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# Liquid chromatographic method for determination of water in soils and the optimization of anion separations by capillary zone electrophoresis

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# Liquid chromatographic method for determination of water in soils and the optimization of anion separations by capillary zone electrophoresis

by

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1994

*This work is dedicated to my parents.*
*I thank them for the values they've instilled in me*
*and for the sacrifices they've made in their lives for me.*

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# GENERAL INTRODUCTION

## Organization of Dissertation

This dissertation begins with a general introduction containing a literature review. This is followed by two research papers which have been published in scientific journals and a third which will soon be submitted for publication. Permission from each publisher extending reproduction and distribution rights is acknowledged. Each paper is preceded by a summary and followed by conclusions. Figures are located at the appropriate location within the text of each paper. References cited within each paper are listed after each section. The three papers are followed by a general conclusions section. References cited in the general introduction are compiled in the literature cited section following the general conclusions.

## Water Determination

The determination of water is an important measurement in many industrial and scientific areas. There have been many methods proposed for water determination. Smith and Mitchell have compiled a comprehensive review of these methods (1-3). The techniques included in this review are chemical, gravimetric, thermal, separation, spectroscopic, as well as a number of miscellaneous methods.

The Karl Fischer (KF) titration method is by far the most extensively used method for determining water. Proof of this fact is the volume Smith and Mitchell dedicated exclusively to applications of the KF method (3). Although the KF method has been used

successfully for many samples, there are several problems with the method. The KF reagent solution which consists of iodine, sulfur dioxide, pyridine, and methanol is unstable and has a relatively short shelf life due to side reactions. Reactions between some classes of compounds and the KF reagents also may occur giving erroneous results. The KF method also requires a relatively large sample size.

Chromatographic methods offer an attractive alternative to the KF method. Sample interference is usually eliminated since separation of sample components occurs before detection. These methods are often simple, fast, sensitive, and require relatively small sample sizes. Instrumentation needed for these analyses is usually readily available in most modern laboratories. Methods based on gas (GC) or liquid chromatography (LC) have been developed.

Water can be determined by GC directly using a universal detector (e.g. thermal conductivity detector (TCD)) or indirectly following a chemical reaction. In the latter approach, a product of the reaction involving water is determined using a more sensitive detector such as the flame ionization detector.

Water has been separated on a packed GC column with subsequent detection using a TCD. Sakano and coworkers developed a relatively simple and rapid GC method for the determination of trace amounts of water in chlorinated solvents containing active chlorine and hydrogen chloride (4). Samples of carbon tetrachloride, chloroform, dichloromethane, and chloroethane containing from 20 to 1000 ppm of water were studied. The GC results were within 10% of those obtained by a KF titration.

Andrawes (5-6) used a capillary column coated with Carbowax and a helium

ionization detector to determine water in a variety of solvents and reagents including toluene, ethanol, and methylene chloride. The detector response was limited to a linear range of up to 700 ppm.

Kolb and Auer introduced headspace gas chromatography (HSGC) for determining water in liquid and soluble and insoluble solid samples (7-8). The liquid and soluble solid samples were dissolved in water-miscible solvents. The insoluble solid samples were suspended in dry, water-miscible solvents such as ethylene glycol monomethyl ether. The water in the solid sample was extracted into the solvent from which it partitioned between the liquid and gas phase. After an equilibration period, headspace samples were analyzed using a fused silica capillary in conjunction with a hot wire detector. Their method was applied to a variety of samples including solvents, engine oils, detergents, paper, and food samples.

A limitation of the direct GC methods described so far is the lack of a standard detector. Methods involving a chemical reaction of water and the subsequent detection of a reaction product take advantage of the attractive features offered by the flame ionization detector (FID). The FID is a sensitive detector which has a linear range of up to 8 orders of magnitude and responds to most organic compounds. The FID has become a standard detector for GC analyses.

A method which converts water to acetylene through a reaction with calcium carbide is often employed (9-12). Latif and coworkers (10) presented a procedure using a simple flow reactor containing calcium carbide. Trace amounts of water in nitrogen gas were converted to acetylene by passing the sample through the flow reactor. The acetylene was

determined using a Porapak column and a FID.

Loeper and Grob (11-12) used the same reaction between water and calcium carbide in their HSGC method using flame ionization detection. The method was applied successfully to a variety of organic solvents containing 60-400 ppm of water.

Fritz et al. used the acid-catalyzed reaction between 2,2-dimethoxy propane (DMP) and water as the basis for their GC method (13). The products of the reaction between DMP and water, acetone and methanol, were detected by a FID. The applicability of this method to a variety of liquid and solid samples was shown.

Later, Fritz and Chen (14) developed a GC method incorporating the acid-catalyzed reaction between triethylorthoformate and water to produce ethanol and the corresponding ethyl ester. This method had several advantages over the previous method. The reaction between water and the ortho ester was faster and more complete than the analogous reaction using DMP. Also, the previous method used a solid acid catalyst (Nafion) which had several drawbacks, whereas the ortho ester method used a liquid acid catalyst (methane-sulfonic acid). The method was applied to a variety of solid and liquid samples. The results obtained by the GC method compared well with those determined by the KF method.

LC methods have also been developed. As with GC, both direct and indirect methods have been proposed. Fehrman and Schnabel determined water in hydrocarbons by gel permeation chromatography with refractive index detection (15). Toluene was used as the eluent to separate water from other low molecular weight compounds.

Bjorkquist and Toivonen reacted phenyl isocyanate with water to form N,N'-diphenylurea which was then analyzed by reverse-phase HPLC using UV detection (16).

Stevens et al. (17) presented a method using a cation-exchange column in conjunction with a methanol eluent containing a low concentration of sulfuric acid. A conductivity detector was employed, showing a decrease in conductivity for the water peak. Although the method was fast and water was separated and detected in ~5 minutes, their data indicated a response factor that varied widely with changing water concentration.

Fritz and coworkers developed a method to determine water using an indirect spectrophotometric detection scheme (18-19). The method was based on the equilibrium between cinnamaldehyde and cinnamaldehyde dimethylacetal in a methanol-acetonitrile eluent. A shift in equilibrium toward the cinnamaldehyde occurred when a sample containing water was injected into the system. An increase in absorbance proportional to the amount of water in the sample was observed. Their system used a cation-exchange column in the $Li^+$ form followed by a catalytic column containing cation-exchange resin in the $H^+$ form. This method was shown to be fast, sensitive, and widely applicable.

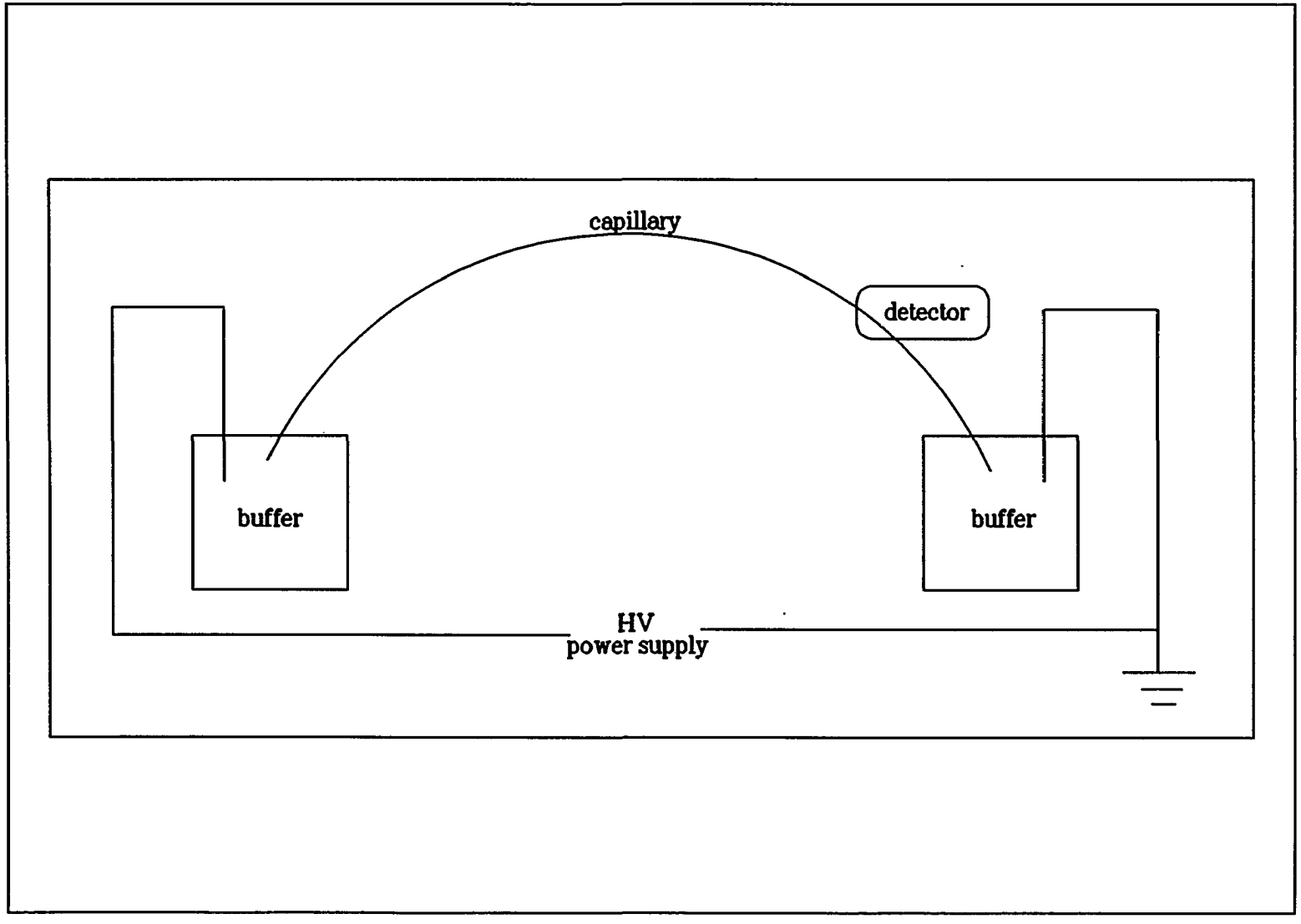
In research presented in Paper I of this dissertation, a variation of this LC method is applied to the determination of water in soil and clay samples. A single cation-exchange column in the $H^+$ form is used as the catalyst of the reaction between *trans*-cinnamaldehyde and methanol in the eluent. Methanol is used to extract water from the samples. The methanol extract is then analyzed for water content. Experimental conditions were optimized for the fast and accurate determination of water in soil and clay samples representing a wide range of water concentrations.

## Capillary Electrophoresis

Electrophoresis is a process based on the migration of charged species under the influence of an electric field. Tiselius introduced moving boundary electrophoresis as a separation technique for proteins in the 1930's (20-21). He found that components of a protein mixture migrated in directions and rates determined by their charge and mobility when placed in a tube between two buffers and an electric field applied. The efficiency of his separations were limited by thermal diffusion and convection. In 1967, Hjerten demonstrated the use of a high field strength in free solution electrophoresis using 3 mm inner diameter (i.d.) tubes (22). In this system, thermal convection was minimized by rotation of the tube along its longitudinal axis. Later, Mikkers described the advantages of using smaller diameter (200 μm) teflon columns to reduce the effect of convection on separation efficiency (23). Perhaps the most significant contribution was by Jorgenson and Lukacs (24-26). They described the theory of dispersion in CE and demonstrated the high efficiency obtained using narrow (75 μm) glass capillaries and a high field strength. Commercial CE instruments were first available in the late 1980's and since that time, significant strides have been made in the development of CE as an analytical technique.

One of the attractive features of CE is the simplicity of the instrumentation. A schematic diagram of a basic CE instrument is shown in Figure 1. The system consists of a high-voltage power supply, two buffer reservoirs, a capillary, and a detector. Narrow-bore capillaries (25-100 μm i.d.) with typical lengths of 25-100 cm are used. The high heat dissipation efficiencies of these narrow columns allow separations to be performed using high voltages of 10-30 kV and thus high field strengths (200-500 V/cm). These conditions

**Figure 1.** Schematic diagram of a basic CE instrument.


capillary
detector
buffer
buffer
HV
power supply

result in short analysis times and separation efficiencies of $10^5$-$10^6$ theoretical plates. Most systems are equipped with on-line detection and automated sample loading of volumes in the nanoliter range.

There are two injection modes used for CE: hydrodynamic and electrokinetic (27). In hydrodynamic injection, sample is introduced into the capillary by applying a pressure across the capillary while one capillary end is immersed in the sample reservoir. The hydrodynamic techniques used for sampling are positive pressure, vacuum, and gravity. The main advantage of hydrodynamic injection is that a representative sample plug is injected. One drawback is the band broadening which may occur due to the parabolic profile of the plug which is characteristic of pressure-driven flow.

Electrokinetic injection is accomplished by applying a voltage across the capillary while one end of the capillary is immersed in the sample solution. The sample is drawn into the capillary by electrophoretic and electroosmotic flow. The amount of each solute injected depends on its corresponding electrophoretic mobility and the electrical conductivity of the sample buffer and running buffer and the electroosmotic flow. Since the amount of each solute injected is a function of several parameters which are often difficult to control, reproducible sample injection is a problem.

There are several modes of CE separations: capillary zone electrophoresis (CZE) (28-30), capillary gel electrophoresis (CGE) (31-33), micellar electrokinetic chromatography (MEKC or MECC) (34-37), capillary isoelectric focusing (CIEF) (38-40), capillary isotachophoresis (CITP) (41-43), and capillary electrochromatography (CEC) (44-46).

