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Performance of charged aerosol detection with hydrophilic interaction chromatography

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ABSTRACT

The performance of the charged aerosol detector (CAD) was investigated using a diverse set of 29 solutes, including acids, bases and neutrals, over a range of mobile phase compositions, particularly with regard to its suitability for use in hydrophilic interaction chromatography (HILIC). Flow injection analysis was employed as a rapid method to study detector performance. CAD response was 'quasi-universal', strong signals were observed for compounds that have low volatility at typical operating (room) temperature. For relatively involatile solutes, response was reasonably independent of solute chemistry, giving variation of 12–18% RSD from buffered 95% ACN (HILIC) to 10% ACN (RP). Somewhat higher response was obtained for basic compared with neutral solutes. For cationic basic solutes, use of anionic reagents of increasing size in the mobile phase (formic, trifluoroacetic and heptafluorobutyric acid) produced somewhat increased detector response, suggesting that salt formation with these reagents is contributory. However, the increase was not stoichiometric, pointing to a complex mechanism. In general, CAD response increased as the concentration of acetonitrile in the mobile phase was increased from highly aqueous (10% ACN) to values typical in the HILIC range (80–95% ACN), with signal to noise ratios about four times higher than those for the RP range. The response of the CAD is non-linear. Equations describing aerosol formation cannot entirely explain the shape of the plots. Limits of detection (determined with a column for solutes of low k) under HILIC conditions were of the order of 1–3 ng on column, which compares favourably with other universal detectors. CAD response to inorganic anions allows observation of the independent movement through the column of the cationic and anionic constituents of basic drugs, which appear to be accompanied by mobile phase counterions, even at quite high solute concentrations.

1. Introduction

An important problem for high performance liquid chromatography (HPLC) is the limited choice of detectors that respond to compounds containing no UV/VIS chromophores. Charged aerosol detection (CAD) is a relatively new type of detector developed for use in HPLC over the last 10 years [1]. About 100 publications concerning the detector have appeared to date (e.g. [2–4]). The detector seems very suitable for the analysis of some pharmaceuticals and compounds of biomedical significance, at least in the reversed-phase (RP) mode [5], however, more detailed study is necessary to further understand its properties. Its response is dependent on the formation of aerosol particles (see Fig. 1), similar to techniques such as evaporative light scattering detection (ELSD) [6] and condensation nucleation light scattering detection (CNLSD) [7]. This dependence results in a response which is supposedly independent of solute molecular structure, giving a signal for any compound that is able to form stable aerosol particles. Therefore, CAD is potentially suitable for impurity analysis, particularly in pharmaceutical development where measurement by UV or mass spectrometry (MS) requires the use of standards that may be unavailable for unknown impurities. In CAD, the aerosol particle becomes charged through collision with positively charged nitrogen gas [8], which differs from MS interfaces which generate molecular ions rather than charged particles [9]. The present work aims to study the performance of the CAD, and investigate to what extent it may fulfil the requirements of a universal detector, particularly with regard to its use in hydrophilic interaction chromatography (HILIC). Clearly some factors influencing CAD behaviour are already understood, although commercial instruments have some differences from the prototype described by Dixon and Peterson [1]. These differences are sometimes ignored

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in the literature in discussions of the mechanism of operation of commercial instruments [10,11]. Nevertheless, the process in both may involve transfer of charge from the sheath gas (e.g. nitrogen) to the solute particles (see Fig. 1), which is distinct from the more direct exposure of the corona discharge to the eluent as occurs in atmospheric pressure chemical ionisation (APCI) sources used in mass spectrometry. As CAD response (along with that of all aerosol detectors) depends on the formation of solid particles, it is limited to solutes that have low volatility at the operating temperature. However, few studies have investigated in detail any relationship between volatility and detector signal. The ability to differentiate between solute and mobile phase determines the detection limit, which has been quoted as 0.1–1 ng sample on-column [2,12]. Salt buffers are often critical additives to HPLC mobile phases in any separation mode, but are potentially detrimental to CAD performance. In HILIC, salt buffers can lead to better peak shape than simple acid solutions [13–15], thus we wished to investigate their influence on CAD sensitivity. Furthermore, as with other aerosol-based detectors, detector response is dependent on organic solvent content. While changing detector response with organic solvent concentration has been investigated for its detrimental effect on response uniformity in gradient elution [16–18], high organic concentrations as used in HILIC may be advantageous for sensitivity as it should facilitate desolvation of particles in the CAD. Aerosol-based detectors are known to produce non-linear calibration curves [19], which can arise for different reasons in different detectors. For instance in the ELSD, it is due to both the non-linearity of aerosol formation and a change in detection mechanism with the size of aerosol particles [20]. The mechanism of detection in CAD is more straightforward than ELSD [5], and CAD calibration curves can be close to linear over small concentration ranges [8]. The detailed mechanism that causes non-linearity of CAD calibration curves and their profile has not been described to date. Detector response for aerosol-based detectors is believed to be mostly independent of solute chemistry [5]. However this factor has also not been investigated in much detail with respect to CAD for a sufficiently broad selection of solute structures.

