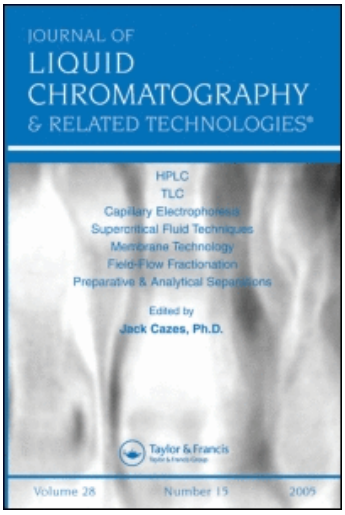

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SELECTED ORGANIC SOLVENTS AS ELECTROOSMOTIC VELOCITY MARKERS IN MICELLAR ELECTROKINETIC CAPILLARY CHROMATOGRAPHY

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ABSTRACT

This study examines typical organic solvents which may be used for analyte dissolution and as reference solvent for determination of electroosmotic velocity. Five solvents were investigated: acetone, acetonitrile, methanol, 1-propanol, and tetrahydrofuran at SDS concentrations of 40, 60, 80, 100, and 120 mM. A precise method for measuring electroosmotic velocity is presented which minimizes run to run variations and yields electroosmotic velocities that are reproducible. Tetrahydrofuran exhibits the greatest electroosmotic velocity at 40 mM SDS, while 1-propanol has the lowest electroosmotic velocity at 40 mM SDS. Acetone, acetonitrile, and methanol are determined to have electroosmotic velocities that are approximately the same at 40 mM SDS. At 60, 80, 100, and 120 mM SDS the migration velocity of each of the five solvents decreased linearly under our operating conditions and are approximately the same at each of these SDS concentrations.

INTRODUCTION

Micellar electrokinetic capillary chromatography (MECC) is a recently developed technique stemming from capillary zone electrophoresis (CZE) for the analysis of

electrically neutral species. MECC utilizes a surfactant system, i. e. , sodium dodecylsulfate (SDS), cetyltrimethylammonium bromide (CTAB), etc. , at concentrations above the critical micelle concentration of the surfactant to serve as a "pseudo" stationary phase. Separation of neutral species is accomplished via partitioning of the solute between the micellar and aqueous phase. MECC has been used to separate various classes of analytes such as PTH-amino acids (1), illicit drugs (2), phenols (3, 4), vitamin B_6 metabolites (5), and nucleic acid constituents (6, 7).

Retention of analytes is limited to a defined time frame designated by the time required for the elution of an unretained species and elution of the micelle. These limits are typically determined by the retention characteristics of specific compounds having known affinity for the micellar phase. For the measurement of the electroosmotic velocity, a compound is employed which is assumed to have no interaction with the micellar phase; micelle elution is determined by the retention of a compound residing exclusively in the micelles.

Typically, the solvents which mark electroosmotic velocity can also serve as aids for dissolution of analytes within a sample. Methanol and acetonitrile have been most popular due to their availability and low water-micelle partition coefficients. However, other methods which are possibly more accurate and yet more time consuming have been demonstrated. Certainly, if one can include a very precise method of measurement for electroosmotic velocity as part of a particular analysis, run-to-run variations in experimental data would be minimized. As mentioned in the preceding paragraph, for measuring the electroosmotic velocity it is assumed that the solvent chosen has no interaction with the micellar phase or walls of the capillary. This is not always the case; however , some electroosmotic velocity markers elute faster than others under the same operating conditions, i.e., they are

not retained as much as others whether it be through interactions with the micellar phase or walls of the capillary. It is therefore desirable to evaluate several common solvents with potential as electroosmotic velocity markers in MECC, to see which of the solvent(s) is/are the least retained and therefore the best/better solvent(s).

For these purposes, we have considered several solvents for use as electroosmotic velocity markers in MECC. The solvents chosen were methanol (MeOH), acetonitrile (ACN), 1-propanol (1-PrOH), acetone, and tetrahydrofuran (THF). These solvents were chosen primarily on the basis of their popularity in MECC and their wide range of hydrophobicities.

