

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/236843223>

ac0493525

DATASET · MAY 2013

---

READS

19

2 AUTHORS:



Qi Lu

Biogen Idec

9 PUBLICATIONS 173 CITATIONS

SEE PROFILE



John Koropchak

Southern Illinois University Carbondale

74 PUBLICATIONS 947 CITATIONS

SEE PROFILE

# Corona Discharge Neutralizer for Electrospray Aerosols Used with Condensation Nucleation Light-Scattering Detection

Qi Lu and John A. Koropchak\*

Department of Chemistry and Biochemistry, Southern Illinois University, Carbondale, Illinois 62901-4409

**To obviate the use of radioactive materials, a simple unipolar electrical neutralizer based on a corona discharge was developed to neutralize electrospray aerosols used when coupling capillary liquid chromatography (CLC) and capillary electrophoresis (CE) to condensation nucleation light-scattering detection (CNLSD). The electrical neutralizer could be operated with either a positive or negative corona to generate ions to neutralize oppositely charged aerosols generated by electrospray and allow sensitive detection with CNLSD. The device could further be operated with organic solvents as well as aqueous buffer. The parameters affecting the corona discharge and neutralization process were studied and optimized. Compared to a CNLSD system using a radioactive neutralizer, the system with the electrical neutralizer showed higher signal and signal-to-noise ratio for test compounds, which indicated better neutralization efficiency of the charged aerosol. Highly reproducible results were obtained with the robust and durable electrical neutralizer with both CLC- and CE-electrospray-CNLSD systems.**

Condensation nucleation light-scattering detection (CNLSD) is an aerosol-based detection method that provides universal and sensitive detection for separations such as HPLC, capillary electrophoresis (CE), capillary electrochromatography (CEC), and supercritical fluid chromatography (SFC).<sup>1,2</sup> In general, the principles of CNLSD involve the conversion of the effluent of the separation process to an aerosol, which undergoes desolvation to ideally produce nanometer-sized dry particles from nonvolatile analytes as they elute from the column. These particles are grown from as small as 2–3 nm to droplets on the order of 10  $\mu\text{m}$  by heterogeneous condensation nucleation, which involves condensation of a saturated solvent vapor (e.g., 1-butanol) onto the dry analyte particles. Because of the greatly increased scattering intensities due to the huge mass gain during the condensation process, on the order of  $10^{11}$ , CNLSD provides much higher sensitivity and wider linear dynamic range compared to evaporative light-scattering detection (ELSD).<sup>1,2</sup>

Capillary liquid chromatography (CLC) and CE are powerful separation techniques that have been successfully coupled to CNLSD to provide sensitive detection for many species including difficult-to-detect ones such as underivatized proteins,<sup>3–5</sup> amino acids and peptides,<sup>5,6</sup> carbohydrates,<sup>7,8</sup> and pharmaceutical drug substances.<sup>9</sup> The principles of CLC- and CE-CNLSD are shown in Figure 1. CLC and CE usually operate at nanoliter/min flow rates that require electrospray aerosol generation for coupling to CNLSD. Since the aerosol generated by electrospray is highly charged, it must be neutralized to prevent electrostatic and other loss processes in order to be sensitively detected by a condensation particle counter (CPC). To date, “static eliminators” based on radioactive materials such as <sup>210</sup>Po, an  $\alpha$ -emitter that ionizes the carrier gas (e.g., air) and generates a balanced weak bipolar plasma, have been commonly used to neutralize these charged aerosol particles.<sup>3–5,7</sup> However, the use of radioactive materials requires special licensing and strict regulation that may not be readily available in all countries or locations, as well as periodic and costly replacement. As a result, a nonradioactive neutralizer is desired for this application.

In this work, we describe an electrical neutralizer based on a corona discharge as an alternative to the radioactive devices. Corona discharges are relatively low-power electrical discharges that take place at atmospheric pressure and are generated by applying a high voltage to a sharp metal point that can create a stream of electrons, ionize the carrier gas molecules, and provide a high concentration of ions. Corona ionizers have been developed for the application of aerosol charge neutralization for years.<sup>10–14</sup> These neutralizers typically contained two unipolar ionizers operated at opposite polarities to produce a high concentration

\* Corresponding author. E-mail: koropcha@siu.edu. Fax: 618-453-4573.

- (1) Koropchak, J. A.; Sadain, S.; Yang, X.; Magnusson, L.; Heybroek, M.; Anisimov, M. *Advances in Chromatography*, Vol. 40; 2000; Chapter 5, pp 275–314.
- (2) Koropchak, J. A.; Sadain, S.; Yang, X.; Magnusson, L.; Heybroek, M.; Anisimov, M.; Kaufman, S. L. *Anal. Chem.* **1999**, *71*, 386A–394A.

- (3) Lewis, K. C.; Dohmeier, D. M.; Jorgenson, J. W.; Kaufman, S. L.; Zarrin, F.; Dorman, F. D. *Anal. Chem.* **1994**, *66*, 2285–2292.
- (4) Lewis, K. C.; Jorgenson, J. W.; Kaufman, S. L.; Skogen, J. W. *J. Microcolumn Sep.* **1998**, *10*, 467–471.
- (5) Szostek, B.; Zajac, J.; Koropchak, J. A. *Anal. Chem.* **1997**, *69*, 2955–2962.
- (6) Szostek, B.; Koropchak, J. A. *Anal. Chem.* **1996**, *68*, 2744–2752.
- (7) Wang, Q. Ph.D. Dissertation, Southern Illinois University, 2002.
- (8) Guo, W.; Koropchak, J. A.; Yan, C. *J. Chromatogr., A* **1999**, *849*, 587–597.
- (9) Lytle, M. L.; Magnusson, L.; Guo, W.; Koropchak, J. A.; Risley, D. S. *LC-GC* **2001**, *19*, 624–631.
- (10) Whitby, K. T. *Rev. Sci. Instrum.* **1961**, *32*, 1351–1355.
- (11) Whitby, K. T.; Peterson, C. M. *Ind. Eng. Chem. Fundam.* **1965**, *4*, 66–72.
- (12) Adachi, M.; Pui, D. Y. H.; Liu, B. Y. H. *Aerosol Sci. Technol.* **1993**, *18*, 48–58.
- (13) Romay, F. J.; Liu, B. Y. H.; Pui, D. Y. H. *Aerosol Sci. Technol.* **1994**, *20*, 31–41.
- (14) Hinds, W. C.; Kennedy, N. J. *Aerosol Sci. Technol.* **2000**, *32*, 214–220.

