Sensitive, universal detection for capillary electrochromatography using condensation nucleation light scattering detection

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Abstract

Condensation nucleation light scattering detection (CNLSD) was coupled with a pressurized capillary electrochromatography (pCEC) system using an electrospray interface. Supplementary pressure from a high-pressure pump was used to stabilize the electrospray and electrochromatography processes. Hydrodynamic injections were made with a 20 nl injection valve, and the inherent dead volume from the valve was successfully minimized, such that plate numbers in the range of 120 000 to 350 000/m were observed. Selectivity tuning using both pressure and voltage with the pressurized capillary electrochromatography system was demonstrated. Good reproducibility, comparable sensitivities for a wide range of compounds, including carbohydrates, and limits of detection down to the 50 ng/ml level, corresponding to 1–2 pg levels, were determined without the need for derivatization, demonstrating that condensation nucleation light scattering detection is a sensitive, universal detection method for pressurized capillary electrochromatography. © 1999 Elsevier Science B.V. All rights reserved.

1. Introduction

Capillary electrochromatography (CEC) can be considered to be a hybrid of capillary electrophoresis (CE) and liquid chromatography that has the potential to become a powerful separation tool for the analysis of complex mixtures, and recently has been the subject of reviews [1–3]. Among the limitations for CEC described currently is the lack of a reliable, sensitive, universal detector [4]. So far, UV ab-

sorbance and laser-induced fluorescence (LIF) are the most common detection methods for CEC. For UV detection, short optical pathlengths of the capillaries limit sensitivity, and not all compounds absorb. Although better sensitivity can be achieved with LIF, even fewer compounds fluoresce. Derivatization techniques can be employed to widen the applicability of UV absorbance and fluorescence, but can introduce detrimental effects on analysis times, separation efficiency, and analytical accuracy.

Condensation nucleation light scattering detection (CNLSD) has been found to be a promising universal detection method for liquid phase separations [5–16]. Both CNLSD and the related technique of

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evaporative light scattering detection (ELSD) are based on the conversion of the effluent of the separation to an aerosol, followed by selective vaporization of the mobile phase, leaving the less volatile analytes behind as dry aerosol particles. With ELSD, the intensity of light scattering from the dry particles is monitored as signal. Suffering from relatively high limits of detection, ELSD has not been used for capillary separations. CNLSD employs an additional step, where the desolvated analyte particles are exposed to a vapor supersaturated environment to cause condensation of that vapor onto the particles, which increase in size from as small as a few nanometers to the micrometer range. As a result, the intensity of scattered light is greatly increased and therefore lower detection limits can be achieved, as long as the level of nonvolatile mobile phase components and contaminants is minimized.

Research with CNLSD has taken two somewhat different directions. In our laboratory, we have focused on the development of CNLSD as a sensitive, general-purpose universal detector for separations of a wide variety of low volatility species including underivatized amines [9], amino acids and peptides [12,13], carbohydrates [6], cations [8], pharmaceuticals [6,10], lipids [11], polymers [7], proteins [12,13], etc. for separations done by ion-exchange [8,9], reversed-phase [6], microbore normal-phase [11], size-exclusion [7], or supercritical fluid chromatography [12], as well as capillary electrophoresis [13,14]. Lewis et al. have focused on macromolecule detection, particularly of proteins, separated by capillary size-exclusion chromatography [15], CE [16], and capillary reversed-phase HPLC [17]; in these works, an electrospray source produced droplets that were small enough (134 nm) that for sufficiently low concentrations the probability of having more than one macromolecule per droplet was low. After desolvation of droplets containing macromolecules above molecular masses of about 10 000, dry particles above the threshold for detection by a condensation particle counter resulted, and counting of these molecules could be accomplished.

CNLSD is preferentially coupled with capillary separations using an electrospray interface [13–17]. In these studies, linear response and limits of de-

tection (LODs) down to the 15 ng/ml level, corresponding to subpicogram or 1–2 fmol levels of underivatized peptides and amino acids [14], or macromolecule counting of proteins with concentration LODs down to 100 ng/ml [15] have been reported. For the latter, Lewis et al. have also reported practical difficulties with the use of this approach for analysis of proteins related to the requirements for volatile mobile phases for CE [16], or molecular clustering with hydrophobic eluents used for reversed-phase separations which limits the linearity of macromolecule counting [17].

The subjects of light scattering detectors for separations [18] and more generally of nanoparticle detection technology for chemical analysis [19] have been summarized in recent reviews.

