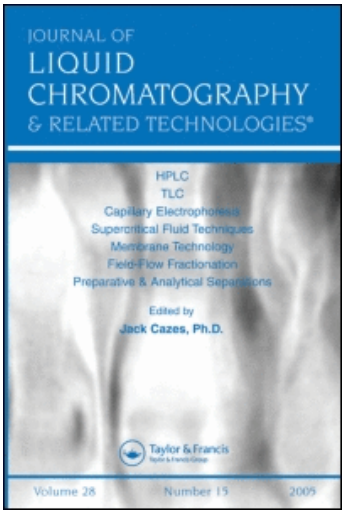

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Quantitative Micellar Electrokinetic Capillary Chromatography: Linear Dynamic Range

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QUANTITATIVE MICELLAR ELECTROKINETIC CAPILLARY CHROMATOGRAPHY: LINEAR DYNAMIC RANGE

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ABSTRACT

The linear dynamic range of micellar electrokinetic chromatography is affected by the concentration of the solute in the injection buffer. The data mimics a model for free solution capillary electrophoresis where the mobility of the analyte is less than the mobility of the buffer ions. This effect causes substantial peak dispersion in a concentration dependent fashion. Good linearity can be obtained by operating at low solute concentrations, e.g., less than 100 μg/mL for solutes such as naproxen, a non-steroidal anti-inflammatory agent. The linear dynamic range can be further improved by employing high ionic strength run buffers. To conpensate for the increase in Joule heating, 25 μm capillaries are employed. These effects are demonstrated with a series of non-steroidal antiinflammatory agents. At the 100 μg/mL level, the migration time % relative standard deviations (% RSD) range from 0.13 -

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0.54%. Peak area % RSD's are approximately 1% using an external standard. Determination of several drugs in the tablet dosage form yield values of 101-104% of the labeled amount.

INTRODUCTION

It has been recognized for some time that the resolving power of capillary electrophoresis (CE) is dependent on among other factors, the concentration and nature of the sample. Mikkers, et. al. (1-2) and Thormann (3) examined this phenomenon from a theoretical basis. They concluded that whenever the mobility of a sample component is greater than that of the carrier or buffer component, the leading edge of the analyte zone will be diffuse, whereas the rear of the zone will be sharp. The converse of this statement also holds as true. Mikkers, et. al. (2) have also shown that this phenomenon is concentration dependent. While absorbance detector linearity as described by Beer's Law is usually obeyed, the CE process may be self-limited in this regard. The implications of this work indicate that CE will have a limited linear dynamic range on the high end of the concentration calibration curve. Since the concentration limits of detection (CLOD) are also poor due to sample loading and detector considerations, the linear dynamic range may be very narrow.

Micellar electrokinetic capillary chromatography (MECC) is a sub-set of capillary electrophoresis. This technique, developed by Terabe, et. al. (4-5) employs an ionic surfactant at a concentration above it's critical micelle concentration (CMC) as a component of the run buffer. MECC has been shown useful for separating a wide variety of neutral, cationic and anionic solutes (6 - 12), a capability that was not possible before

its inception. The separating power is based on the partitioning of a solute between the micellar and the bulk aqueous phases. If an anionic micelle like sodium dodecyl sulfate is employed at neutral to alkaline pH, the micellar aggregate will migrate towards the anode or positive electrode, whereas the bulk electroendosmotic flow is directed towards the cathode. When a solute is partitioned into the micelle, its overall migration velocity is slowed relative to the bulk electroendoosmotic flow. Because of this complex separation process, the arguments describing concentration mediated bandbroadening (1-3) may need modification as only direct electrophoretic processes were considered there.

The purpose of this paper is to describe the sample mediated dispersion found in MECC. Potential solutions to this fundamental problem will be described from two perspectives: ensuring that analyses are conducted within the linear range of the assay, and examining a general solution to the problem.

A series of non-steroidal anti-inflammatory drugs will be used to illustrate some of the problems and solutions when using MECC as a quantitative technique. Selectivitity differences between free solution CE, MECC and reverse-phase LC will also be described.

