Water Condensation Nucleation … a Universal detection technique opening up new HPLC applications

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Introduction

Water Condensation Nucleation (or Water Condensation Particle Counting) is a technology originally developed to detect aerosol particles in the application field of environmental monitoring. Using this technique air was drawn through an instrument and any particles counted to produce a global particle count for the environment.

By introducing a liquid stream rather than a gas flow the technology has been now been refined for use in Liquid Chromatography. Since Water Condensation Nucleation requires no knowledge of chemical structure to detect compounds it has found use in the area of universal detection. This article was written to demonstrate how the Water Condensation Nucleation technique has been developed into a LC detector with the ability to detect sub-nanogram levels that operates with a superior linear response over a wide dynamic range (3-4 orders of magnitude) and offers near universal detection for compounds that are less volatile than the mobile phase.

What is universal detection?

'Universal detection in Liquid Chromatography' is the ability to detect compounds eluting through an LC system without the need to use any of the chemical structure of a molecule for detection – typical of these types of detector are UV, fluorescence, mass spectroscopy and electrochemical.

The 'original' universal detector was refractive index, although used in many applications its limitations were lack of sensitivity and could only be used with isocratic mobile phases.

The need to enhance sensitivity of universal detection lead first to the development of the evaporative light scattering detector (ELSD), then the charged aerosol detector (CAD) and now the Water Condensation Particle Counter (WCPC).

Water Condensation Nucleation

Water condensation nucleation is a technique where particles are grown to a size in which they can be individually detected and counted. Particles are grown by exposing them to a chamber of water saturated air. This causes water to condense on the surface of the particle much like water will condense on a cold drinks can sitting outside on a hot day. The result is a dry particle with a shell of water around it. This particle is much larger than the