Typically with CEC, the packed capillary column serves as the injector, the pump, the separation column, and the detection cell. Unlike HPLC, where pumps control the mobile phase flow without affecting selectivity, electroosmotic flow (EOF) in CEC is affected by characteristics of the stationary phase, properties of the mobile phase, ionic strength and pH of the buffer, and temperature, all of which also influence selectivity. The addition of a high-pressure pump to augment the flow through and pressurize CEC capillaries has been used to manipulate separations [20], to perform both CEC and micro-HPLC [21,22], to overcome problems of bubble formation [23], for analysis of pharmaceutical compounds [24], to improve the separation of co-eluting species [25], and to develop a sheathless electrospray mass spectrometry (ESI–MS) interface [26]. In the latter study, Schmeer et al. [26] also found that the eluent was mainly transported by the EOF and pressure was only employed to stabilize the EOF at high electrical field strength. Wu et al. [27] reported a pressurized CEC–ESI–MS system to fully separate a tryptic digest of bovine cytochrome c, where a supplementary pressure was used to suppress bubble formation and also allow the tuning of the elution of peptides using the electrical field.

In this study, we present the first demonstration of CNLSD for CEC, using pressurized CEC (pCEC) and an electrospray interface for detection of underivatized substances, including a series of typically difficult-to-detect carbohydrates.

2. Experimental

2.1. Apparatus

Fig. 1 schematically depicts the apparatus used in these studies. An ISCO Model 3850 capillary electropherograph (Lincoln, NE, USA) was used for CEC operation. The CNLSD system included a TSI (St. Paul, MN, USA) Model 3025A Ultrafine condensation particle counter (CPC) operated in low-flow mode (aerosol uptake at 300 ml/min), an electrospray aerosol generator, spray chamber and neutralizer, as described previously for use with CE [14]. The CPC provides output of the number of detected particles per unit gas-phase volume (ml), here represented as No./ml. A 20 nl injector (Valco Instruments, Houston, TX, USA) was used for sample injection. In some experiments, smaller injections were made by switching the injection valve back to the load mode after a certain time interval (typically 5 s, corresponding to ~5 nl injection at 1200 p.s.i.; 1 p.s.i.=6894.76 Pa). A high-voltage power supply (Series 230, Bertan, Hicksville, NY, USA) was used to power the electrospray. A laboratory written Basic program was used to transfer data (No./ml) from the CPC to an IBM 386 computer at 1 Hz.

2.2. Pressurized CEC

Coupling CNLSD with CEC presented new problems compared with our experience with CE–CNLSD. Relatively low CEC flow-rates and the higher volatility of the mobile phase compared to those for CE made stable electrospray operation more difficult with our system, and a stable electrospray is necessary to get stable background for CNLSD. Typically, we observed that the flow evaporated rapidly enough that we could not observe a droplet at the tip of the electrospray capillary, making normal electrospray CNLSD operation impossible. Our first attempt to deal with these problems consisted of a sheath flow assisted interface. With that system, increased background and background noise limited the sensitivity and LODs obtained. Therefore, we developed a CEC system to reach a reasonable flow-rate for electrospray operation and retain the sensitivity of CNLSD. Another advantage of the pressurized system was that it minimized the bubble formation that is commonly encountered in CEC when high ionic strength buffers or high voltages are used. Bubbles in the flow stream also destabilize electrospray operation. This approach was taken with CEC–ESI-MS for similar reasons [27].

As shown in Fig. 1, a high-pressure syringe pump (Model 100 DM, ISCO) was used to provide supplementary flow to the CEC column. The high voltage for CEC operation was applied to the injector after the injection was made. A stainless steel union was grounded to protect the pump from possible damage
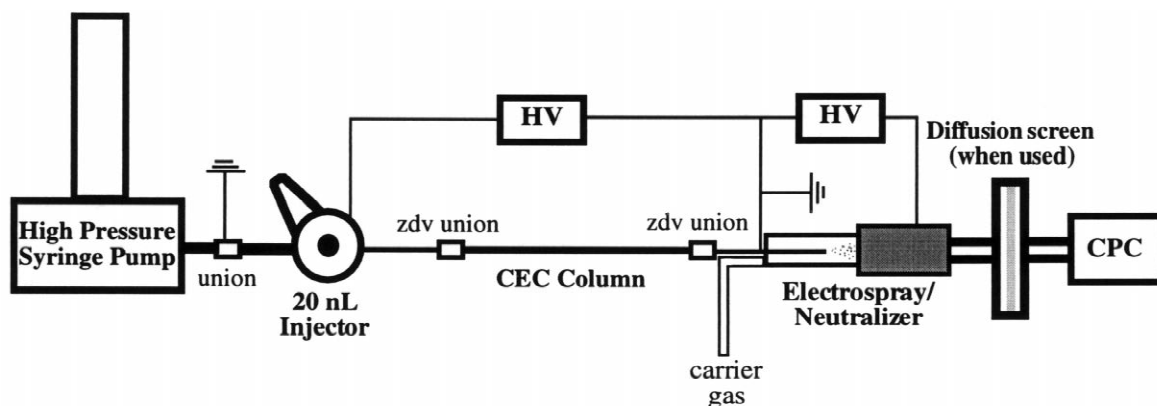

