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Hydrostatic Flow Injection and Diffusional Injection in Reverse Direction Micellar Electrokinetic Capillary Chromatography: Theory and Application

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ABSTRACT

The effect of injection time on separation in reverse direction micellar electrokinetic capillary chromatography is investigated. In this study, hydrostatic and diffusion injection were studied. The empirical data have been compared with theoretical models for both cases.

INTRODUCTION

In previous work, reverse direction capillary zone electrophoresis was studied (1). The analog of reverse direction in micellar electrokinetic capillary chromatography (MECC) has been reported by Rasmussen and McNair (2). In the present work, the effect of hydrostatic injection and diffusional injection on separation in reverse direction MECC is examined. The effect of hydrostatic injection has been studied for conventional capillary zone electrophoresis (CZE) (3, 4). However, no studies on hydrostatic injection and diffusional injection in reverse direction MECC appear to have been conducted. This study will help aid in elucidating the mechanism of hydrostatic injection and its effect on quantitative analysis, number of theoretical plates, and resolution. Diffusional injection is another mode of injection examined here.

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THEORY

It is observed that during the sample injection phase of CZE, the actual insertion or withdrawal of a capillary into (or from) the sample can cause extraneous injection of solute into a CZE capillary (3). Existing theoretical explanations suggest that when the injection tip of the capillary is held higher than the other side of the capillary, sample injection occurs based on the Poiseuille equation for flow in a circular tube (5).

$$V_{hd} = (\rho g r^2 \Delta h)/(8\eta L) \tag{1}$$

where V_{hd} is the hydrodynamic velocity, ρ is the solution density, g is the gravitational force constant, r is the radius of the capillary, Δh is the difference in heights between the two ends of the capillary, η is the solution viscosity, and L is the capillary length. Further, when t_i is the time of injection, the length of the sample zone, l, is defined as

$$l = V_{hd} t_i \tag{2}$$

By substituting Eq. (1) into Eq. (2), one obtains the length of the sample zone as

$$l = (\rho g r^2 \Delta h t_i)/(8\eta L) \tag{3}$$

The injected amount may then be determined by multiplying the sample zone length by the cross-sectional area, πr^2, of the capillary, and the concentration, C, of the analyte:

$$Q_{nd} = (\rho \pi r^2 \Delta h t_i C)/(8\eta L) \tag{4}$$

At this juncture a different type of injection, diffusional injection, is proposed. Diffusion-type injection is present during the hydrostatic injection process but is overlooked because it is a minor contributor therein. However, it is the major component of the injection mechanism when the injection tip is on the same level as the other tip of the capillary. In such a situation, the diffusion equation is applicable (6).

$$\delta C/\delta t = D[(\delta^2 C)/(\delta x^2)] \tag{5}$$

If the boundary condition is $C(0, t) = C_s$, the concentration of sample would be an error function and the solution to the diffusion is

$$C(x, t) = C_s \operatorname{erfc}[(x)/(2\sqrt{Dt})] \tag{6}$$

The amount of solute injected into the capillary by diffusion would be

$$Q_d = \pi r^2 \int_0^\infty C(x, t)\, d_x \tag{7}$$

and

$$Q_d = 2r^2 \sqrt{\pi D C t^{1/2}} \quad (8)$$

Experimentation was done to determine how well the theoretical model predicts actual behavior. These experiments are outlined in the next section. The area under the peak is proportional to the amount of sample injected.

Results of these experiments will show how these peaks change with variation in injection times in the two schemes; the first being when the injection tip is higher than the other side of the capillary, and the second where both the injection tip and the capillary exit tip are on the same level. The impact of injection time on theoretical plates and resolution are studied. A short discussion follows concerning the comparison of the experimental data and the theoretical model.

EXPERIMENTAL

Instrumentation

A Bertan power supply (Bertan Associates, Inc., Hicksville, NY), whose range of operation is 0 to 30 kV, was used for MECC experiments. The current was monitored using the built-in current meter (ammeter). The fused silica capillaries used in the experiments (51.36 cm by 75 μm i.d. and 363 μm o.d.) were purchased from Polymicro Technologies, Inc. (Phoenix, AZ). An ISCO CV[4] capillary electrophoresis absorbance detector was used. The detection wavelength was fixed at 214 nm and the other settings (sensitivity, 0.02; rise time, 0.8 seconds; time constant, 0.36 seconds) were also held constant. Data were collected with an Omniscribe chart recorder (Houston Instrument, Houston, TX).