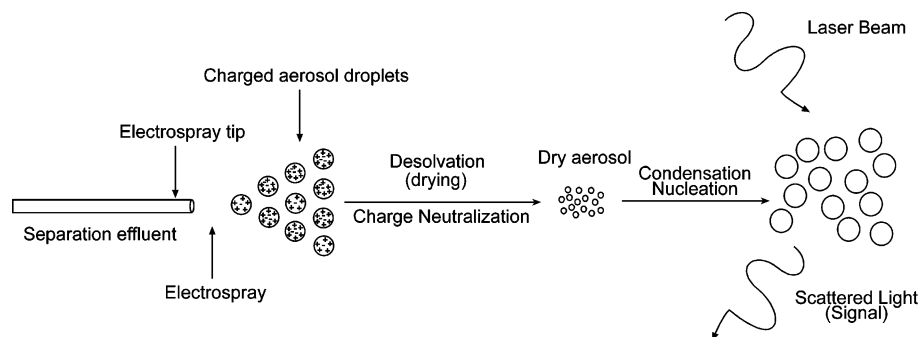


Figure 1. Schematic diagram of capillary LC (CLC)- and CE-electrospray-CNLS.

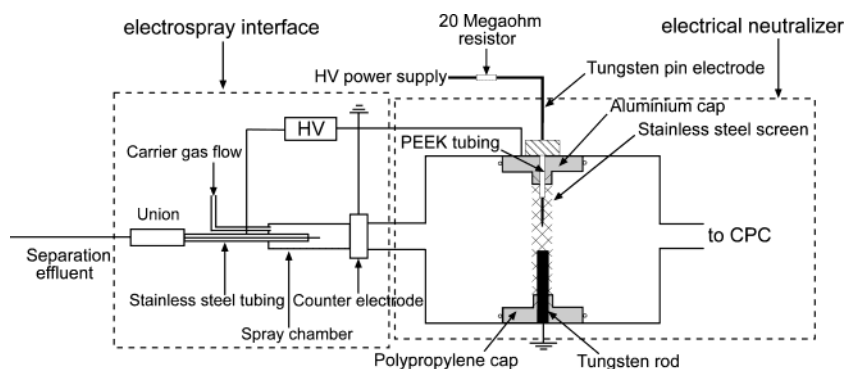


Figure 2. Schematic diagram of CLC- or CE-electrospray-CNLS system interfaced with the electrical neutralizer.

of bipolar ions to neutralize high unipolarly or bipolarly charged aerosol particles to obtain the Boltzmann charge distribution for both micrometer-sized and submicrometer-sized aerosol particles. More recently, a unipolar corona ionizer was applied to charge reduction electrospray mass spectrometry for the analysis of complex mixtures of biological samples such as proteins and oligonucleotides.<sup>15</sup>

In this report, a simple unipolar electrical neutralizer based on a "point-to-plane" geometry corona discharge was developed. The variables affecting the corona discharge and neutralization processes were studied. The optimized device allowed sensitive detection for CLC- and CE-electrospray-CNLS systems. Different combinations of modes of electrospray and corona discharge were also investigated. The performance of the electrical neutralizer was compared with that of the radioactive neutralizer. The electrical neutralizer showed enhanced signals for both CLC- and CE-electrospray-CNLS detection systems, which indicated excellent neutralization efficiency of the electrical neutralizer. The electrical neutralizer is a robust and durable device with simple maintenance and will potentially obviate the use of radioactive materials from the CLC-, CE-, and even CEC-electrospray-CNLS systems.

## EXPERIMENTAL SECTION

**CLC-Electrospray-CNLS System.** An ISCO model 100DM syringe pump (Lincoln, NE) was used to provide the nanoscale liquid delivery for CLC using the "constant flow" mode. A Tylan model 280 mass flow controller (Torrance, CA) was used for the gas flow control. A VICI 20-nL injector (Valco Instrument Co.) was used for the sample injection. A Bertan series 230 high-voltage

power supply (Hicksville, NY) provided the high voltage for the electrospray. An electrical neutralizer was directly connected to the glass electrospray chamber to neutralize the highly charged aerosol generated by electrospray (Figure 2). A separate Bertan series 230 high-voltage power supply was used to apply the dc voltage for the corona discharge. An NRD (Grand Island, NY) polonium-210 disk static eliminator ( $\alpha$ -emitter, 5 mCi), was used to obtain comparison data for a radioactive neutralizer. A TSI 3025A ultrafine CPC operated in the low-flow mode (300 cm<sup>3</sup>/min aerosol intake flow) was used as the particle detector. The condenser temperature of the CPC was set to 10 °C unless otherwise noted. Diffusion screens were added in the aerosol pathway when necessary. A home-written Microsoft Qbasic program was used with a PC to collect data from the CPC at 1 Hz through an RS-232 interface. A home-written "CNLS Integrator" program in Microsoft Visual Basic 6.0 was used for data evaluation and integration.