EXPERIMENTAL

Apparatus

An Applied Biosystems, Inc.(San Jose, CA) Model 270A Capillary Electrophoresis System was used for all studies. Both 25 μm and 50 μm capillaries (Polymicro Technologies, Phoenix, AZ) were used. The capillary lengths were adjusted as appropriate for individual experiments and are described in the

individual figure captions. The instrument, equipped with a variable wavelength UV detector, was set at 230 nm. Vacuum injection of 1 or 2 seconds was used for 50 and 25 μm capillaries respectively. The voltage was set at 20 or 25 kV as specified in the individual figure captions. The temperature was set at 50°C for single drug applications and 30°C for multi-component separations. Data was collected on a recording integrator (Spectra-Physics, San Jose, CA).

Materials

Dibasic sodium phosphate, phosphoric acid, sodium borate and sodium hydroxide were reagent grade and used without further purification. Sodium dodecyl sulfate was from Aldrich Chemical (Milwaukee, WI). Methanol was HPLC grade. Water was obtained from an in-house reverse osmosis, ion-exchange purification system (Hydro, Durham, NC). Drug standards from Sigma Chemical (St. Louis, MO) were used as received. Tablet dosage forms were obtained from a local physician.

For multi-component separations, phosphate buffers were employed. The buffers were prepared by titrating 100 mM dibasic sodium phosphate with 100 mM phosphoric acid to pH 7.0. That buffer was either diluted 5 times with water or used as is. The appropriate amount of SDS was added to the phosphate buffer to prepare the working 25 mM MECC buffer solution. For single drug determinations, a 20 mM borate buffer with 50 mM SDS, pH 9.2 was employed. The buffers were filtered through a 0.21 μm membrane prior to use.

STANDARD CURVE

A 100 mg/mL solution of naproxen was prepared in methanol. A ten-fold dilution of the above was prepared in run buffer. The standard curve was prepared by making two-fold serial dilutions starting with the 10 mg/mL solution. The impact of up to 10% methanol in the injection solution was assessed and found not to affect peak shape, peak width or the retention characteristics of naproxen.

SAMPLE PREPARATION

For single tablet determinations, a tablet was placed in 100 mL methanol and sonicated for about 10 minutes. An aliquot of the suspension was added to the run buffer to yield a drug concentration of 100 μg/mL. Tablet composites were run for naproxen with the final concentration being about 10 μg/mL of the drug.

RESULTS AND DISCUSSION

SELECTIVITY

Non-steroidal antiinflammatory agents are a structurally diverse class of compounds as illustrated in Figure 1. The only common structural element within the class is the carboxylate functional group. Free solution electrophoretic separations are based only on the combined features of charge and size. Even so, a good separation (Figure 2) is obtained in a 20 mM, pH 7.0 phosphate buffer.

