Aerosol-based detectors for liquid chromatography: do they have a role in bioanalysis?

Analysis of the components of biological samples can be extremely challenging due to low concentrations of the compounds of interest, high concentrations of other compounds and the complexity of samples. In order to obtain quality measures of the concentrations of the analytes of interest, instrument-based methods must show good selectivity, high sensitivity and the ability to calibrate the instrument for the analytes of interest. Sample preparation steps and HPLC separations provide some of the selectivity needed.

Before LC-MS instruments became readily available, analysts often faced a choice of using UV detection or a more sensitive and selective alternative, such as fluorescence or electrochemical detectors. This choice would depend on whether the analysis was aimed more at specific compounds or comprehensive analysis. For many compounds, especially compounds without chromophores, conversion of the compounds to fluorescent or UV-absorbing derivatives was an additional step, adding to sample-preparation complexity but resulting in greater selectivity, with only a fraction of the compounds eluting from the column being detected. The increasing availability and performance of LC-MS instruments has changed the focus of many analyses; LC-MS gives excellent sensitivity and selectivity and responds to a wide range of analytes, it

has therefore become increasingly important in bioanalysis. The main shortcomings of LC-MS are the higher equipment and operation costs, difficulties in ionizing some analytes and variability in response (which makes the quantitative determination of analytes without standards difficult). Variability in response is one limitation that can be overcome through the use of a more universal-type detector.

In the early period of HPLC development, one apparent limitation was the lack of a detector that was economical, sensitive and universal in the way that the flame ionization detector (FID) is for GC. Refractive index detectors have been used as universal detectors, although they have limited sensitivity and are not compatible with gradient elutions. UV detectors are also used as universal detectors, despite the wide range of sensitivities for compounds, including compounds without chromophores. The earliest aerosol-based (AB) detectors – evaporative light scattering (ELS) detectors – were first described in the 1960s [1] but were further developed in the late 1970s and early 1980s to meet the need for universal detection [2–5]. While neither inexpensive nor highly sensitive compared with FIDs for GC, ELS detectors are universal for compounds of low volatility. These detectors have seen a gradual increase in sensitivity and usage since the early 1980s. Many of the early applications were aimed at compounds of biological origin with weak chromophores, such as lipids and carbohydrates [3,6]. More recent reviews of ELS detection have shown some analysis of biological samples including serum and urine, although more common samples are food and pharmaceuticals [7]. Numerous companies sell ELS detectors. More

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recently, two more advanced AB detection methods have been developed, condensation nucleation light-scattering (CNLS) detection [8,9] and charged aerosol (CA) detection (originally termed aerosol charge detection) [10,11], which have greater sensitivity than ELS detection.

AB detectors, a term adapted from Koropchak *et al.*, all function by converting the effluent exiting a HPLC column into an aerosol and then detecting some property of the resultant aerosol with an 'aerosol detector' (FIGURES 1 & 2) [12]. The conversion phase typically occurs through pneumatic spraying of the effluent into a stream of nitrogen, followed by evaporation of the spray droplets in an evaporation chamber. It is often said that AB detectors can respond to any compound that is less volatile than the solvent. However, it should be pointed out that response is best for 'nonvolatile' compounds and attention must be paid to ensure that nonvolatile additives or contaminants are not present in solvents. In ELS detectors, the aerosol property detected is light scattering. The general principle is that when higher concentrations of analyte are present, the resulting aerosol particles are larger and scatter more light. When concentrations are low (under $\sim 1 \mu\text{g ml}^{-1}$), residual particles are too small to scatter light efficiently, thus limiting sensitivity [8].