Fig. 1. Schematic diagram of pressurized capillary electrochromatography–condensation nucleation light scattering detection system. ZDV=Zero dead volume; HV=high voltage.

caused by the high voltage as current can also flow through the solution back from the high voltage end of the CEC power supply to the pump. Two 10 cm lengths of 25 μm I.D.×150 μm O.D. fused-silica capillary were connected to the inlet and outlet of the CEC column with two identical microtight zero dead volume unions (Upchurch, Oak Harbor, WA, USA).

2.3. CEC–electrospray interface

To fabricate the capillary terminus, 2–3 cm of the outlet capillary were heated to remove the polyimide coating and painted with a gold paint (OG 805 Premium Gold, Duncan Enterprises, Fresno, CA, USA). Unlike the previous study with electrospray coupled to CNLSD [14], the capillary was not drawn in this case. The gold paint was then heated using a heat gun to form a very smooth coating on the capillary surface, which is required for stable electrospray operation. The end of the capillary was cut with a capillary cutter (Supelco, Bellefonte, PA, USA) yielding a flat cross section surface. The remaining part of the capillary was painted with copper paint (Quick Grid Repair Resin, Loctite, Cleveland, OH, USA) for connection to the power supply.

The outlet of the transfer capillary with a fabricated electrospray tip at its terminus was placed in the cylindrical, glass spray chamber (1.5 cm I.D., 2 cm long) through a length of stainless steel tubing, which was used to position the capillary within the spray chamber. The aerosol was carried with a 900 ml/min flow of air regulated with a rotameter. A cylindrical flow-through neutralizer (Model P-2021 SS Nuclecel in-line ionizer, NRD, Grand Island, NY, USA) was placed directly at the end of spray chamber. The negative high voltage necessary for the electrospray process was directly applied to the neutralizer. The neutralizer contains polonium-210 (α emitter) of 10 mCi activity whose decay creates a weak bipolar plasma. The electrons from the plasma neutralize the charge from the highly charged droplets resulting of the electrospray process [28]. This approach is similar to that employed by Lewis et al. [15] for electrospray condensation particle counting of macromolecules separated by microbore size exclusion chromatography. When used, one diffusion screen (Model 376060 particle size selector, TSI) was used to modify the size distribution of the particles introduced to the CPC for background reduction [6,15]. In some experiments, the diffusion screen was not used.

2.4. Reagents and materials

Fused-silica capillaries were purchased from Polymicro Technologies (Phoenix, AZ, USA). CEC columns, 250 mm×75 μm I.D.×375 μm O.D., packed with 3 μm C_{18} derivatized silica particles, were from Unimicro Technologies (Pleasanton, CA, USA). Mobile phases were acetonitrile–water (20:80) mixtures containing 2.5 mM ammonium acetate. Buffer constituents and acetonitrile were obtained in the highest purity available. Water was obtained from a Barnstead (Dubuque, IA, USA) NANOpure water system. All sample solutions were prepared using the mobile phase as the solvent.

3. Results and discussion

3.1. Injection optimization

The syringe pump was operated in a constant-pressure mode. The operating pressure and voltage were optimized in order to obtain reasonable flow-rates for efficient separations and stable electrospray. Electrospray was operated in the silver bullet mode [14,27], which was shown to provide high sensitivity and efficiency in the previous study [29]. Our initial attempt to employ the injection valve was via direct connection of the separation column to the valve. Broad, but basically symmetrical peaks and moderate sensitivity were observed, which suggested that there existed a dead volume acting as a mixing chamber before the column. With a microscope, a void volume in the sample port of the valve cap was observed. The inner diameter of the sample port becomes smaller to 180~200 μm at the sealing surface of the cap so that the column, which had a 375 μm outer diameter, could not get through the port to approach the rotor (Fig. 2a). The dead volume forms between the inlet of the column and the rotor surface.

