Characteristics and Calibration of Conductivity Detection in Capillary Electrophoresis

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Abstract

The characteristics of end-column conductivity detection in capillary electrophoresis are investigated using solutes and background electrolytes that possess a range of mobilities. Indirect detection is most sensitive for low- to medium-mobility solutes. Detection limits are 1×10^{-6}M for hexane sulfonate, and the response is linear for over two orders of magnitude. A generalized response expression is developed for conductivity detection based on the similarities in conductivity and electrophoretic mobility.

Introduction

Most capillary electrophoresis (CE) studies use spectroscopic detection techniques such as absorbance and fluorescence (1). Electrochemical techniques are used to a much lesser extent, despite their inherent advantages of low cost and ease of miniaturization (2). A conductivity detection system (3,4) based on the end-column design originally introduced by Huang et al. (5,6) has been commercialized. This detector monitors the conductivity difference between the sample zone and the background electrolyte using a disk electrode positioned 24 μm from the outlet end of the capillary.

Introductory studies with the commercial end-column conductivity detector demonstrated sensitivities for inorganic ions more than a factor of 10 better than achievable by indirect ultraviolet (UV) absorbance detection (3,4). However, these studies provided few details regarding development or optimization of the electrophoretic buffer system. They simply stated that, because conductivity is a bulk solution property, most analytes are best detected using direct conductivity. In this mode, the background electrolyte has a lower mobility than the analyte ions. Zwitterionic buffers are thus recommended. Paradoxically, organic surfactants were later analyzed using indirect conductivity detection.

This work probes some of the characteristics of conductivity detection using anionic surfactants as test analytes. These studies emphasize the effect of the background electrolyte buffer composition on the resultant detection sensitivities.

Experimental

Apparatus

Studies were performed with a Crystal 300 CE system (Thermo CE, Boston, MA) and a Crystal 1000 direct conductivity detector. A standard ConCap I uncoated fused-silica capillary (52 cm × 50-μm i.d. × 375-μm o.d.) was used. All separations were performed at 25°C. The applied voltage was 20 kV, unless otherwise noted. The outlet end of the capillary was connected to a ConTip I conductivity sensor. Readers are referred to references 3 and 4 for greater details regarding the detector construction.

Capillaries were conditioned by 0.1M NaOH for 2 min followed by 2 min of rinsing with water and a 2-min rinse of running electrolyte buffer before sample introduction.

Analytes dissolved in distilled water were introduced onto the capillary by pressure injection using 20 mbar for 12 s. The strong negative (low conductivity) peak due to the water was used as the electroosmotic flow marker. Data acquisition was performed at 5 Hz with a CHROM-AT data acquisition board (Keithley MetraByte, Taunton, MA) modified for faster acquisition. Data analysis was performed using Lab-Calc software (Galactic, Salem, NH) on a 486-based microcomputer. The reproducibility of migration times was 2%, and that of corrected peak areas was 3.3%.

Chemicals

Tris was from Schwarz/Mann Biotech (Cleveland, OH). All other chemicals used were of analytical grade from Aldrich (Milwaukee, WI) or Sigma Chemicals (St. Louis, MO) and were used as received. The surfactants used as standard analytes were ethane sulfonate, butane sulfonate, hexane sulfonate, octane sulfonate, and dodecyl sulfate. Distilled deionized (18 MΩ) water from a Nanopure System (Barnsted, NY) was used to prepare all electrolyte buffers and standards. All electrolyte solutions were vacuum-filtered (0.45-μm Millicup, Millipore, Bedford, MA) before use.