A 75/360- $\mu$ m (i.d./o.d.) 150-mm long fused-silica capillary column packed with 5- $\mu$ m Lichrospher diol packing materials (Alltech, Deerfield, IL), packed by Unimicro Technologies, Inc. (Pleasanton, CA), was used for carbohydrates separation. An optimized mobile phase containing 90% acetonitrile and 10% methanol at a flow rate of 0.5  $\mu$ L/min was used for the separation of carbohydrates with this column.<sup>7</sup> In order to reduce the dead volume, the column (360- $\mu$ m o.d.) was adapted to the sample port of the microvolume injector (180–200  $\mu$ m) by using a 75/150- $\mu$ m (i.d./o.d.) fused-silica capillary (Polymicro Technologies, Phoenix, AZ). The other end of the column was connected to another length of the same fused-silica capillary that was gold painted at the outlet to allow for electrospray generation. Microtight zero dead volume (ZDV) unions (Upchurch, Oak Harbor, WA) were used for connections between the capillary column and the capillary. The

(15) Ebeling, D. D.; Westphall, M. S.; Scalf M.; Smith L. M. *Anal. Chem.* **2000**, *72*, 5158–5161.

last 6–8 cm of the outlet capillary were painted with gold paint (OG805 Premium Gold, Duncan Enterprise, Fresno, CA) to provide electrical contact with the stainless steel tube. A stainless steel ring mounted on the neutralization chamber was used as the counter electrode for the electrospray. A voltage of 1.5–1.8 kV was applied between the spray tip and counter electrode to convert the effluent into electrospray aerosol droplets (Figure 2). HEPA-filtered air (or CO<sub>2</sub> or N<sub>2</sub>) was used both as the carrier gas for the aerosol and for the bath gas of the corona discharge. A molecular sieve was added before the gas entering the electrospray chamber to remove possible water vapor and organic contaminants from the carrier gas.

**CE-Electrospray-CNLS System.** An ISCO model 3850 capillary electropherograph (ISCO, Lincoln, NE) was used to provide the high voltage for CE and sample introduction. The ground of the CE power supply was floated at the high voltage of the electrospray applied on the spray tip of the capillary.<sup>16</sup> The CE separation voltage was the potential difference between the CE voltage shown on the electropherograph and the applied electrospray voltage. The electrospray interface and neutralization chamber were the same as those of the CLC-electrospray-CNLS. The separation capillaries used were nominal 50/155- $\mu$ m (i.d./o.d.) fused-silica capillaries, which were 80–85 cm long. Capillaries were conditioned with 1M NH<sub>4</sub>OH in water for at least 30 min and then flushed with 10 mM ammonium acetate aqueous solution prior to use. The applied CE voltage was 17.5 kV. A 10 mM ammonium acetate aqueous solution was also used as the running buffer unless otherwise noted. The buffer was prepared each day by diluting 1M ammonium acetate stock solution. The stock solution was prepared from 99.999% ammonium acetate (Aldrich, Milwaukee, WI). Samples prepared in the same buffer were manually injected by electrokinetic injection at 5 kV for 5 s unless otherwise noted.

**Corona Discharge Electrical Neutralizer.** The corona discharge electrical neutralizer is depicted in Figure 2. The discharge was housed in a 5.10 cm  $\times$  3.85 cm  $\times$  3.85 cm (length  $\times$  width  $\times$  height) neutralization chamber made of polypropylene. There were 1.90-cm-diameter holes cut into the top and bottom of the rectangular chamber and casing, into which an aluminum cap (top) and a polypropylene cap (bottom) were inserted with “O-rings” holding the corona discharge electrodes. The corona discharge was generated in the “point-to-plane” geometry by using a pure tungsten electrode (top, 0.5-mm diameter) ground to a sharp point and a grounded flat pure tungsten rod electrode (bottom, 3.2-mm diameter). The pure tungsten electrodes were chosen because of reduced surface sputtering and electrode degradation.<sup>17</sup> A 20-M $\Omega$  resistor was connected to the pin electrode to limit the discharge current. A positive or negative voltage could be applied to the pin electrode to obtain a positive or negative corona as desired. The electrode gap could be adjusted by sliding the top tungsten pin electrode inside a length of PEEK tubing that was fixed on a finger-tight fitting. A 4.0-mm electrode gap was most commonly used unless otherwise indicated. A stainless steel cylindrical screen that fit both caps was used to shield the high electrical field generated by the corona discharge

from the neutralization chamber. The screen allowed partial penetration of ions generated by the corona discharge.

**Standards.** All the chemicals utilized were reagent grade unless otherwise noted. The 5000  $\mu$ g/mL stock solutions of all carbohydrates were prepared in Nanopure water. All the standard solutions of carbohydrates were prepared by diluting stock solutions with acetonitrile. The 1000  $\mu$ g/mL LiCl and tryptophan stock solutions were also prepared in water. The standard solutions were prepared by diluting stock solution with 10 mM ammonium acetate.

**Sample Preparation.** The Coca-Cola and orange juice were purchased locally. The Coca-Cola samples were diluted 100 times with pure water and then diluted with acetonitrile to the final dilution factor. The orange juice samples were centrifuged for 3 min at 3000 $\times g$  and filtered through a 0.45- $\mu$ m membrane filter. The pretreated orange juice was diluted via the same procedure as the Coca-Cola sample.

## RESULTS AND DISCUSSION

**Coupling Capillary Liquid Chromatography-Electrospray-CNLS with an Electrical Neutralizer. (A) High-Voltage Scheme.** In principle, the electrospray can be operated in either positive ion or negative ion mode. The charged aerosol has to be neutralized in order to be detected by CNLS because of electrostatic loss of charged aerosols and conversion of analyte molecules into gas phase ions induced by Rayleigh-charge-limit fission. The radioactive neutralizer containing <sup>210</sup>Po provides bipolar ions to neutralize the charged aerosol regardless of the polarity of the aerosol. In our case, a unipolar corona ionizer was used to produce one dominant polarity of ions to neutralize the opposite polarity aerosol. The negative or positive neutralizing ions can be generated by applying a negative or positive high voltage on the pin electrode of the electrical neutralizer.