EXPERIMENTAL

Apparatus

The chromatographic system consisted of a Quanta 4000 capillary electrophoresis system (Waters Chromatography Division, Millipore, Corp. , Milford, MA) equipped with ultraviolet detection at 254 nm. Data were collected with an IBM-AT computer using Omega-2 software (Perkin-Elmer, Norwalk, CT). Fused silica capillary columns were purchased from Alltech Associates, Inc. (Deerfield, IL, USA) with dimensions of 50 μm i. d. and 170 μm o. d.. These untreated fused silica capillaries (75. 0 cm from injection to detection) were activated by washing with 0. 1 N NaOH for two hours followed by a 20 min rinse with H_2O, a 20 min rinse with the operating buffer, and finally filling the column with the desired surfactant solution. Purges with the operating buffer were performed after each run for 5 min using a vacuum of ~14 inches Hg at the outlet reservoir.

Materials and Methods

Five solvents were chosen as possible electroosmotic velocity markers; acetone, acetonitrile, 1-propanol, tetrahydrofuran, and methanol. Methanol (MeOH), acetone, and acetonitrile (MeCN) were obtained from Mallinkrodt, Inc. (Paris, KY, USA). 1-Propanol (1-PrOH) and tetrahydrofuran (THF) were obtained from J. T. Baker Chemical Co. (Phillipsburg, NJ, USA). Each neat solvent was injected hydrodynamically for a duration of 1 sec followed by the application of a voltage of 10 kV. For the length of column employed in this study (82. 5 cm total length), this voltage and the resultant current translates into a power per unit length of no more than 0. 1 W/m. The elution time of each solvent was determined by a baseline disturbance due to the difference in refractive index of the solvent and surfactant solution and recorded at two points where inflection of the signal occurred.

Stock phosphate buffer was prepared with $NaH_2PO_4 \cdot H_2O$ and NaOH to give a 100 mM concentration of pH 6.85. This buffer was diluted to a 10 mM concentration and used for preparation of surfactant solutions. The surfactant employed in this study was the anionic surfactant sodium dodecyl sulfate (SDS). Electrophoresis-grade SDS was purchased from Bethesda Research Laboratories (Gaithersburg, MD, USA). Distilled water was deionized and redistilled with a Corning Mega-Pure™ Water Purification System (Corning, Inc., Corning, NY, USA). Weighed amounts of SDS were dissolved in 100 mL of 10 mM phosphate buffer to yield SDS solutions with concentrations of 40, 60, 80, 100, and 120 mM.

Determination of Electroosmotic Velocities

Selecting a solvent for use in MECC as an electroosmotic velocity marker requires that the solvent be detectable by UV detection, either by a change in refractive index or absorption of ultraviolet light, as it is the primary means for solute and solvent detection. Since many of the solvents used in MECC do not

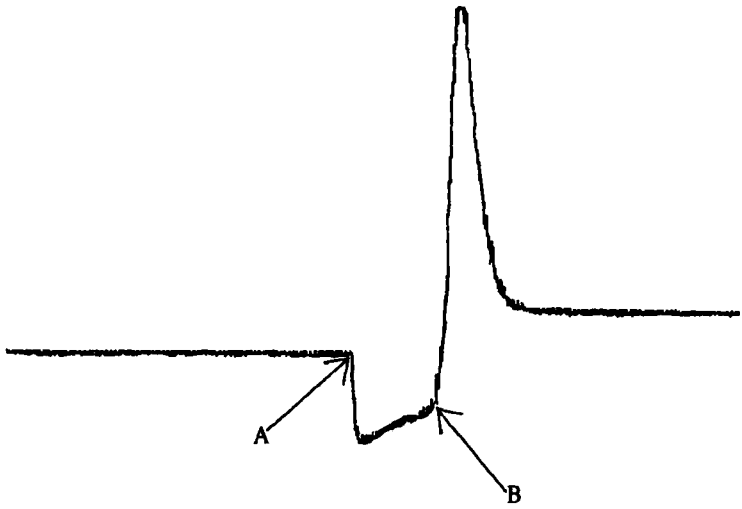

FIGURE 1. A typical chromatogram generated by an electroosmotic velocity marker in MECC. The elution times for the electroosmotic velocity markers were measured at point A and point B.

contain UV absorbing chromophores, detection and hence determination of electroosmotic velocity is done through analysis of the inverted peak generated by a slight change in refractive index (8).