In order to overcome this dead volume, a 10 cm fused-silica capillary with smaller outer diameter was
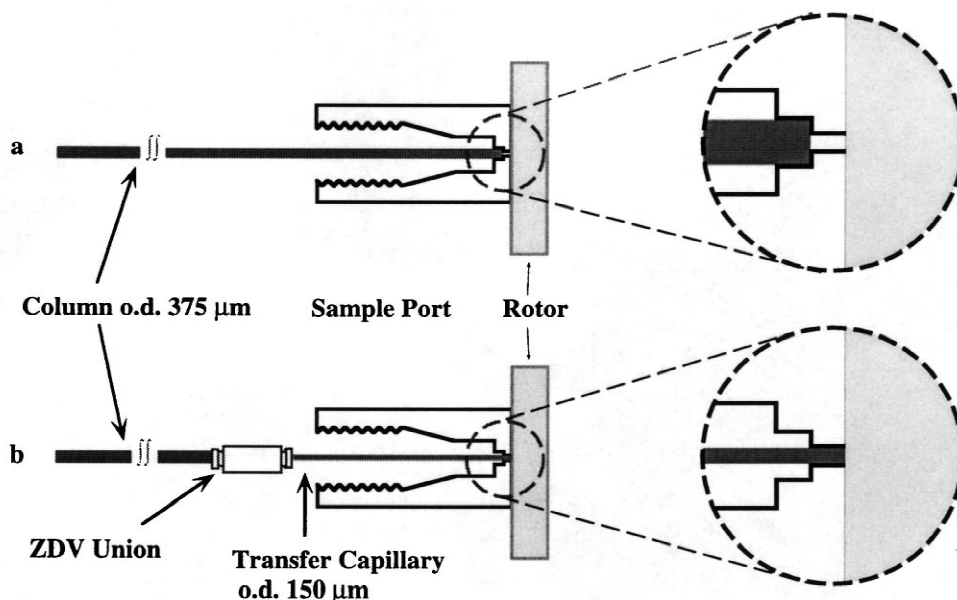
Fig. 2. Connections to the sample port of the injection valve. ZVD=Zero dead volume. For (a) and (b), see text.

used to connect the valve and column. This capillary had an outer diameter of 150 μm, which was slightly smaller than the inner diameter of the mixing chamber, so it could get through the port to nearly touch the rotor (Fig. 2b). Comparison of chromatograms obtained for 200 μg/ml thiourea without this capillary connection and with the capillary in line showed peak areas for both cases are at the same level (14 700 and 14 500, respectively). However, significantly better sensitivity (2×) and separation efficiency (4×) were obtained with the smaller O.D. capillary in line.

Although the 20 nl injector is the smallest commercially available, this volume was suspected to exceed the column capacity. Smaller volume injections were made by switching the injector valve back after 5 s, giving rise to an~5 nl injection based on the flow-rate at 1200 p.s.i. Chromatograms obtained for both cases are shown in Fig. 3. As expected, compared to the 20 nl injection (plate number: 18 000/m), much higher efficiency (plate number: 130 000/m) was achieved for the 5 s injection which was comparable to typically reported values for CEC [2]. Comparison of the peak areas

(154 000 and 39 000, respectively) confirmed the estimation of the injection volume; that is ~1/4 of 20 nl. It is also notable that the peak height for the 5 s injection was not much less than that for the 20 nl injection, which indicates that for the smaller volume
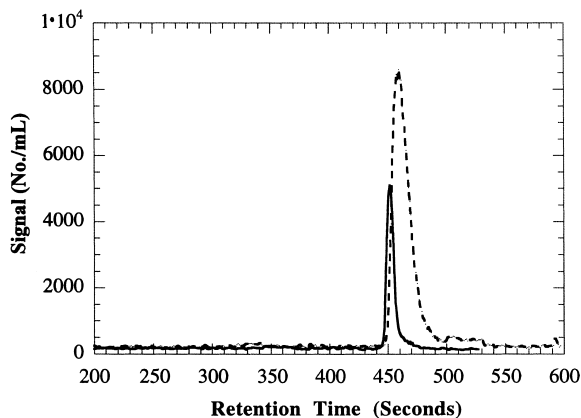

Fig. 3. Comparison of peak shapes for 20 nl injection (dashed line) and 5 s injection (solid line). CEC column: 250 mm×75 μm I.D., C_{18} 3 μm; mobile phase: acetonitrile–water (20:80) containing 2.5 mM ammonium acetate; applied pressure: 1200 p.s.i.; applied voltage: 22 kV; sample: 40 μg/ml sucrose.

injections, comparable concentration LODs and lower mass LODs based on peak heights should be obtained. However, the peak height reproducibility for the timed injections was substantially poorer than for the full injections. As a result, quantitative data reported herein are based on the full 20 nl injections, while efficiency data are based on the timed injections. In principle, automation of the timed injection process, or availability of a smaller volume injector should improve the reproducibility of the small volume injections.