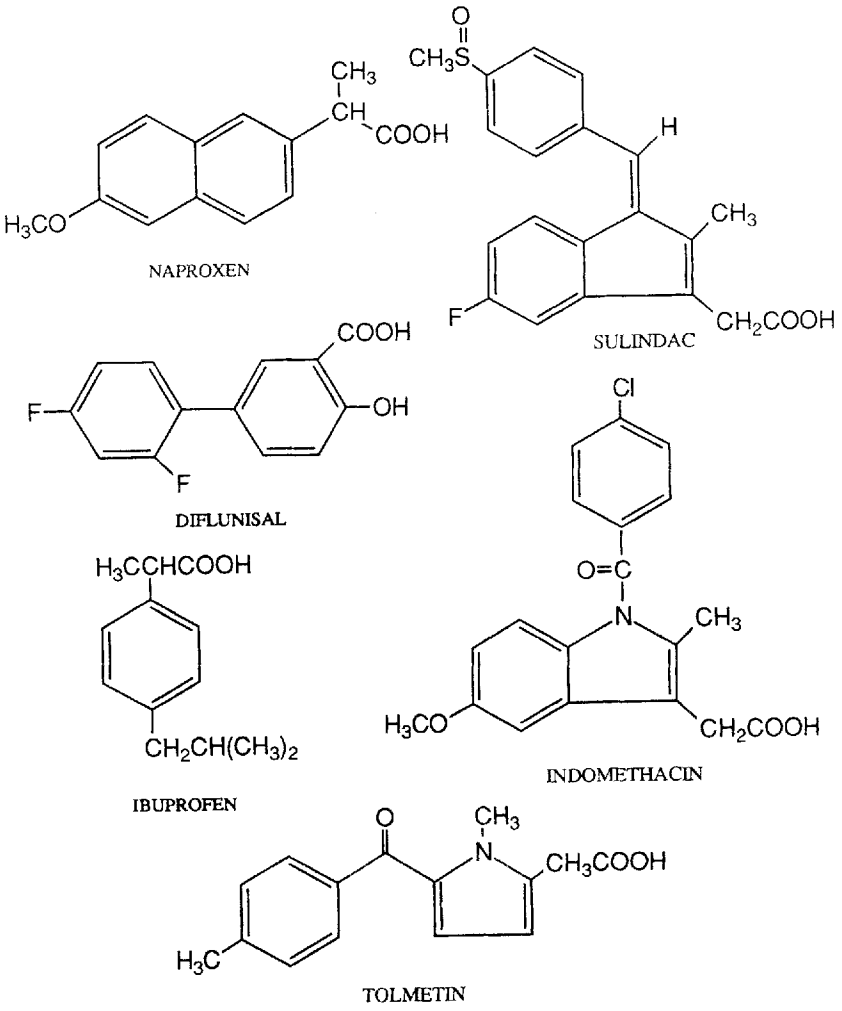

Figure 1. Structures of the non-steroidal antiinflammatory drugs.

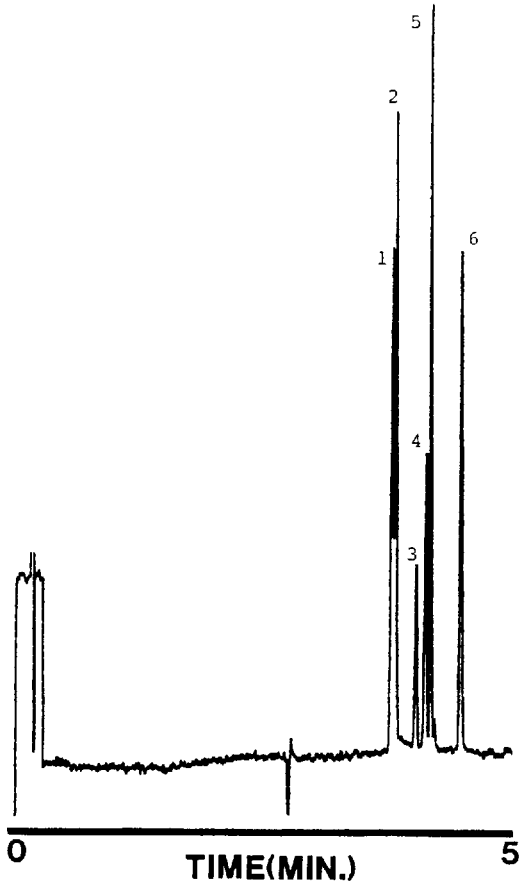

Figure 2. Free Solution Capillary Electrophoresis of Non-Steroidal Antiinflammatory drugs. Capillary: 42.5 cm to detector x 25 μm; buffer: 20 mM phosphate, pH 7.0; temperature: 30°C; voltage: 25 kV; current: 5 μA; injection: vacuum, 2 sec.; detection: UV, 230 nm. Key: 1) sulindac, 100 μg/mL; 2) indomethacin, 100 μg/mL; 3) tolmetin, 100 μg/mL; 4) ibuprofen, 100 μg/mL; 5) naproxen, 10 μg/mL; 6) diflunisal, 50 μg/mL.