Figure 1 shows a typical chromatogram in which the solvent chosen as an electroosmotic velocity marker exhibits a slight change in refractive index upon elution and generates an inverted peak. The elution times of all the solvents in this study were measured at two points, labeled A and B, on the inverted and asymmetric peak shown in Figure 1. Electroosmotic velocities for each solvent studied were calculated with respect to the solvent elution times at point A and point B.

The relationship between electroosmotic velocity and the elution time of a component residing exclusively in the mobile aqueous phase is given by equation (1),

$$v_{eo} = \frac{t_o}{L} \quad (1)$$

where v_{eo} is the electroosmotic velocity , L is the capillary length from the injection end to the detector cell, and t_o refers to the elution time of the unretained component residing exclusively in the aqueous mobile phase (9). All electroosmotic velocities in this study were calculated using eq. (1).

RESULTS AND DISCUSSION

The first objective of this study centered on finding a method to measure electroosmotic velocity which would yield highly reproducible measurements. As mentioned earlier, in choosing an electroosmotic velocity marker it is assumed that the marker will reside exclusively in the aqueous mobile phase. If our assumption is valid then a method which yielded highly precise results would also yield an accurate result. With this in mind, measurements of t_o were made at points A and B as shown in Figure 1 and electroosmotic velocities were calculated using eq. (1).

Table 1 presents the average elution times of the five solvents studied measured at points A and B. Nearly half of the solvent elution data measured at point B could not be obtained, for reasons illustrated in **Figure 2** which shows the solvent peak generated by acetone at various concentrations of SDS. As the concentration of SDS increases, point B disappears. At higher concentrations of SDS, the inverted solvent elution peak results from an even smaller change in refractive index as compared to the peak generated at lower concentrations of SDS. The detection of slight changes in refractive index is caused by irradiation of UV light at right angles to the axis of the capillary (8). At increased SDS concentrations this refraction is not as intense, as the irradiation is limited by the increase in surfactant molecules. The occurrence of this phenomenon is seen in Figure 2, which clearly

TABLE 1

Average Elution Time (minutes) Of Solvents At Various SDS Concentrations

Measured at Point A

[SDS],M	Acetone	Methanol	Acetonitrile	1-Propanol	Tetrahydrofuran
0.000	22.93	N/A	24.35	28.83	28.22
0.040	15.75	15.75	15.71	16.03	15.52
0.060	15.71	15.81	15.81	15.76	15.87
0.080	16.23	16.23	16.16	16.14	16.15
0.100	16.62	16.60	16.57	16.64	16.63
0.120	16.99	17.06	16.97	17.07	17.04

Measured at Point B

[SDS],M	Acetone	Methanol	Acetonitrile	1-Propanol	Tetrahydrofuran
0.000	N/A	N/A	N/A	N/A	N/A
0.040	16.21	N/A	16.07	17.07	16.65
0.060	16.67	16.33	16.56	17.28	17.84
0.080	17.94	17.20	17.46	18.64	N/A
0.100	19.19	18.08	N/A	N/A	N/A
0.120	N/A	N/A	N/A	N/A	N/A