Reproducibility (based on five trials) of retention time, peak height and peak area for full injections (20 nl) and constant time interval injections were compared for sucrose and caffeine, and are shown in Table 1. Relative standard deviations (RSDs) for retention times were less than 0.5% in both cases, indicating good reproducibility for this CEC system. RSDs for peak height and peak area with constant time interval injections were about four times worse than with constant volume injection, which was due to the unavoidable random errors resulting from the manual operation of the time interval mode.

3.2. Sensitivity

Fig. 4 shows a log–log plot peak area calibration plot over two orders of magnitude for thiourea, galactose and sucrose concentrations ranging from 1 to 50 µg/ml with one diffusion screen in line. The injection volumes were 20 nl. The calibration plots were best fit (correlation coefficients >0.999) with power curves having exponents ranging from 1.1 to 1.2, suggesting slightly nonlinear response over this
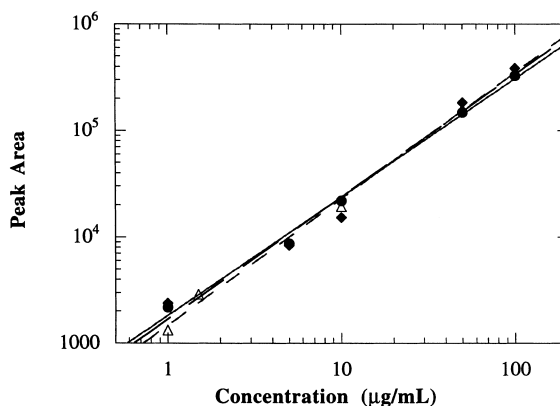

Fig. 4. Calibration curves for peak areas. Column and mobile phase, applied pressure and voltage as in Fig. 3; 20 nl injection. Sample: thiourea (filled circles), sucrose (filled diamonds) and galactose (open triangles).

concentration range. Similar responses for the different compounds on a mass basis were observed. Table 2 gives a summary of estimated limits of detection (3σ) and lowest measured concentration and amount [signal-to-noise ratio $(S/N) \geq 10$] for a variety of sugars and pharmaceutical compounds. The LODs are on the order of subhundreds of nanograms per milliliter and the mass LODs are in the picogram range, corresponding to femtomole levels. These levels are comparable to those reported for CE–CNLSD when a diffusion screen was utilized [14]. Notable as well are similar mass LODs calculated for this wide range of substances, including a series of carbohydrates, without the need for derivatization. The reason for the five times lower sensitivity for caffeine, which has been previously observed with HPLC–CNLSD [6], is currently uncertain, but under investigation.

Background signal levels, which limit LODs, are determined with CNLSD by the overlap of the desolvated particle size distribution resulting from the mobile phase, and the growth efficiency curve for the condensation process [5,14,15]. The sizes of background particles increase as the level of dissolved solids in the mobile phase increases, increasing the number of detectable particles, and the background signal level. The mobile phases used in this work are typical for CEC, and generally provided relatively low background that required the use of only one diffusion screen for background particle

Table 1
Reproducibility of retention time, peak height and peak area*

Analyte	RSD (%)		
	Retention time	Peak area	Peak height
Sucrose[a]	0.18	2.4	7.9
[b]	0.21	8.9	12.1
Caffeine[a]	0.20	1.4	5.9
[b]	0.28	7.6	9.1

*($n=5$).
[a] Full 20 nl injection.
[b] 5 s injection.