Table 1. Selectivity of Capillary Electrophoresis: Comparisons with Liquid Chromatography.

FREE SOLUTION CE	MECC	RPLC[1]	SILICA LC[1]
1	3	3	1
2	5	1	4
3	4	5	5
4	1	2	3
5	6	4	2
6	2	nm	nm

Key: 1) sulindac; 2) indomethacin; 3) tolmetin; 4) ibuprofen; 5) naproxen; 6) diflunisal. [1]See reference 13 for conditions.
nm = not measured.

The addition of 25 mM SDS dramatically alters the selectivity of the separation (Figure 3). Comparisons between these 2 modes of electrophoresis and two LC methods are shown in Table 1.

There is no obvious correlation that explains the comparative elution order observed for the 4 separation methods described in Table 1. For the MECC data, the selectivity is based on the relative contributions of hydrophobic interaction and electrostatic repulsion between the analyte and the micelle. In micellar media, this interaction is further complicated by pH differences between the bulk solution and the surface of the micelle. Since the SDS micelle is anionic, a proton rich double layer forms at the surface that lowers the pH by as much as 3 pH units (14). For the compounds studied, the micelle's surface pH may fall below the pK_a of the molecule. This may result in differences in molecular ionization of the analyte when in free solution or bound to a micelle.

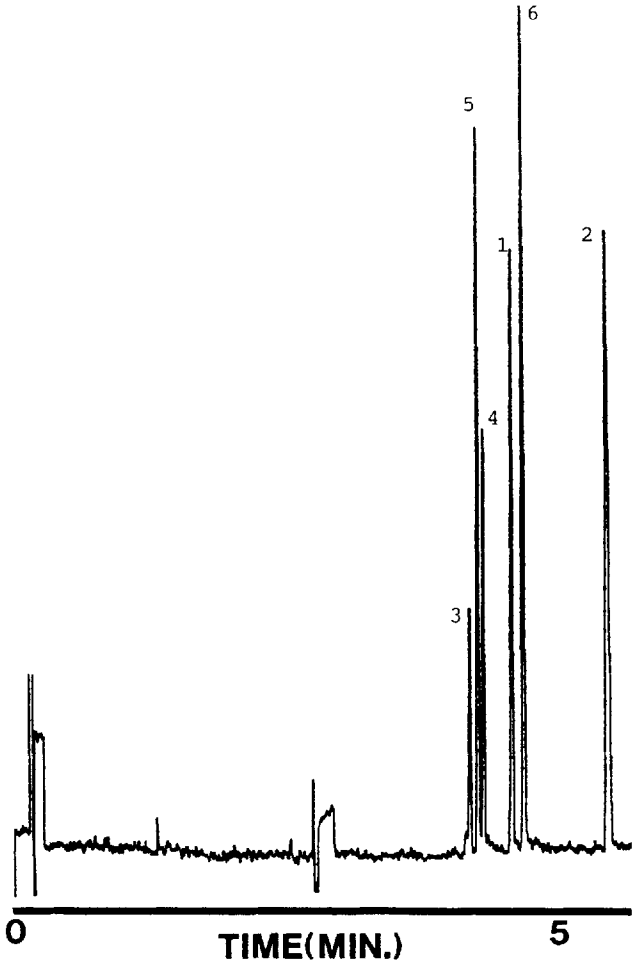

Figure 3. Micellar Electrokinetic Capillary Chromatography of Non-Steroidal Antiinflammatory Drugs. Buffer: 20 mM phosphate, pH 7.0, 25 mM SDS. Other conditions given in Figure 2.