Initial attempts were made to operate the electrospray in the positive mode to generate positively charged aerosol by grounding the electrospray tip and applying negative high voltage to the counter electrode (Figure 3a). The mesh screen was floated. However, particles could be detected with mobile phase (or injection of 10  $\mu$ g/mL glucose sample) only when the applied corona voltage was lower than –3.40 kV which was well below the breakdown voltage of air in these experiments (around –2.1 kV). In addition, the baseline and the analyte peak were not stable. Our rationale for these effects is that an electrical field ( $\Delta V$ ) is established between the counter electrode of the electrospray and the mesh screen due to the relatively higher potential of mesh screen, which prevented the positively charged aerosol from approaching the screen, reducing the effectiveness of the neutralization by the negative ions generated by the corona discharge (Figure 3a). An alternative explanation could be that positive charge builds up on the “floated” mesh screen, repelling the subsequent droplets of the same polarity. To establish a field-free region between the counter electrode and mesh screen and remove the possible charge built up on the mesh screen, a positive high voltage was applied to the electrospray tip instead of the negative high voltage on the counter electrode, and the counter electrode and the mesh screen were connected and grounded (Figure 3b). In this layout, the charged aerosol would readily reach the screen and be neutralized by the oppositely charged ions generated by the corona discharge. For the negative ion mode of

(16) Wu, J. T.; Qian, M. G.; Li, M. X.; Liu, L.; Lubman, D. M. *Anal. Chem.* **1996**, *68*, 3388–3396.

(17) Murray, K. K.; Ainsworth, G. F.; Gross, V. P. *1998 Electr. Overstress/Electrost. Discharge Symp. Proc. 20th* **1998**, 195–200.



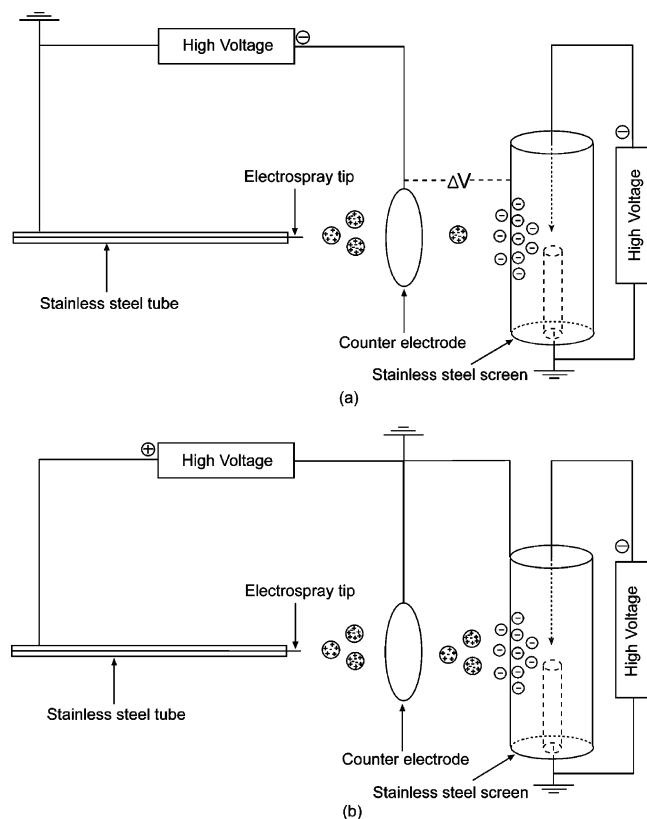


Figure 3. Voltage setup of the electrospray interface and corona electrical neutralizer: the problematic setup (a); the functional setup (b).

electrospray, the polarity of the high voltage applied to the electrospray tip and corona pin electrode was simply changed to negative and positive, respectively.

**(B) Electrospray Mode.** Negative ion mode electrospray combined with a positive corona was studied in detail first with CLC-CNLS. As for most corona ionizers, compressed air was used as both the carrier gas and bath gas since air has a low breakdown voltage. Previous studies showed that there are several modes of electrospray operation in terms of shape of the “Taylor cone”: the “cone-jet”, the “silver bullet”, and the “pulsating” modes that are useful with CNLS. Both “cone-jet” and “silver bullet” modes generate a nearly monodisperse particle size distribution, which results in a significant reduction of the baseline noise and thus an increased signal-to-noise ratio. With the pulsating mode, larger droplets with much wider size distribution were obtained. The “cone-jet” mode was easily achieved for pressure-driven CLC at a working flow rate of 0.5  $\mu\text{L}/\text{min}$ . Consequently, the “cone-jet” mode of electrospray was used for the studies of the electrical neutralizer. The electrospray voltage applied was usually between  $-1.50$  and  $-1.80$  kV. A stable “cone-jet” mode spray was achieved without introduction of a  $\text{CO}_2$  sheath flow around the capillary.

**Studies of Parameters Affecting the Corona Discharge and Charge Neutralization. (A) Corona Discharge Voltage and Gas Flow Rate.** The corona discharge requires an applied voltage higher than the breakdown voltage of the bath gas. However, a voltage too high will create a spark, which gives an unsteady

current and baseline noise.<sup>19</sup> The air flow rate was one factor affecting the charge neutralization process and efficiency by influencing the residence time of the charged aerosol in the neutralization chamber. With the glucose as the test compound and an electrode gap of 3.0 mm, the signal for 10  $\mu\text{g}/\text{mL}$  glucose increased as the corona voltage increased to 2.4 kV, reached a plateau, and then declined above 2.8 kV for a flow rate ranging from 0.4 to 0.6 L/min. At higher flow rates (0.8–1.0 L/min), the signals peaked at 2.4 kV and then decreased to a steady level. The highest signals of glucose were achieved at a flow rate of 0.6 L/min. This result was reasonable because the higher the air flow rate, the shorter amount of time the aerosol resided in the neutralization chamber, which decreased the interaction time between the charged aerosol and neutralizing ions and thus decreased the efficiency of neutralization. The highest signal-to-noise ratio was achieved at 2.4 kV for all tested air flow rates. Higher corona voltages did not improve the signal and signal-to-noise ratio. As a result, 2.4 kV and 0.6 L/min were chosen as the optimized conditions for the detection of glucose when the corona electrode gap was 3.0 mm.

**(B) Effect of Electrode Gap.** The corona electrode gap was another parameter we investigated since it may affect the discharging current and the number of ions diffusing out of the mesh screen for neutralization. The effects of applied corona discharge voltage on the signal and the signal-to-noise ratio of 10  $\mu\text{g}/\text{mL}$  glucose at different electrodes gaps was studied when the air flow was 0.6 L/min. With a 3.0-mm gap, the signal increased as voltage increased. However, the signals were relatively stable in the range of tested voltages for 4.0- and 5.5-mm gaps, with the highest signal achieved at 2.5 kV. The corona ionizer gave the highest signals and signal-to-noise ratios with a 4.0-mm electrode gap. On the basis of the above results, a 2.5-kV corona discharge voltage and 4.0-mm electrode gap were chosen as the optimized conditions for the detection of carbohydrates.