Free solution CZE is the most commonly used mode due to its versatility and

simplicity of operation. CZE is used to separate ionic species based on differences in their electrophoretic mobilities and hence their velocities. The velocity of an ion (v) is given by:

$$v = \mu_e E \qquad (1)$$

where

E = applied electric field (volts/cm)

$\mu_e$ = electrophoretic mobility of the ion given by:

$$\mu_e = q/(6\pi\eta r) \qquad (2)$$

where

q = ion charge

$\eta$ = solution viscosity

r = ion radius

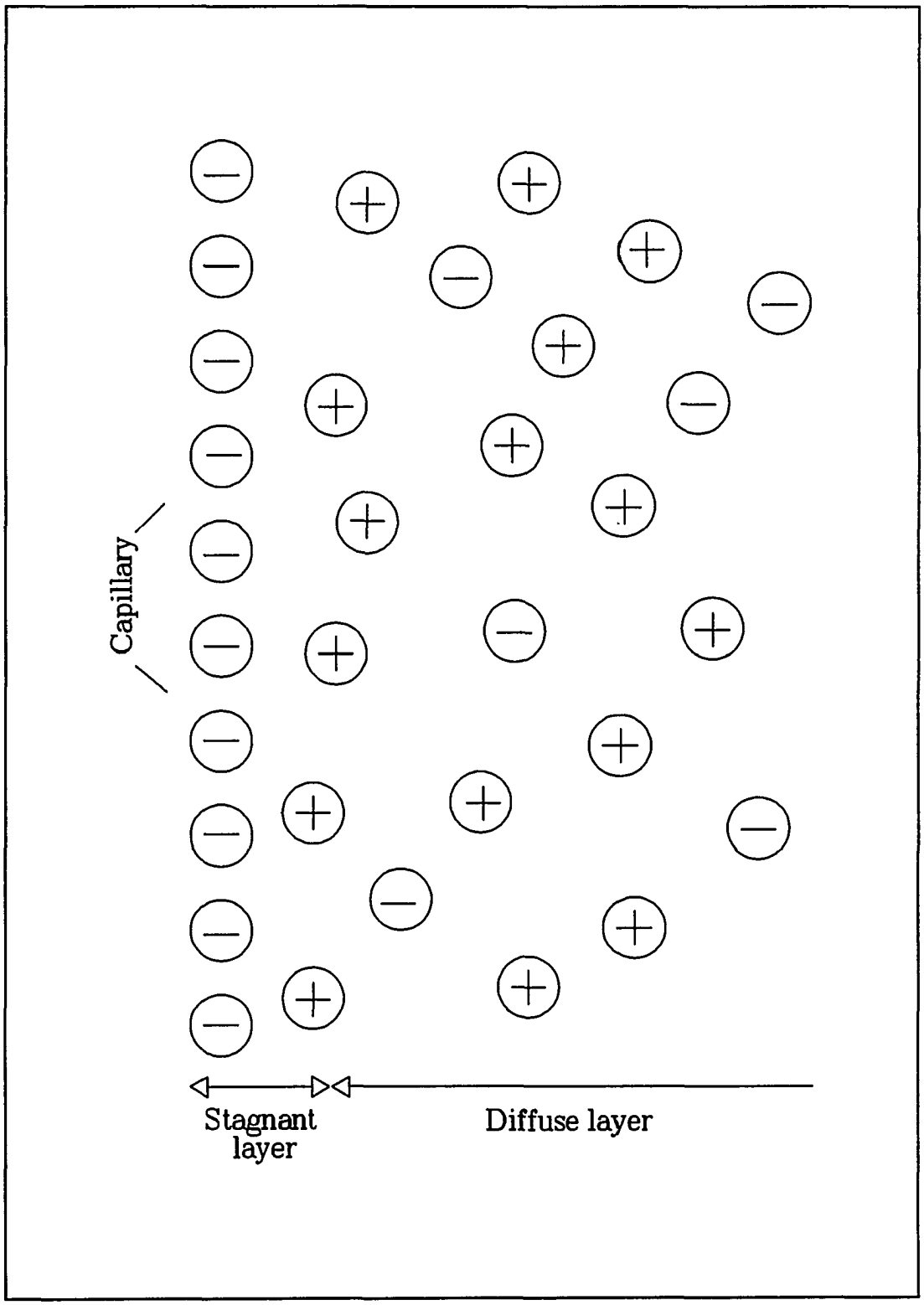
Small highly charged species have high mobilities whereas, minimally charged species have low mobilities.

An important process in CE is electroosmotic flow (EOF). EOF is the bulk flow of the liquid in the capillary. Fused silica capillaries are usually used for CZE. Under typical experimental conditions, the inner capillary wall is negatively charged from the ionized silanol groups. Counter cations which are electrostatically attracted to the capillary surface are in the stagnant double layer adjacent to the capillary walls. This cationic charge extends into the diffuse mobile layer (Fig 2). The potential difference across the layers is called the zeta potential, $\zeta$, and is given by the equation:

$$\zeta = 4\pi\eta\mu_{EOF}/\varepsilon \qquad (3)$$

where

**Figure 2.** Schematic diagram of the double layer of a silica surface.


Capillary
Stagnant layer
Diffuse layer

$\eta$ = solution viscosity

$\mu_{EOF}$ = electroosmotic flow coefficient

$\varepsilon$ = solution dielectric constant

When an electric field is applied to the capillary, the counter cations migrate toward the cathode and since they are solvated, they drag the bulk solution in the capillary along with them. The EOF velocity is given by:

$$v_{EOF} = \zeta\varepsilon E/(4\pi\eta) \qquad (4)$$

where

$\zeta$ = zeta potential

$\varepsilon$ = solution dielectric constant

E = electric field strength

$\eta$ = solution viscosity

Figure 3 shows a schematic diagram of the direction of EOF and electrophoretic flow of anions and cations under typical experimental conditions. In the configuration shown, injection occurs at the anode and detection at the cathode. The electrophoretic mobilities of cations are toward the cathode, whereas the mobilities of anions are toward the anode. Neutral species move with the EOF toward the cathode. Only the anions having mobilities with magnitudes greater than the magnitude of the EOF will not be detected under this scheme. The apparent mobility ($\mu_a$) observed for an ion is the sum of the effective electrophoretic and electroosmotic mobilities.

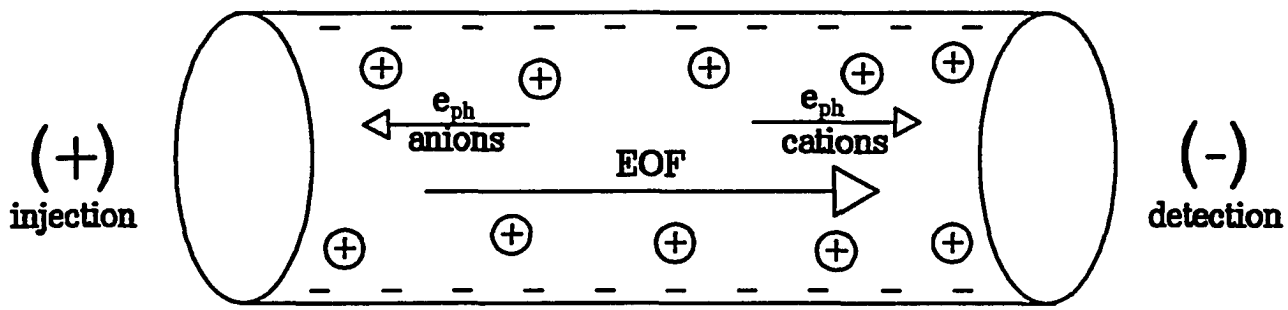
$$\mu_a = \mu_e + \mu_{EOF} \qquad (5)$$

where

$\mu_e$ = effective electrophoretic mobility

**Figure 3.** Schematic diagram of EOF and electrophoretic mobilities of anions and cations.

# Electroosmotic & Electrophoretic Flow:




Negatively charged wall: $SiOH \rightleftharpoons SiO^- + H^+$

⊕ = Hydrated cations

$\mu_{EOF}$ = electroosmotic mobility

The apparent electrophoretic mobility can be calculated using the migration time of the ion and the following equation:

$$\mu_a = lL/(tV) \qquad (6)$$

where

l = effective capillary length (injection to detector) (cm)

L = total capillary length (cm)

t = migration time of ion (sec)

V = applied voltage (v)

A neutral species such as acetone, benzene, or water may be used to measure the EOF. The electroosmotic mobility can be calculated using the migration time of the neutral species in equation 6. Thus, the effective electrophoretic mobility can be obtained by subtracting the EOF component from the apparent electrophoretic mobility.

$$\mu_e = lL/[(t-t_{EOF})V] \qquad (7)$$

where

t = migration time of ion (sec)

$t_{EOF}$ = migration time of neutral species (sec)

EOF is beneficial for some separations but not for others. For these latter instances it is necessary to control or eliminate EOF. There are several experimental parameters which may be modified to accomplish this. Several reviews have been published which describe the effects of these parameters on EOF (47-52).

The electric field may be varied resulting in a proportional increase in the EOF with

increasing applied voltage as given by equation 4 (53). There have been attempts to control EOF by electronic means. Lee et al. changed the direction and rate of EOF with the application of an external electric field to control the zeta potential (54-55). Ewing and coworkers presented a scheme for electronic control of EOF which applied a radial voltage field with a conductive polymer sheath (56-57).

Coated capillaries are used to control or eliminate EOF. These capillaries are primarily used for separations of biological compounds which adsorb to the walls of uncoated capillaries. Hjerten eliminated EOF in glass capillaries with coatings of non-cross-linked polyacrylamide (58). He demonstrated the utility of these capillaries by separating organic acids and proteins. Hydroxypropyl cellulose (59) and polyethylene glycol-modified (60) capillaries have also been used for the separation of proteins.

Temperature control is now available in many commercial CE instruments. This control is necessary in part because of the effect of temperature on EOF. As temperature is increased, the viscosity of the electrolyte solution decreases resulting in an increase in the EOF (eq 4).

EOF also changes with the ionic strength of the buffer solution. EOF decreases with increasing ionic strength due to a decrease in the zeta potential (eq 4). EOF varies linearly with the natural logarithm of the concentration of the electrolyte solution.

A convenient method of modifying EOF is to change the buffer pH. Lukacs and Jorgenson studied the effect of pH on the EOF in pyrex, fused silica, and teflon capillaries (61). In each case, the EOF increased with increased pH due to the presence of more ionized groups at the capillary wall. Varying the pH of the buffer solution may also change

the charge on the solute thus, affecting electrophoretic mobility.

Another way to modify the EOF is by adding cationic surfactants to the electrolyte solution. This method is effective not only for the suppression of EOF but also for its reversal. The most commonly used surfactants are quaternary ammonium salts (62-63). Tsuda studied the effect of the concentration of cetyltrimethylammonium bromide (CTAB) on EOF (64). Later, Yoshida et al. examined the effect of the chain length of the alkyl group on the quaternary ammonium salt using decyl-, dodecyl-, and tetradecyltrimethyl ammonium bromide (65). As the alkyl chain length increased the change in EOF increased and a reversed EOF was observed at a lower concentration. Chang and Yeung demonstrated the self-regulating dynamic control of EOF using CTAB (66). The final EOF in this system was not zero but was equal and opposite to the electrophoretic mobility of the CTAB. In order to achieve this effect, two buffer solutions that by themselves cause EOF in opposite directions were used with the more concentrated buffer at the anode. Applications using this method were limited to analyte ions that did not adsorb to or ion-pair with the CTAB.

A popular way to modify EOF is to add water-miscible organic solvents to the electrolyte solution (49,67-73). All of these studies used methanol and acetonitrile although higher aliphatic alcohols (68,71,73-74) and diols (70,72) were also used. When organic solvents are added in increasing amounts, the viscosity of the resulting solution initially increases while the dielectric constant decreases (74). The magnitude of the zeta potential also decreases (72). According to equations 3 and 4, the changes described should combine to reduce EOF. While there is general agreement of this relationship, there is not a clear understanding of how the surface charge, zeta potential, and double layer are affected by the

organic solvent.

A variety of explanations have been presented. First, it has been suggested that when alcohols are added to the electrolyte solution, they interact to different degrees with the capillary wall, depending on the alcohol chain length (70-71,73). This interaction leads to a masking of the surface charge and an increase in the viscosity in the double layer. Van Orman et al. (71) showed that alcohol-containing electrolyte solutions with the same bulk viscosity resulted in different decreases in EOF, depending on the interaction of the particular alcohol with the capillary wall.

Salomon (49) conjectured that the addition of methanol to the electrolyte solution does not affect the surface charge equilibrium, but does increase the number of ionized silanol groups on the capillary wall by shielding the charged ions from each other, thus, allowing more ions to exist in a given area. This should increase the zeta potential and thus EOF. However, this increase is offset by the decrease in EOF due to the shrinking of the double layer resulting from the addition of methanol.

Schwer and Kenndler (73) attributed the observed changes to a decrease in the zeta potential due to a combination of effects. First, the $pK_a$ of the silanol group was shifted to higher values in the presence of organic solvents. Also, the dielectric constant of the solution was reduced whereas, the viscosity increased. The adsorption of solvent molecules to the silica surface caused changes in the viscosity and dielectric properties of the double layer. This adsorption resulted in the substitution of solvent molecules for water molecules and thus decreased the number of adsorbed hydroxy ions, since the solvents studied were less able to stabilize anions via hydrogen bonding compared to water.

In contrast, Issaq et al. concluded from their studies of acetonitrile, methanol, ethanol, and isopropyl alcohol that changes in EOF solely parallel changes in the solution viscosity irrespective of the nature of the organic solvent (74).

Research presented in Papers II and III of this dissertation use the effects of pH, quaternary ammonium salts, and organic solvents on the EOF and electrophoretic mobilities to optimize separations of several anions. Paper II describes the effect of a low concentration of a quaternary ammonium salt and 1-butanol on the separation of several inorganic and short-chain organic acid anions. Paper III examines the effect of pH and concentration of acetonitrile in the electrolyte solution on the separation of several alkyl-substituted phenolate anions.