Buffers and Reagents

Buffer at pH 4 was used throughout the experimentation: 0.0056 M citric acid, 0.0068 M sodium hydroxide, and 0.0044 M sodium chloride (Fluka Chemika buffer solution pH 4.0, Fluka Chemie AG Ch-9470 Buchs. Switzerland). Dodecyl sulfate, sodium salt 98% (SDS) (Aldrich, Milwaukee, WI) was added to the buffer solution in an amount greater than the critical micellar concentration, 0.1 M. The sample analytes, toluene (MCB Reagents, Cincinnati, OH) and *p*-xylene (Fisher Scientific Co., Fair Lawn, NJ) were carried in the buffer-SDS solution at concentrations of 0.1 M.

System Operation

Samples were injected both hydrostatically and diffusionally. Hydrostatic injection was accomplished by submersion of the capillary tip in the sample solution container followed by elevation of the container. The sample container and capillary were raised 5 cm above the detector window. The injections were repeated for periods of 2 to 100 seconds.

To perform the diffusional injection, the capillary tip was submerged in the sample container, then the container and the capillary were kept on the ground. The injection was repeated from the sample container for periods of 2 to 20 seconds.

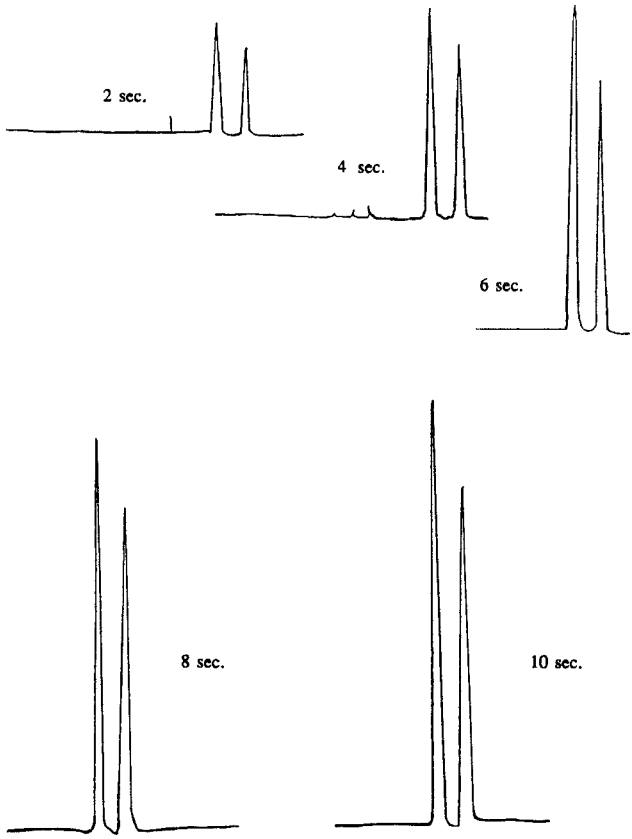

FIG. 1 Electropherograms of toluene and xylene at various short hydrostatic injection times.

All sample injections were made at the cathode end of the capillary, 20.5 cm from the point of detection. After injection, the capillary tip was wiped clean and placed in buffer solution.

RESULTS AND DISCUSSION

In Fig. 1 the electropherograms of xylene and toluene for injection times from 2 to 10 seconds are demonstrated. The hydrostatic injection was done by submerging the capillary tip in the sample container and raising the capillary tip 5 cm above the detector window. The first peak belongs to xylene, the second is that of toluene. This figure depicts an increase in the peak heights as injection times were increased. The widths of the peaks, however, remain relatively constant. This indicates that the injected zone does not contribute to band broadening. Figure 2 shows the

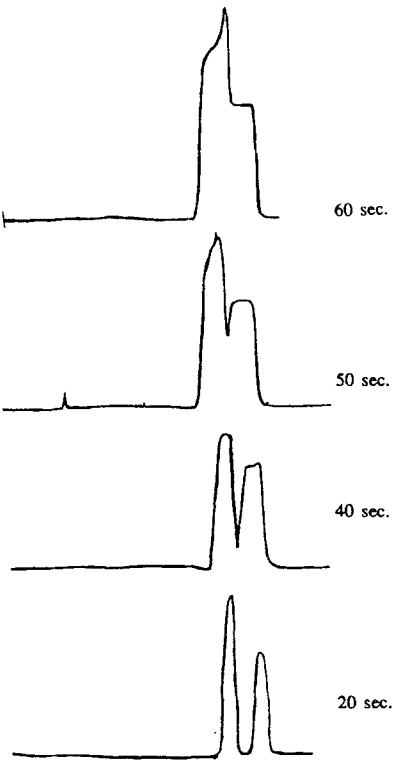

FIG. 2 Electropherograms of toluene and xylene at various long hydrostatic injection times.

electropherograms of xylene and toluene obtained under the same conditions except for the injection time, which was increased from 20 to 100 seconds. This figure clearly demonstrates that the injection time will contribute to band broadening. This band broadening can even cause the overlapping of two xylene and toluene peaks for injection times greater than 50 seconds. Figures 3a and 3b are plots of the peak areas of Fig. 1 and 2 versus injection time. Figure 3a demonstrates the trend predicted by Eq. (4) but, for reasons yet to be clarified, the relation between peak area and time for longer injection times is not entirely linear as was predicted in Eq. (4). A linear relationship between the peak area and time for longer injection times is depicted in Fig. 3b just as predicted in Eq. (4). Figure 4 demonstrates that the number of theoretical plates is relatively independent of short injection time. At longer injection time, the number of theoretical plates decreases by increasing the injection time. Figure 5 demonstrates that the resolution remains constant at short injection time and starts decreasing at longer retention times.

