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ARTICLE *in* ANALYTICAL CHEMISTRY · APRIL 2013

Impact Factor: 5.64 · DOI: 10.1021/ac400370f · Source: PubMed

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Design and Adaptation of an Interface for Commercial Capillary Electrophoresis—Evaporative Light Scattering Detection Coupling

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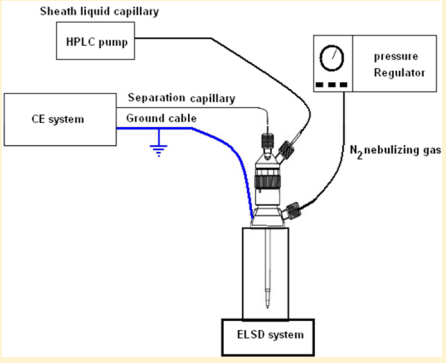
Supporting Information

ABSTRACT: In this technical note, an interface for coupling commercially available capillary electrophoresis (CE) equipment to an evaporative light scattering detector (ELSD) is described. The nebulization process was identified as the most critical parameter for performing the reliable coupling between both pieces of equipment. Therefore, appropriate modifications in the nebulization chamber and in the conventional nebulizer were brought to make ELSD fully compatible with CE. The impact of the customized interface on CE separation and detection was evaluated in terms of resolution and sensitivity. ELSD can be considered as an attractive alternative to other CE detection systems (e.g., UV−vis, fluorescence, electrochemical detection, or even MS detection), particularly those in which derivatization is needed. This advantage is due to the versatility and the quasi-universality of ELSD. Thus, sensitive and fast separations of several compounds were performed using this CE−ELSD customized arrangement, which opens up an interesting analytical potential for the determination of compounds not presenting sensitive UV−vis chormophore, fluorophore, or electroactive groups. Carbohydrates were selected in this work to demonstrate the applicability of CE−ELSD coupling.



Capillary electrophoresis (CE) has evolved into a large family of high-resolution separation techniques over the past two decades. CE can surpass high-performance liquid chromatography (HPLC) in terms of simplicity, resolution, and economy. The extremely small inner diameter of the capillary used, results in typical advantages, such as the ability to use small volumes of sample and reagents, low mass detection limits, and easy miniaturization.[1] For analytes with one or more UV−vis-absorbing chromophore groups, UV−vis detection is commonly the most widely used detection method for CE, due to its appropriate sensitivity for many compounds, broad linear range, relatively low cost, ease of use, and its compatibility with most buffers. 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, electrochemical detection (ED),[2−4] mass spectrometry (MS),[5−7] and evaporative light scattering (ELSD)[8−10] could be used. ED techniques are useful for detection in CE in general, and specifically for carbohydrates.[2] Particularly, by using pulsed amperometric detection (PAD) because the electrode surface was constantly maintained fresh by the repeated application of a series of pulses of potential. However, PAD used as a CE detection mode for carbohydrates can present the limitations imposed by the alkaline conditions needed for sensitive detection and differential electromigration, which restrict the useful pH to a very narrow range (i.e., pH >12) as well as the response produced by other organic compounds, which may negatively affect the accuracy of the results.[11] 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 CE is currently less robust and the high price of the instruments limits its use for routine analysis.[12]

ELSD has largely been developed, and it can be considered today as a reliable, economic, and versatile mode of detection in liquid chromatography.[13−15] ELSD can be considered a complementary detector to UV−vis.[16,17] The principles, properties, advantages, and drawbacks of ELSD are well-known.[18] The basic principle is to create droplets that are easily evaporated by a Venturi atomization process, thanks to the addition of a gas to the liquid phase at the chromatographic column outlet.[19,20] This step is carried out in a nebulizing chamber. The aerosol is then forced, by the nebulizing gas, into

Received: February 6, 2013
Accepted: April 23, 2013
Published: April 23, 2013

   dx.doi.org/10.1021/ac400370f | *Anal. Chem.* 2013, 85, 4858−4862

a drift evaporative tube, which induces a drying of the droplets to form suspended particles. These particles, considered as spherical, pass through a light beam. The light deflected by this particle stream is detected by a photomultiplier or a photodiode. The droplet (or particle) size is of critical importance in the light scattering process. Indeed, different types of interactions between light and particles were reported for ELSD. These interaction types (Rayleigh, Mie, and reflection/refraction) depend on the ratio between particle diameter and wavelength of the incident light. Depending on this ratio, changes in the scattering intensity (i.e., in the peak area) are observed.