# PAPER I. LIQUID CHROMATOGRAPHIC METHOD FOR DETERMINATION OF WATER IN SOILS

# LIQUID CHROMATOGRAPHIC METHOD FOR DETERMINATION OF WATER IN SOILS

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## SUMMARY

A simple, rapid, and sensitive liquid chromatographic (LC) method for the determination of water in soils was developed. In this method, water is extracted from soil with anhydrous methanol and injected into an LC system including a cation-exchange column in the $H^+$ form. The eluent is 1.0 m*M trans*-cinnamaldehyde in acetonitrile-methanol (40:60). The detection scheme is based on the effect of water on the equilibrium established when *trans*-cinnamaldehyde and methanol react in the $H^+$ column to form *trans*-cinnamaldehyde dimethylacetal and water. When water is introduced into the column, the equilibrium of the reaction is shifted towards the *trans*-cinnamaldehyde, which absorbs strongly at the detection wavelength. The extent of the shift and the resulting change in absorbance are proportional to the amount of water present in the sample.

Application of the method to a wide range of soils and of clay minerals containing from 0.7 to 25% water showed that the results of the LC method agreed closely with those obtained by a gravimetric method. The LC method is accurate, precise, relatively free from interference, requires a small sample size, and gives a linear calibration graph over approximately three orders of magnitude of water concentrations.

# INTRODUCTION

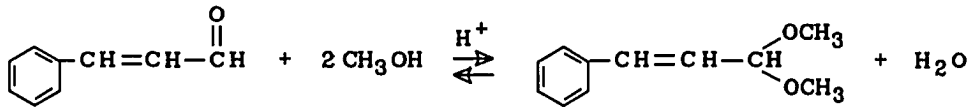
The determination of water content in soils is one of the most widely used measurements. Knowledge of the water content of soil or clay samples is essential before chemical or physical analyses are performed. The amount of water in soil usually is determined gravimetrically by heating a known weight of sample at about 105°C for 12 to 24 hours to remove the water. This method was proposed by Whitney (1) and since has been modified by Davisson and Sivaslian (2), and by Zamyatina and Chernikova (3). Other methods proposed for the determination of water in soils include the use of heat generated by propane gas instead of an electric furnace or by using a volumetric method in combination with the specific gravity of soil (4,5). More recently, the use of a microwave oven as a source of heat for the determination of water content in soils has been proposed by Miller et al. (6) and modified by Hankin and Sawhney (7). All these methods are tedious, time consuming, or require a large sample size for accurate determination of water.

Although no results were presented, Bouyoucos (8) reported that water, down to considerably below air-dried concentrations, can be extracted from soils almost instantaneously with alcohol and determined in less than five minutes by using a hydrometer to determine the difference in specific gravities of pure alcohol and the alcohol-water mixture. Bouyoucos claimed that results by this method were similar to those obtained by a gravimetric method.

In recent years, liquid chromatography (LC) has become a rapid and sensitive technique for analyzing complex mixtures, and LC systems now are available in many

laboratories. Water has been determined chromatographically via an ion-exclusion mechanism using conductivity detection (9). Recently, Fortier and Fritz (10), and Chen and Fritz (11,12), developed a liquid chromatographic method for the determination of water that combines separation by ion-exclusion chromatography with a novel and sensitive spectrophotometric method of detection. The method is very fast and has few interferences. A linear calibration plot is obtained over a wide range of water concentrations.

The chromatographic separation of water takes place on a cation-exchange column. The water is separated from any sample matrix compounds as it partitions between the eluent and the occluded liquid in the resin. The eluent contains a low concentration of *trans*-cinnamaldehyde in an acidic acetonitrile-methanol mixture. The following equilibrium is established in the eluent:



*trans*-Cinnamaldehyde + 2 $CH_3OH$ $\overset{H^+}{\rightleftharpoons}$ *trans*-Cinnamaldehyde dimethyl acetal + $H_2O$ (1)

Because the eluent is essentially water free after a drying step, the equilibrium lies far to the right. Thus, the background absorbance is due primarily to the acetal, which has only a weak absorbance at the detection wavelength. Within the sample zone, which contains the water, the equilibrium is forced to the left, towards the cinnamaldehyde. Because the cinnamaldehyde absorbs strongly at the detection wavelength, an increase in absorbance is observed as the sample zone passes through the detector cell.

The objective of this work was to apply this method to the determination of water in soil and clay samples.

# MATERIALS AND METHODS

***Soils:*** The nine soils used in this study were surface samples selected to represent a wide range of pH values (5.9 to 7.5), textures (18 to 36% clay and 4 to 40% sand), organic C (1.5 to 4.4%), and moisture contents (3.8 to 25%). Before use, the soil samples were passed through a 2-mm mesh sieve. Subsamples of four soils were ground and passed through a 100 mesh sieve (150 μm).

***Clay Minerals:*** The three clay minerals used, Bentonite (Volclay), Kaolinite (Peerless), and Panther Creek Bentonite, were stored under air-dried conditions. The clay samples were ground to a 100 mesh sieve (150 μm) size.

***Liquid Chromatograph:*** The chromatographic system consisted of a Gilson Model 302 HPLC pump, a Model 802B Gilson manometric module (Gilson, Middleton, WI), a Model LP-21 Scientific Systems pulse dampener (Scientific Systems, State College, PA), a Model 7000 Rheodyne injection port (Rheodyne, Berkeley, CA) equipped with a 10-μL sample loop, a Kratos Spectroflow 783 UV-Vis detector (Applied Biosystems, Ramsey, NJ), a Hitachi D-2000 Chromato-Integrator (EM Science, Cherry Hill, NJ), and a Fisher Recordall recorder (Fisher Scientific/Instrument Lab, Itasca, IL).

***Reagents:*** *trans*-cinnamaldehyde, 99% (Aldrich Chemical, Milwaukee, WI), anhydrous trimethyl orthoformate (Aldrich), HPLC grade acetonitrile (Fisher Scientific, Pittsburgh, PA), anhydrous methanol (Mallinckrodt, St. Louis, MO), and reagent-grade sulfuric acid all were used without purification.

***Column Preparation:*** The Aminex Q-150S resin, 20-30 μm particle size, (Bio-Rad,

Richmond, CA) was received in the $Na^+$ form and converted to the $H^+$ form using a 1.0 *M* $H_2SO_4$ methanolic solution. No special precaution was taken to protect the resin from atmospheric moisture during equilibration. The resin was packed into a stainless steel column (27.5 mm x 4.6 mm I.D.) by using a Shandon single piston packing pump (Shandon Southern, Sewickley, PA) and an upward slurry packing method using acetonitrile-methanol (40:60) as the packing solvent. The packed column in the $H^+$ form was equilibrated further by pumping a 0.1 *M* $H_2SO_4$ methanolic solution through the column for approximately two hours.

***Eluent and Standards:*** A weighed amount of *trans*-cinnamaldehyde was dissolved in dry acetonitrile-methanol (40:60) to prepare a 1 m*M* solution. Approximately 5 m*M* $H_2SO_4$ was added to the eluent. The eluent was passed through a Nylon-66 filter (Rainin, Woburn, MA) before degassing. The eluent was dried each day by adding an appropriate amount of trimethyl orthoformate (TMOF) as it was being pumped through the detector. The following reaction occurs between the water and the TMOF:

$$\underset{\text{TMOF}}{CH(OCH_3)_3} + H_2O \xrightarrow{H^+} HCO_2CH_3 + 2CH_3OH \qquad (2)$$

The TMOF was added approximately 0.25 mL at a time, using a disposable pipet. After each addition, the eluent was mixed thoroughly. The eluent was protected from the atmosphere between TMOF additions by capping the container. As TMOF was added to the eluent, the detector signal decreased. Sufficient TMOF had been added when the absorbance of the eluent no longer decreased. The total amount of TMOF required to dry a volume of eluent varies depending upon how dry the acetonitrile and methanol are

initially. Typically 1-3 mL of TMOF may be added to 2 L of eluent. After being dried, the eluent was stored under nitrogen to avoid any atmospheric contact.

Standard samples were prepared by adding accurately weighed amounts of water to anhydrous methanol. The concentrations of the standards used for preparation of the calibration plot ranged from $5.0 \times 10^{-4}$ g/mL to $5.0 \times 10^{-2}$ g/mL.

***Chromatographic Conditions:*** The following chromatographic conditions were employed for all experimental work: flow rate of 1.5 mL/min, detector wavelength of 300 nm, and chart speed of 0.1 in/min.

***Procedure:*** Special care was taken throughout the procedure to avoid unnecessary exposure of the soil sample, methanol, and extract to the atmosphere. Any adsorption of atmospheric moisture or evaporation of methanol could lead to erroneous results. A soil sample (*ca.* 3 g of field-moist or partly-dried soil or 1 g of air-dried soil or clay) was rapidly transferred to and weighed in a septum-capped (Supelco, Bellefonte, PA) vial. A volume of anhydrous methanol (5 mL for 1 g sample or 25 mL for 3 g sample) was transferred, with a gas-tight syringe, from a septum-capped bottle to the vial containing the soil. The vial containing the soil sample and methanol was placed on a wrist-action shaker and shaken for 12 h at room temperature (23-25°C). A shaking time of 12 h was used to obtain the experimental results shown in this paper. A shaking period of 30 min later was found sufficient, however, for quantitative extraction of water from soil and clay mineral samples. After shaking, the tube was centrifuged for approximately 1 min or until the supernatant was clear of soil particles. A gas-tight syringe was used to remove the liquid layer from the vial. A syringe filter was attached to the syringe tip, and the needle attached to the filter. Two different sized syringe

filters were used, depending on sample size: 25 mm, 0.45 μm pore size PTFE (Cole-Parmer, Chicago, IL); or 13 mm, 0.45 μm pore size PTFE (Supelco, Bellefonte, PA). The liquid layer was filtered into another septum-capped vial. The samples were injected into the column and analyzed using the chromatographic conditions described previously.

The results obtained by the LC method (expressed on a water-free basis) were compared with those by a gravimetric method (105°C for 72 h). Unless otherwise indicated, all results by the gravimetric method are averages of five determinations and are expressed on an oven-dried basis.

## RESULTS AND DISCUSSION

The various factors affecting the chromatographic method for determining water have been studied previously (10-12). In applying this method to the determination of water in soils, we studied systematically the factors affecting extraction of water from soils by anhydrous methanol. These factors included the soil/methanol ratio and the shaking time.

Separation and detection of water peaks by the method described can be accomplished in about 5 min with excellent resolution and baseline stability. Chromatograms of successive injections of standard water samples prepared in anhydrous methanol and anhydrous methanol extracts obtained from soils are shown in Figs. 1A and 1B, respectively.

Calibration graphs prepared for standard water samples in anhydrous methanol analyzed by the LC method described were linear, with the detector response up to 50 $mgL^{-1}$. The peak heights were highly reproducible, with a stable baseline and peak resolution in five min (Fig 2).

Several soil and clay samples representing a wide range of water concentrations were studied. Air-dried clay minerals and soil samples containing from 0.6 to 13.8% water and field-moist soil samples containing from 12-25% water were analyzed. Comparisons of the results obtained by the LC and gravimetric methods for < 2 mm particle size field-moist and partly-dried soil samples are shown in Tables I and II, respectively. Table III shows the water contents obtained for < 80 mesh and < 2 mm samples of air-dried soils. Three clay minerals were also studied and a comparison of the results are shown in Table IV. In

**Figure 1.** Chromatograms of successive injections of standard water samples (A): a, $4.0 \times 10^{-3}$ g/mL; b, $5.0 \times 10^{-3}$ g/mL; c, $6.0 \times 10^{-3}$ g/mL, and of water in methanol extracts obtained from soils (B): a, 22.6%; b, 18.4%; c, 14.6%.

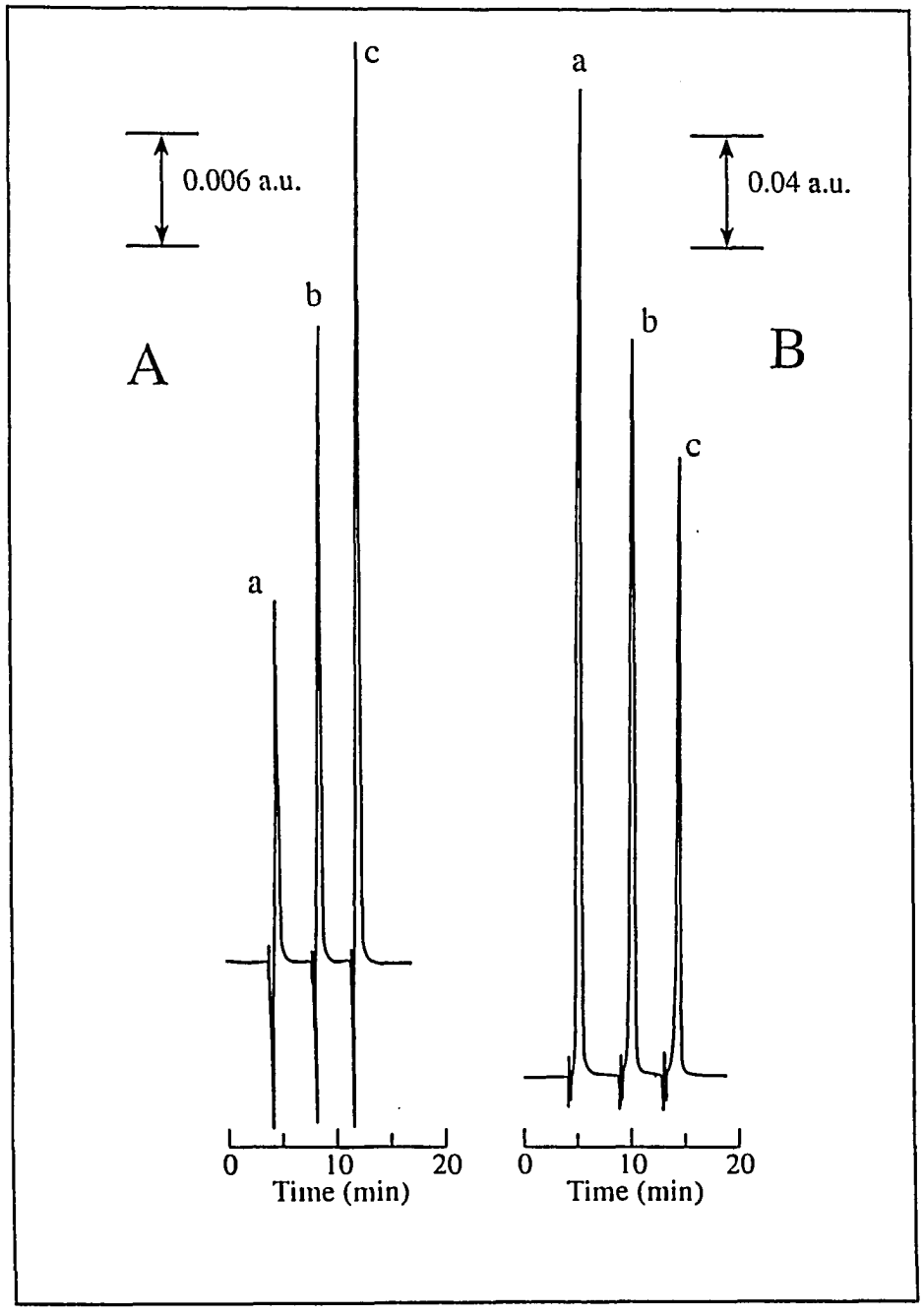

0.006 a.u.
0.04 a.u.
A
B
a
b
c
a
b
c
0
10
20
Time (min)
0
10
20
Time (min)

