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Review

Review of operating principle and applications of the charged aerosol detector

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ABSTRACT

Recently a new detection method, based upon aerosol charging (the charged aerosol detector (CAD)) has been introduced as an alternative to evaporative light-scattering detector (ELSD), chemiluminescent nitrogen detector and refractive index detector for detection of non-ultraviolet and weakly ultraviolet active compounds and for UV-absorbing compounds in the absence of standards. The content of this review article includes description of operation principle, advantages and disadvantages of CAD system, and short reports of selected applications of this detector. The main advantages of CAD detector are unique performance characteristics: better sensitivity than ELSD system, a dynamic range of up to 4 orders of magnitude, ease of use and constancy of response factors. Both detectors are mass dependent and the response generated does not depend on the spectral or physicochemical properties of the analyte. This attractive feature of a detection technique generating universal response factors is the potential use of a single, universal standard for calibration against which all other compounds or impurities can be qualified. CAD also has the same limitation as ELSD, namely, the response is affected by mobile-phase composition. This problem has been resolved by using inverse gradient compensation as is done for high pressure liquid chromatography and supercritical fluid chromatography. CAD has been applied for the analysis of structurally diverse compounds used in the pharmaceutical, chemical, food, and consumer products industries and in life science research. They include nonvolatile and semivolatile neutral, acidic, basic, and zwitterionic compounds, both polar and nonpolar (e.g. lipids, proteins, steroids, polymers, carbohydrates, peptides).

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1. Introduction

For analytes with one or more UV-absorbing chromophores, ultraviolet (UV) detection is probably the most widely used detection method in liquid chromatography due to its high sensitivity, broad linear range, relatively low cost, ease of use, and the fact that it is compatible with most solvents used as the mobile phase in isocratic or gradient elution mode [1]. As an alternative to UV/Vis detection in many applications, in particular for the analysis of compounds lacking strong UV chromophores such as many amino acid derivates, carbohydrates, lipids, polymers and surfactants, which also include some drug substances and natural products, mass spectrometry (MS), evaporative light-scattering (ELSD), chemiluminescent nitrogen (CLND), and refractive index (RID) detectors could be used. Mass spectrometry is considered to be a specific and universal detection method but, as the response depends on the ionization process, quantitative analysis using MS coupled with liquid chromatography is currently less robust [2] and the high price of the instruments limits its use for routine analysis [3]. In some applications ELSD detectors exhibit significant limitations in precision, sensitivity, dynamic range and the nature of calibration curves [4–6]. Like ELSD, the relatively new condensation nucleation light-scattering detector (CNLSD system) is appropriate for detecting any compounds, provided their volatility is low enough, and has recently become commercially available. It reportedly offers better sensitivity than ELSD [5,7,8]. CLND systems may have poor precision, require high maintenance and are not compatible with nitrogen-containing mobile phases such as those containing acetonitrile and triethylamine [4]. RID detectors have significant limitations in sensitivity and are not compatible with gradient elution [9]. Nevertheless, these limitations do not effect most of the application and can be successfully used for analysis. However, a new detection method, based on aerosol charging (the charged aerosol detector), has recently been introduced as an alternative to ELSD, CLND and RID systems for HPLC detection of non-UV active and weakly UV active compounds and for UV-absorbing compounds for which the standards are not available. The main positive characteristics of CAD system are: universal detection of nonvolatile analytes, a response independent of chemical properties, a broad dynamic response range with high sensitivity from low ng to high μg amounts of analytes, good precision for a diverse range of analytes, and simple and reliable operation [10]. However, as CAD system is an aerosol-based detector, it has the same main limitation as ELSD detector – the response of the detector varies with the mobile-phase composition [11]. Another drawback of this type of detector is that no spectral information is acquired so it is not possible to identify a certain peak or perform peak purity analysis as in UV-diode array detectors or MS detectors [1].

The comparison of the main characteristics of CAD and ELSD systems is compiled in Table 1.