In this work, we describe a simple approach for using ELSD as a detector for CE. It is commercial in nature, since it directly uses a triple-tube nebulizer to facilitate the connection of the commercial CE equipment with the nebulizer chamber of ELSD. A study on the influence of the shape chamber nebulization on separation was carried out. Then, the determination of noneasily detectable analytes, by performing changes in nebulizer chamber length to ensure a compatible connection between ELSD and CE systems (at least to obtain the best sensitivity and resolution), was achieved. In addition, the developed interface can easily be implemented. To this point, no reports describing the direct use of commercial ELSD with CE equipment have appeared to date. However, Koropchak et al. described two different means for interfacing condensation nucleation light scattering detection (CNLSD) with CE:[20] the first one requires a Nafion membrane to ground the CE circuit and to connect the system to a microconcentric pneumatic nebulizer, and the second system consists of the use of a coated polyether ether ketone capillary, which is extended to the tip of the nebulizer, finally completing the CE circuit. Then, the authors improved the first coupling systems by the development of a new method which employs an electrospray aerosol.[21] The arrangement proposed in this work uses aqueous volatile buffers commonly used in CE−MS (aqueous ammonium bicarbonate and diethylamine). The equipment coupled are commercially available equipment, and the developed interface works in a very simple way. The significant advantages of the coupled CE−ELSD system were demonstrated through the separation of underivatized carbohydrates.

RESULTS AND DISCUSSION

Interface Description. The conventional ELSD detector, commercialized as a detector for HPLC, does not work if it is directly connected to the capillary of the CE equipment. It is necessary: (i) to close the electrical circuit for the electrophoretic separation; (ii) to accommodate the flow requirements for an efficient formation of the electrospray; and (iii) some, additional, minor adjustments. Figure 1(A) shows the interface designed for CE−ELSD, meeting these requirements. It is a stainless steel triple-tube nebulizer, with some similarities to those used in CE−MS interfaces by the ESI mode,[22] which was accommodated in a customized ELSD nebulizer chamber with stainless head connected to the CE chassis with a ground cable (Figure 1B), in order to avoid electrical discharges. The triple tube nebulizer consists of a central tube (the CE capillary) surrounded by a second stainless steel tube, the sheath-liquid tube (see details in Figure 1C). The sheath-liquid flows between this tube and the inner CE capillary. Between the sheath-liquid tube and the third outer tube, or gas tube, flows the nebulizing gas controlled by an additional pressure-regulator compatible with the CE−ELSD interface that

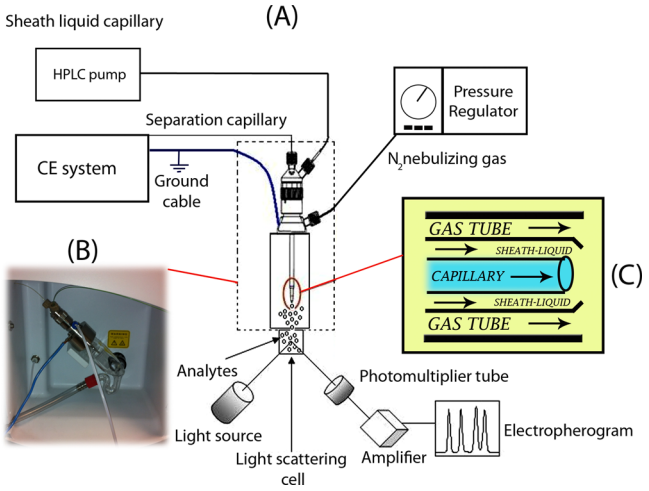


Figure 1. (A) Scheme of the interface for coupling the CE equipment with the ELSD detector via a stainless steel triple-tube nebulizer. (B) Picture of the nebulizer accommodated in a customized nebulizer chamber and scheme of the different connections. (C) Details of the flow tubes to produce an efficient nebulization.