**Figure 2.** Chromatograms of successive injections of a standard water sample (2.5 x $10^{-3}$ g/mL).

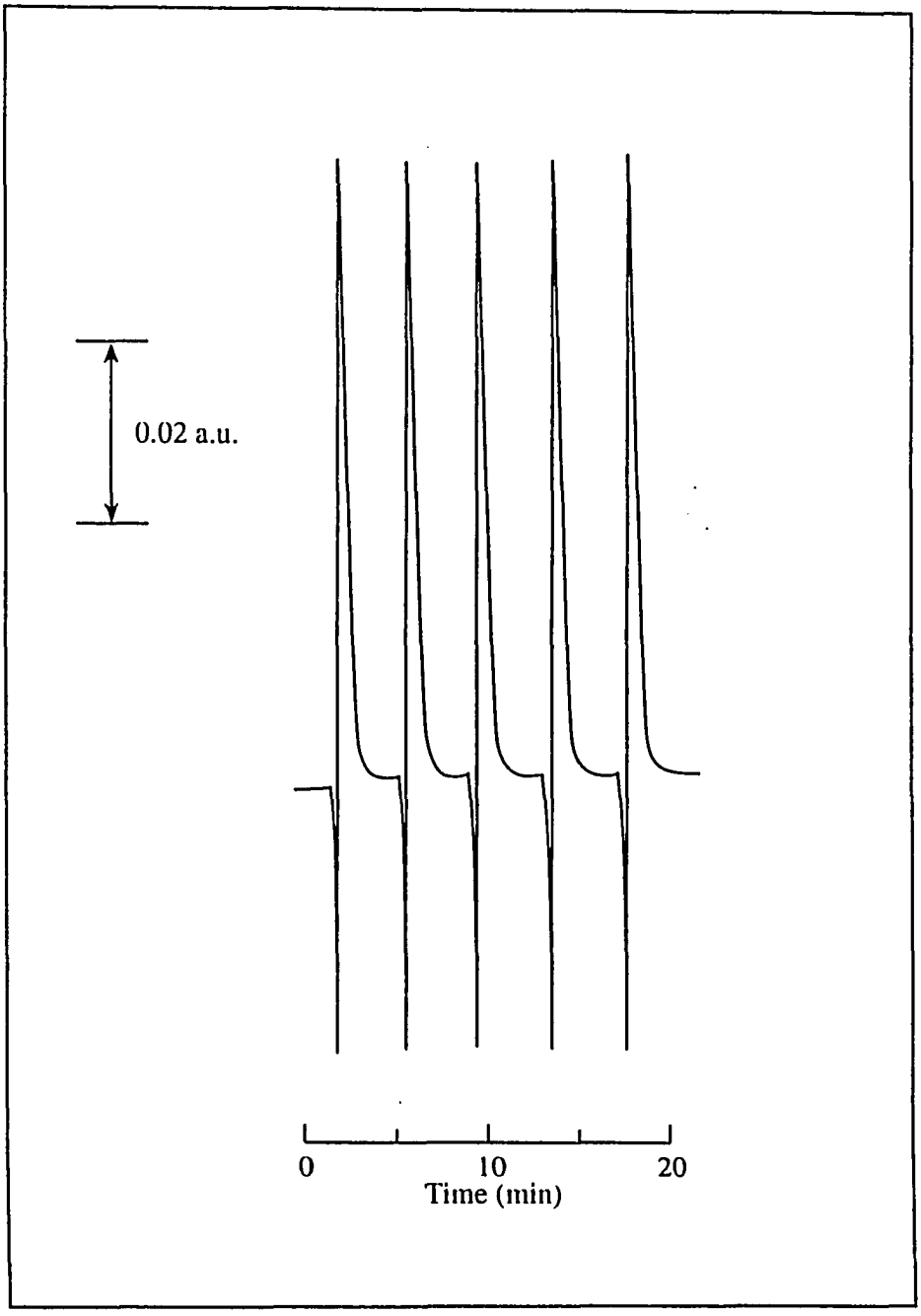

0.02 a.u.
0
10
20
Time (min)

**Table I.** Comparison of results obtained by the LC and gravimetric methods for field-moist soil samples.

| Soil Identity | LC Results[a] < 2 mm | Gravimetric Results[b] < 2 mm |
|---|---|---|
| Grundy | $\overline{X}$ = 22.9%<br>s.d. = 0.4<br>R = 1.2 | $\overline{X}$ = 22.6%<br>s.d. = 0.4<br>R = 1.3 |
| Okoboji | $\overline{X}$ = 22.2%<br>s.d. = 0.7<br>R = 1.9 | $\overline{X}$ = 20.8%<br>s.d. = 0.4<br>R = 0.9 |
| Weller | $\overline{X}$ = 19.4%<br>s.d. = 0.3<br>R = 1.0 | $\overline{X}$ = 18.3%<br>s.d. = 0.3<br>R = 0.8 |
| Fayette | $\overline{X}$ = 24.2%<br>s.d. = 0.7<br>R = 2.1 | $\overline{X}$ = 23.3%<br>s.d. = 0.4<br>R = 1.2 |
| Pershing | $\overline{X}$ = 21.9%<br>s.d. = 0.5<br>R = 1.4 | $\overline{X}$ = 20.3%<br>s.d. = 0.3<br>R = 0.8 |

[a] Water content determined by the liquid chromatographic method. Statistics of eight replicated determinations: $\overline{X}$, mean; s.d., standard deviation; R, range.

[b] Water content determined by the gravimetric method. Statistics of five replicated determinations.

**Table II.** Comparison of results obtained by the LC and gravimetric methods for partly-dried soil samples.

| Soil Identity | LC Results[a] < 2 mm | Gravimetric Results[b] < 2 mm |
|---|---|---|
| Grundy II | $\overline{X}$ = 17.3%<br>s.d. = 0.3<br>R = 0.8 | $\overline{X}$ = 17.2%<br>s.d. = 0.4<br>R = 0.9 |
| Okoboji II | $\overline{X}$ = 19.4%<br>s.d. = 0.4<br>R = 1.2 | $\overline{X}$ = 19.2%<br>s.d. = 0.2<br>R = 0.6 |
| Weller II | $\overline{X}$ = 12.0%<br>s.d. = 0.3<br>R = 0.7 | $\overline{X}$ = 11.8%<br>s.d. = 0.1<br>R = 0.2 |
| Fayette II | $\overline{X}$ = 19.4%<br>s.d. = 0.2<br>R = 0.4 | $\overline{X}$ = 20.0%<br>s.d. = 0.2<br>R = 0.5 |
| Pershing II | $\overline{X}$ = 15.4%<br>s.d. = 0.4<br>R = 1.0 | $\overline{X}$ = 15.2%<br>s.d. = 0.1<br>R = 0.3 |

[a] Water content determined by the liquid chromatographic method. Statistics of eight replicated determinations: $\overline{X}$, mean; s.d., standard deviation; R, range.

[b] Water content determined by the gravimetric method. Statistics of five replicated determinations.

**Table III.** Comparison of results obtained by the LC and gravimetric methods for air-dried soil samples.

| Soil Identity | LC Results < 2 mm | LC Results < 80 mesh | Gravimetric Results < 2 mm | Gravimetric Results < 80 mesh |
|---|---|---|---|---|
| Harps | $\overline{X}$ = 6.1%<br>s.d. = 0.2<br>R = 0.5 | $\overline{X}$ = 6.3%<br>s.d. = 0.1<br>R = 0.3 | $\overline{X}$ = 5.7%<br>s.d. = 0.2<br>R = 0.4 | $\overline{X}$ = 5.1%<br>s.d. = 0.2<br>R = 0.5 |
| Clarion | $\overline{X}$ = 4.7%<br>s.d. = 0.1<br>R = 0.2 | $\overline{X}$ = 4.6%<br>s.d. = 0.1<br>R = 0.3 | $\overline{X}$ = 4.3%<br>s.d. = 0.04<br>R = 0.1 | $\overline{X}$ = 3.7%<br>s.d. = 0.3<br>R = 0.8 |
| Webster | $\overline{X}$ = 4.3%<br>s.d. = 0.05<br>R = 0.1 | $\overline{X}$ = 4.5%<br>s.d. = 0.1<br>R = 0.3 | $\overline{X}$ = 3.8%<br>s.d. = 0.1<br>R = 0.2 | $\overline{X}$ = 3.3%<br>s.d. = 0.1<br>R = 0.3 |
| Nicollet | $\overline{X}$ = 4.0%<br>s.d. = 0.1<br>R = 0.2 | $\overline{X}$ = 3.8%<br>s.d. = 0.2<br>R = 0.6 | $\overline{X}$ = 3.8%<br>s.d. = 0.2<br>R = 0.4 | $\overline{X}$ = 2.7%<br>s.d. = 0.2<br>R = 0.4 |

[a] Water content determined by the liquid chromatographic method. Statistics of eight replicated determinations: $\overline{X}$, mean; s.d., standard deviation; R, range.

[b] Water content determined b for all separatc method. Statistics of five replicated determinations.

**Table IV.** Comparison of results obtained by the LC and gravimetric methods for air-dried clay minerals.

| Clay Identity | LC Results < 80 mesh | Gravimetric Results < 80 mesh |
|---|---|---|
| Kaolinite (Peerless #2) | $\overline{X}$ = 0.7%<br>s.d. = 0.1<br>R = 0.2 | $\overline{X}$ = 0.6%<br>s.d. = 0.02<br>R = 0.04 |
| Bentonite (Volclay) | $\overline{X}$ = 7.0%<br>s.d. = 0.3<br>R = 0.9 | $\overline{X}$ = 7.2%<br>s.d. = 0.2<br>R = 0.5 |
| Panther Creek (Bentonite) | $\overline{X}$ = 13.9%<br>s.d. = 0.3<br>R = 0.9 | $\overline{X}$ = 12.8%<br>s.d. = 0.1<br>R = 0.3 |

[a] Water content determined by the liquid chromatographic method. Statistics of eight replicated determinations: $\overline{X}$, mean; s.d., standard deviation; R, range.

[b] Water content determined by the gravimetric method. Statistics of five replicated determinations.

general, the results obtained by the LC method agreed well with those obtained with the gravimetric method as shown by the 1:1 plot in Figure 3.

A shaking period of 12 h initially was thought necessary for complete extraction of the water from the soil samples. But several shaking times from 0.5 to 12 h were studied, and the results obtained with 0.5 h shaking were consistent with those with longer shaking times (Fig 4). An investigation into the need for continuous shaking also was conducted. An extraction time of 0.5 h with occasional shaking (i.e. brief shaking by hand every 10 min) was satisfactory for complete extraction of the water from the soil. Several extraction times with no shaking also were studied. The extraction of water from these samples was incomplete, and low values were obtained.

**Figure 3.** Comparison of water contents of soils and clay minerals obtained by the gravimetric method and the LC method.