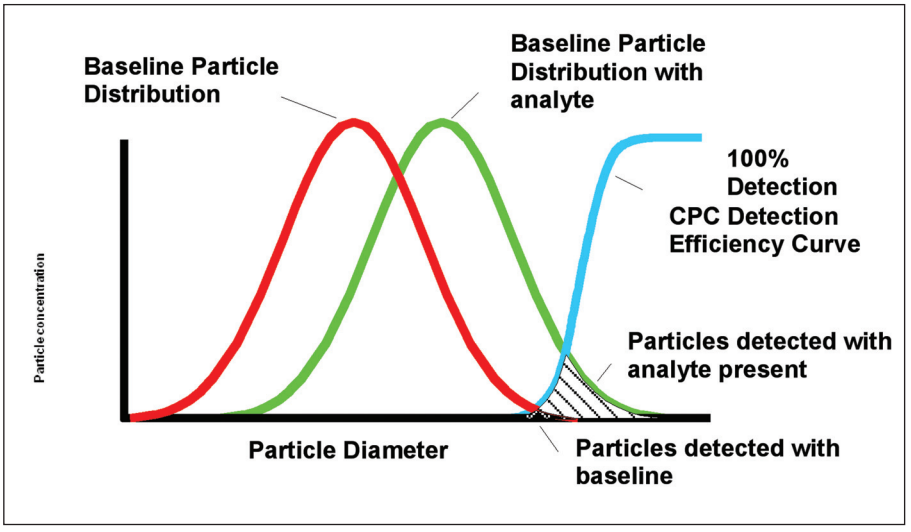

Figure 1: CPC Efficiency Detection Curve

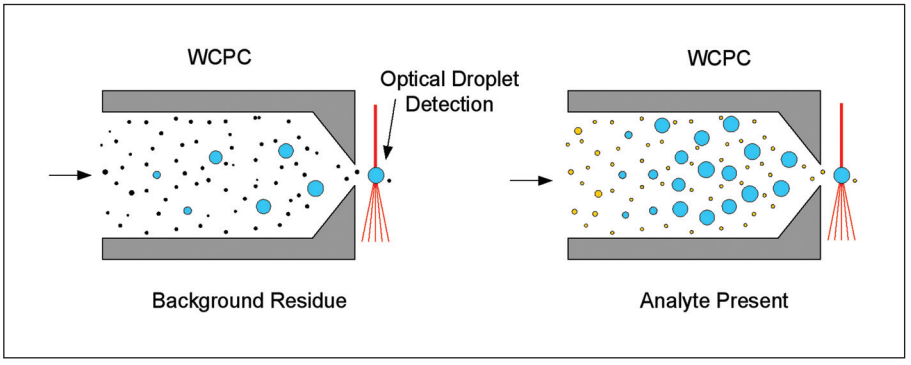

Figure 2: WCPC Particle Counting

dry particle. In fact, many of these particles are large enough to be individually sensed and counted. After water is condensed on the particles, the particles are streamlined so that they pass through a laser beam one by one. When a particle passes the laser beam, it will scatter light. A photodetector will count every time it sees scattered light. What is important about this step, is that only some particles are large enough to be detected (Figure 2).

What the WCPC detector will see and what it will not see is dependent on a factor parameter called the CPC detection efficiency curve as shown in light blue in the graph (Figure 1). Anything that lies to the right of this CPC detection efficiency curve is large enough for the detector to see it. Anything that lies to the left of the CPC detection efficiency curve is too small to be detected. Since background particles that only contain mobile phase residue are small, only a small fraction of these particles cross the CPC detection efficiency curve and will be detected (shown in black). These particles contribute to the baseline of the chromatogram. When there is analyte and mobile phase residue present, the particles are much larger in diameter. Because of this shift in size, many more of these particles are large enough to cross the CPC detection efficiency curve and be detected. These particles are show in yellow and will contribute to the analyte signal on the chromatogram.

How does WCN detect in LC?

WCN starts the same way as any other aerosol detector on the market. First, the mobile phase is continuously nebulized as it comes off the column. Then, it is evaporated leaving dry residue suspended in air or nitrogen. The dried residue then undergoes an additional step called Water Condensation Nucleation. In this step, the dry particles are swept through a chamber of water saturated air or nitrogen. This causes water to condense on the surface of the particle, effectively growing the particles to a size in which they can be individually sensed and counted. WCN is highly sensitive because individual particles are counted. This is similar to counting photons (if you could do that) rather than measuring light intensity such as in the ELSD.

Enhanced user benefits

The Water Condensation Particle counting technology makes a highly useful tool. In Universal detection the limit of detection of these detectors is always quoted as amounts on column rather in amounts/volume.

Sub nanogram sensitivity is possible, when using columns with low bleed and pure solvents, making it the most sensitive detector in the field today. This, coupled with a wide dynamic range, a linear range that generally spans 3-4 orders of magnitude (Figure 3) and reproducibility less than 2%, even at low concentrations making WCPC ideal for a wide range of application areas, particularly for the pharmaceutical, food/beverage, polymer and cosmetic industries.

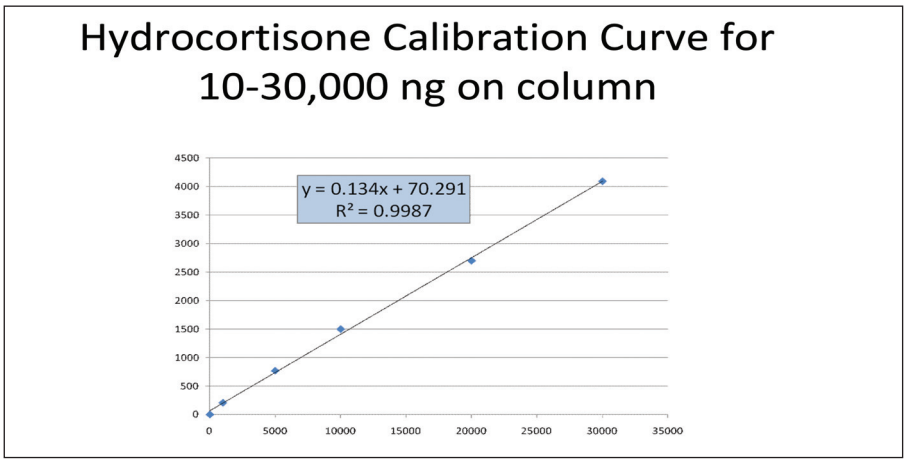

Figure 3: WCPC Linear range (Uracil)