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Results and Discussion

Huang et al. (7) studied the relationship between peak sensitivity and migration time using on-column conductivity
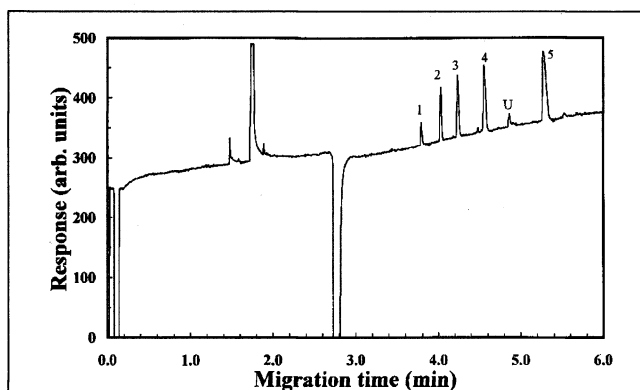

Figure 1. Electropherogram of five surfactants in low-mobility Tris electrolyte: $C_{12}SO_4^-$ (1), $C_8SO_3^-$ (2), $C_6SO_3^-$ (3), $C_4SO_3^-$ (4), unknown (U), and $C_2SO_3^-$ (5). Experimental conditions: applied voltage, 20 kV; buffer, 20mM Tris–0.1M glycine (pH 8.4); analyte concentration, 5×10^{-5}M.
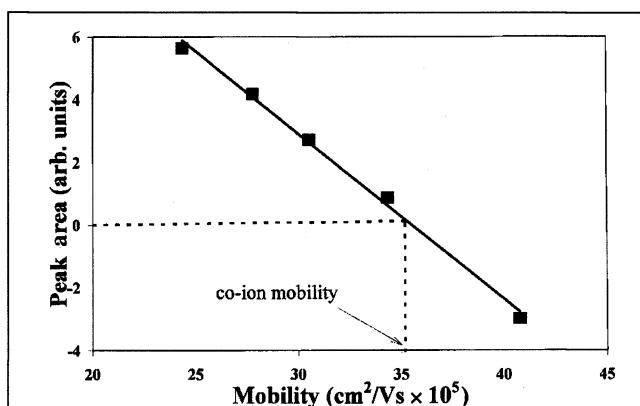

Figure 2. Detector response versus experimentally measured mobility for a series of anionic surfactants. The line is from linear regression of the experimental points. Experimental conditions: applied voltage, 20 kV; buffer, 2.5mM sodium salicylate and 0.1M glycine (pH 5.8); analyte concentration, 5×10^{-5}M.

Table I. Background Electrolyte Mobilities Determined by Extrapolation of Conductivity Detector Response*

Background electrolyte	pH	Measured mobility $(cm^2/Vs \times 10^4)$	Literature mobility[†] $(cm^2/Vs \times 10^4)$
20mM Tris	8.4	2.05 ± 0.03	[‡]
2.5mM Tris	7.5	2.5 ± 0.2	[‡]
Benzoate	5.9	3.4 ± 0.1	3.36
Salicylate	5.8	3.5 ± 0.2	3.7
Acetate	6.5	4.3 ± 0.2	4.24
Formate	5.6	5.7 ± 0.2	5.66
Bromide	5.8	8.0 ± 0.4	8.09

* Experimental conditions: buffer, as above with 0.1M glycine; applied voltage, 20 kV; analytes, 5×10^{-5}M of each analyte in Figure 1. Mobilities are based on triplicate measurements.
† From equivalent conductances listed in reference 17. Equivalent conductances converted to mobilities by dividing by Faraday's constant.
‡ Value not available.

detection of six carboxylic acids comigrating with the electroosmotic flow. They found that the peak areas measured in both the direct and indirect conductivity modes correlated linearly ($r > 0.98$) with the migration times. Peak areas decreased with migration time in the direct mode and increased with migration time in the indirect mode. Similarly, Haber et al. noted that, "The characteristic appearance of [direct] conductivity electropherograms underlines the decreasing peak sensitivity with increasing migration time" (3).