contributes to control the nebulizing process. The sheath liquid serves a dual function in the sprayer. First, it provides the means to complete an electrical circuit between the anode in the inlet vial and the metal of the sprayer, which is, in effect, the cathode. The sheath liquid contacts both the metal sprayer and the buffer flowing out of the CE capillary. Second, the electrospray process is optimal at flow rates in the microliters per minute range and because the electroosmotic flow (EOF) in CE is on the order of 20−200 nL/min, there exists an obvious discrepancy between the EOF and the electrospray requirements. In order to match the effluent flow to the requirements for electrospray, a makeup liquid is provided by the sheath liquid. The capillary must be placed in the triple-tube sprayer and positioned in the ELSD nebulization chamber so that the capillary exit and spray are arranged to optimize transfer efficiency of the generated gas-phase ions into the ELSD. The degree to which the CE capillary exits the tube is also important and should not be more than approximately 1 mm. It is a simple arrangement in which the capillary end is at ground and the voltage is applied to the end plates. In this setup, the applied field strength will be the voltage applied by the CE instrument divided by the capillary length. This arrangement is simple and not require any special electrical connections between the CE and ELSD instruments, such as the use of Nafion membrane or nebulizer coated with a metal for the grounding of the CE circuit system.[20] It is enough to connect a ground cable between the CE chassis and the head of the stainless steel nebulizer chamber (Figure 1A). A complete description of the experimental procedures is included in the Supporting Information.

Detection by ELSD is influenced by CE instrumental parameters associated with the ELSD instrument [like nebulizing gas flow-rate (pressure), evaporating temperature, and nebulizer chamber length], the composition of buffer, and flow-rate of the employed sheath liquid. On the basis of previous investigations in CE−MS,[23,24] we adapted the condition of separation and detection of caffeine and theophylline in order to test the reliable work of the CE−ELSD-coupled system. Then, the separation and determination of mixtures of carbohydrates was selected as an example of