2. Operating principle of CAD system

The operating process of CAD system is illustrated in Fig. 1 and is broadly comparable to that of the ELSD and CNLSD. In CAD, CNLSD and in ELSD the eluent of a chromatographic system is nebulized, using a flow of nitrogen, and the resulting aerosol is transported through a drift tube where the volatile components and solvents are evaporated. In the last step in CAD the dried particle stream is charged with a secondary stream of nitrogen that has passed a high-voltage platinum wire and the resulting charged particle flux is measured by an electrometer. In case of ELSD the signal is proportional to the number of photons scattered from the residual solid fraction that has been introduced into a detection cell [3,5]. Because the mobile phase is converted to gas before the detection of the solutes in CAD, it belongs to the category of so-called “open cell detectors” [12].

Table 1
Comparison of the main characteristics between CAD and ELSD systems.

	CAD system/ELSD system
Operating principle	Both detectors are aerosol-based HPLC detectors. The operating principle is the same in the first phase (nebulizing the column eluent, evaporating the mobile phase from the droplets), but different in the detection (ELSD systems measure light scattering of the particles, CAD system measures charged particle flux).
Response	Both detectors detect semivolatile compounds regardless of their spectral or physicochemical properties. The CAD system gives parabolic calibration curve and when the concentration level is very low or when the concentration range is small, the calibration curve is close to linear. The nature of ELSD response is polynomial and a sharp decrease in signal at lower concentrations is an expected result.
Dynamic Range	CAD system shows a dynamic range of up to 4 orders of magnitude, ELSD system may show some limitations.
Sensitivity	According to the published applications better with CAD system.
Main limitations	The response of both detectors varies with the mobile-phase composition (this could be eliminated with mobile-phase compensation). No spectral information of both detectors is acquired.

Operation is simple, requiring only setting of few controllable parameters, among them the gas input pressure, the temperature or the temperature range and signal output range [13]. However, this could actually limit the room for optimization of detection [14]. CAD detector has been coupled with HPLC [3,5,10], with packed column supercritical fluid chromatography (pSFC) [15] and with micro high-temperature liquid chromatography (μHTLC) [16].

3. The response of CAD system

3.1. Mass-dependent detector

Like ELSD, CAD system is a mass-dependent detector and the generated response does not depend on the spectral or physicochemical properties of the analyte as in a specific UV detector, which is a concentration-dependent detector. Theoretically this means that CAD and ELSD systems as bulk property detectors generate a similar response for identical amounts of different analytes. For example, only a slight variation of the response for equal amount of compounds analyzed, was observed by Gamache et al. over a test set of 17 chemically different compounds under isocratic elution conditions. However this variation was about 7% relative standard deviation (RSD) between all responses of all 17 chemically different compounds, which indicates that CAD response depends upon analyte volatility [10]. The relationship between signal and amount of analyte is nonlinear in CAD, as in ELSD, in which the relationship between area response and analyte mass can be described by:

$$A = aM^b \tag{1}$$

where A is the area response of the detector, M the mass of the analyte, and a and b are values that depend on the analyte and chromatographic conditions.

Eq. (1) can be transformed into:

$$\log A = b \log M + \log a \tag{2}$$

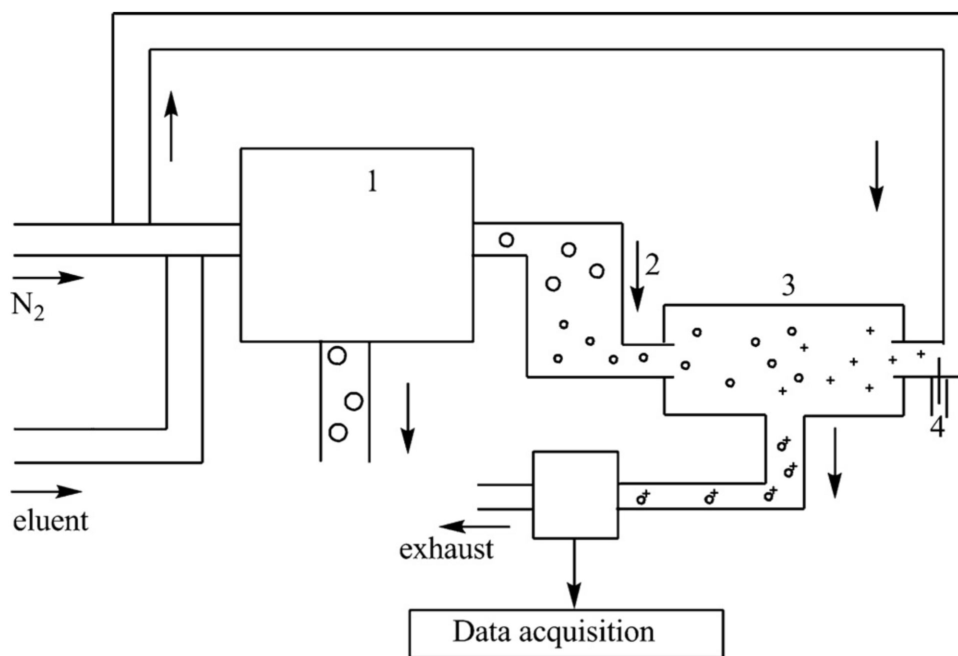

Fig. 1. Schematic diagram of the operation of the CAD system: 1, nebulizer and impactor (removal of large droplets); 2, drying tube; 3, charge transfer chamber; 4, discharge needle; 5, detection cell.

which can be used for calibration as a linear log–log plot of peak area versus quantity of analyte. This allows accurate quantification when using a two- or three-point calibration curve with CAD. The study of recovery performed on 10 components by Vervoort et al. [1], showed that recovery for a high concentration sample was always in the 98–102% interval when using a two- or three-point calibration curve. The same was true for low concentrations, except for one compound (isoconasole). With the latter, the area response of the data point with the lowest concentration used for calibration (0.005 mg/ml) deviated markedly from the values based on the other data points, for no obvious reason [1]. However, when the concentration level is very low or when the concentration range is small, the calibration curve is close to linear [17]. In HPLC determination of enantiomer ratios [18], in contrast to the ELSD response, the CAD signal is nearly linear in the range of interest for many routine analytical studies (5–250 μg/ml). This study also proved the higher sensitivity of CAD relative to ELSD [18], as the signal generated by CAD is much less influenced by the aerosol droplet size or its size distribution, which is influenced by the analyte concentration of the droplets. The sensitivity of CAD was poorer than with CNLSD in some experiments, however it was improved and was comparable to the CNLSD when CAD was coupled with reversed-phase HPLC as shown in the study of Dixon and Peterson [5]. Furthermore, over a narrow concentration range, good linearity of the CAD response was observed when determining the relative response factors of paclitaxel-related impurities [19].

3.2. Mobile-phase compensation

As with many aerosol processes, the response of CAD system is influenced by the diameter of the generated particles, which is given by the equation:

$$d_p = d_d \left(\frac{C}{\rho_p} \right)^{1/3} \quad (3)$$

where ρ_p is the density of the particle (given by the density of the analyte), C is the concentration, d_p the particle diameter, and d_d the droplet diameter [5].

Since the droplet diameter is related to several other factors, including density and viscosity of the mobile phase, it is also dependent on the mobile-phase composition. In gradient elution chromatography the response factor will vary significantly with the mobile-phase composition, which is the main drawback of this detector [3]. Higher organic content in the mobile phase leads to greater transport efficiency of the nebulizer, which results in a larger number of particles reaching the detector chamber and a higher signal [20]. Gorecki et al. [3] proposed an elegant approach based on mobile-phase compensation to solve this problem. The principle is to provide the detector at all times with a constant composition of the mobile phase. In this method, a secondary stream of the mobile phase of exactly reverse composition is provided by a second pump, and was added to the column effluent to ensure a constant mobile-phase composition at the detector inlet. This resulted in constant response, independent of the mobile-phase composition in the column [3].

3.3. The effect of additives to the mobile phase

The response of CAD system is also sensitive to contaminants or additives to the mobile phase. With varied concentrations of ammonium acetate (5, 10 and 20 mM) in the mobile phase (water/acetonitrile, 60/40), CAD system performed significantly better than ELSD system at low buffer concentrations but, at higher buffer concentrations the S/N ratio for CAD system dropped markedly. Using volatile acids such as formic or acetic acid did not pose any problem for CAD [1].