**Reproducibility.** The reproducibility of the signals obtained when using the electrical neutralizer was evaluated with nine consecutive injections of 10  $\mu\text{g}/\text{mL}$  glucose under the optimized conditions described above. The RSD levels for peak height and peak area were 1.0% and 3.2%, respectively, with electrical neutralizer, versus 2.7% and 3.4%, respectively, with the radioactive neutralizer. The system with the electrical neutralizer gave higher signals and signal-to-noise ratios than the system with the radioactive neutralizer.

**Effect of the Diffusion Screen.** For the carbohydrates separation system, the background was over 10 000 particles/ $\text{cm}^3$  with a high noise level. Diffusion screens are often employed with CNLS to improve the signal-to-noise ratio and sensitivity of detection when high background and noise levels are present.<sup>1</sup> Diffusion screens are fine mesh screens, where the smaller particles with high diffusion coefficient are more likely to be captured on the surface of the screen and removed. The mesh size and number of diffusion screens have to be optimized so that a compromise between the reduction of background and analyte signal loss can be reached for the most sensitive detection. A 400-mesh diffusion screen was first added to the pathway of the neutralized aerosol right after the neutralization chamber. It turned

(18) Chen, D. R.; Pui, D. Y. H.; Kaufman, S. L. *J. Aerosol Sci.* **1995**, 26 (6), 963–977.

(19) Chang, J. S.; Lawless, P. A.; Yamamoto, T. *IEEE Trans. Plasma Sci.* **1991**, 19 (6), 1152–1166.

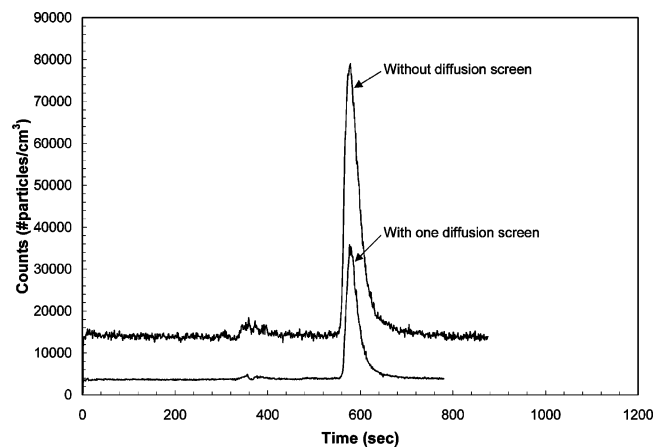


Figure 4. Chromatograms of 10  $\mu\text{g/mL}$  glucose obtained by using the electrical neutralizer without and with a 200-mesh diffusion screen. Mobile phase  $\text{CH}_3\text{CN}/\text{CH}_3\text{OH}$  90:10; air flow rate 0.6 L/min; pump flow rate 0.5  $\mu\text{L/min}$ ; electrode gap 4.0 mm; electrospray voltage  $-1.63$  kV; corona discharge voltage  $+2.50$  kV.

Table 1. Comparison of the CNLSD Response and Reproducibility for 10  $\mu\text{g/mL}$  Glucose Using the Electrical and Radioactive Neutralizers with a 200-Mesh Diffusion Screen

	electrical neutralizer		radioactive neutralizer	
	average	RSD%	average	RSD%
peak height (no. of particles/ $\text{cm}^3$ )	25620	2.2	12687	3.0
peak area	859295	2.0	446051	2.9
signal-to-noise ratio	351	5.7	152	5.9

out that the loss of analyte signal overwhelmed the reduction of background. Hence, a 200-mesh diffusion screen was used instead. Figure 4 shows chromatograms of 10  $\mu\text{g/mL}$  glucose with and without a 200-mesh diffusion screen. With the use of the diffusion screen, the background and noise level were greatly decreased, which greatly improved the signal-to-noise ratio for the analyte. Consequently, a 200-mesh diffusion screen was selected. Due to the addition of the diffusion screen, the carrier gas flow rate affects not only the aerosol's residence time in the neutralization chamber but also the interaction time with the diffusion screen. The highest signal-to-noise ratio was achieved at 0.6 L/min. A stable signal-to-noise ratio level was seen in the range between 0.8 and 1.2 L/min, and it decreased when the flow rate increased to 1.4 L/min. Identical experiments were also carried out with the radioactive neutralizer. The peak heights and peak areas of signals obtained with the electrical neutralizer were 66%–129% higher than those with the radioactive neutralizer at the same flow rate. The background and noise level were similar for both neutralizers. Signal-to-noise ratios achieved with the electrical neutralizer were more than twice those obtained with the radioactive neutralizer. A comparison of the signals and reproducibility for 10  $\mu\text{g/mL}$  glucose using the electrical and radioactive neutralizers with one diffusion screen is shown in Table 1. Peak height, peak area, and signal-to-noise ratio obtained with the electrical neutralizer were 2 or more times higher than those obtained with radioactive neutralizer, which indicated that the electrical neutralizer provided even better neutralization efficiency and higher sensitivity than

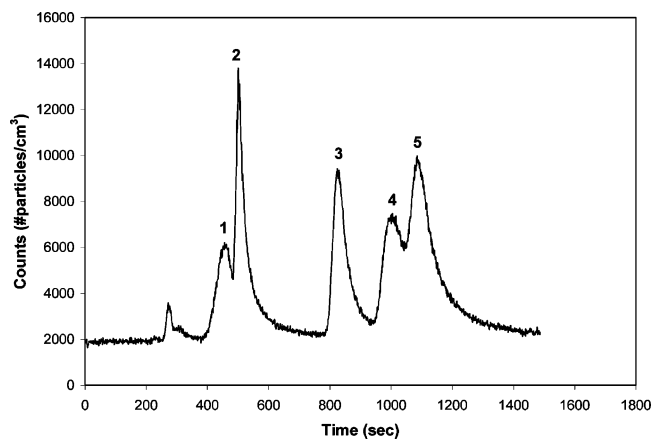


Figure 5. Chromatogram of 10  $\mu\text{g/mL}$  fructose (1), glucose (2), sucrose (3), lactose (4), and maltose (5) with a 200-mesh diffusion screen using the electrical neutralizer. Same conditions as in Figure 4.