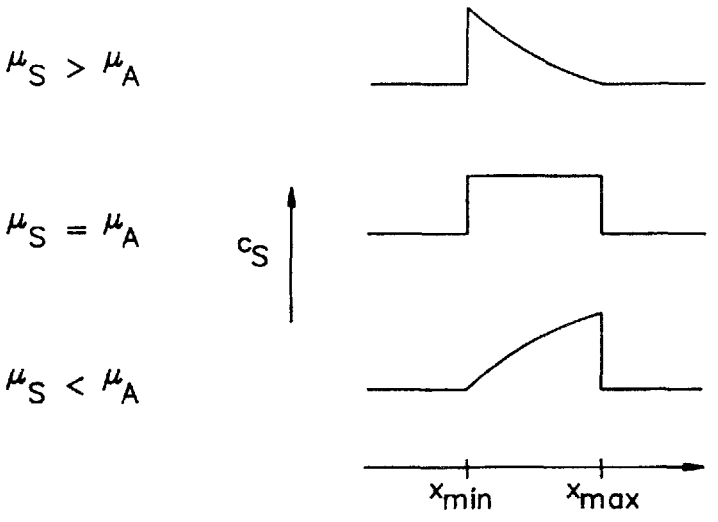

Figure 4. Peak shape dependence on analyte concentration. Reproduced with permission from reference 3.

CONCENTRATION DISTRIBUTIONS IN ELECTROPHORESIS

According to Mikkers, et. al. (1-2) and Thormann (3), the peak shape in capillary electrophoresis is dependent on the relative mobilities of the solute and the carrier in free solution. This phenomenon which is also dependent on the concentration of the analyte is illustrated in Figure 4. For example, if the sample ion (μ_s) has a greater mobility than the carrier ion (μ_a), the leading edge of the zone will be diffuse whereas the rear will be sharp. The converse is also true and if the mobilities are identical, the peak will resemble a square wave. The peak representations in Figure 4 are in the spatial domain. In the time domain, the square wave representation would be illustrated as a Gaussian peak.

In MECC, the situation is more complex since the separation has electrophoretic, electrostatic and hydrophobic mechanistic contributions. The data shown in Figure 5, at high solute concentrations, resembles the situation where $\mu_s < \mu_a$. It appears likely that this is caused by a concentration dependence on the electrophoretic mobility of the solute within the injection buffer. It has been reported that electrophoretic mobility increases in a linear fashion with concentration (1, and reference 10 therein). This results in a net decrease in the solute's migration velocity since the electrophoretic vector is directed towards the positive electrode. It appears that the electrophoretic mobility differences of the analyte in the injection and run buffer is responsible for concentration dependent bandbroadening. As the electrophoretic mobility increases, the observed migration velocity decreases. As the edge of the analyte band electroosmotically flows and mixes (dilutes) with the run buffer, its migration velocity increases, leaving behind the balance of the band remaining in the injection buffer. As a result, the band becomes diffuse. The other edge of the band is sharp since only diffusional broadening is possible. An alternative argument that the electroosmotic flow is reduced is not likely since the injection buffer occupies perhaps 2.5 mm of a 50 cm capillary. Furthermore, the electroosmotic flow must be constant, or averaged out over the entire length of the capillary since fluids can be considered incompressible at atmospheric pressure.

DRUG DETERMINATIONS

With a general understanding of the linear dynamic range of the methodology, the appropriate dilutions can now be