Moreau [21] designed two experiments to measure the effect of various common HPLC solvents on the CAD baseline at a constant flow rate at 0.5 ml/min, without a column and without injecting any samples. In the first experiment, methanol produced the highest CAD background of the four solvents hexane, isopropanol, methanol and water. In the second, acetonitrile produced the highest CAD background of hexane, isopropanol, acetonitrile and water. However, more studies are needed to fully investigate the effects of these solvents on the noise and performance of CAD system [21].

Table 2
Comparison of validation data of CAD system versus ELSD system available from the published applications.

Application	Criteria	CAD system	ELSD system
Levamisole, liazorole, domperidone, flubendazole, azaconazole, ketoconazole, isoconazole, itraconazole, sabeluzole, cinarizine, enilconazole [1]	Limit of detection	Under gradient conditions the CAD is reported to be somewhat more sensitive than the ELSD system. Data not shown.	
	Linearity (R^2)	0.9951–0.9995	0.9975–0.9995
	Accuracy (results of recovery)	The recovery of the low concentration sample was always in between the limits of 98% and 102%, except for one component (see the text).	For the low concentration sample the recovery fell outside the 98-102% range for three compounds and in two cases no recovery could be calculated.
	Precision (RSD of 6 injections)	In terms of system repeatability the CAD system always seems to perform somewhat better than the ELSD system. Data not shown.	
Polyketide: 6-deoxyerythronolide B, erythromycin [23]	Limit of detection	0.012 mg/L	0.091 mg/L
	Within-run precision	1 mg/L: RSD = 7.6% 10 mg/L: RSD = 4.2%	1 mg/L: RSD = 2.8% 10 mg/L: RSD = 3.1%
	Dynamic range	4.2	3.1
	Linearity (R^2)	0.909	0.9997
R-enantiomer of tosyl-protected α-methylbenzylamine [18]	Response	Nearly linear in the range of interest.	Not linear in the range of interest.
	Sensitivity	Higher as ELSD system	Lower than CAD system
	Accuracy	In very good alignment with the actual value.	The results were less accurate as obtained results with CAD system.
Free fatty acids (linolenic, linoleic, polmitic, oleic and stearic) [43]	Specificity	Comparable with the ELSD system.	Comparable with the CAD system.
	Precision (RSD of 3 or 6 injections)	0.4–3.0%	0.2–11.2%
	Accuracy (results of recovery)	90.8–108.5%	80.4–109.3%
	Linearity (R^2)	0.99–1.00	0.98–0.99
	Limit of detection	0.26 μg/ml–0.75 μg/ml	0.96 μg/ml–3.4 μg/ml
Saponins from Radix et Rhizoma Notoginseng [14]	Accuracy (results of recovery)	94.4–103.2%	Data not shown.
	Linearity (R^2)	0.9978–0.9996	0.9916–0.9994
	Limit of detection	0.01–0.15	0.04–0.19
Synthetic polymer [72]	Limit of detection for the same component	0.01%	0.1%

3.4. The effect of column bleed

Teutenberg et al. [22] used CAD system for detecting column bleed, as an indicator of induced degradation of the stationary phase. Five HPLC columns (from Phenomenex, ZirChrom, Thermo, Polymer Laboratories, and ZirChrom-Sachtleben) were heated to 200 °C using a homemade heating system. The results were also evaluated by ultraviolet diode array detection at different wavelengths. CAD system was better for detecting HPLC column bleed than the UV detector because, in charged aerosol detection, peak area is not dependent on the extinction coefficient of the analytes.

4. Selected applications of CAD

CAD has been applied for the analysis of nonvolatile and semivolatile neutral, acidic, basic, and zwitterionic compounds, both polar and nonpolar. These include lipids, proteins, steroids, polymers, carbohydrates, peptides and other compounds with weak chromophores used in the pharmaceutical, chemical, food, and consumer products industries and in life science research [13]. Some applications are described below and the comparison of validation data of CAD versus ELSD system available from the published applications is compiled in Table 2.