the radioactive neutralizer when using a 200-mesh diffusion screen. The results for the electrical neutralizer also showed better reproducibility than those for the radioactive neutralizer. The calibration data for glucose using the electrical neutralizer under optimized conditions showed good linearity for both peak height ( $R^2 = 0.998$ ) and peak area ( $R^2 = 0.994$ ) from 0.2–10  $\mu\text{g/mL}$  glucose. The calculated LOD ( $3\sigma$ ) was 77 ng/mL.

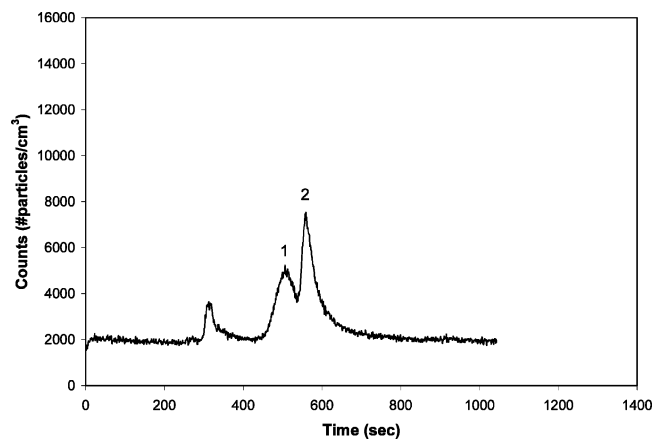
A representative chromatogram of the separation of 10  $\mu\text{g/mL}$  each of fructose, glucose, sucrose, lactose, and maltose obtained with the electrical neutralizer is shown in Figure 5.

**Long-Term Stability.** After the electrical neutralizer was operated for several hours, a very thin brown deposit typically appeared on the surface of the bottom electrode. However, the CPC response remained stable. After several days of operation, the pin electrode developed a white deposit on the tip, causing an increase in background and noise level. In that case, both electrodes could be polished with abrasive paper and sonicated with acetone and then water for 5 min each, after which the original performance could be reestablished.

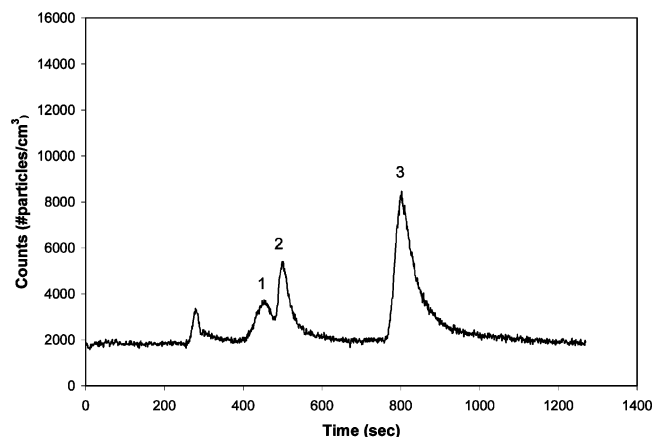
**Alternative Carrier Gases.**  $\text{N}_2$  and  $\text{CO}_2$  were also investigated as carrier gases. It was found that  $\text{N}_2$  provided similar responses and signal-to-noise ratios to those obtained with compressed air, but poorer reproducibility ( $\sim 10\%$  RSD). Since the breakdown voltage of  $\text{CO}_2$  is much higher than that of air, the corona discharge voltage had to be over 3.0 kV to get a CNLSD response with  $\text{CO}_2$ . The CPC's condenser temperature also had to be decreased to 6  $^\circ\text{C}$  to achieve similar sensitivity as that achieved using air.<sup>20</sup> At 3.4 kV corona voltage and 0.6 L/min  $\text{CO}_2$  flow rate, similar signal-to-noise ratios and reproducibility were obtained compared to air. As a result,  $\text{CO}_2$  could be an alternative carrier gas to air with the electrical neutralizer.

**Negative Corona Mode.** All of the above experiments were carried out with the negative ion mode electrospray operation. The positive ion mode operation of electrospray was also studied with the electrical neutralizer, which when operated in the negative corona mode with air also showed good reproducibility with 2.7% and 3.3% RSD ( $n = 7$ ) for peak height and peak area for 10  $\mu\text{g/mL}$  glucose.  $\text{CO}_2$  also turned out to be a good alternative bath gas for the negative corona.

(20) Magnusson, L. Ph.D. Dissertation, Southern Illinois University, 2002.



(a)



(b)

Figure 6. Chromatograms of 10 000 $\times$  diluted Coca-Cola sample (a) and 10 000 $\times$  diluted orange juice sample (b) obtained by using the electrical neutralizer with a 200-mesh diffusion screen. Fructose (1), glucose (2), sucrose (3). Same conditions as in Figure 4.

The electrical neutralizer can be operated with positive or negative corona to couple with either negative or positive ion mode electrospray, respectively, using air or CO<sub>2</sub> as carrier gases, and it provided higher signals and signal-to-noise ratios than the radioactive neutralizer.