Figure 6 demonstrates the difference in the size of peaks for two cases of hydrostatic injection and diffusion injection. The size of peak areas are

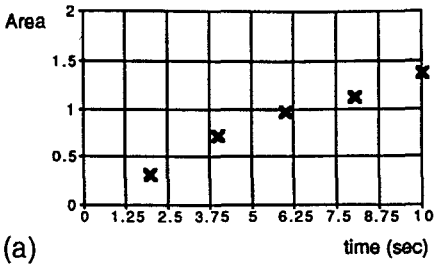

FIG. 3a Peak area versus time for short hydrostatic injection times.

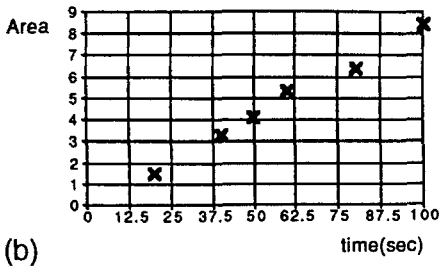

FIG. 3b Peak area versus time for long hydrostatic injection times.

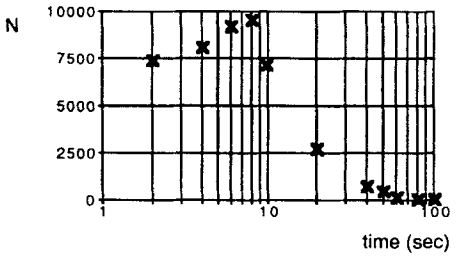

FIG. 4 Number of theoretical plates versus hydrostatic injection times.

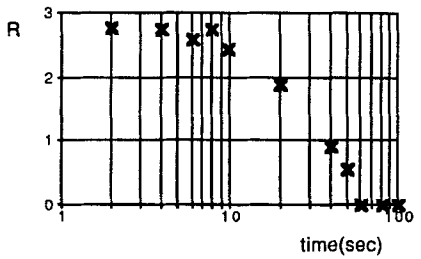

FIG. 5 Resolution versus hydrostatic injection times.

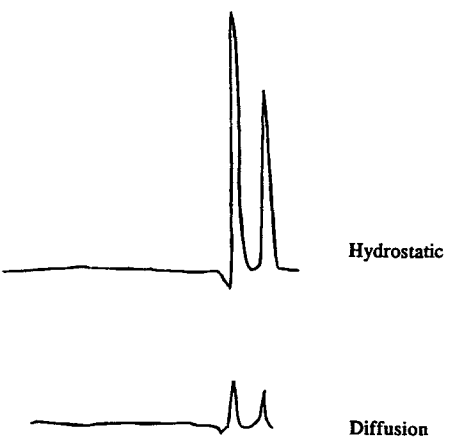

FIG. 6 Electropherograms of toluene and xylene for hydrostatic and diffusional injections in the same scale.

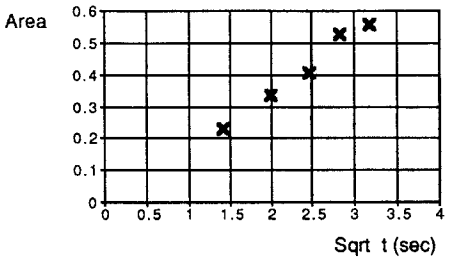

FIG. 7 Peak area versus square root of time for diffusional injection.

one order of magnitude greater for hydrostatic injection in comparison to diffusional injection.

Figure 7 demonstrates a linear relation between peak area and square root of time. The peak area is proportional to the amount of sample injected by diffusion. The experimental results are in good agreement with what Eq. (8) predicts.

CONCLUSION

In this work the effect of injection time on band broadening for reverse direction MECC was investigated. It was shown that band broadening is insignificant for short injection times, yet it becomes important when longer injection time periods are applied. The theory can explain the data for longer times in hydrostatic injections but it cannot explain the nonlinear relation of the amount injected as a function of time for shorter injection times in hydrostatic injections.

The diffusional types of injections were examined experimentally. There is a good agreement between what the model predicts and the experimental results.

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