However, the relationship between peak area and migration times noted above is not strictly correct. The response of the conductivity detector is directly related to the difference in the *mobility* of the analyte ion relative to the *mobility* of the background electrolyte. Thus, the peak sensitivity in conductivity detection is more correctly related to the *mobility* of the analyte ion rather than the migration times for a given electrolyte buffer. To demonstrate the significance of this distinction, five anionic surfactants ranging from C_2 to C_{12} were studied under counter-electroosmotic flow conditions. Figure 1 shows a typical electropherogram under these conditions. The correlation (r^2) between peak area and migration time was 0.988, whereas that between peak area and analyte mobility was 0.9995. Similar results were obtained for the same solutes in a 2.5mM formate–0.1M glycine pH 5.6 buffer (peak area versus migration time, $r^2 = 0.989$; peak area versus mobility, $r^2 = 0.997$). Thus, although peak sensitivity varies with migration time, it is more appropriate to relate the detection sensitivity to analyte mobility.

The conductivity detector measures the difference between the mobilities of the analyte and background electrolyte buffer. Thus no peak is detected when the mobility of the analyte matches that of the buffer co-ion. Figure 2 illustrates this behavior. The ordinate of this figure is the peak area observed for the surfactants studied in Figure 1 using a salicylate buffer. The abscissa is the experimentally measured surfactant mobilities. The mobility corresponding to zero response ($3.5 \pm 0.2 \times 10^{-4}$ cm²/Vs) is a measure of the mobility of the background electrolyte co-ion (salicylate). In this manner, mobilities of various background electrolytes were experimentally determined. Table I shows the measured mobilities for a number of the electrolyte systems. The agreement between the measured mobilities and those in the literature was quite good.

Intuitively, the greatest detector sensitivity would be achieved if the buffer had zero mobility (that is, if the buffer were composed solely of a zwitterionic species). Haber et al. recommend the use of high-ionic-strength buffers composed of zwitterions such as CHES (2-[cyclohexylamino]-ethanesulfonic acid) or MES (2-morpholinoethanesulfonic acid) (3). However, these "zwitterions" were used at approximately the pK_a value of the amine functionality. Thus, they possess a net charge of about –0.5. We attempted to use zwitterionic buffers at a pH at which they were truly zwitterionic (i.e., a net charge of 0). At best, these efforts resulted in severely broadened peaks (0.1M glycine, pH 5.4); at worst, no peaks at all resulted (10mM CHES, pH 4.8). These poor results stem from the severe electrodispersion experienced by the analyte under such conditions.

Thus, it is necessary that the co-ions within the background

electrolyte buffer possess some intrinsic mobility. However, this still begs the issue of whether it is better to use direct or indirect conductivity detection when dealing with moderate- (C_2 sulfonate) to low-mobility (C_{12} sulfate) solutes. The question is whether it is better to use a low- or high-mobility background electrolyte. To investigate this, a series of electrolytes possessing mobilities from 2.0×10^{-4} to 8.1×10^{-4} $cm^2/$Vs were prepared. Glycine (0.1M) was added to each of these electrolytes to ensure high ionic strength, as recommended by Haber et al. (3). Tris was a low-mobility electrolyte, thus all of the alkyl sulfonates yielded a direct response, as can be seen in Figure 1. Alternatively, high-mobility electrolytes such as bromide, formate, and acetate resulted in an indirect response, as shown in Figure 3 for the acetate electrolyte. Intermediate mobility electrolytes such as benzoate and salicylate resulted in some analytes responding directly and others indirectly (i.e., both positive and negative peaks were observed).

Figure 4 shows the detector sensitivity for the various surfactants versus the mobility difference between the analyte and the co-ion of the background electrolyte. Detector sensitivity was reported as peak area divided by migration time, as is appropriate for a concentration-based detector in CE (8). As expected from the above discussion, there was a direct relationship between the detector sensitivity and mobility difference. More importantly, however, much greater sensitivity was achieved in the indirect detection mode than via direct detection for these low- to moderate-mobility analytes. This was due to a greater mobility difference between the analyte and background electrolyte being achievable in the indirect detection mode. The mobility of the analyte was smaller in magnitude than the mobility difference between the analyte ion and the high-mobility electrolyte co-ion. Thus, despite the inherent attraction toward performing conductivity in the direct mode, the indirect mode will yield greater sensitivity for many analytes such as surfactants.