4.1. Analysis of drugs without a natural UV chromophore

CAD has been applied for the analysis of polyketide [23] and bisphosphonate compounds [24] that do not possess a natural chromophore. Mass spectrometry is a powerful tool for quantifying low-titer and poorly identified polyketide compounds, as it has a low limit of detection and is able to provide sample molecular weight information. Pistorino and Pfeifer [23] compared the analytical capabilities of MS, ELSD and CAD analyzers for quantifying a model polyketide target compound (6-deoxyerythronolide B (**1**)). The limit of detection (LOD), within-run precision, dynamic range and linearity obtained with all three detection systems were compared. The results showed that CAD is a cost-efficient alternative to MS for research and commercial use challenged by low natural product titers, since the limit of detection is lower than that of MS while maintaining a comparable dynamic range. In addition, the aerosol detectors were more precise and provided greater accuracy over the measurement range. However, ELSD exhibited a well-defined calibration curve over its analytical range and consistently the best precision of the three analyzers, but it is restricted by its limit of detection. ELSD could be primarily applicable for titers ≥1 mg/L [23].

Traditional analysis of bisphosphonates is very time-consuming and various methods are needed to analyze for assay and degradation products. These involve indirect UV detection [25–27] or pre-column derivatization to obtain better sensitivity and selectivity [28–35]. Fang and co-workers [24] developed a rapid, direct and stability-indicating method for analysis of etidronate, a model bisphosphonate compound without a UV chromophore. A mixed-mode column was used to separate etidronate (**2**) from its impurities and no time-consuming derivatization was needed with CAD [34,36,37]. The method was successfully validated for specificity, linearity, accuracy, precision, sensitivity and stability, and it may be used to analyze dissolution samples as well as

assay/degradation products of etidronate for both release and stability testing purposes. Similar methodologies may be applied for pharmaceutical analysis of other bisphosphonates with significantly improved analytical efficacy and accuracy.

4.2. Analysis of enantiomer ratios

CAD has been applied successfully for determining enantiomeric ratios of non-UV active compounds [18]. Wipe et al. [18] applied CAD, ELSD and UV spectroscopy to measuring the enantiomeric ratios of mixtures of (R) and (S) isomers of tosyl-protected α-methyl-benzylamine (**3** (**S**), **3** (**R**)). The results obtained by the CAD were as accurate as those from UV. In contrast, it was evident that ELSD should not be used for determining enantiomeric ratios by chiral HPLC, as the data obtained were considerably less accurate than those obtained with UV spectroscopy and CAD. This can be attributed to the nonlinear response and higher detection limit of ELSD, even after generating a double logarithmic calibration curve [18].

4.3. Analysis of lipids, triacylglycerols and free fatty acids

Moreau [21] applied CAD for analysis of lipids with HPLC. For quantitative analysis of lipids, so-called "mass" or universal detectors, such as flame ionization detectors (FID systems) and ELSD systems, are used because most lipid extracts consist of mixtures of saturated and unsaturated molecules. Moreau evaluated CAD system with several normal phase and reverse phase HPLC systems commonly used for the quantitative analysis of lipid classes and lipid molecular species. However, in the last 15 years the sensitivity of ELSD has improved greatly and numerous methods have been developed for analyzing lipids by HPLC–ELSD [38,39]. The minimum limits of detection with early ELSD systems, 10–20 μg per peak, have been improved to 50–100 ng [38].

Francese and co-workers [40] developed a new HPLC method coupled with CAD system for detecting lipids in liposomal formulations. Compared to UV detection, no interference with the lipid signals due to absorption of the organic solvents in the mobile phase were observed with CAD. The method was reproducible and reliable for the most important evaluation parameters such as linearity, precision and accuracy. The authors recommend using HPLC–CAD system for investigating lipids typically used for preparing liposomes. Furthermore, they recommended using CAD to analyze liposomal preparations bearing a drug substance in order to determine whether there are any difficulties in the parallel detection of lipids and drugs [40].

Chaminade and co-workers [41] developed a simple, sensitive, accurate and reproducible HPLC–CAD method for quantifying a pegylated phospholipid, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-(methoxy(polyethyleneglycol)-2000) (**4**). This is the first quantitative method for the analysis of pegylated phospholipids associated with polymeric microcapsules. The main positive characteristics of this method are that sample preparation for quantification does not require any complicated extraction of the phospholipids, it has a short run time (20 min) and provides adequate quantitative data. The results were evaluated with linear and power models and, from the comparison of correlation coefficients, it was concluded that, for pegylated phospholipids with a CAD system, the power model describes experimental values better than the linear model [41].