One of the major benefits of aerosol based detectors is their ability to detect compounds that do not have a chromophore such as lipids, polymers, surfactants, carbohydrates, and impurities/degradation products. Detecting these compounds without derivatization greatly reduces sample preparation time and allows for less complicated chromatography.

WCPC technology is uHPLC and HPLC compatible, Figure 4 is an example of a PEG separation on HPLC and uHPLC. Baseline resolution was still achieved when the peaks were separated in approximately 1 minute as opposed to the 7 minutes required for the HPLC separation. uHPLC minimizes sample analysis time, and can minimize waste consumption by up to 80%.

WCPC detectors are also SFC compatible. Generally, to be successful with SFC applications, the nebulizer temperature should be heated to 40C in order to keep a stable baseline during the adiabatic expansion of the CO2 gas. Later models of WCPC detectors are most suited for SFC applications since these models allow control of the nebulizer temperature.

Gradient elution is often an important requirement to separate compounds of interest. However, for some aerosol based detectors, the response is mobile phase dependent. This results in a higher response with a higher organic concentration. Ultimately, when estimating the concentrations of sample components using these detectors,

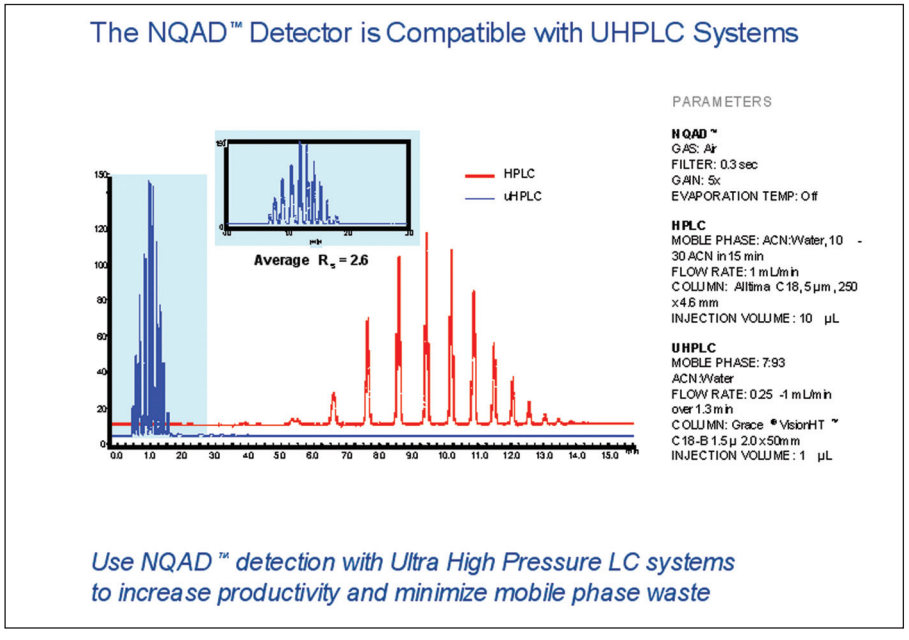

Figure 4: WCN Compatibility with HPLC and UHPLC Systems

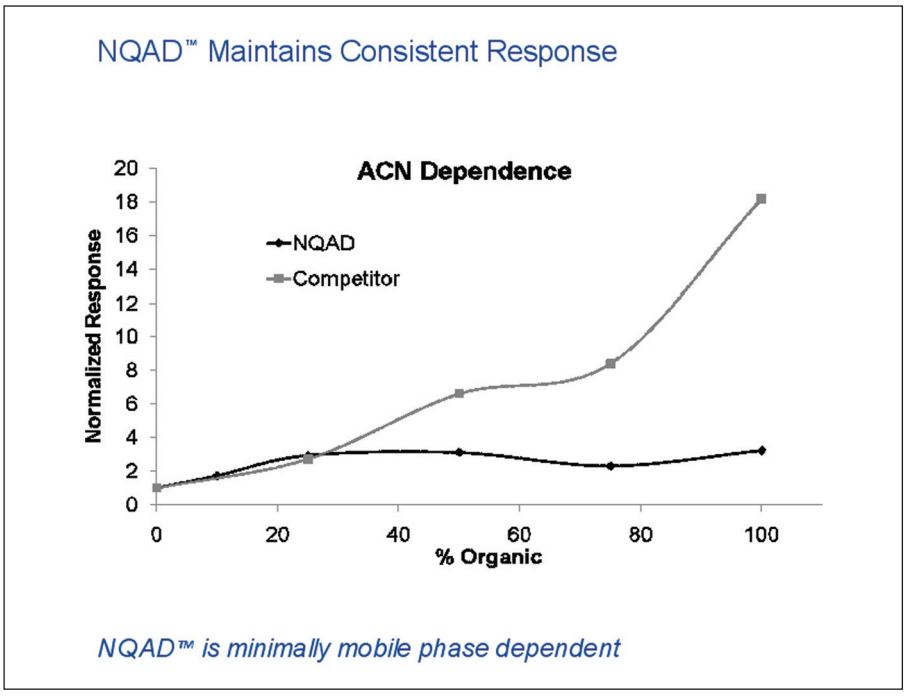

Figure 5: Gradient Elution Effect of Different Universal Detectors

analytes that elute later in the chromatogram (with a higher concentration of organic) are greatly exaggerated. Because the response from the NQAD is not as dependent on mobile phase composition, a much more accurate representation of analyte concentrations can be achieved, even during gradient elution methods (Figure 5).

Areas of Application

Given below are the typical areas of application that the NQAD is being used for:

- Impurity Analysis