Table 2
Lowest measured concentrations and estimated limits of detection using CEC–CNLSD with one diffusion screen

Compound	Lowest measured concentration		3σ LOD		
	μg/ml	S/N	μg/ml	pg	fmol
Galactose	1.0	13.2	0.31	6.3	35
Fructose	1.0	12.6	0.44	8.7	48
Sucrose	1.0	13.1	0.29	5.7	17
Glucose	1.5	13.8	0.45	9.1	50
Thiourea	1.0	11.0	0.40	8.1	110
Sulfanilamide	1.0	10.8	0.37	7.3	43
Sulfanilic acid	2.0	12.9	0.51	10	53
Caffeine	10	10.6	2.6	52	270
Saccharin	2.0	13.4	0.45	9.0	49
Theophylline	1.5	10.7	0.48	9.5	53

removal and low background signal levels. However, with sufficient column conditioning, background levels were sufficiently low to eliminate the need for the diffusion screen. As reported previously for CE–CNLSD [14], further improvement in sensitivity was obtained with removal of the diffusion screen. Although this resulted in a higher background level, lower limits of detection were still observed. Table 3 lists the lowest measured concentrations and LODs for measurements made without the diffusion screen, which are about 5–8 times lower than those in Table 2 for the same species. Again, this observation is consistent with that reported for CE–CNLSD [14]. As an example of the sensitivity of the method, Fig. 5 shows a chromatogram obtained for 0.3 μg/ml of thiourea in the absence of a diffusion screen.

These detection limits compare favorably with those reported for CEC with other detection techniques. For example, Banholczer and Pyell [30] compared in-column and on-column UV absorbance detection and report LODs for a series of benzoates at 1–2 μg/ml levels. Lurie et al. [31] comment on
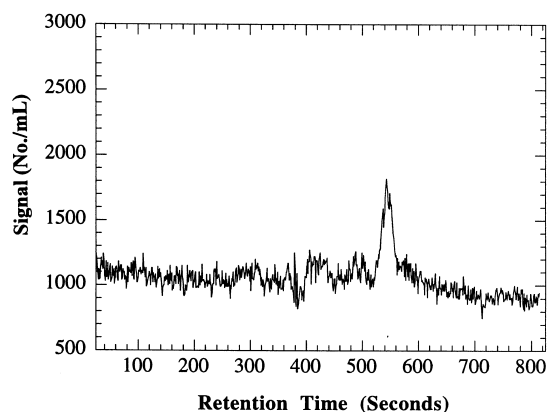

Fig. 5. Chromatogram for 0.3 μg/ml thiourea obtained without a diffusion screen using a pressure of 800 p.s.i. and an applied voltage of 22 kV.

the limited concentration sensitivity of UV absorbance with CEC for application to cannabinoid detection, and countered this limitation using with an extended pathlength detection cell and a large 32 s×5 kV sample injection, which allowed a 3 sigma

Table 3
Lowest measured concentrations and estimated limits of detection using CEC–CNLSD without a diffusion screen

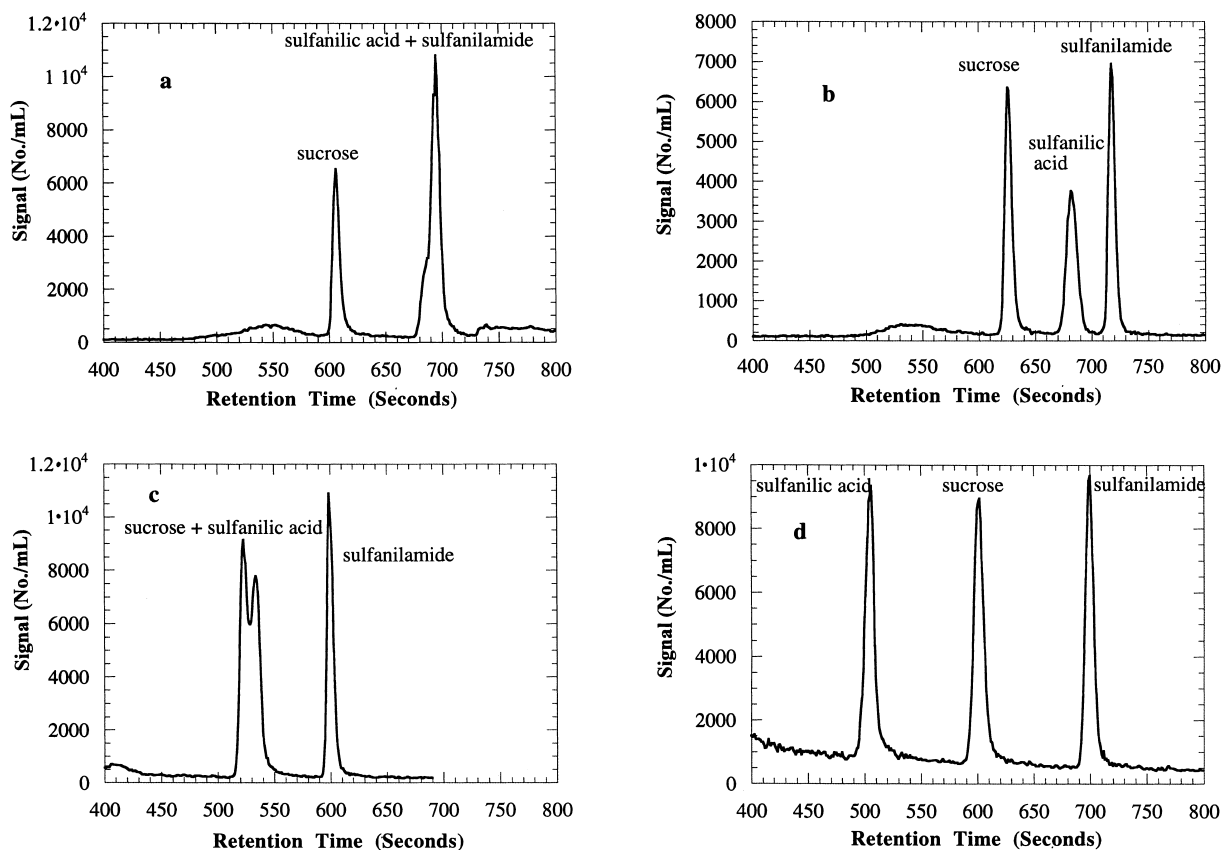| Compound	Lowest measured concentration		3σ LOD		
	μg/ml	S/N	μg/ml	pg	fmol
Thiourea	0.30	12.8	0.056	1.1	15
Sulfanilic acid	0.50	15.4	0.074	1.5	7.8
Sulfanilamide	0.25	10.8	0.052	1.0	6.1
Caffeine	2.5	13.6	0.457	9.1	47