An additional observation made during this study was that there was an optimum for the zwitterion concentration. All of the surfactants studied displayed the maximum signal-to-noise ratio in the presence of 0.1M glycine. For instance, with the C_{12} sulfate, the signal-to-noise ratio increased 20% as the glycine concentration increased from 0 to 0.1M and then decreased 40% as the glycine concentration further increased to 0.5M.

Two intrinsic advantages of conductivity detection are its sensitivity and wide linear response (3). In the indirect detection mode using 2.5mM acetate–0.1M glycine (pH 6.5) as an electrolyte (Figure 3), the detection limit (3 times the baseline noise) for $C_6SO_3^-$ (peak 3) was 1×10^{-6}M. This is approximately one order of magnitude better than the corresponding detection limits achieved using indirect UV detection (9–11). Calibration curves were constructed for $C_4SO_3^-$, $C_6SO_3^-$, $C_8SO_3^-$, and $C_{12}SO_4^-$ from 5×10^{-6} to 5×10^{-4}M for 2.5mM lithium acetate–0.1M glycine (pH 6.5) buffer. All plots yielded correlation coefficients in excess of 0.9998. However, the American Society for Testing and Materials provided a more rigorous test of linear detector response. For a detector response to be truly linear, the response factor should not vary by more than 5% (12). The response factor is the detector response divided by the corresponding solute concentration.
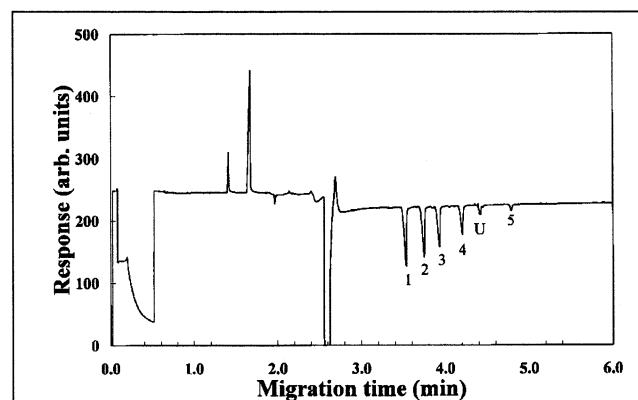

Figure 3. Electropherogram of five surfactants in high-mobility acetate electrolyte: $C_{12}SO_4^-$ (1), $C_8SO_3^-$ (2), $C_6SO_3^-$ (3), $C_4SO_3^-$ (4), unknown (U), and $C_2SO_3^-$ (5). Experimental conditions: applied voltage, 20 kV; buffer, 2.5mM lithium acetate–0.1M glycine (pH 6.5); analyte concentration, 5×10^{-5}M.
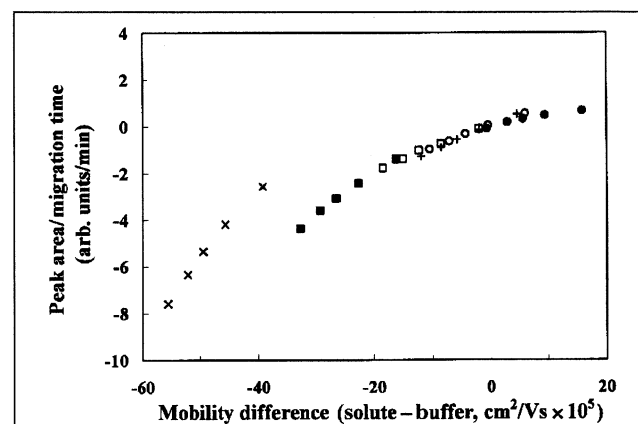