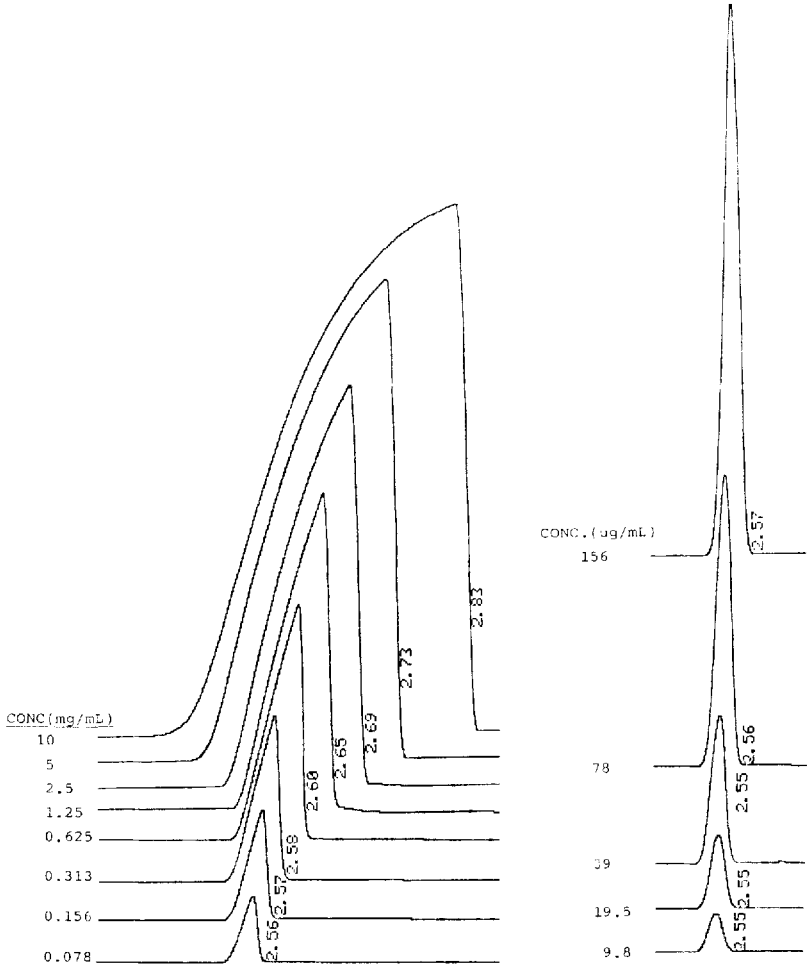

Figure 5. Band Profile Dependence on Solute Concentration in MECC. Solute: naproxen; Capillary: 38 cm to detector x 50 μm; buffer: 20 mM borate, pH 9.2; temperature: 50°C; voltage: 25 kV; current: 29 μA; injection: vacuum, 1 sec.; detection: UV, 230 nm.

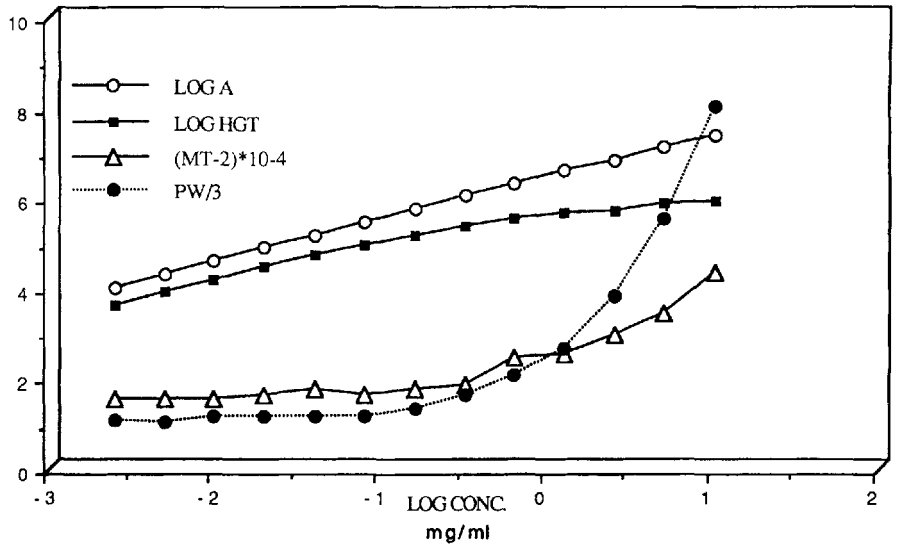

Figure 6. Impact of Solute Concentration on the Analytical Figures of Merit. Key: Log A = Log Peak Area; Log HGT = log peak height; ((MT-2)x10)-4 = scaled migration time in minutes; PW/3 = scaled peak width in seconds.

employed to ensure the appropriate analytical behavior. The figures of merit for the data presented in Figure 5 are illustrated in Figure 6. At concentrations below 100 μg/mL, both peak width and height are linear with concentration and, migration time and peak width are constant. The analytical figures of merit for the test compounds at the 100 μg/mL concentration are given in Table 2. The data for peak area and peak height are reported using external standard computations. It is expected that the peak area and peak height %RSD's would be somewhat improved if an internal standard is used (15). The limit of detection (LOD) data reported in Table 2 was measured

Table 2. Analytical Figures of Merit[1].