Sandra and co-workers [42] have developed a simple approach to the quantitation of triacylglycerols in complex natural mixtures from plant oils, using non-aqueous reversed-phase HPLC in the gradient mode, combined with universal CAD system and mobile-phase compensation. The latter improved the uniformity of response of the analyzed triacylglycerols under gradient elution conditions and there was therefore no need to determine response factors (RFs) for the analysis of complex natural triacylglycerol mixtures from plant oils. The calibration curve was constructed using a linear model, with a good correlation coefficient (≥0.997) for all analyzed triacylglycerol standards. The method showed good reproducibility, excellent limits of detection and is cheaper than previously published quantitative methods, since no standards are needed, faster since there is no need to determine RFs, and because the precision is acceptable for most analytical purposes [42].

An HPLC method coupled with CAD system was developed by Nair and Werling to quantify free fatty acids resulting from the hydrolysis of phospholipids in a pharmaceutical suspension formulated with phospholipids as stabilizing agent. The CAD method, as compared with ELSD, provided better sensitivity, precision, recovery and linearity for the parameters evaluated. This method could be a valuable tool for screening phospholipid based formulations and has been used in various formulation development studies [43]. Traditional methods included gas chromatographic techniques, in most cases with pre-column sample derivatization to improve volatility and detectability of the analytes [44,45], HPLC-UV detection techniques with pre-column sample derivatization to improve sensitivity [46–48], capillary electrophoresis with indirect photometric detection [49] or enzyme assay to determine free fatty acids in food in blood [50]. CAD was also proved to be a good complementary technique in the investigation of lipids in a study of changes in meat phospholipid composition and products of induced oxidation [51].

4.4. Analysis of natural products from plants (saponins) and humans (bile acids)

CAD can be applied to quantitate saponins, which are common effective ingredients in traditional Chinese medicines (TCMs) and botanical medicines, and have therefore been considered as important chemical markers for the quality control of TCMs. The detection of saponins has proved difficult as they usually lack chromophores, and their ultraviolet absorption is weak [14]. Several methods have been developed for the separation and analysis of saponins, HPLC coupled with various detectors being the most commonly used [52–60]. Tu and co-workers [14], established an HPLC–CAD method, which was successfully applied to the analysis of seven saponins (notoginsenoside R_1, ginsenosides Rg_1, Re, Rb_1, Rg_2, Rh_1, and Rd) (**5**) in 30 batches of samples *Radix et Rhizoma Notoginseng*. LODs and limits of quantitation (LOQs) of UV, ELSD and CAD were compared and it was found that the response of CAD system was generally higher than that with other detectors. Additionally, CAD system exhibited a steadier baseline in gradient elution compared with UV detection at 203 nm. Also the validation data of the developed HPLC–CAD method showed it to be precise, accurate and sensitive for simultaneous quantitative evaluation of seven major saponins in *Radix et Rhizoma Notoginseng* [14].

Over the last three decades various methods have been proposed for determining levels of intraluminal bile acids (chenodeoxycholic acid (**6**)), ranging from enzymatic determination [61–63] to determination by LC with RID system [64], UV detector [65,66], ELSD system [67,68], HPLC–MS/MS [69] and, recently, GC [70]. Only concentrations higher than about 200 μM could be quantified by enzymatic determination, imposing limitations on the determination of bile acids in the fasted stomach, especially after administration of a glass of water, typically administrated in oral drug absorption studies [63]. The limitations of RID, UV, ELSD systems for determining bile acids are the same as those noted in the Introduction. GC requires appropriate sample preparation procedures and cannot distinguish conjugated from non-conjugated bile acids [70]. No LC–MS data on analytical characteristics in aspirates from the gastrointestinal lumen have been reported [71]. Vert-