Figure 4. Detector sensitivity versus mobility difference between the analyte and co-ion in the background electrolyte for the five solutes in Figure 3. Buffers: 2.5mM bromide, pH 5.8 (x); 2.5mM formate, pH 5.6 (■); 2.5mM acetate, pH 6.5 (□); 2.5mM salicylate, pH 5.8 (+); 2.5mM benzoate, pH 5.9 (O); and 2.5mM Tris (●), each also containing 0.1M glycine. Experimental conditions: applied voltage, 20 kV; analyte concentration, 5×10^{-5}M.
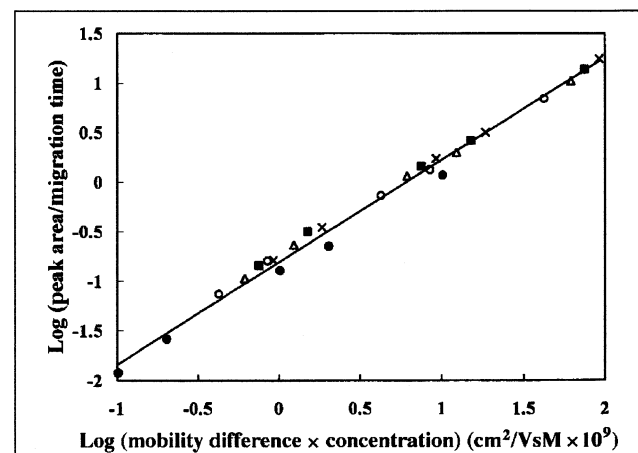

Figure 5. Universal calibration for the linear alkyl surfactants in conductivity-based CE. $C_{12}SO_4^-$ (x), $C_8SO_3^-$ (■), $C_6SO_3^-$ (△), $C_4SO_3^-$ (O), and $C_2SO_3^-$ (●). Experimental conditions: applied voltage, 20 kV; analyte concentration, 5×10^{-6}–5×10^{-4}M; and buffer, 2.5mM lithium acetate–0.1M glycine (pH 6.5).

The $C_6SO_3^-$ and $C_8SO_3^-$ response fulfilled this criterion over the two orders of magnitude studied. $C_4SO_3^-$ and $C_{12}SO_4^-$ showed some fluctuation in sensitivities below 5×10^{-5}M.

A second noteworthy feature of Figure 4 is that the response for all of the surfactants overlaps much of the mobility difference range. This suggests that it may be possible under these conditions to generate a universal calibration in conductivity-based CE. In essence, the observed mobility of an ion relative to that of the background electrolyte is precisely the phenomenon that governs the response in conductivity-based CE. The generalized response relationship has the form:

$$\text{Corrected peak area} = k\Delta\mu \, [\text{analyte}] \qquad \text{Eq 1}$$

where corrected peak area is the peak area divided by the migration time (t_m), k is a constant, $\Delta\mu$ is the mobility difference between the analyte and the co-ion of the background electrolyte, and the analyte concentration is in molarity. The constant in Equation 1 also has a dependence on the mobility of the counter-ion within the buffer (13). The failure to allow for the counter-ion mobility is believed to be the cause of the deviation observed in Figure 4 when responses from six different buffers were compared. However, if only a single buffer is used, as is normally the case in a practical analysis situation, the counter-ion can be ignored.

Figure 5 presents the universal calibration plot for the five surfactants. This log–log plot is rectilinear with a slope of 1.026 and a correlation coefficient (r^2) of 0.992. With such a calibration, it would be possible to determine the concentration of analytes for which no calibration standards were available. However, two caveats must be noted. Such a universal calibration is only valid for analytes possessing the same transfer ratio (14,15) (that is, analytes possessing the same charge). Secondly, this universal calibration will only apply to strong electrolytes. With weak electrolytes, the response becomes much more complex, as has recently been shown by Gebauer et al. (16).

Conclusion

On-capillary conductivity detection provides sensitive, linear detector response in CE. The detector responds to the difference in mobility between the analyte and the co-ion of the background electrolyte. Because the mobilities of the analyte and electrolyte co-ion are readily obtained from the migration behavior, it is possible to construct a universal calibration curve for conductivity detection in CE. This is demonstrated for a group of five anionic surfactants. For low- to medium-mobility solutes such as low-molecular-weight surfactants, the best sensitivity is achieved using indirect detection. Detection limits achieved using indirect conductivity detection with 2.5mM acetate–0.1M glycine (pH 6.5) were 1×10^{-6}M, and the response was rectilinear to the maximum concentration studied (5×10^{-4}M).

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