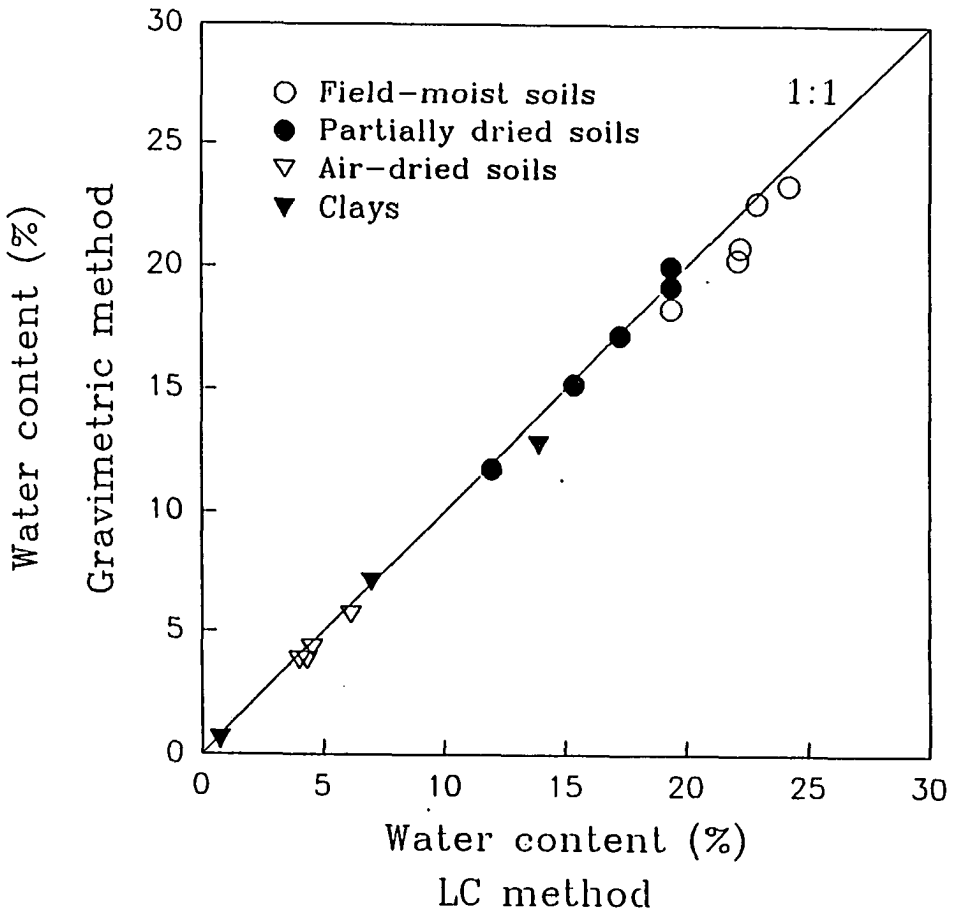
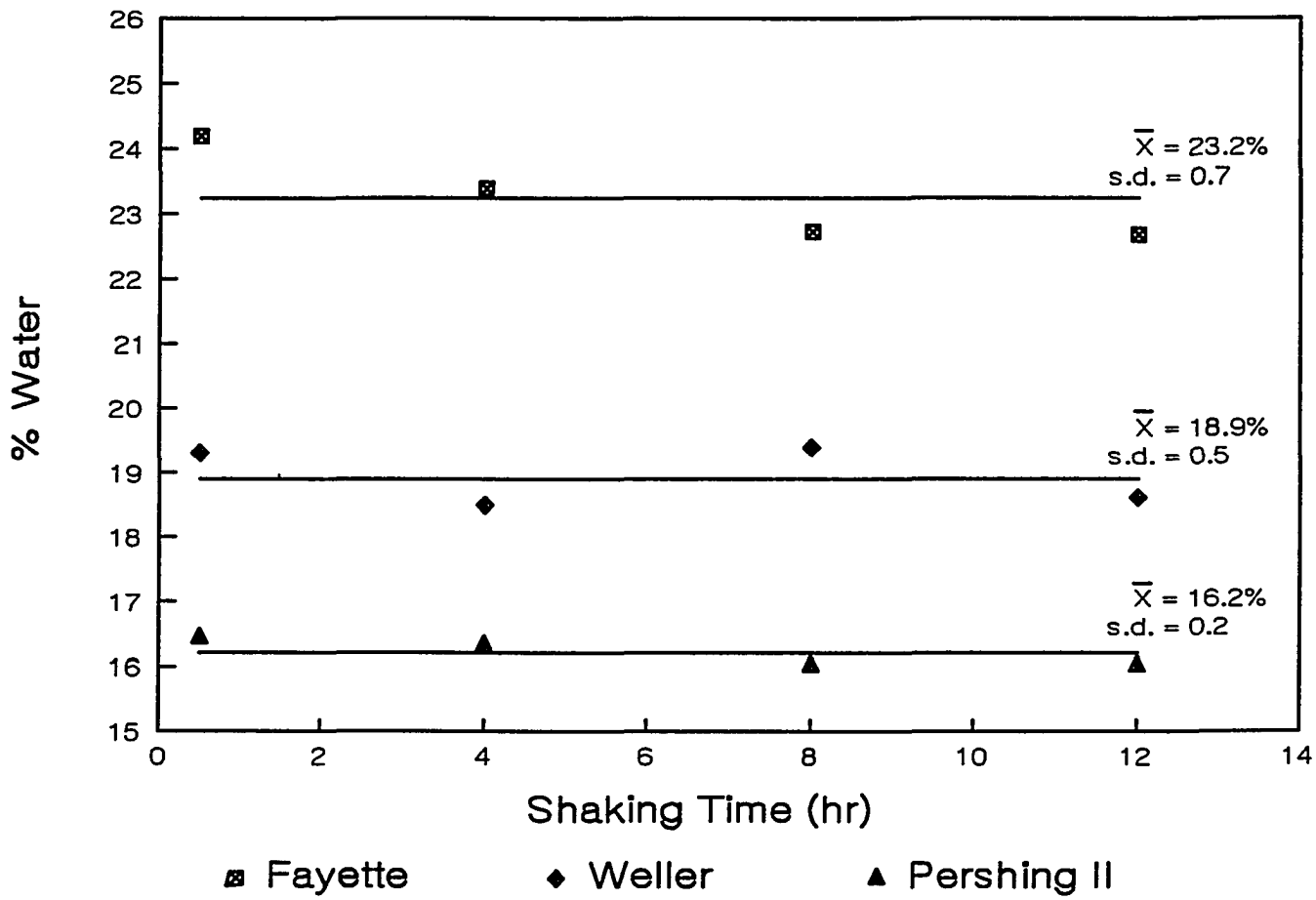

Water content (%)
Gravimetric method
30
25
20
15
10
5
0
Field-moist soils
Partially dried soils
Air-dried soils
Clays
1:1
0
5
10
15
20
25
30
Water content (%)
LC method

**Figure 4.** Comparison of water contents obtained using different shaking times.

# Effect of Shaking Time on Water Recovery



# CONCLUSIONS

The water contents obtained by the LC method corresponded quite well with those obtained by the gravimetric method. In general, results by the LC method were as precise as those by the gravimetric method. It may be noted, however, that the LC method measures only water, whereas loss in weight on drying is less specific. The LC method is much faster than the gravimetric procedure, especially for clay samples that may require as long as 96 h to dry. The LC method also can be used to determine very low concentrations of water.

## ACKNOWLEDGEMENTS

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# PAPER II. STUDIES ON THE DETERMINATION OF INORGANIC ANIONS BY CAPILLARY ELECTROPHORESIS

# STUDIES ON THE DETERMINATION OF INORGANIC ANIONS BY CAPILLARY ELECTROPHORESIS

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# SUMMARY

In order to separate anions effectively by capillary zone electrophoresis (CZE) it is usually necessary to eliminate or reverse the direction of electroosmotic flow (EOF) resulting from attraction of cations to the negatively charged surface of a silica capillary. Previous workers have accomplished this by adding a quaternary ammonium salt ($Q^+$) as an EOF "modifier". Under typical experimental conditions, a concentration of $Q^+ > 0.25$ mM is required to reverse the EOF direction. Addition of a low percentage of 1-butanol to the aqueous electrolyte was found to reduce the EOF. A combination of butanol and a very low concentration of $Q^+$ (typically 0.03 mM) was found to be particularly effective in controlling EOF and in giving good separations of complex mixtures of anions.

# INTRODUCTION

Capillary zone electrophoresis (CZE) is an efficient technique for separating inorganic and organic anions. Several separations of inorganic and short-chain organic acid anions have been reported using indirect UV detection (1-5) and conductivity detection (6-7).

In conventional CZE using a fused-silica capillary, the electroosmotic flow (EOF) is toward the cathode where detection is performed. Anions, however, have electrophoretic mobilities toward the anode. Only anions having mobilities with magnitudes less than that of the EOF are detected with this configuration. Thus, it is necessary to eliminate or reverse the direction of the EOF for most anion separations.

There are several ways to control EOF. These include altering the buffer pH (8-10) or the electrolyte composition by adding surfactants (6,11-12) or organic solvents (10,13-14). Other methods include coating the inner capillary walls (15-17) or applying an external electric field (18-20). Several studies of the effects of these parameters on anion separations have been published (21-23).

The most common method used to reverse the EOF for anion separations is to add a quaternary ammonium salt to the electrolyte solution. The positively charged compound is electrostatically attracted to the ionized capillary wall, thus creating a net positive charge on the wall. With the use of a negative power supply, all anions are detected at the anode.

In HPLC, Morris and Fritz (24) found that chromatographic behavior of polar compounds can be dramatically modified by use of a suitable mobile phase additive. A concentration of 4-5% 1-butanol in water was found to be particularly useful. A dynamic

equilibrium of butanol exists between the mobile phase and the resin, thus coating the resin with a thin film of butanol.

It was thought that a similar effect was likely to occur in CZE. Again, a dynamic equilibrium of butanol is expected between the aqueous electrolyte and the walls of the silica capillary. Several anion separations have been reported which use electrolyte solutions with organic solvent additives (2,22-23). Most of these studies have been limited to low molecular weight solvents such as methanol, ethanol, and acetonitrile.

In the present work, it is shown that a combination of low concentrations of a quaternary ammonium salt and 1-butanol added to the electrolyte solution affects anion separations. EOF can be controlled more easily when butanol is present and excellent anion separations are possible.

# EXPERIMENTAL

The CZE system used for all experiments was the Waters Quanta 4000 (Waters Chromatography Division of Millipore, Milford, MA, USA). Fused-silica capillaries (Polymicro Technology, Phoenix, AZ, USA) with lengths of 70 cm and inner diameters of 75 μm were used. At 62.5 cm from the injection end of the capillary, the polyimide coating was burned off to create a detection window.

New capillaries were conditioned by rinsing with 1 M NaOH for approximately 1 h, followed by a 15-min rinse with deionized water. A 5-min rinse with NaOH, followed by a 5-min rinse of deionized water was used to wash the capillaries at the start of each day and between runs with different electrolyte solutions.

Electrokinetic sampling was used with a sample voltage of 10 kV for 5 s (unless otherwise specified) or the hydrostatic sampling mode was used with a sampling time of 20 s and height of 10 cm. The capillary was purged with electrolyte solution for 2 min before each run. All separations were carried out at room temperature. The negative or positive power supply was used at 30 kV for each experiment.

On-column indirect UV detection at 254 nm was used for all separations of inorganic and short-chain organic acid anions. The electrolyte solutions for these separations contained 5 mM sodium chromate as the visualization reagent. Direct UV detection at 254 nm was used for the separation of the aromatic organic acid anions.

All solutions were prepared using deionized water from a Barnstead Nanopure II system (Sybron Barnstead, Boston, MA, USA). All reagents and solvents used were reagent grade.

Stock solutions of the inorganic anions were prepared from their corresponding sodium salts. Stock solutions of the organic acids were prepared from the organic acid and the pH raised to form the anion. The chromate electrolyte solutions were prepared from a stock solution of 100 mM sodium chromate (Fisher Scientific, Fair Lawn, NJ, USA). The borate buffer solutions were prepared from a stock solution of 20 mM sodium tetraborate decahydrate (Fisher Scientific). A 20 mM stock solution of OFM Anion-BT, a proprietary reagent from Waters Chromatography Division of Millipore, identified as $R(CH_3)_3N^+Br^-$, where R is a long-chain alkyl group (25), was used as the quaternary ammonium salt. All alcohols used were obtained from Aldrich (Milwaukee, WI, USA). Adjustments of pH were made with dilute solutions of reagent grade HCl or NaOH.

# RESULTS AND DISCUSSION

## *Effect of $Q^+$ on EOF*

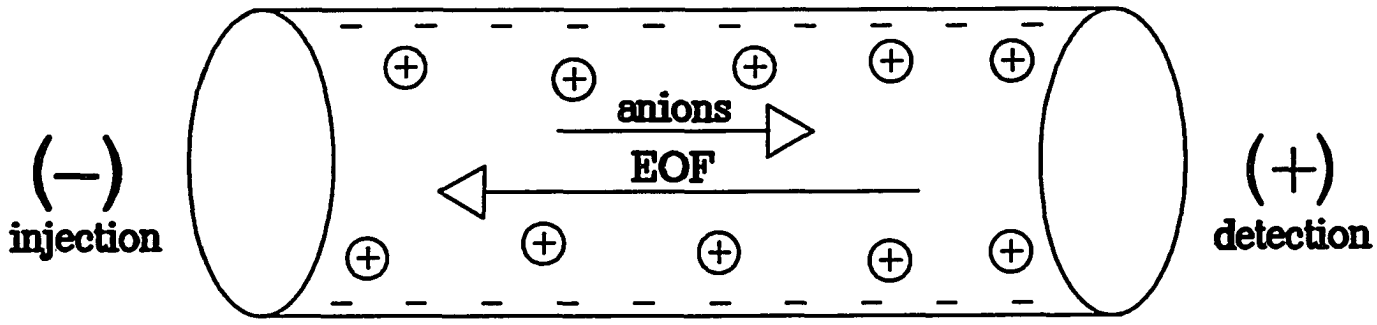
Separation of inorganic and short-chain organic acid anions by CZE requires the reversal or elimination of EOF. A proprietary reagent developed by Waters, OFM Anion-BT (which will be referred to as $Q^+$), was used in our work as an EOF modifier. When there is no $Q^+$ present in a 5 mM chromate electrolyte solution and a negative power supply is used, the EOF is strong and toward the cathode (Fig 1). However, as increasing amounts of $Q^+$ are added to the electrolyte solution, the magnitude of the EOF to the cathode is decreased until its direction is eventually reversed to the anode. As the concentration of $Q^+$ in the electrolyte solution is increased, the charge on the capillary wall becomes less negative before obtaining a net positive charge (Fig 2). Under the experimental conditions stated previously, a concentration of ~0.25 mM is required to reverse the EOF. A dynamic equilibrium is likely to exist between the $Q^+$ on the surface of the capillary wall and the $Q^+$ in the electrolyte solution.