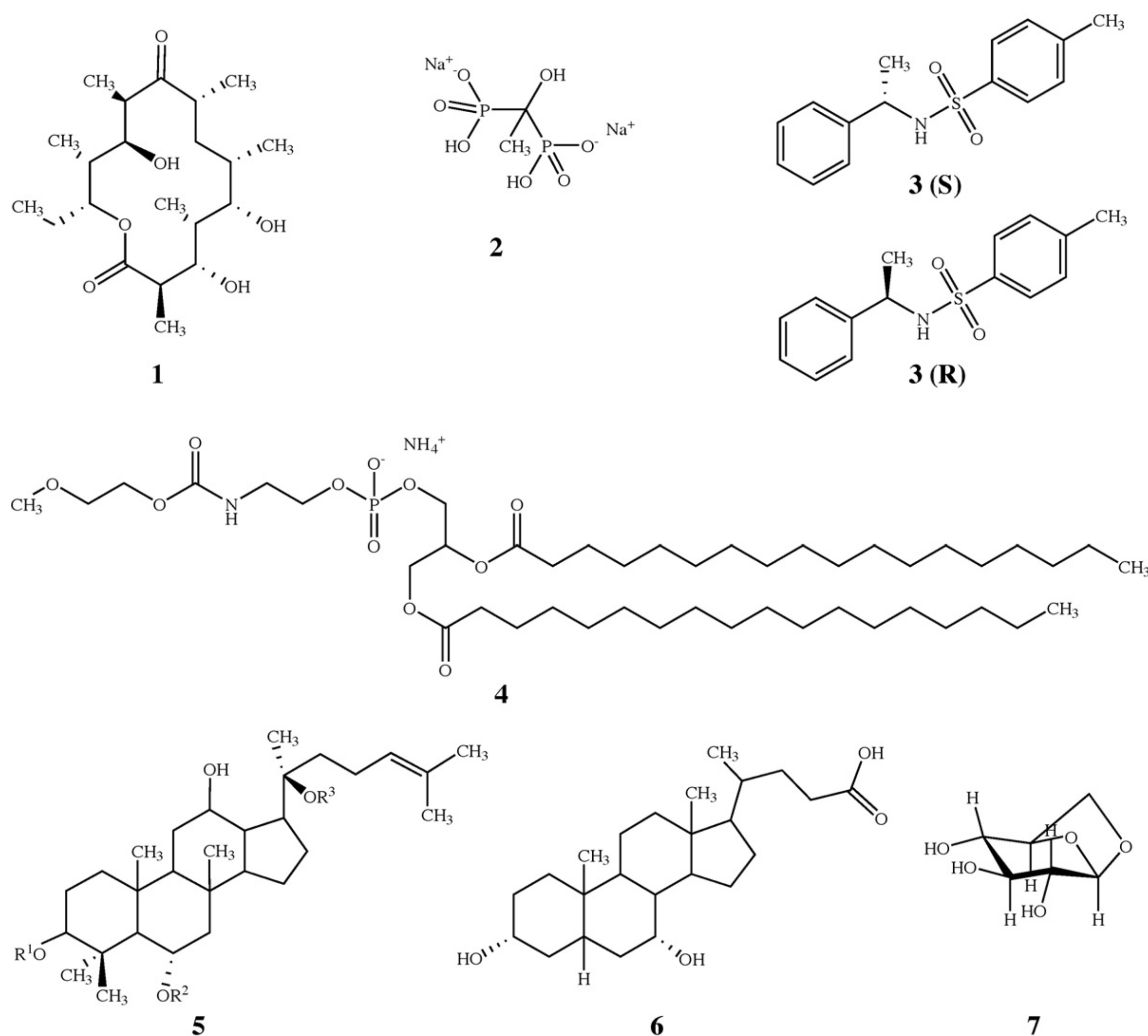

Fig. 2. Structures of selected compounds, analyzed with CAD.

zoni et al. developed, validated and applied an isocratic HPLC–CAD method for determining individual bile acids in human gastric and duodenal aspirates [71]. The method has shown a number of advantages compared with previously proposed HPLC–UV or HPLC–ELSD methods, including simple preparation procedure, low intra-day precision (<6%), high recovery (98.2%), small sample volume (≤100 μL), and low (<0.60 μM) LOD. The simple preparation procedure is important, especially when aspirates are collected in the fed state, as is the low LOD when aspirates are collected from the fasted stomach or from the colon [71] (Fig. 2).