**Real Sample Tests.** The optimized system operated with negative ion electrospray mode was used to directly determine the sugar contents in beverages. Samples of 10 000 $\times$  diluted Coca-Cola and orange juice samples were analyzed. Their chromatograms are shown in Figure 6. Fructose and glucose were found in both Coca-Cola and orange juice, but sucrose was found only in orange juice. The resulting calculated concentrations of fructose and glucose in the original Coca-Cola sample were 72.6 and 42.1 mg/mL with total sugars of 114.7 mg/mL, which is in good agreement with the values reported on the label (112.5 mg/mL). For the orange juice, the calculated concentrations of fructose, glucose, and sucrose were 13.4, 11.2, and 49.2 mg/mL with total sugars of 73.8 mg/mL, which is comparable to the sugar contents (90 mg/mL) provided by the manufacturer. The system showed very good reproducibility for these samples. For the orange juice sample, the RSDs of peak height for fructose, glucose, and sucrose were 3.1%, 2.3%, and 1.7%, respectively. As a result, the electrical

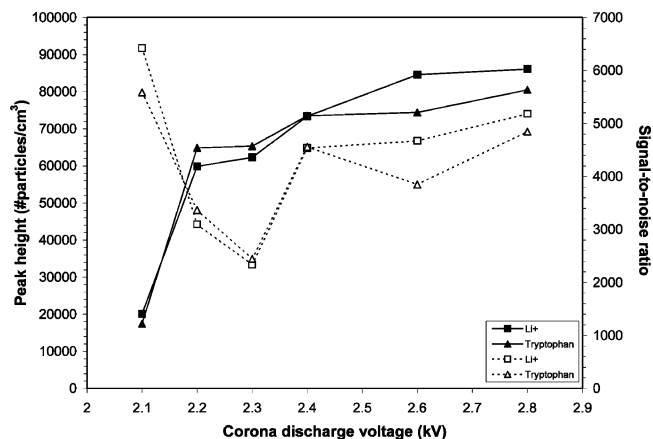


Figure 7. Effects of corona discharge voltage on the signals (a) and signal-to-noise ratios (b) of 0.49  $\mu$ g/mL Li<sup>+</sup> and 3  $\mu$ g/mL tryptophan with CE-electrospray-CNLS. Electrode gap 4.0 mm; air flow rate 1.15 L/min; electrospray voltage +3.15 kV.

neutralizer interfaced CLC-CNLS system showed good performance with these samples.

**Coupling Capillary Electrophoresis-Electrospray-CNLS with an Electrical Neutralizer.** The setup of the CE-electrospray-CNLS with the electrical neutralizer was similar to that of the CLC-electrospray-CNLS. For the CE electrospray, the ground of the CE was floated at the high voltage of the electrospray on the spray tip, resulting in an effective separation voltage being the potential difference between the applied CE voltage and the electrospray voltage. A 10 mM NH<sub>4</sub>Ac aqueous buffer was used for the CE separation. Li<sup>+</sup> and tryptophan were chosen as test compounds.

The "silver bullet" mode is a common operation mode of CE-electrospray with CNLS. The system was tested with both positive and negative ion modes of electrospray. However, the long-term stability of the "silver bullet" mode could only be obtained with the positive ion mode electrospray at a relatively low voltage of 2.7–3.3 kV. Since the "silver bullet" mode gave a low background and a high signal-to-noise ratio, it was employed for all the CE experiments. Air was used as the carrier gas. For the negative mode, the effective voltage on the CE was higher than with the positive mode because the CE ground was floated at negative high voltage applied on the electrospray tip. The electroosmotic flow would be increased at higher separation voltage, which may give a higher than optimal flow rate for electrospray. Nevertheless, even by decreasing the CE voltage, a stable "silver bullet" mode was still not attainable. As a result, no further experiments were done under the negative mode of electrospray with CE.

With the positive mode of electrospray, the negative corona was created by applying a negative high voltage on the pin electrode of the ionizer. The effects of the corona discharge voltage on the signals and signal-to-noise ratios of 0.49  $\mu$ g/mL Li<sup>+</sup> and 3  $\mu$ g/mL tryptophan are shown in Figure 7 (solid lines for peak height; dashed lines for signal-to-noise ratio). The voltage had a similar effect on the peak height of both compounds. Low signal was obtained at a low voltage of 2.1 kV, which was due to the low concentration of the neutralizing ion generated at low voltage. The signal gradually increased as the voltage increased. However, the highest signal-to-noise ratio was achieved at 2.1 kV.

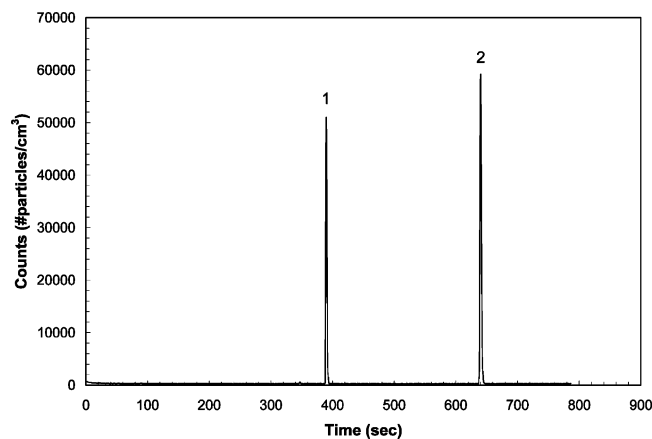


Figure 8. Electropherogram of 0.33  $\mu\text{g/mL}$   $\text{Li}^+$  (1) and 2  $\mu\text{g/mL}$  tryptophan (2) in 10 mM  $\text{NH}_4\text{Ac}$  buffer obtained by using the electrical neutralizer. Electrospray voltage +3.30 kV; corona discharge voltage -2.40 kV; air flow rate 0.87 L/min.

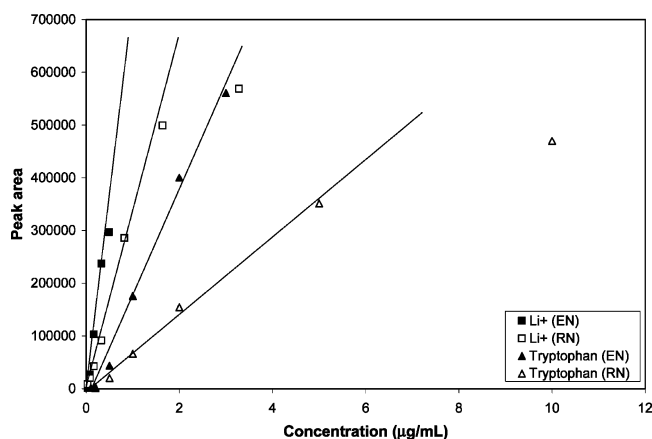


Figure 9. Calibration curves of peak area for  $\text{Li}^+$  and tryptophan in 10 mM  $\text{NH}_4\text{Ac}$  buffer with the electrical neutralizer (EN) and the radioactive neutralizer (RN). Same conditions as in Figure 8.

