DENSITY AND OTHER CONSIDERATIONS

A density correction is infrequently applied because dried particle diameter varies only by the cube root of solute density, as follows[1]:

$$\text{dried particle diameter} = \mathrm{D}\left(\frac{C_S}{\rho_S}\right)^{1/3}$$

Where ρ_S = solute density, C_S = novolatile solute mass concentration, and D = primary aerosol droplet diameter

Density data is sparse and, because density is not a major factor affecting response and this equation only applies to round particles, it is generally not practical to address this issue. However, if solutes have widely disparate densities, one can simply multiply the CAD response for each analyte by the cube root of its density (or density of analyte salt).[1] Other considerations for response:

- purity of the material
- changes during storage and preparation (e.g., adsorption of water)
- analyte degradation
- analyte loss on the column
- weighing and dilution errors

EFFECTS OF SALT FORMATION, NON-VOLATILES

Salt formation by non-volatile ionizable compounds can lead to decreased response uniformity. The use of mobile phase additives with low molar mass, such as formic acid and ammonium formate, minimizes this effect. Response can be normalized as shown in Figure 4 and in the equation below (dopamine example):

Corrected Response =

$$\text{Response} * \frac{M_w(\text{dopamine})}{M_w(\text{dopamine+HCl})}$$

The dopamine-HCl salt is seen because they co-elute when analyzed by flow injection.

EFFECTS OF SALT FORMATION; VOLATILES

Intentional salt formation can broaden the range of compounds that produce a CAD response (see also Thermo Fisher Technical Note 72806).[2] Salt forms of volatile analytes are generally non-volatile. An example of response normalization after salt formation by semivolatile substances is shown in Figure 4 for oxalic acid. The corrected response is described, similar to the dopamine example, by:

Corrected response = Response * M_w(oxalic acid) / [M_w(oxalic acid) + M_w(TEA)]

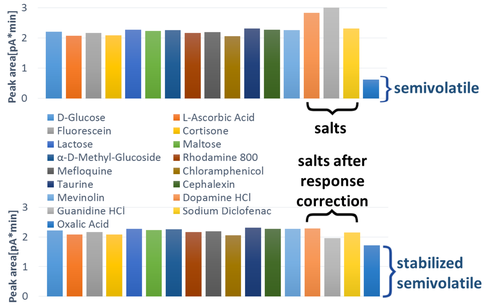


Figure 4. Examples of response after mathematical correction for salt formation (dopamine, guanidine, and diclofenac) and stabilization of a semivolatile substance (oxalic acid) after TEA addition in charged aerosol detection.

EFFECTS OF ANALYTE VOLATILITY

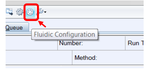
The ability to predict whether the CAD can measure a particular analyte is of considerable interest. Several studies have described approximate cut-offs beyond which all analytes behave as non-volatiles.[1] Suggested cut-offs have characterized non-volatiles as substances with boiling points above 400 °C or with enthalpies of vaporization above 65 kJ/mol and molecular weight above 350 g/mol. Suggested cutoffs for vapor pressure also exist. These rough guidelines depend on instrument design and conditions, especially evaporation temperature. Some differences arise due to salt formation. Research into spray drying and gas-to-particle partitioning will help improve predictions of LC-CAD response.

Although high evaporation temperatures will reduce background current and noise, evaporation temperature should be set as low as possible to maximize the response for semivolatiles.

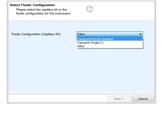
INVERSE GRADIENT WIZARD IN CHROMELEON

Starting with version 7.2.8, Thermo Scientific™ Chromeleon™ Chromatography Data System Software features an inverse gradient wizard that facilitates design and implementation of inverse gradient methods. There are two stages to the wizard. The first stage allows the user to define the fluidic configuration. The second stage is initiated when the user programs an instrument method. The two stages are detailed below.

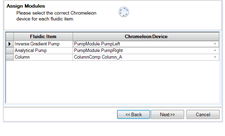
1) The valve icon opens the fluidic configuration dialog.



2) The user selects relevant workflows based on system configuration and capillary kit.



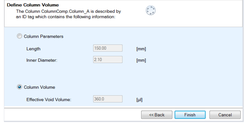
3) The user assigns pump heads to the analytical or inverse gradients



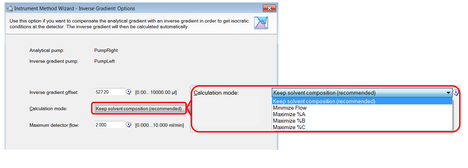
4) The wizard calculates column volume (in µL) using the equation:

$$\frac{\pi}{4} * d^2 * L * 63\%$$

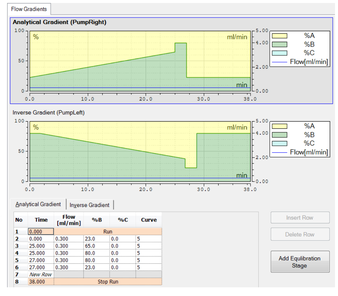
where d and L are the column's inner diameter and length in mm, and 63% is the interparticle volume factor.



5) The user selects an option for calculating the inverse gradient. The user can minimize flow or maximize %A, %B, or %C.



6) When the user edits the analytical gradient, Chromeleon automatically calculates and updates the inverse gradient. The gradient delay is automatically calculated from the fluidic configuration.



CONCLUSIONS

Inherent universal response of non-volatile and most semi-volatile compounds is a superior feature of the CAD compared to classic detection options like UV-Vis. We have presented simple considerations and techniques to further increase analyte response uniformity:

- The Vanquish Duo Inverse Gradient Workflow can compensate the analytical gradient with a second low-pressure gradient pump to avoid bias occurring from different solvent compositions. This approach gives a more uniform response and more reliable quantitation.
- Salt formation between ionizable analytes can influence response uniformity. This effect can be minimized by choosing low molar mass mobile phase additives and response can be normalized using the described calculations.
- Volatility is a crucial consideration in CAD response. It is best to use the lowest evaporation temperature that consistently produces the required sensitivity limits. This should provide the most uniform response between analytes. The formation of salts can markedly improve the response for compounds that are both ionizable and volatile compounds.

REFERENCES

1. Charged Aerosol Detection for Liquid Chromatography and Related Separation Techniques. Gamache, P. H. (ed.). Wiley. Chapters 1 and 3.
2. Thermo Fisher Scientific Technical Note 72806, Charged Aerosol Detection – factors affecting uniform analyte response, 2018.
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5. Thermo Fisher Scientific Application Note, Quantitation of tenofovir and impurities in multi-component drug products by ternary gradient reversed phase chromatography with charged aerosol detection, 2019.

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