The concentrations of $Q^+$ used in this work are far below the critical micelle concentration ($3.51 \times 10^{-3}$ M) thus, there is no micelle formation (27). Ion-interaction between the $Q^+$ and analyte anions is unlikely to occur in the solution phase because of the very low concentrations of $Q^+$ being used. Furthermore, the relative migration times of the anions studied were unchanged when the concentration of $Q^+$ was varied from 0.03 to 0.1 mM.

Fairly high concentrations of $Q^+$ (e.g. 0.5 mM) have been used for several applications

**Figure 1.** Schematic diagram of EOF and electrophoretic mobility of anions using the negative power supply.

## Negative Power Supply :




silica surface:

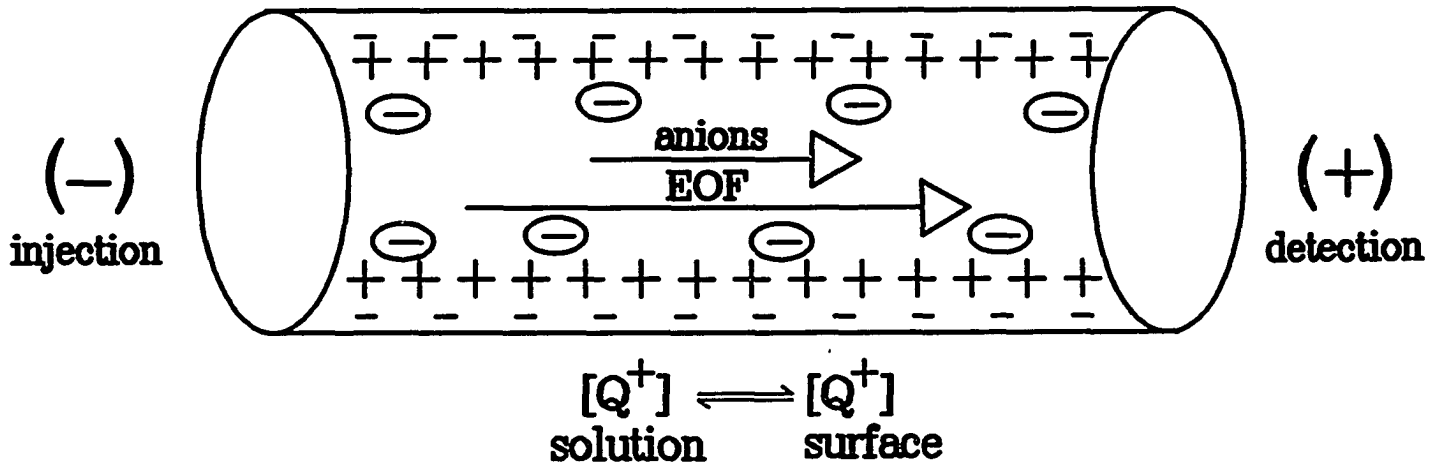
$$SiOH \rightleftharpoons SiO^- + H^+$$

⊕ = hydrated counter-cations

**Figure 2.** Schematic diagram of reversed EOF.

# Reversal of EOF:

- Add $Q^+$; quaternary ammonium salt
  (e.g. tetradecyltrimethyl ammonium bromide)
- Negative power supply




⊖ = hydrated counter-anions

(2-5). However, these concentrations of $Q^+$ in the electrolyte solution may result in a build-up of $Q^+$ on the capillary from run to run, thus making it necessary to clean the capillary more often. Hydrophobic alkyl ammonium salts, such as $Q^+$, also have limited solubility and may form insoluble pairs with some electrolyte components (23,26). Therefore, a lower concentration of $Q^+$ in the electrolyte solution may be beneficial for the analysis of some samples.

### *Effect of Organic Solvents on EOF*

The effects of several organic solvents on the EOF were studied. Addition of alcohols such as methanol, ethanol, 1-propanol, 1-butanol, and 1- and 2-pentanol to a 5 mM chromate solution (no $Q^+$) decreases the EOF toward the cathode. However, even high concentrations (e.g. 20%) of methanol, ethanol, and 1-propanol do not result in satisfactory decreases in the EOF. Low concentrations of butanol (3-5%) and pentanol (1-2%) result in significant decreases in the EOF. Since pentanol had limited solubility in the aqueous electrolyte solution and less controllable effects on the EOF, 1-butanol was chosen as the most promising alcohol to use as an EOF modifier.

### *Effect of 1-Butanol on EOF*

The effect of 1-butanol on the EOF was studied by measuring the EOF at increasing butanol concentrations. The EOF was measured using a positive power supply, a 5 mM chromate electrolyte solution at pH 8.0, and deionized water as the neutral marker. Electrolyte solutions containing greater than 8% butanol were not studied due to solubility

limitations of butanol in the aqueous solution. Figure 3 shows the effect of increasing butanol concentrations on the EOF. The EOF coefficient was calculated from the expression $\mu_{eo}=(LL_d)/(Vt)$, where L = capillary length (cm), $L_d$ = capillary length from injection to detector (cm), V = applied voltage (volts), and t = time (s). Although butanol does not reverse the EOF, there is a significant decrease in the EOF to the cathode as the concentration of butanol is increased. This effect is due in part to the butanol interacting with the capillary surface and changing the characteristics of the double layer. It is likely that a dynamic equilibrium is established between the butanol in solution and the butanol on the capillary surface (Fig 4).

***Effect of $Q^+$ and Butanol on EOF***

Since neither a low concentration of $Q^+$ or butanol alone reversed the EOF, a combination of the two modifiers was investigated. Figure 5 shows the effect of increasing $Q^+$ concentrations on the EOF in electrolyte solutions containing 0, 3, or 5% butanol. The EOF direction is reversed at a much lower concentration of $Q^+$ when butanol is added to the electrolyte solution.

The butanol and $Q^+$ are likely to interact with the capillary surface by a dynamic equilibrium (Fig 6). The adsorbed butanol would shift the $Q^+$ equilibrium so that the surface achieves a net positive charge at a significantly lower $Q^+$ concentration. This mechanism is supported by the work of Scott (28) on the interaction of several polar solvents with a silica gel surface. He concluded that polar solvents interact with the silica surface via hydrogen bonding and a layer of solvent is formed. Scott and Simpson (29) also

**Figure 3.** Variation of EOF coefficient versus percentage 1-butanol added to an electrolyte solution of 5 mM chromate at pH 8.0. Negative coefficients indicate flow toward the cathode.

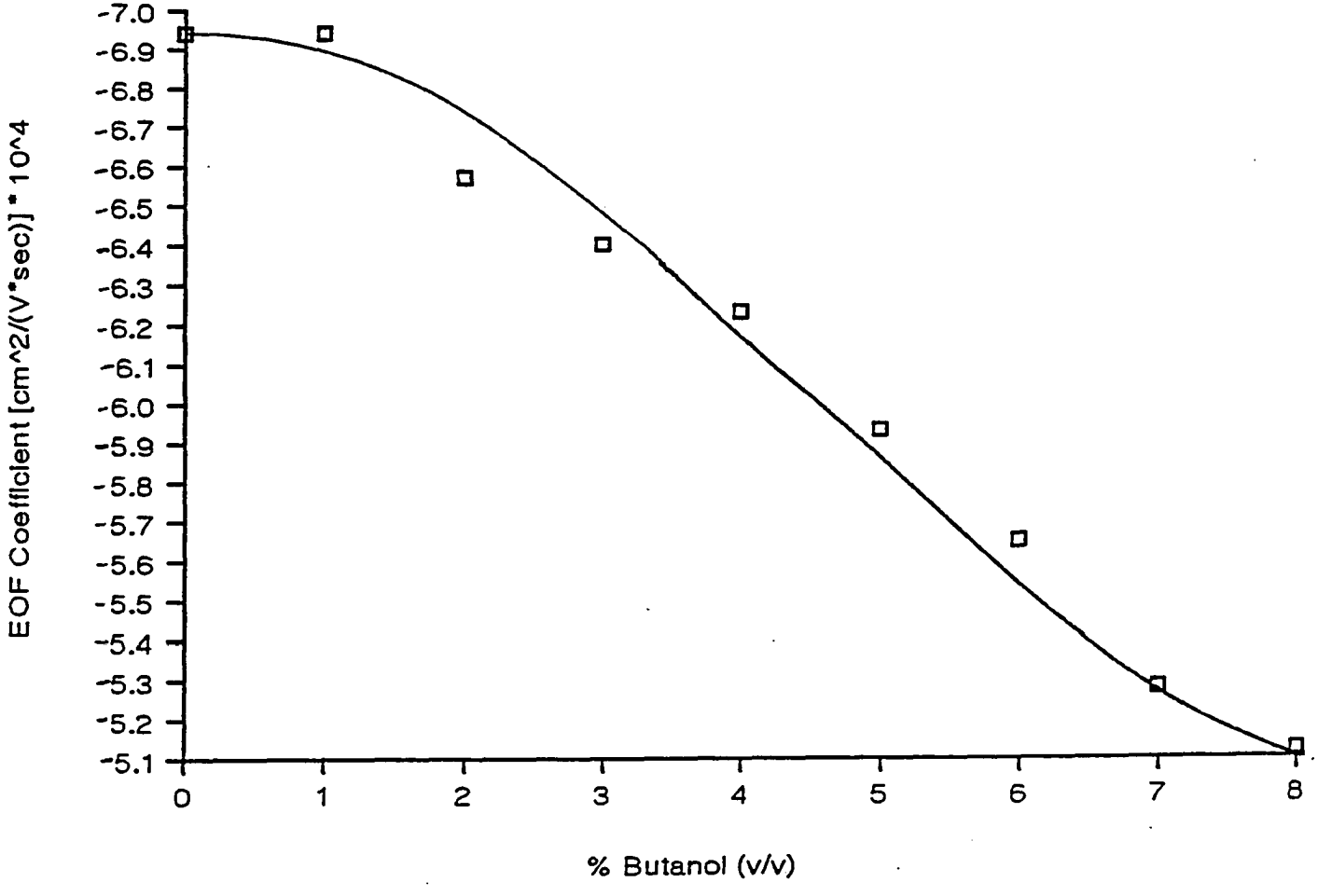
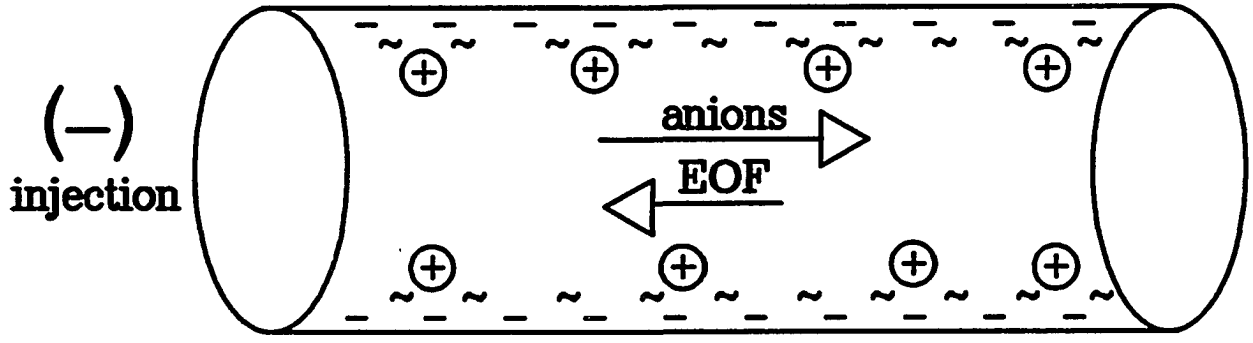

EOF Coefficient [cm^2/(V*sec)] * 10^4
-7.0
-6.9
-6.8
-6.7
-6.6
-6.5
-6.4
-6.3
-6.2
-6.1
-6.0
-5.9
-5.8
-5.7
-5.6
-5.5
-5.4
-5.3
-5.2
-5.1
0
1
2
3
4
5
6
7
8
% Butanol (v/v)

**Figure 4.** Schematic diagram of the effect of 1-butanol on the EOF and electrophoretic mobility of anions.

# Addition of Butanol:




[Butanol] solution ⟸ [Butanol] surface

⊕ = hydrated counter-cations
~ = butanol

**Figure 5.** EOF coefficient as a function of concentration of $Q^+$ added to electrolyte solutions of 5 mM chromate at pH 8.0 containing 0, 3, or 5% 1-butanol. Negative coefficients indicate flow toward the cathode.