Fig. 6. Chromatograms for separations at different pressures and voltages. Column and mobile phase as in Fig. 3; injection: 5 s at each applied pressure. Sample: 50 μg/ml sucrose, sulfanilic acid and sulfanilamide. Applied pressure and voltage: 800 p.s.i., 22 kV (a), 800 p.s.i., 18 kV (b), 1000 p.s.i., 18 kV (c) and 900 p.s.i., 0 kV (d).

LOD of 0.5 μg/ml for [^{2}H$_9$]tetrahydrocannabinol (THC). These values are comparable to or above most of the values reported in Table 1 for pCEC–CNLSD with the use of the diffusion screen, and significantly higher than most values obtained without the diffusion screen. Without derivatization, the LODs by UV absorbance for substances such as carbohydrates would be much higher than those reported for these UV-absorbing substances. Substantially higher sensitivity of CNLSD compared to UV absorbance has previously been reported for underivatized amino acids separated by CE [14], or proteins separated by capillary size-exclusion chromatography and detected in the macromolecule counting mode [15]. With open-tubular CEC and preconcentration, Wu et al. reported LODs by ESI–

MS for peptides at the 1 μM level, which they estimated to correspond to 1–2 fmol [32], while with pCEC, sample stacking and injection volumes between 1 and 1.5 μl, the same group reported lower sensitivity and a working concentration range of 5–10 μM [27]. Ding and Vouros suggest similar detection at about the 1 μM level for DNA adduct mixtures using CEC–ESI–MS after ten-fold preconcentration [33]. In this work, without preconcentration/stacking/focusing and excluding the values for caffeine, the molar LODs obtained by pCEC–CNLSD (i.e., the substances and conditions in Table 1) range from 0.8 μM for sucrose to 5.3 μM for thiourea with the diffusion screen present, while without the diffusion screen (i.e., Table 2), the values range from 0.3 μM for sulfanilamide to 0.7

μ*M* for thiourea. With preconcentration, stacking or focusing, even lower LODs for CNLSD would be anticipated.