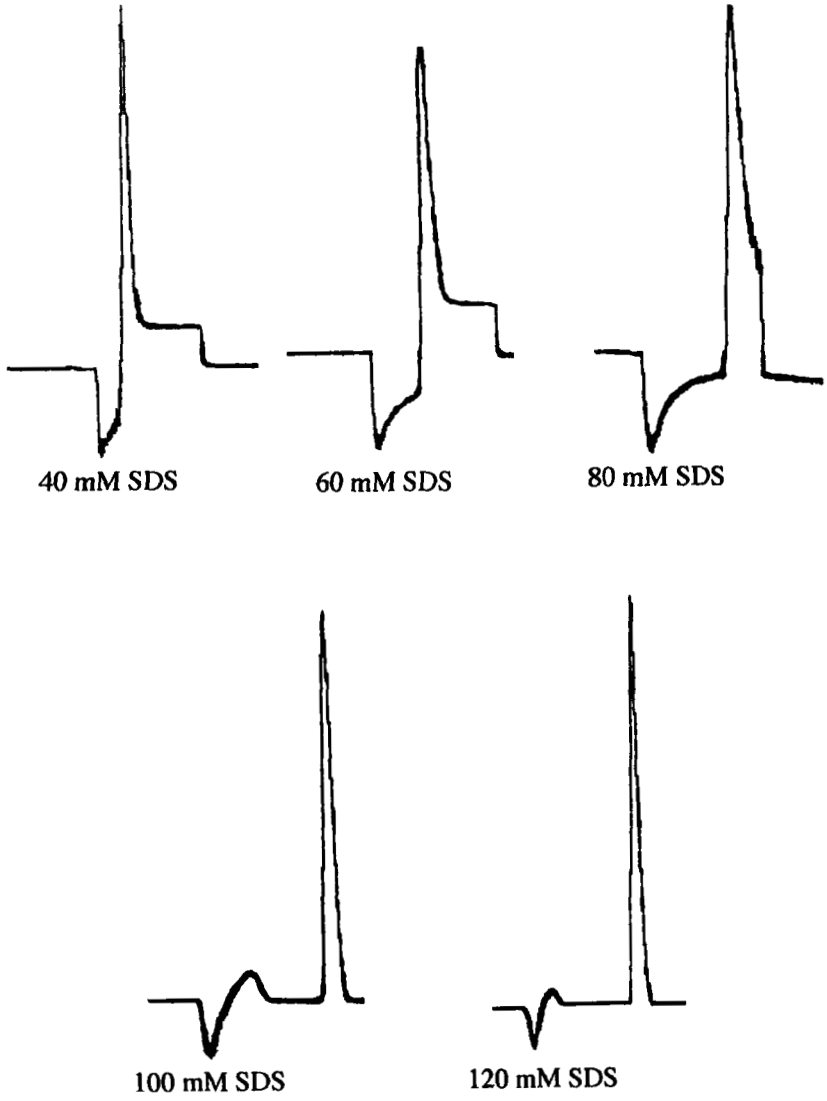

FIGURE 2. Comparison of chromatograms generated for acetone at various concentrations of SDS. As can be seen, measurement of the elution time at both points A and B is possible at the lower concentrations of SDS, but at the higher concentrations of SDS such as 100 mM and 120mM SDS measurement of the elution time at point B is no longer possible.

shows the decreasing intensity of the inverted solvent peak and subsequent loss of point B as SDS concentration increases. The determination of the average elution time of each solvent measured at point A was still possible at higher concentrations of SDS.

All the experimental runs were done in triplicate so as to address the issue of reproducibility in t_o measurements. **Table 2** presents the relative standard deviations for each solvent's elution times at the various concentrations of SDS. The method of measuring t_o at point A provides the most precise results, since the % RSD for any of the solvents is less than 0. 50% at concentrations of SDS from 40-120 mM. Measurements of t_o made at point B were limited due to the reasons stated earlier.

The results presented here clearly show the benefit of measuring the elution time of the electroosmotic velocity marker at point A in Figure 1. Run to run variations were minimized considerably at all concentrations of SDS using this method. Measuring t_o at point A is a very precise method, and keeping in mind that the solvent chosen to serve as an electroosmotic velocity marker is assumed to be completely unretained in the aqueous mobile phase, it is also an accurate method.

The second purpose of this study centered on looking at some of the most popular solvents employed in MECC as electroosmotic velocity markers and attempt to determine which solvent or solvents are better suited to serve as t_o markers.

Table 3 presents the calculated average electroosmotic velocity of each solvent at various concentrations of SDS. For the sake of consistency, these velocities were reported for both methods of t_o measurement presented earlier. However, analysis of the results obtained in the search for the best suited t_o marker(s) was carried out only for electroosmotic velocities calculated with measurements of t_o

TABLE 2

Reproducibility of Elution Times of Solvents, (% RSD)

Measured at Point A

[SDS],M	Acetone	Methanol	Acetonitrile	1-Propanol	Tetrahydrofuran
0.000	0.42	N/A	2.09	0.69	1.48
0.040	0.47	0.20	0.11	0.27	0.10
0.060	0.04	0.07	0.11	0.19	0.13
0.080	0.07	0.16	0.16	0.04	0.16
0.100	0.09	0.04	0.07	0.07	0.10
0.120	0.12	0.15	0.15	0.09	0.07