To achieve a high signal level and lower detection limit, 2.4 kV was chosen as the optimized corona discharge voltage. The experimental evaluation of the effect of gas flow showed that the signal decreased as the gas flow rate increased. Because of the high noise level at low flow rate, the best signal-to-noise ratio was obtained at 0.87 L/min. As a result, 0.87 L/min was chosen for the following experiments. Figure 8 shows a representative electropherogram for 0.33  $\mu\text{g/mL}$   $\text{Li}^+$  and 2  $\mu\text{g/mL}$  tryptophan obtained using the electrical neutralizer under optimized conditions with a high sensitivity and stable background over the course of the experiment.

Peak area calibration data for  $\text{Li}^+$  and tryptophan obtained by using the electrical neutralizer at the optimized condition is shown in Figure 9. For comparison, the calibration data obtained with the radioactive neutralizer was also included. With the electrical neutralizer, the peak area for  $\text{Li}^+$  showed good linearity from 0.033 to 0.49  $\mu\text{g/mL}$  and for tryptophan from 0.2 to 3  $\mu\text{g/mL}$ . Since the signals for 0.82  $\mu\text{g/mL}$   $\text{Li}^+$  and 5  $\mu\text{g/mL}$  tryptophan were above the upper limit of the scale of the CPC ( $10^5$  particles/ $\text{cm}^3$ ), higher concentrations were not tested with the electrical neutralizer. The radioactive neutralizer showed a wider linear dynamic range for peak area up to 0.82  $\mu\text{g/mL}$  for  $\text{Li}^+$  and 5  $\mu\text{g/mL}$  for tryptophan. The slopes of the peak area calibrations with the electrical

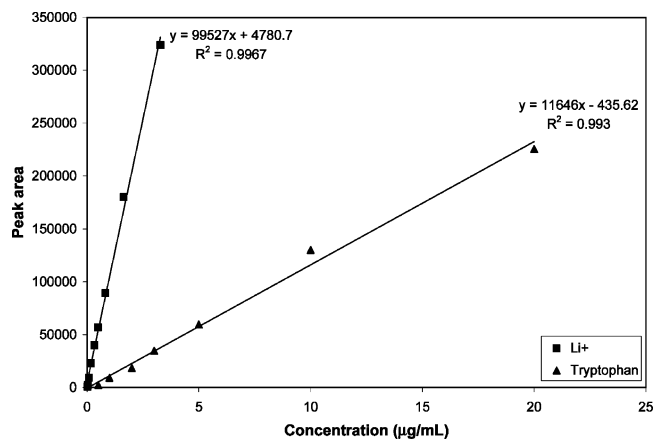


Figure 10. Calibration curves of peak area for  $\text{Li}^+$  and tryptophan in 10 mM  $\text{NH}_4\text{Ac}$  buffer obtained by using the electrical neutralizer with a 200-mesh diffusion screen. Same conditions as in Figure 8.

neutralizer were much larger than those with the radioactive neutralizer, suggesting that the system with the electrical neutralizer provided better neutralization efficiency of the charged aerosol. Similar results were obtained for the peak height.

The higher sensitivity achieved by using the electrical neutralizer provided the possibility to investigate the extension of the linear dynamic range of detection by suppressing the signal with the diffusion screen. Figure 10 shows the calibration curves of peak area for  $\text{Li}^+$  and tryptophan with a 200-mesh diffusion screen. Very good linearity was observed from 0.033 to 3.3  $\mu\text{g/mL}$  for  $\text{Li}^+$  and from 0.2 to 20  $\mu\text{g/mL}$  for tryptophan, which was wider (2 orders of magnitude) than that observed without the diffusion screen. Similar results were observed for peak height. Another possibility for extending the linear dynamic range was to operate the electrical neutralizer at a low corona voltage (e.g., 2.1 kV). However, the result did not show an obvious improvement of the linear range although lower signal levels were obtained.

The reproducibility (RSD) of the peak height and peak area for 0.33  $\mu\text{g/mL}$   $\text{Li}^+$  ( $n = 7$ ) were 6.0% and 4.7%, respectively, with the electrical neutralizer. For 2  $\mu\text{g/mL}$  tryptophan, they were 5.1% and 6.9%, respectively. Good stability of the electrical neutralizer was observed by collecting background in the experiment for as long as 3 h.

## CONCLUSIONS

A simple unipolar electrical neutralizer based on a corona discharge was developed for the CLC- and CE-electrospray-CNLS systems. The electrical neutralizer can be operated with either positive or negative corona to generate ions to neutralize oppositely charged aerosols generated by electrospray. The electrical neutralizer could be operated with both organic solvents with CLC and aqueous buffers with CE. The parameters related to corona discharge and neutralization were investigated and optimized. In comparison to a radioactive neutralizer, the system with the electrical neutralizer showed higher signals and signal-to-noise ratios for test compounds, which indicated better neutralization efficiency of the charged aerosol and higher sensitivity detection with CNLS. The results obtained with the electrical neutralizer were reproducible. The electrical neutralizer was also confirmed to be useful for real sample analysis. The robust and durable electrical neutralizer is an effective alternative to



radioactive materials for charge neutralization with CLC- and CE-electrospray-CNLS systems.

#### ACKNOWLEDGMENT

The authors acknowledge Dr. Shirshak Dhali of the Electrical and Computer Engineering department, Southern Illinois University, for helpful discussion and Mr. Greg Moroz of the Central

Research Machine shop, Southern Illinois University, for construction of the electrical neutralizer chamber.

Received for review May 2, 2004. Accepted July 1, 2004.

AC0493525