3.3. Chromatography

With the addition of augmented flow from the syringe pump, stable, long-term performance for chromatographic separations was obtained, as exemplified by the chromatograms reported herein. In addition, Fig. 6 demonstrates the added freedom of adjusting the separation in the pCEC. A mixture of sucrose and two sulfa drugs were tested. A 5 s injection was made for each pressure, which explains the slightly different responses for the same compound as the pressure changed. In Fig. 6a, 800 p.s.i. and 22 kV were applied on the column. Sulfanilic acid and sulfanilamide coeluted. Since in ammonium acetate buffer sulfanilic acid exists as a negatively charged species whose electrophoretic mobility is opposite to the flow direction, it was expected that changing voltage would influence the separation of sulfanilic acid and sulfanilamide. A slight change of voltage from 22 kV to 18 kV was employed for Fig. 6b. The retention times for sucrose and sulfanilamide became longer due to the decreased EOF, while sulfanilic acid had a shorter retention resulting from its decreased electrophoretic mobility and the three compounds were successfully separated. Further attempts to increase the flow-rate by increasing pressure led to the coelution of sucrose and sulfanilic acid as shown in Fig. 6c. The reason for the greater dispersion for sulfanilic acid in CEC is currently unknown but suspected to be related to the more complicated retention mechanism for charged com-
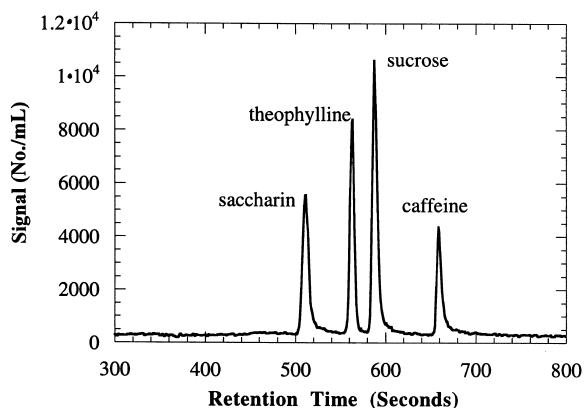

Fig. 7. Chromatogram for separation of sample test mixture. Column and mobile phase as in Fig. 3; injection: 5 s at 900 p.s.i.; applied pressure: 900 p.s.i.; applied voltage: 22 kV; Sample: saccharin (50 μg/ml), theophylline (50 μg/ml), sucrose (50 μg/ml) and caffeine (200 μg/ml).

pounds. Fig. 6d shows a separation that would occur as for capillary HPLC (i.e. voltage off) where the elution order of the compounds was changed and lower plate numbers (116 000/m, 100 000/m and 192 000/m for sucrose, sulfanilic acid and sulfanilamide, respectively) were obtained compared to the cases (Fig. 6a–c) where voltage was applied (225 000/m, 126 000/m and 300 000/m for sucrose, sulfanilic acid and sulfanilamide, respectively). The source of the broad peak at short retention is unknown, but reproducible as evident from this series of chromatograms.

Fig. 7 presents an example chromatogram obtained at 22 kV and 900 p.s.i. for a mixture of substances (note that the column employed in this study cannot satisfactorily resolve carbohydrates, and

Table 4
Estimated plate numbers. (based on peak width at half height)

Analyte	Pressure (p.s.i.)	Voltage (kV)	Concentration (μg/ml)	Retention time (s)	Plate number (*N*/m)
Sucrose	800	18	50	626	225 000
Sulfanilic acid	800	18	100	682	130 000
Sulfanilamide	800	18	100	718	294 000
Saccharin	1200	22	100	536	120 000
Thiourea	1200	22	100	475	161 000
Theophylline	1200	22	50	567	314 000
Caffeine	1200	22	100	583	348 000

hence the example chromatogram with the species indicated). A 5 s injection was made at 900 p.s.i. It is estimated that some dispersion of peaks might result from the two transfer capillaries. Regardless, plate numbers of 120 000~350 000/m were calculated for all the species listed in Table 4, comparable to those typically reported for CEC [1].

4. Conclusions

In this paper, we report the first demonstration of the use of CNLSD detection for CEC-type separations. Stable, long-term, easy-to-use performance on a routine basis can be obtained with a pCEC system coupled to CNLSD using an electrospray interface. As has been previously shown, such a system also allows the fine adjustment of both voltage and pressure to manipulate the selectivity of a particular separation. The volatile mobile phase species typically used for CEC provide low background and are almost ideally suited for CNLSD, as mobile phases containing nonvolatile additives or contaminants lead to high background and background noise, and higher LODs with CNLSD. Without preconcentration or derivatization, LODs for a variety of sugars and pharmaceutical compounds were at sub-microgram per milliliter levels. These LODs appear to substantially exceed those attainable for CEC of favorably absorbing substances with high sensitivity UV absorbance, and are at least comparable to, if not lower than, those reported for CEC–ESI-MS. The sensitive detection of underivatized carbohydrates by the method is particularly notable. Good reproducibility and plate numbers of 120 000–350 000 were obtained for all of the compounds, indicating that the use of the CNLSD detection system did not significantly affect the separation efficiency of the CEC process. These results show that CNLSD can be a relatively simple, effective, universal and sensitive detector for CEC.

Acknowledgements

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