# EOF Coefficients vs. mM EOF Modifier

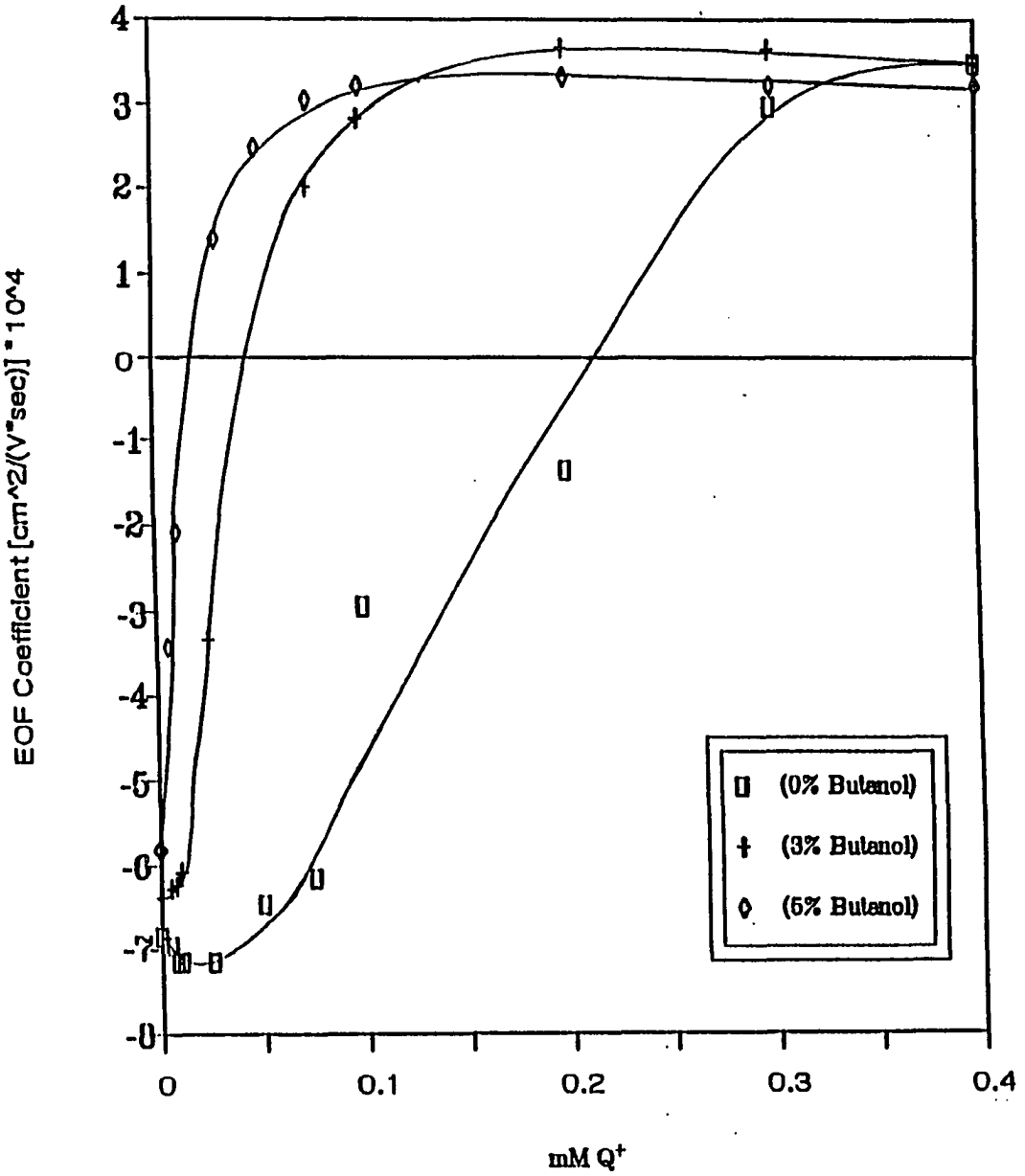
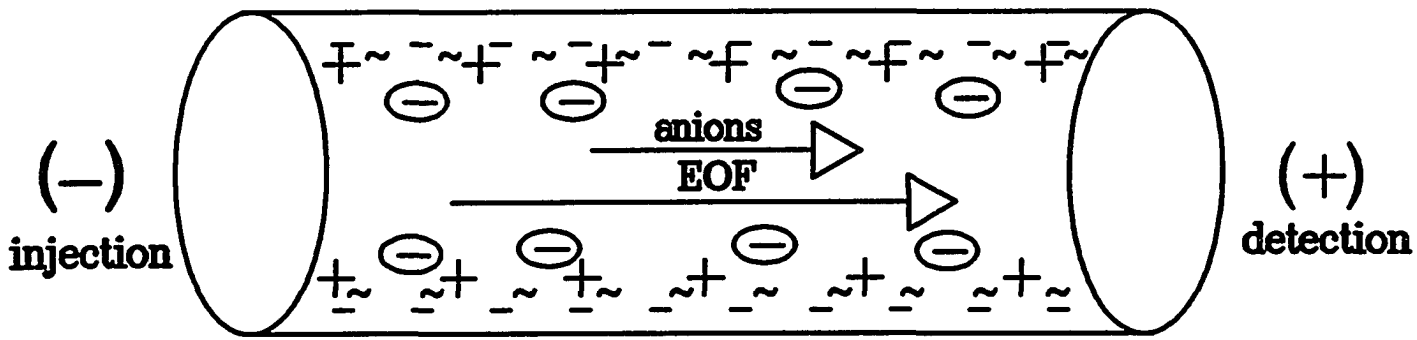


**Figure 6.** Schematic diagram of the effect of $Q^+$ and 1-butanol on the EOF and electrophoretic mobility of anions.

# $Q^+$ and Butanol:




[Butanol] solution $\rightleftharpoons$ [Butanol] surface

$[Q^+]$ solution $\rightleftharpoons$ $[Q^+]$ surface

⊖ = hydrated counter-anions

~ = butanol

studied the distribution of several solvents, including 1-butanol, between an aqueous phase and a reversed phase silica surface. They concluded that a dynamic equilibrium exists in which there is a monolayer coating of butanol on the surface.

The effect of using low concentrations of both $Q^+$ and butanol is more impressive when actual CE separations are viewed. Figure 7A shows an attempted separation of an anion mixture using 0.075 mM $Q^+$ in an aqueous electrolyte solution containing 5 mM chromate at pH 8.0. Migration of the anions was so slow that only the first few anions had appeared after an hour. Using the same experimental conditions except for the addition of 3% butanol to the electrolyte solution, an excellent separation (Fig 7B) was obtained in less than 5 min. The separation (Fig 7C) observed when 5% butanol was added was even faster.

Several anions were separated using a combination of low concentrations of $Q^+$ and butanol. A typical separation of some of these anions using 0.03 mM $Q^+$ and 4% butanol is shown in Figure 8. Without butanol, a much higher concentration of $Q^+$ was required to obtain a comparable separation.

Several aromatic carboxylic acid anions were separated using conditions similar to those used previously (Fig 9). Direct UV detection at 254 nm was used for these anions along with a borate buffer solution. Separation of this mixture was also attempted using the positive power supply and a borate buffer solution with no additives. Under these conditions, the EOF was toward the cathode with the electrophoretic mobilities of the anions in the opposite direction. The separation was unsuccessful, resulting in poor resolution of the less mobile anions and long migration times for the most mobile anions.

**Figure 7.** Separation of inorganic and short chain organic acid anions using varying concentrations of 1-butanol. Peaks: 1 = $Br^-$ (5 ppm), 2 = $Cl^-$ (5 ppm), 3 = $SO_4^{2-}$ (6 ppm), 4 = $NO_2^-$ (7 ppm), 5 = $NO_3^-$ (8 ppm), 6 = $F^-$ (8 ppm), 7 = $HCOO^-$ (10 ppm), 8 = $CO_3^{2-}$ (7 ppm), 9 = acetate (10 ppm), 10 = propionate (10 ppm), 11 = butyrate (10 ppm), 12 = valerate (10 ppm). (A) Electrolyte: 5 mM chromate, 0.075 mM $Q^+$, pH 8.0; applied voltage: -30 kV, current: 23 μA, electromigration injection 6s/10kV. (B) Electrolyte: 5 mM chromate, 0.075 mM $Q^+$, 3% 1-butanol, pH 8.0; applied voltage: -30 kV, current: 21 μA, electromigration injection 6s/10kV. (C) Electrolyte: 5 mM chromate, 0.075 mM $Q^+$, 5% 1-butanol, pH 8.0; applied voltage: -30 kV, current: 21 μA, electromigration injection 10s/10kV.

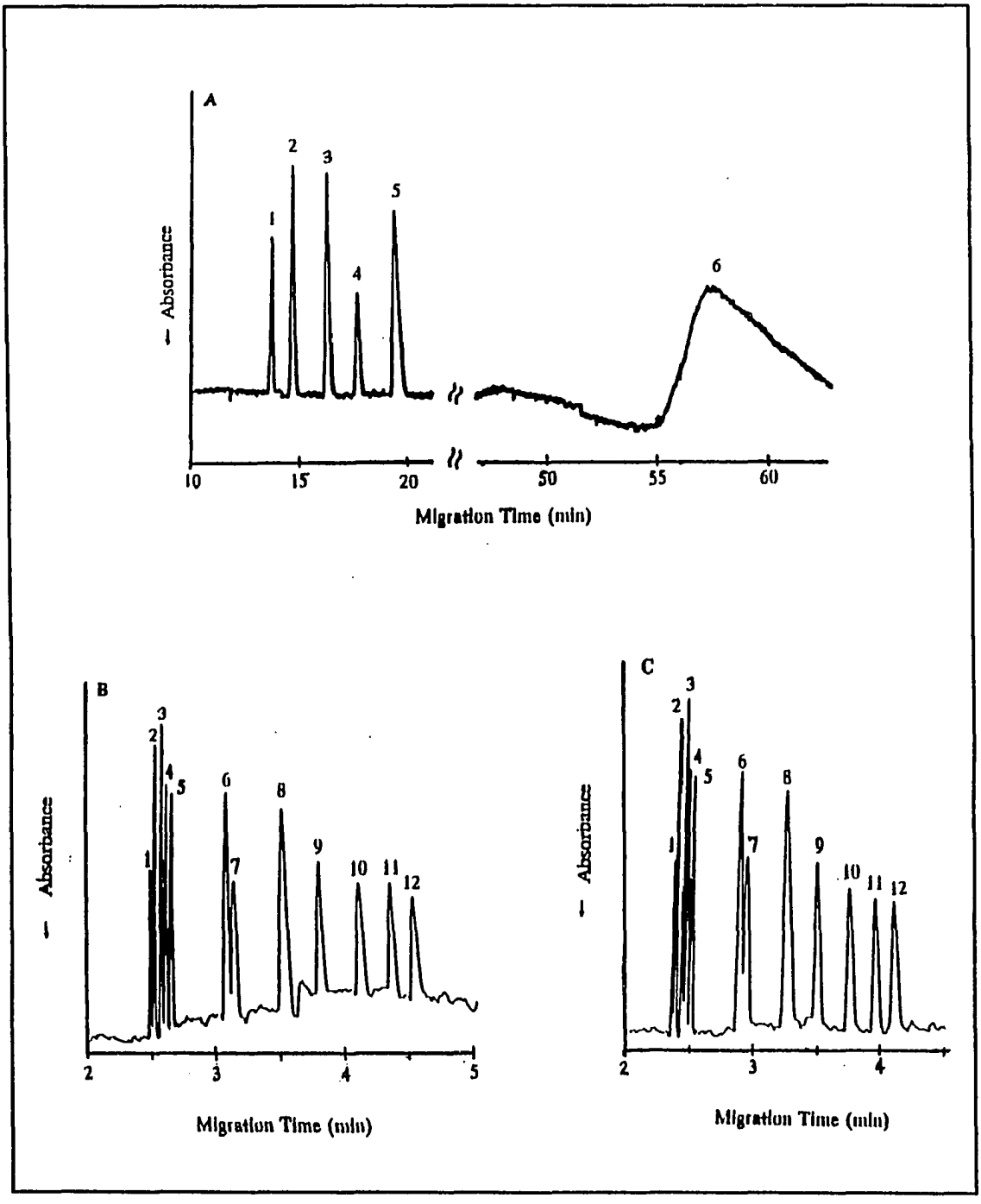

A
1
2
3
4
5
6
Absorbance
10
15
20
50
55
60
Migration Time (min)
B
1
2
3
4
5
6
7
8
9
10
11
12
Absorbance
2
3
4
5
Migration Time (min)
C
1
2
3
4
5
6
7
8
9
10
11
12
Absorbance
2
3
4
Migration Time (min)

**Figure 8.** Separation of inorganic anions. Electrolyte: 5 mM chromate, 0.03 mM $Q^+$, 4% 1-butanol, pH 8.0; applied voltage: -30 kV, current: 23 μA, electromigration injection 5s/10kV. Peaks: 1 = $Br^-$ (5 ppm), 2 = $Cl^-$ (5 ppm), 3 = $SO_4^{2-}$ (6 ppm), 4 = $NO_2^-$ (7 ppm), 5 = $NO_3^-$ (7 ppm), 6 = $MoO_4^{2-}$ (10 ppm), 7 = $N_3^-$ (10 ppm), 8 = $ClO_3^-$ (8 ppm), 9 = $F^-$ (8 ppm), 10 = $HCOO^-$ (8 ppm), 11 = $ClO_2^-$ (8 ppm), 12 = $CO_3^{2-}$ (7 ppm).