dx.doi.org/10.1021/ac400370f | Anal. Chem. 2013, 85, 4858−4862

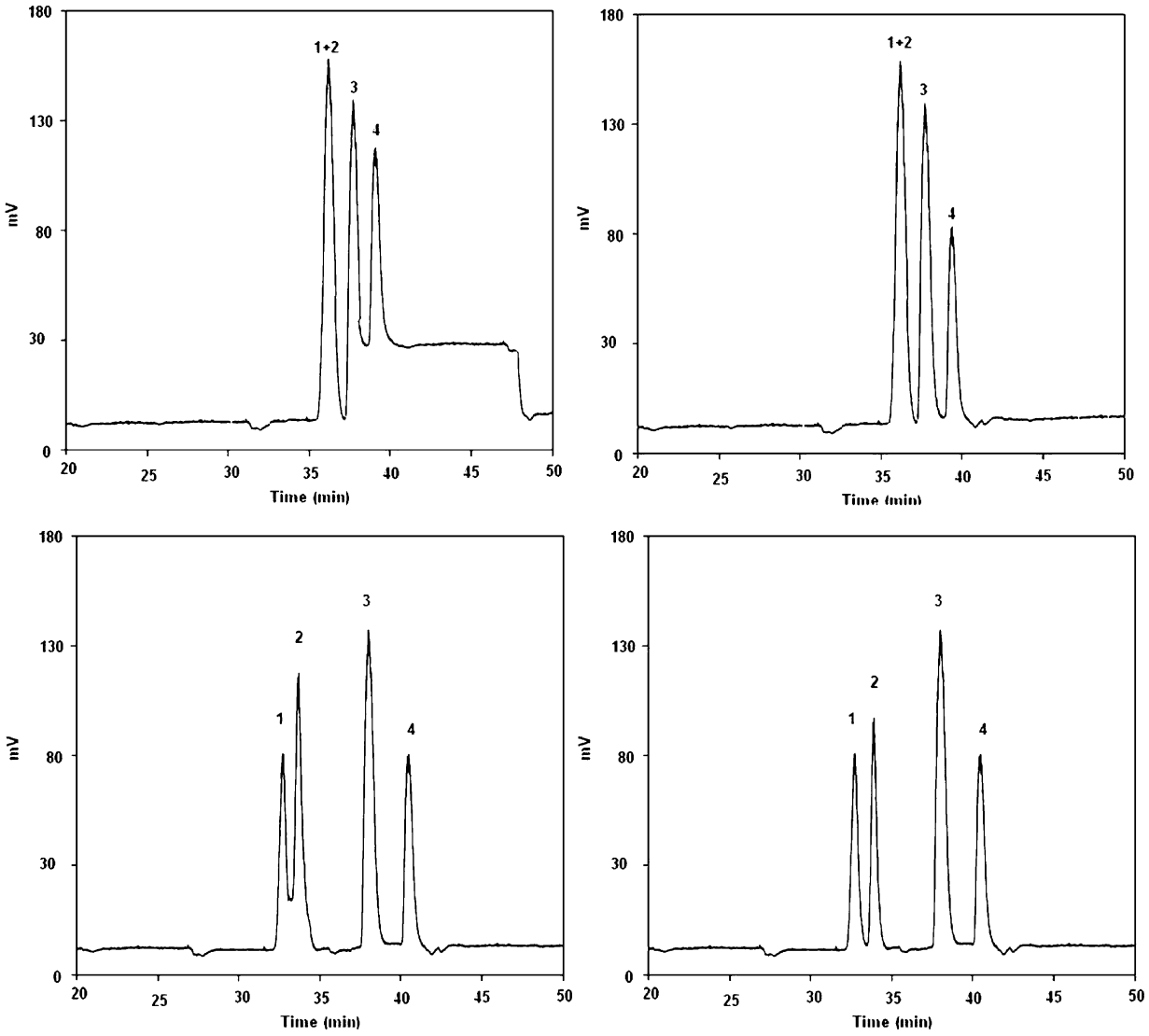


Figure 2. Electropherogram of a 50 μg/mL standard solution of carbohydrates by the proposed CE−ELSD at different nebulizer chamber lengths: (A) 14.0 cm; (B) 13.5 cm; (C) 11 cm, and (D) 9.5 cm. Conditions: capillary, 50 μm i.d. × 110 cm total length, capillary temperature, 30 °C; 20 s hydrodynamic injection (50 mbar); applied voltage, 15 kV; the running electrolyte, 300 mM diethylamine (DEA) (pH = 12); sheath liquid, 5 mM diethylamine:methanol (1:1 v/v) at 0.5 μL min^{-1}; nebulizer pressures, 6 psi; evaporative tube temperature, 70 °C; photomultiplier gain, 12. Peak 1 (sucrose), peak 2 (glucose), peak 3 (maltose), and peak 4 (fructose).

analytical application, using analytes presenting difficulties to be detected by other detectors (UV−vis, fluorescence, electrochemical) without any derivatization. A summary of optimization parameters are described below.

Optimization of the Experimental Conditions for the CE−ELSD Method. In order to verify the correct operation of CE−ELSD, an optimized test procedure was used. The electrophoretic conditions used in this test are those described previously by N.A. Guzman et al.[23] and detailed in the CE−ELSD conditions section. In ELSD, under these fixed electrophoretic conditions, nebulizing gas flow rate (pressure) and evaporating temperature are the major instrumental adjustments available for giving good resolution and maximizing detector response. The evaporating temperature should be appropriate to evaporate the mobile phase and to maintain uniformity of particle size.