In CNLS detection, sensitivity is greatly increased by 'growing' particles through condensation of a liquid (water or butanol) to sizes where detection by light scattering becomes efficient [8,9,12]. The aerosol detectors used in CNLS detection are used for clean rooms where particles in the order of nanometers can be detected at concentrations as low as one particle per liter. This indicates that, in principle, an extremely sensitive detector is possible. In practice, sensitivity is often limited by solvent impurities (solvents of the lowest listed residue concentrations should always be

used) and column bleed [9]. CNLS detection was found to result in detection limits two orders of magnitude less than ELS detection [13]. Sensitive CNLS detection has been demonstrated in the analysis of sugars in urine [14], biogenic amines in fish samples [15] and lipids in egg yolk samples [16]. A commercial CNLS detector for HPLC has recently been produced [101].

“Aerosol-based detectors ... all function by converting the effluent exiting a HPLC column into an aerosol and then detecting some property of the resultant aerosol with an 'aerosol detector'.”

Small aerosol particles are also efficiently charged through diffusion of molecular ions to particles' surfaces; this is the basic principle behind smoke detectors. Although the exact charging mechanism is somewhat different in the first detector described [9] and the commercial instrument [10], in both cases a corona discharge supplies the molecular ions and the charged particle flux is measured by collecting charged particles on a particle filter electrically connected to an electrometer. While it is not possible to detect single charged particles, charging is most efficient for small particles, giving CA detectors sensitivity much higher than ELS detectors and comparable with CNLS detectors [9]. Sensitive CA detection has been observed for lipids [17] and sugars [18,19], as well as degradation products [20].

“Concentrations of unknown compounds can be estimated when standards are not available or prohibitively expensive...”

Both CNLS detection and CA detection have been applied to microbore columns, with results showing that mass detection limits can be reduced to picogram levels by using smaller bore columns [16,21]. Presumably, this is because conversion to an aerosol is more efficient at lower flow rates.

While all of the aforementioned AB detection methods described are universal, it is conceivable that future AB detectors could use other aerosol properties that depend on aerosol components, thus yielding a selective AB detector. Besides the AB detectors mentioned previously, other detectors, such as atomic spectrometers and LC mass spectrometers, also involve aerosol production, although only as a step in converting analytes to products detected in the gas phase. Other

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Figure 1. Generic aerosol-based detector.

Figure 2. System used by the author for converting HPLC effluent to an aerosol.

detection strategies could use aerosol technology to separate aerosol particles from surrounding volatilized solvent, which could lead to a new type of FID.

Application to biological samples

In the application of AB detectors to biological samples, the goals of the project must be kept in mind. If the goal is to perform a comprehensive analysis of a compound class that makes up a significant fraction of the nonvolatile sample mass, AB detectors are practical alternatives to other methods. Since the AB response is not highly dependent on compound composition, concentrations of unknown compounds can be estimated when standards are not available or prohibitively expensive. This is commonly the case when examining metabolites or complex biological compounds such as glycosides or glycoprotein oligosaccharides. On the other hand, if the goal is to determine one to several compounds present at trace levels in complex samples, insufficient selectivity can limit the ability to meet the goal. This author and a student unsuccessfully attempted to apply a method developed for measuring the concentration of a sugar in atmospheric aerosols to urine samples [18]. In aerosol samples, the sugar was present at the percent levels, but, in urine, other components overwhelmed the analyte peak. While the sensitivity of CNLS detection and CA detection may be sufficient for trace analysis, the method selectivity must also be high. However, in cases where

sample preparation methods can greatly reduce sample complexity (e.g., extraction of lipids from biological samples), trace level constituent analysis should be possible. Additionally, improvements in chromatographic resolution, including the move to smaller-packing particle sizes and advanced methods such as 2D chromatography may make a less selective detector more successful in the analysis of complex samples.

Conclusion

AB detection methods will work best for samples with relatively high fractions of analyte or with selective separation methods. However, with improved sample preparation methods and more efficient chromatographic separations, it should be possible to apply AB detection to somewhat more challenging biological samples. There is also an advantage when there are calibration challenges (e.g., no standards) that make selective MS detection difficult for quantification.

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