Approximately 50% of drug active pharmaceutical ingredients (API) are salts [21], and many salt counter ions do not contain chromophores. An important benefit of CAD is the ability to detect solutes which do not contain chromophores, and thus it should respond to these counterions [22].

2. Experimental

2.1. Chemicals and reagents

A set of 29 probe compounds comprising acids, bases and neutrals (as used in a previous study [23]) was obtained from Sigma Aldrich (Poole, UK) and used as probes. Structural and physicochemical data are provided in Table 1. Log D values were calculated as the average from three different software packages: ACD version 12.0 (ACD Labs, Toronto, Canada), Marvin (Chem Axon, Budapest Hungary) and MedChem Designer (Simulations Plus, Lancaster, USA). Standards were diluted in the exact mobile phase from stock solutions typically at 10,000 mg/L made up in 50% ACN containing 0.1% FA. ACN (HPLC gradient grade), ammonium formate (AF), formic acid (FA) (LCMS grade), ammonium acetate (AA) and acetic acid (HPLC grade), were purchased from Fisher Scientific (Loughborough, UK).

2.2. Equipment and methodology

A Thermo UltiMate 3000 Rapid Separation Liquid Chromatography system was used for all experiments, comprising a quaternary pump, diode array detector (DAD) and either a Corona Ultra or Corona Veo CAD, with Chromeleon 7.2 software (Thermo, Germering, Germany). The CAD is a destructive detector, therefore the DAD and CAD detectors were connected in series in some experiments, with flow first through the DAD. Thermo Viper tubing (0.13 mm ID) was used as connection tubing. Data collection rates were 100 Hz for both DAD and CAD, due to narrow peak widths (typically 1 s at half height in flow injection analysis (FIA)). The Corona Ultra nebuliser (cross flow design similar to that used in atomic absorption spectrometry) was controlled at 22 °C with the evaporator tube at ambient temperature, while the Veo (concentric flow design similar to those used in mass spectrometry) nebuliser was at ambient temperature and the evaporator tube set to 30 °C. The Veo had a power function (PF) designed to 'linearise' data, which was set to either 0.67 (this simulates 'off'), 1.00 (the default) or 1.2 (optimised setting using experimental data, see below).

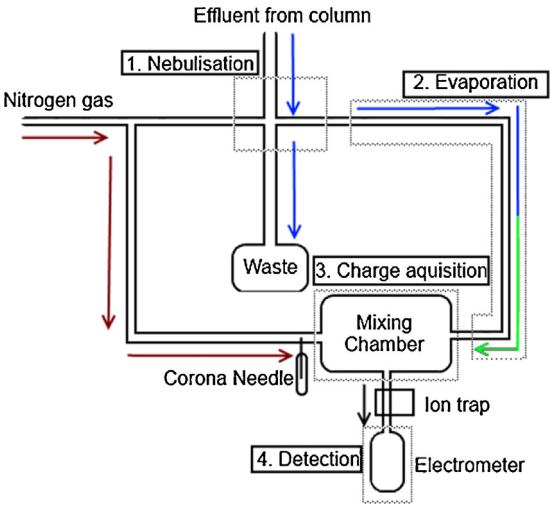

Fig. 1. Simple schematic of CAD operation.

An ethylene bridged hybrid (BEH) amide column (150 × 4.6 mm, particle size = 3.5 μm, Waters, Milford, USA) was used for determination of the detection limit, linearity and for the salt separation experiments. An Atlantis bare silica column (250 × 4.6 mm ID, particle size = 5 μm, Waters) was used for some salt composition experiments. The mobile phase was ACN-5 mM ammonium formate or ammonium acetate buffer (80:20, w/w) unless otherwise stated. The pH meter was calibrated in aqueous buffers and formic or acetic acid was used to adjust the aqueous portion to $_{w}{}^{w}$pH 3 or 5. Solutions at $_{w}{}^{w}$pH 6.8 were unadjusted 5 mM ammonium acetate. Care is necessary as pH calibration buffers can be a major source of non-volatile contaminants in the mobile phase.

In flow injection analysis (FIA), narrow bore tubing (75 μm × 1100 mm) was used in place of the chromatographic column to maintain sufficient backpressure. Samples for FIA were prepared at a concentration of 300 mg/L; injection volumes were 1 μL unless otherwise stated. Flow rate was 1 mL/min.

For calculation of retention factors, toluene is generally used as a void volume marker in HILIC with UV detection [14], but is too volatile for use with the CAD. Naphtho [2,3-a] pyrene appeared to be a suitable alternative for CAD.

3. Results and discussion

3.1.1. Detection limits (HPLC)

When applied to the impurity profiling of amino acid mixtures in nutritional infusion bags, CAD limits of quantitation (LOQ) were reported at 10 ng on-column (1 μg/mL; 10 μL injection) [22]. The