	MIGRATION TIME(%RSD)	PEAK AREA (%RSD)	PEAK HEIGHT (%RSD)	LIMIT OF[2] DETECTION (ng/mL)
SUL	0.16	1.1	0.49	386
IND	-	-	-	371
TOL	-	-	-	1100
IBU	0.13	0.86	0.91	595
NAP	0.54	0.91	0.96	65
DIF	0.27	1.06	1.9	176

[1]From 6 or more runs employing the single drug experimental conditions.

[2]Determined at S/N = 2 using the single drug protocol with a 50 μm capillary at 230 nm.

%RSD = relative standard deviation.

at 230 nm. Superior LOD's can be achieved by operating at each compounds' actual absorption maximum.

Drug determinations in some commercial tablets were performed using the single drug protocol. These data are shown in Table 3. The recoveries were consistant with the label claims for the reported tablet determinations. Application of the protocol for diflunisal (Dolobid Tablets) gave incomplete recoveries of the drug due to problems with matrix solubility. No such problems were apparent with the data reported in Table 3.

EXTENDING THE LINEAR DYNAMIC RANGE

Extending the linear dynamic range at the high end is attractive since less dilution is required for these tablet

Table 3. Drug Determinations in Tablets

DRUG	TRADE NAME	LABEL AMOUNT (mg)	AMOUNT FOUND (mg)	% OF LABEL
SULINDAC	CLINORAL	200	203	101.5
NAPROXEN	NAPROSYN	250	254	101.6
		375	387	103.2
		500	520	104.0
IBUPROFEN	NUPRIN	200	207	103.5

determinations. Since it appears that the non-linearity is related to the analytes' contribution to the overall ionic strength of the injection buffer, increasing the ionic strength of the run buffer should minimize the impact of the analyte. The limitation of this approach is that high ionic strength buffers draw high currents which result in Joule heating problems. The solution is to use 25 μm capillaries. The lower diameter capillary passes only 1/4 the current of the 50 μm capillary. Comparative data employing this approach are shown in Figures 7a and 7b.

In Figure 7a, a normal 20 mM buffer is used. With the 25 μm capillary, the current is only 5 μA with a voltage of 25 kV. At a concentration of 1 mg/mL for each substance, there is substantial dispersion compared to a 250 μg/mL run. The fronting effect previously illustrated in Figure 5 is clearly evident in Figure 7a, peak 3 even though the time axis is condensed.

The data in Figure 7b show the same samples run with a 100 mM phosphate buffer. To reduce the overall runtime, the capillary length was reduced from 42.5 cm to 20 cm. Several issues can be extracted from these electropherograms:

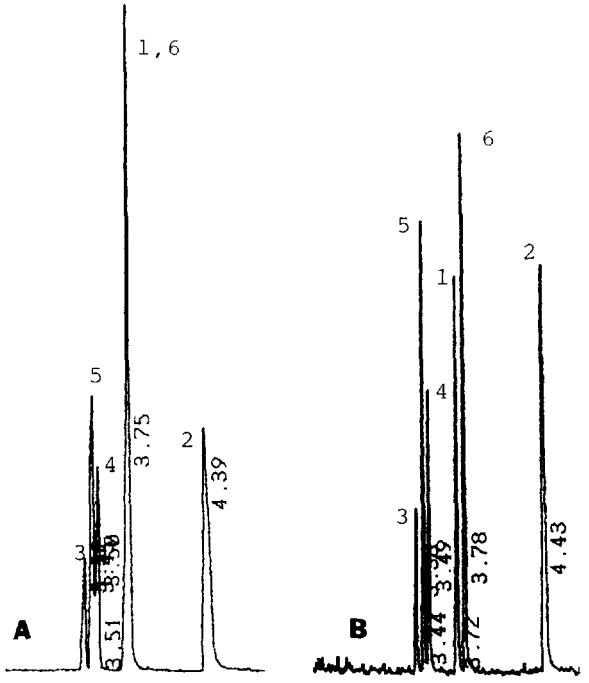

Figure 7a. Band profile dependence on solute concentration and buffer strength in MECC - low ionic strength buffer. Conditions given in Figure 3. A) drug concentrations: 1) sulindac, 1 mg/mL; 2) indomethacin, 1 mg/mL; 3) tolmetin, 1 mg/mL; 4) ibuprofen, 1 mg/mL; 5) naproxen, 100 μg/mL ; 6) diflunisal, 500 μg/mL; B) 4X dilution of A.

-A selectivity shift for peak 3 (Tolmetin) occurs in the high conductivity buffer.

-The high ionic strength buffer clearly reduced the electroosmotic flow (not shown).

-The current is 48 μA at 20 kV. If a 50 μm capillary was used, the current would rise to 192 μA causing substantial bandbroadening.

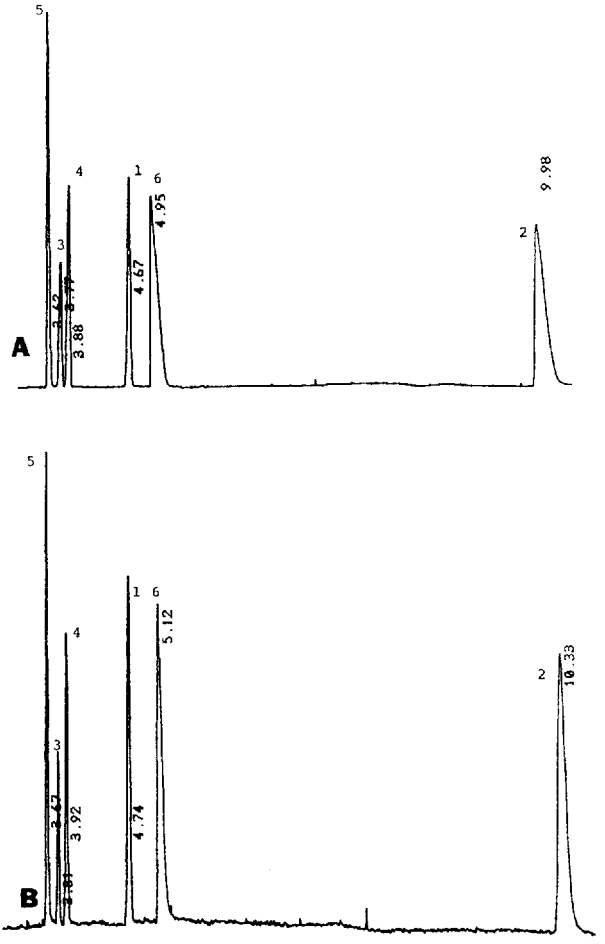

Figure 7b. Band profile dependence on solute concentration and buffer strength in MECC - high ionic strength buffer. Conditions as per Figure 3 except: capillary: 20 cm to detector x 25 μm; buffer: 100 mM phosphate, 25 mM SDS, pH 7.0; A) drug concentration given in Figure 7a; B) 4X dilution of A.

-The high salt concentration probably reduced the solubility of the drugs in the bulk solution as well. The last 2 peaks were badly tailed possibly indicating a solubility problem. Perhaps that could be solved by adding a small amount organic modifier to the run buffer.

- While the electrophoretic efficiency is lower in high ionic strength buffer (compare Figures 7a and 7b at the 250 μg/mL concentration), the first 4 peaks are now linearized between 250 μg/mL and 1 mg/mL, a substantial improvement compared to the work in the lower conductivity buffer.

While 25 μm capillaries are not usually employed in capillary electrophoresis because of poor concentration limits of detection, the lower current drawn by these capillaries may create unique opportunities for buffer selection, thereby expanding the capability of the technique.

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