In conventional ELSD systems coupled to capillary liquid chromatography (CLC), ca. 3.5 bar is a gas pressure that could be used while still enabling proper nebulizer operation. When used with CE, no peak resolutions were obtained (see Figure S-1A of the Supporting Information), and hence, it was necessary to optimize the nebulizer pressure. Nebulizer pressure ranging between 10 and 30 psi was tested in term of resolution and sensitivity (see Figure S-1 of the Supporting Information). A nebulizer pressure value of 10 psi was chosen as a compromise between sensitivity and peak resolutions for caffeine and theophylline.

In accordance with the theories of nebulization and light scattering, the intensity of light-scattering mainly depends on the size of the particle in the drift tube that passes through the detector, and this depends on the size of the aerosol formed in the nebulization process.[18] Very high temperatures decrease the signal of the analytes because it produces the loss of semivolatile analytes and smaller particles would enter the detector. Temperatures ranging from 30 to 80 °C were tested by comparing peak area values. Baseline stability and sensitivity

dx.doi.org/10.1021/ac400370f | Anal. Chem. 2013, 85, 4858−4862

Table 1. Regression Data, LODs, and LOQs for the Carbohydrates Determined by CE−ELSD

compound	$Y = (a \pm S_a) + (b \pm S_b)X$	R^2	$S_{x/y}$	LOD (ng)	LOQ (ng)	RSD (%)
sucrose	(2.091 ± 0.004)+(0.421 ± 0.008) X	0.9983	0.0070	1.07	1.25	4.9
glucose	(2.185 ± 0.004)+(0.417 ± 0.007) X	0.9985	0.0065	1.07	1.24	3.4
maltose	(2.385 ± 0.002)+(0.386 ± 0.003) X	0.9996	0.0030	1.03	1.11	2.5
fructose	(2.395 ± 0.003)+(0.399 ± 0.005) X	0.9992	0.0045	1.05	1.17	4.2

increased progressively when evaporative temperature was raised to 60 °C. When the temperature was higher than 60 °C, a significant decrease in the analytical signal was obtained. Then, 60 °C was selected as optimal for separation and detection of caffeine and theophyline, due to a complete solvent evaporation and an acceptable baseline noise. Moreover, photomultiplier gain of the ELSD was also tested in a range from 1 to 12. Separation of analytes was not affected by varying the gain, but the signal of all analytes increased as photomultiplier gain value was higher. Thus, photomultiplier gain was set at a value of 12.

For the separation of studied carbohydrates, and according to their pK_a values which ranged between 11.94 and 12.51, strongly alkaline carrier electrolytes with a pH above 12 are needed for their separation.[22] In order to maintain compatibility with the ELSD, only volatile buffers like diethylamine (DEA) can be used to obtain such BGEs. DEA concentrations between 50 and 400 mM, corresponding to pH values in the range of 12.0−12.5, were tested for the separation of the selected analytes. The separation in the presence of high-pH electrolyte systems can be explained by the lability of the proton of the monosaccharides and by the charge-to-mass ratio. At this pH, the carbohydrates become negatively ionized. The stronger acid compound, fructose (pK_a = 12.03), is moving at a higher velocity upstream against the EOF, thus eluting at the end of the group of carbohydrates. Glucose (pK_a = 12.35), a weaker acid, eluted before fructose. Disaccharides presented a lower electrophoretic mobility than monosaccharides of the same acidity. This fact is due to the higher size-to-charge ratio of the disaccharides compared to monosaccharides. Sucrose (pK_a = 12.51), a disaccharide, moves upstream against the flow at a lower velocity than glucose due to its higher molecular weight, thus eluting first. Finally, maltose (pK_a = 11.94), moves upstream against the flow at a higher velocity than glucose and lower velocity than fructose due to its higher molecular weight and acidity.