Measured at Point B

[SDS],M	Acetone	Methanol	Acetonitrile	1-Propanol	Tetrahydrofuran
0.000	N/A	N/A	N/A	N/A	N/A
0.040	0.41	N/A	0.13	0.31	0.12
0.060	0.06	0.09	0.06	0.19	0.19
0.080	0.06	0.20	0.14	0.05	N/A
0.100	0.03	0.03	N/A	N/A	N/A
0.120	N/A	N/A	N/A	N/A	N/A

TABLE 3

Average Electroosmotic Velocity (mm/sec) Of Solvents At Various SDS Concentrations

Measured at Point A

[SDS],M	Acetone	Methanol	Acetonitrile	1-Propanol	Tetrahydrofuran
0.00	0.545	N/A	0.513	0.434	0.443
0.04	0.794	0.794	0.796	0.780	0.806
0.06	0.796	0.791	0.791	0.793	0.788
0.08	0.770	0.770	0.773	0.775	0.774
0.10	0.752	0.753	0.754	0.751	0.752
0.12	0.736	0.733	0.736	0.732	0.733

Measured at Point B

[SDS],M	Acetone	Methanol	Acetonitrile	1-Propanol	Tetrahydrofuran
0.00	N/A	N/A	N/A	N/A	N/A
0.04	0.770	N/A	0.780	0.730	0.750
0.06	0.750	0.770	0.750	0.720	0.700
0.08	0.700	0.730	0.720	0.670	N/A
0.10	0.650	0.690	N/A	N/A	N/A
0.12	N/A	N/A	N/A	N/A	N/A

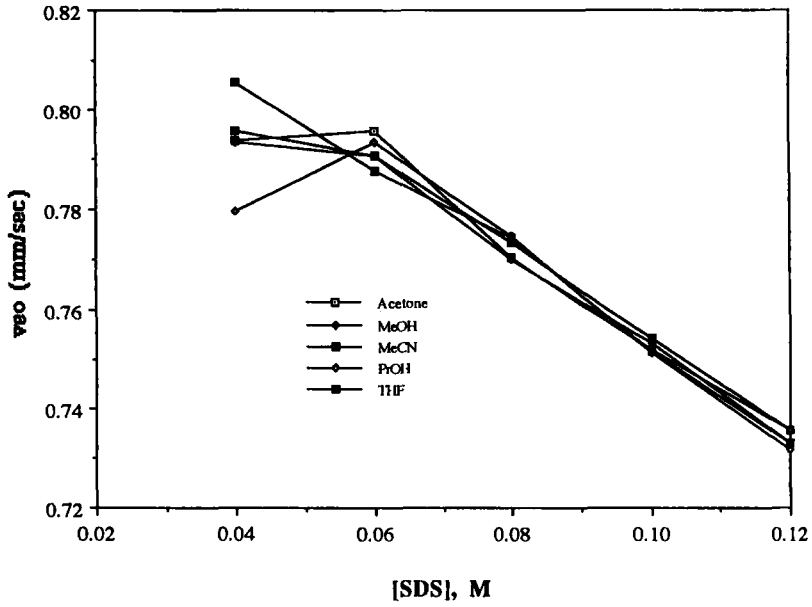

FIGURE 3. Comparison of v_{eo} (mm/sec) vs. concentration of SDS for each of the electroosmotic velocity markers investigated. Electroosmotic velocity for each of the solvents studied decreases at SDS concentrations greater than 60 mM. For specific run conditions see Experimental Section.

made at point A as illustrated in Figure 1 since it proved to be the better method. At an SDS concentration of 40 mM the average velocity was greatest for tetrahydrofuran, indicating that it was the least retained. Acetone, methanol, and acetonitrile had approximately the same velocity at 40 mM SDS, while 1-propanol was the retained the most. At all other concentrations of SDS studied, the average electroosmotic velocity calculated for each solvent remained approximately the same at each concentration of SDS. However, we see a decrease in electroosmotic velocity with an increase in SDS concentration except for the deviations reported at 40 mM SDS. This is illustrated in **Figure 3**.