4.5. Analysis of polymers

Takahashi et al. [72], have compared CAD with ELSD in an SFC system used for quantifying and determining the molecular mass distribution of uniform polyethylene glycols with various degrees of polymerization, as well as the certified reference material PEG 1000. The study showed that the repeatability of CAD is greater than that of ELSD, that CAD exhibits better signal-to-noise at very low concentrations and a lower minimum detectable quantity than ELSD. A 10 times more dilute solution of uniform oligomers could be detected with CAD than with ELSD systems. Since the molecular mass distribution of synthetic polymers is usually a function of the conditions of synthesis, its determination is important for controlling their properties [72].

4.6. Analysis of monosaccharide anhydrides

Monosaccharide anhydrides such as levoglucosan (1,6-anhydro-β,D-glucopyranose) (**7**) appear to be good tracers for biomass combustion smoke [73]. The most commonly used methods for their determination in atmospheric aerosols involve extraction with organic solvents, derivatization to trimethylsilyl ethers, and analysis by GC or GC–MS [74–77]. More recent studies have employed electrospray ionization mass spectrometry (ESI-MS), ion chromatography with pulsed amperometric detection [78], microchip capillary electrophoresis with pulsed amperometric detection (CE-PAD) [79], HPLC with ESI-MS [80], HPLC using ion-exclusion chromatography with low wavelength (94 nm) UV detection [81], and HPLC–CAD [82]. HPLC–CAD method was directly compared with GC–MS method for determination of levoglucosan in a study by Ward et al. [83]. Results were statistically evaluated and they showed that the GC–MS and HPLC–CAD approaches to levoglucosan analysis were statistically equivalent. Both methods could easy detect levoglucosan in the smoke impacted samples. However, the GC–MS method was more sensitive at lower concentrations [83]. The main advantage of HPLC–CAD [77] is the simple sample handling that involves extraction of filters into aqueous solutions with no derivatization or concentration steps. This method appears to compare favorably with the more recent liquid based analytical methods [78–81]

in terms of simplicity of sample treatment, chromatographic selectivity and concentration detection limits. Only HPLC–MS [80] had superior sensitivity and selectivity, although the costs are expected to be higher. The HPLC–CAD method has been applied successfully in routine analysis.

4.7. Other interesting applications of CAD

As CAD generates a nearly constant response under isocratic conditions for compounds at similar concentrations, the relative magnitude of the CAD system response correlates with the relative mass of the analytes. Wang and co-workers [19] used this characteristic in developing an HPLC–UV–CAD method involving multi-standard calculation to determine the UV relative response factors (RRFs) of impurities of paclitaxel-related impurities in paclitaxel drug at the appropriate wavelength. The UV RRFs of 8 known impurities (10-deacetylbaccatin III, baccatin III, 10-deacetyl-7-xylosyltaxol C, photodegradant, cephalomannine, 10-deacetyl-7-epitaxol, taxol C, 7-epi-taxol) were also determined by the conventional way (linear calibration curves) by analyzing pure compounds in different quantities under the same UV detection conditions and calculating the ratio of slope of the linear calibration curves for each impurity to that of paclitaxel. The estimated RRFs of known impurities were comparable with accurate values obtained from linearity data. The study showed that this approach could be a fast, convenient, and accurate method to determine RRFs of known and unknown impurities and is important for accurate quantitation of impurities when pure standards cannot be obtained [19].

5. Conclusion

In this review we have highlighted the increased use of CAD over the few past years. It can be a powerful tool for fast quantitative or semiquantitative analysis of analytes for which, for example, no pure standards are available. With mobile-phase compensation it could prove an excellent addition to the toolbox of analytical chemists requiring universal HPLC detectors with uniform response for all analytes [3]. However, there are still some aspects of sample identification and quantification that have not been entirely satisfied. For example, there are only few controllable parameters, among them gas pressure, temperature and response range on CAD system, which limits the room for optimizing detection. In addition, the flow rate cannot be as high as with UV, because at high flow rates it is not possible to ensure that a certain degree of volatilization of the mobile phase is not transported into the detectors in CAD [14]. Additionally it does not respond to all species and therefore the detector should be used in addition to, rather than instead of, other detectors [3].

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