Due to the high current obtained with the 300 mM BGE, a separation voltage of only +15 kV was used for these experiments. A nebulizer pressure of 6 psi and drift tube temperature of 70 °C were optimum for separation and detection of carbohydrates. Nebuliser chamber length has also been optimized. When a conventional cell (14.0 cm length) was used, no resolution was obtained between peak 1 and 2 (see Figure 2), whereas shorter lengths increased the resolution. A value of 9.5 cm produced the best resolution. This was the final nebulizer chamber length used for the separation and determination of carbohydrates by CE−ELSD.

Application to Carbohydrates Determination. Combined CE−ELSD can be an excellent way to determine compounds lacking strong UV chromophore/fluorophore groups, such as carbohydrates. As explained in our previously published work,[14] with ELSD, the peak output signal (peak area, Y) is a function of the mass of the scattering particles and generally follows an exponential relationship. In different recent works, ELSD was used for analyte quantification with a logarithmic linearization.[14] Such mathematical transformation is allowed by ICH[25] but leads to an experimental error distortion by simple data flattening.[26] Individual calibration graphs were run with standard mixtures of the four carbohydrates within the linear ranges (10−100 μg/mL). Each solution was injected by triplicate. Table 1 reports the figures of merit of the method, namely, linear range and sensitivity (as the limit of detection for each individual carbohydrate, using a linear model described). The precision of the method, expressed as relative standard deviation (RSD), for the determination of each carbohydrate, was found within the 2.5−4.9% (n = 10) range in all cases. The limit of detection (LOD), defined as the concentration of analyte giving a signal equivalent to the blank signal plus three times its standard deviation, was calculated for each individual carbohydrate. In this case, because the coincidence of the background signal with the blank signal, intercept values, and their corresponding standard deviation from the calibration equations were taken for LOD calculations. Thus, the LODs obtained for the proposed method were in the 1.03−1.07 ng range. The limit of quantification (LOQ), defined as the concentration of analyte giving a signal equivalent to the blank signal plus ten times its standard deviation, were in the 1.11−1.25 ng.

The applicability of the proposed method was checked by analyzing samples containing a mixture of carbohydrates. The results obtained are shown in Table S-1 of the Supporting Information. As can be seen in the table, the concentrations added and found were generally in good agreement. The sensitivity was sufficient for detection of carbohydrate levels in such complex matrices as milk and coffee,[27] juice samples,[28] and wine.[24] Appropriated preconcentration or cleanup techniques can be used in other cases in which higher sensitivity or selectivity were required.

CONCLUSIONS

ELSD has been coupled as a detector to a CE equipment. The developed interface is based on a triple-tube design sprayer. With the use of such a sprayer the sheath liquid provides a further point of optimization that contributes to a high reliability. One of the most salient advantages of the proposed approach is the fact that both coupled systems are commercially available equipment and that the customized interface is very easy to perform. With regard to buffers that are suitable for use with CE−ELSD, the applications presented above indicate that there is a more extensive range of buffer choice than initially expected. ELSD detection in CE opens interesting possibilities, especially for analytes nonsensitive to other common detectors used in CE, such as UV−vis, fluorimetric, or electrochemical. ELSD can be considered to be universal, as in principle the only analyte characteristic required for response is low volatility compared to the buffer solution. It is expected that CE−ELSD will open new possibilities for the analytical characterization of polymers and biopolymers, macromolecules, nanoparticles, and in general, big molecules or aggrupation of molecules.

dx.doi.org/10.1021/ac400370f | Anal. Chem. 2013, 85, 4858−4862

■ ASSOCIATED CONTENT

Supporting Information

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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These authors have contributed equally.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Financial support from the Spanish Ministry of Economy and Competitiveness (CTQ2010-15027) is gratefully acknowledged. The support given through an "INCRECYT" research contract to M. Zougagh is also acknowledged.

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