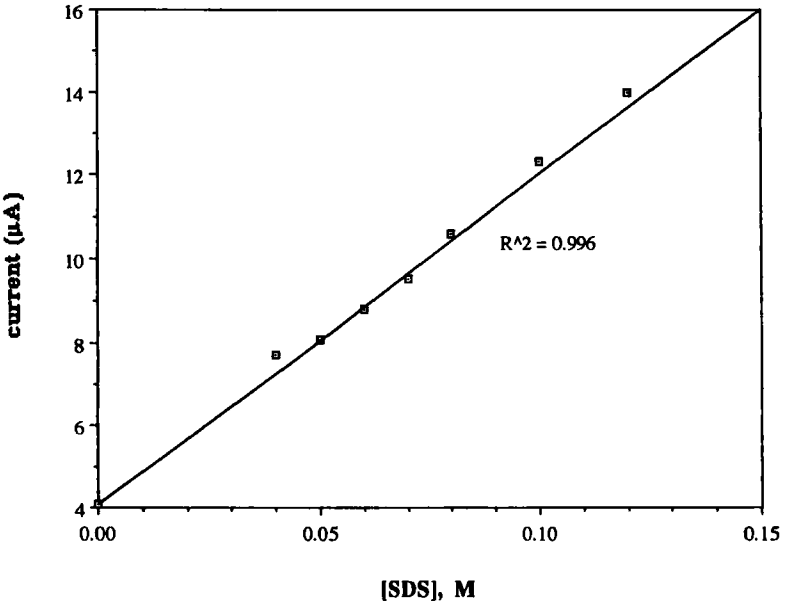

FIGURE 4. Comparison of current (μA) vs. SDS concentration showing linear relationship. It should be noted that at all concentrations of SDS studied the current generated was less than 15 μA (operating voltage 10 kV).

In contrast to these results, Terabe et al. (8) observed that the electroosmotic velocity is not affected by changes in concentrations of SDS. They accounted for their results by examining the classical formula given for electroosmotic velocity which is described by equation (2),

$$v_{eo} = -\frac{\varepsilon\zeta}{\eta}E \qquad (2)$$

where ε is the permittivity of the liquid, ζ is the zeta potential, η is the viscosity of the liquid, and E is the electrical field strength (10). The SDS concentration range they studied was 0. 03M to 0. 15M for which currents ranged linearly from 22 μA

to 45 μA. These high currents generated were reported to have caused a temperature rise in the capillary by Joule heating. The explanation reported for the independence of electroosmotic velocity from SDS concentration suggested that the viscosity η remains approximately unchanged due to the effect of the temperature rise generated by increasing current on η which is accidently compensated for by the increasing SDS concentration.

In our case, the currents generated ranged linearly with SDS concentration from 7. 7 μA to 14 μA as shown in **Figure 4.** Operating under these conditions would tend to limit the effect of Joule heating that was reported by Terabe et al (9). Therefore, we may be seeing the effect of increasing viscosity η due to increasing SDS concentration which would result in a decrease in electroosmotic velocity. It is also possible that the zeta potential ζ may be changing since the electroosmotic velocity was not constant at all the SDS concentrations studied. Further investigations are needed to accurately explain which of these possibilities is occurring.

CONCLUSIONS

A precise method for measuring the elution time of an unretained component serving as an electroosmotic velocity marker was demonstrated to yield values of excellent precision (Table 1 and Table 2). Tetrahydrofuran exhibits the greatest electroosmotic velocity at an SDS concentration of 40 mM while 1-propanol had the smallest electroosmotic velocity at 40 mM SDS. Acetone, acetonitrile, and methanol had electroosmotic velocities that were approximately the same at 40 mM SDS and between the electroosmotic velocities that were calculated for tetrahydrofuran and 1- propanol. At all other concentrations of SDS studied, each solvent exhibited approximately the same electroosmotic velocity. This makes

deciding which solvent(s) better serve(s) as electroosmotic velocity markers in MECC rather difficult when looking only at the electroosmotic velocities that have been calculated. In choosing one of these solvents to serve as an electroosmotic velocity marker, the ability of the solvent to solubilize solutes of interest may make the decision easier. Finally, at operating currents of less than 15 μA, it was noted that the electroosmotic velocity decreases with increasing SDS concentration.

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