  - The high sensitivity coupled with the wide dynamic range possible with WCPC allows for impurities to be detected along with the main analyte peak in a single run, a time saving benefit.
  - The additional benefit of fraction collection is available for the isolation of impurity peaks so they may be characterized using alternative instrument techniques.
- Excipients Characterization
  - Many excipients do not contain a chromophore which makes WCPC an ideal orthogonal detector to UV detection. Seeing more sample components per run saves time and money as less runs need to be performed and less organic solvents are used reducing disposal costs.
- Method Transfer
  - WCPC detectors are UHPLC/HPLC/SFC compatible. This allows the use of the same detector for multiple sample analyses using several separation techniques, providing expected results and easy method transfer.
- Cleaning Validations and Solvent/Column Quality Control
  - The high sensitivity of WCPC allows the chromatographer to detect small changes in solvent purity and column bleed over time. In addition, it is an ideal tool for cleaning validation where trace amounts of analyte need to be detected.
- Difficult Analyte Detection
  - The NQAD is an ideal detector for analytes that were previously difficult to detect such as phosphotidylcholine, a molecule which is challenging to separate and detect reproducibly because it contains both polar and nonpolar ends. Reproducibility detecting challenging compounds saves the customer time

A typical instrument configuration would include the WCPC detector as an orthogonal detector to UV detector and/or a Mass Spectrometer.

Commercial products based on WCPC

The NQAD™ or Nano Quantity Analyte Detector is the first aerosol based detector in the field of Universal Detector for Liquid Chromatography to utilize Water Condensation Nucleation. The detector is optimized for use in UHPLC, HPLC and SFC.

The NQAD detector is designed and manufactured by Quant Technologies, Blaine, MN, USA and is exclusively distributed by Dorton Analytical Ltd in the United Kingdom.

The author thanks Quant Technologies for the use of the diagrams included in this article.