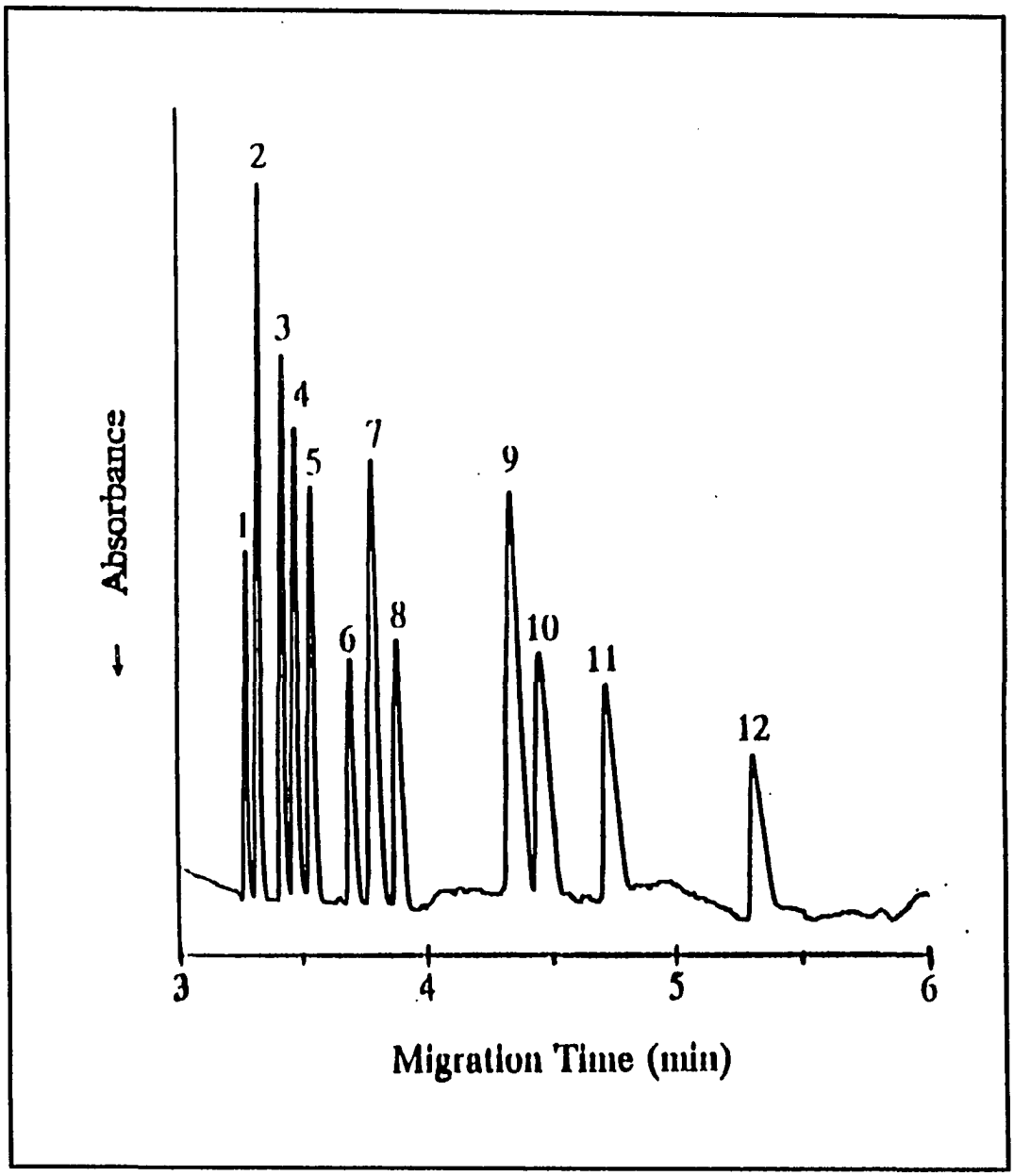
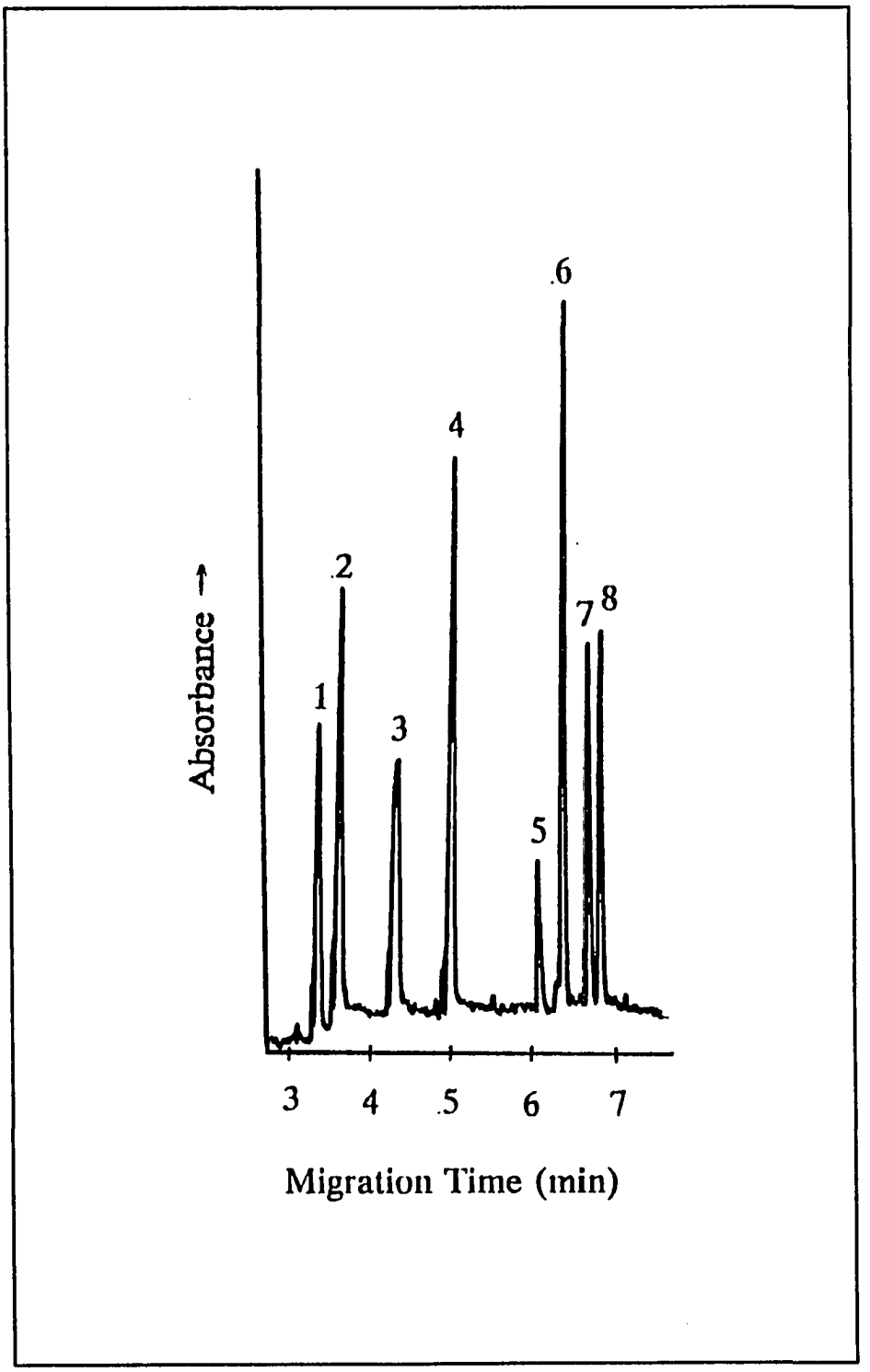

2
3
4
5
7
9
1
6
8
10
11
12
Absorbance
3
4
5
6
Migration Time (min)

**Figure 9.** Separation of aromatic organic acid anions. Electrolyte: 1 mM borate, 0.03 mM $Q^+$, 4% 1-butanol, pH 8.5; applied voltage: -30 kV, current: 5.2 μA, hydrostatic injection: 20 sec. Peaks: 1 = 1,2,4,5-benzenetetracarboxylate (5.9 x $10^{-5}$ M), 2 = 1,2,4-benzenetricarboxylate (7.2 x $10^{-5}$ M), 3 = 3-nitrophthalate (7.1 x $10^{-5}$ M), 4 = 2,4-dihydroxybenzoate (6.5 x $10^{-5}$ M), 5 = benzoate (1.2 x $10^{-4}$ M), 6 = 4-nitrobenzoate (4.8 x $10^{-4}$ M), 7 = 3,5-diaminobenzoate (6.6 x $10^{-5}$ M), 8 = 1,2-napthoquinone-4-sulfonate (4.2 x $10^{-5}$ M).


Absorbance →
1
2
3
4
5
6
7
8
3
4
.5
6
7
Migration Time (min)

# CONCLUSIONS

The combination of low concentrations of 1-butanol and a quaternary ammonium reagent in aqueous solutions is an excellent method for modifying EOF in the CZE separation of anions. Dynamically coating the capillary surface with butanol and $Q^+$ is an attractive alternative to other methods that have been proposed for adjusting EOF. Separations using this system are less noisy and more reproducible.

## ACKNOWLEDGEMENTS

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# PAPER III. OPTIMIZATION OF SEPARATIONS OF ALKYL-SUBSTITUTED PHENOLATE ANIONS BY CAPILLARY ZONE ELECTROPHORESIS

# OPTIMIZATION OF SEPARATIONS OF ALKYL-SUBSTITUTED PHENOLATE ANIONS BY CAPILLARY ZONE ELECTROPHORESIS

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To be submitted

## SUMMARY

Separations of alkyl-substituted phenolate anions were optimized by CZE by varying the pH and concentration of acetonitrile in the buffer solution. The narrow range represented by the $pK_a$s and the small differences in size of the alkyl-substituted phenols result in their similar electrophoretic mobilities. This is especially true for some structural isomers. By studying electrophoretic mobility as a function of pH and percentage acetonitrile, optimum separation conditions were determined. The addition of 40% acetonitrile to the buffer solution increased migration times but improved separation resolution. The migration behavior and optimum separations of a set of alkyl-substituted phenols are presented.

# INTRODUCTION

Capillary zone electrophoresis (CZE) has proven to be an effective method for separating ionic species. The separation is due to the differential migration of the charged species in an electric field. Although CZE is a highly efficient technique, it is often difficult to separate compounds with very similar electrophoretic mobilities. For these separations, it is often necessary to carefully manipulate experimental parameters to improve the separation resolution (1-4).

Terabe and coworkers (1) separated three isotopic benzoic acids based on slight differences in their dissociation constants. The effects of several factors including pH, applied voltage, capillary length, and the suppression of electroosmotic flow (EOF) were studied.

Nielen (2) examined the influence of pH, electrolyte, ionic strength, addition of alcohols, counter-ion, and temperature on the electrophoretic and electroosmotic mobilities. Two positional isomers of aminobenzoic acid were used as model compounds.

Gonnard and Collet (3) optimized the separation of a mixture of chlorophenols by varying buffer pH and concentration along with the applied voltage. Zare et al. (4) separated low molecular weight carboxylic acids under conditions of reversed EOF using 30% methanol added to the buffer solution to aid the separation. Fujiwara and Honda (5) studied the effect of organic solvents on the separation of positional isomers of substituted benzoic acids.

Changing the buffer solution pH is one of the most effective methods for optimizing the

separation of weak acids. EOF as well as electrophoretic mobilities are affected by changes in pH. By taking advantage of differences in the degrees of dissociation of weak acids (or $pK_a$) under particular conditions, an optimum operating pH may be found.

Smith and Khaledi (6) developed a mathematic model based on $pK_a$ values which predicts the optimum buffer pH for the separation of a mixture of substituted phenols.

Yeung and coworkers explored pH gradients for the separation of organic acids (7,8) and fluorescent dyes (9). They presented three procedures for creating pH gradients: voltage programming (7), temperature programming (8), and externally using an HPLC gradient pump (9).

In the present work, separations of alkyl-substituted phenolate ions were optimized by varying the pH and the percentage of acetonitrile in the buffer solution. The alkyl-substituted phenols chosen for this study represent a narrow range of $pK_a$ values and have small differences in size, thus resulting in similar electrophoretic mobilities. The effect of pH and acetonitrile on EOF and electrophoretic mobility is presented.

# EXPERIMENTAL

The Waters Quanta 4000 CZE system (Waters Chromatography Division of Millipore, Milford, MA, USA) was used for all experiments. Fused-silica capillaries (Polymicro Technology, Phoenix, AZ, USA) were used with lengths of 70 cm and inner diameters of 75 μm. At 62.5 cm from the injection end of the capillary, the polyimide coating was burned off to create a detection window. New capillaries were conditioned by rinsing with 1 M sodium hydroxide for approximately 1 hr, followed by a 15-min rinse with deionized water. A 2-min rinse with 0.1 M sodium hydroxide, followed by a 2-min rinse of deionized water was used to clean the capillary at the start of each day and between runs with different buffer solutions. The capillary was purged with buffer for 1 min before each run.

The positive power supply was used at 30 kV for each experiment. Hydrostatic sampling was used with a sampling time of 20 s and a height of 10 cm for all separations. Direct UV detection at 254 nm was used for all experiments. All separations were carried out at room temperature. EOF measurements were made using water as the neutral marker.

All solutions were prepared using deionized water from a Barnstead Nanopure II system (Sybron Barnstead, Boston, MA, USA). All reagents used in this work were reagent grade. Buffer solutions of 1 mM borate were prepared from a 100 mM stock solution of sodium tetraborate decahydrate (Fisher Scientific, Fair Lawn, NJ, USA). Adjustments of pH were made with dilute solutions of hydrochloric acid or sodium hydroxide. HPLC-grade acetonitrile (Fisher Scientific) was added to the buffer. Stock solutions of the phenolate ions were prepared from their corresponding phenols and the pH raised to form the anion.

## RESULTS AND DISCUSSION

Under the experimental conditions stated previously, EOF was toward the detector, whereas electrophoretic flow was in the opposite direction, toward the anode. A scheme using low concentrations of a quaternary ammonium salt and 1-butanol to reverse EOF was initially investigated. The migration order of the anions was reversed to that observed using the standard mode. The conventional scheme (non-reversed EOF) was chosen for in-depth studies because the separations of phenolate ions obtained with this method were both more reproducible and showed better resolution due to the counter-flows.

Electroosmotic mobility is a function of several parameters as given by:

$$\mu_{EOF} = \zeta\varepsilon/(4\pi\eta) \qquad (1)$$

where

$\zeta$ = zeta potential

$\varepsilon$ = solution dielectric constant

$\eta$ = solution viscosity

The magnitude and direction of the EOF may be measured using a neutral marker and the EOF coefficient calculated from:

$$\mu_{EOF} = lL/(Vt_{EOF}) \qquad (2)$$

where

l = effective capillary length (injection to detector) (cm)

L = total capillary length (cm)

$t_{EOF}$ = migration time of neutral marker (sec)

V = applied voltage (v)

The electrophoretic mobility of an ion is given by:

$$\mu_e = q/(6\pi\eta r) \qquad (3)$$

where

q = ion charge

$\eta$ = solution viscosity

r = ion radius

Electrophoretic mobilities are calculated using the observed migration time and the following equation.

$$\mu_e = lL/[(t-t_{EOF})V] \qquad (4)$$

where

t = migration time of ion (sec)

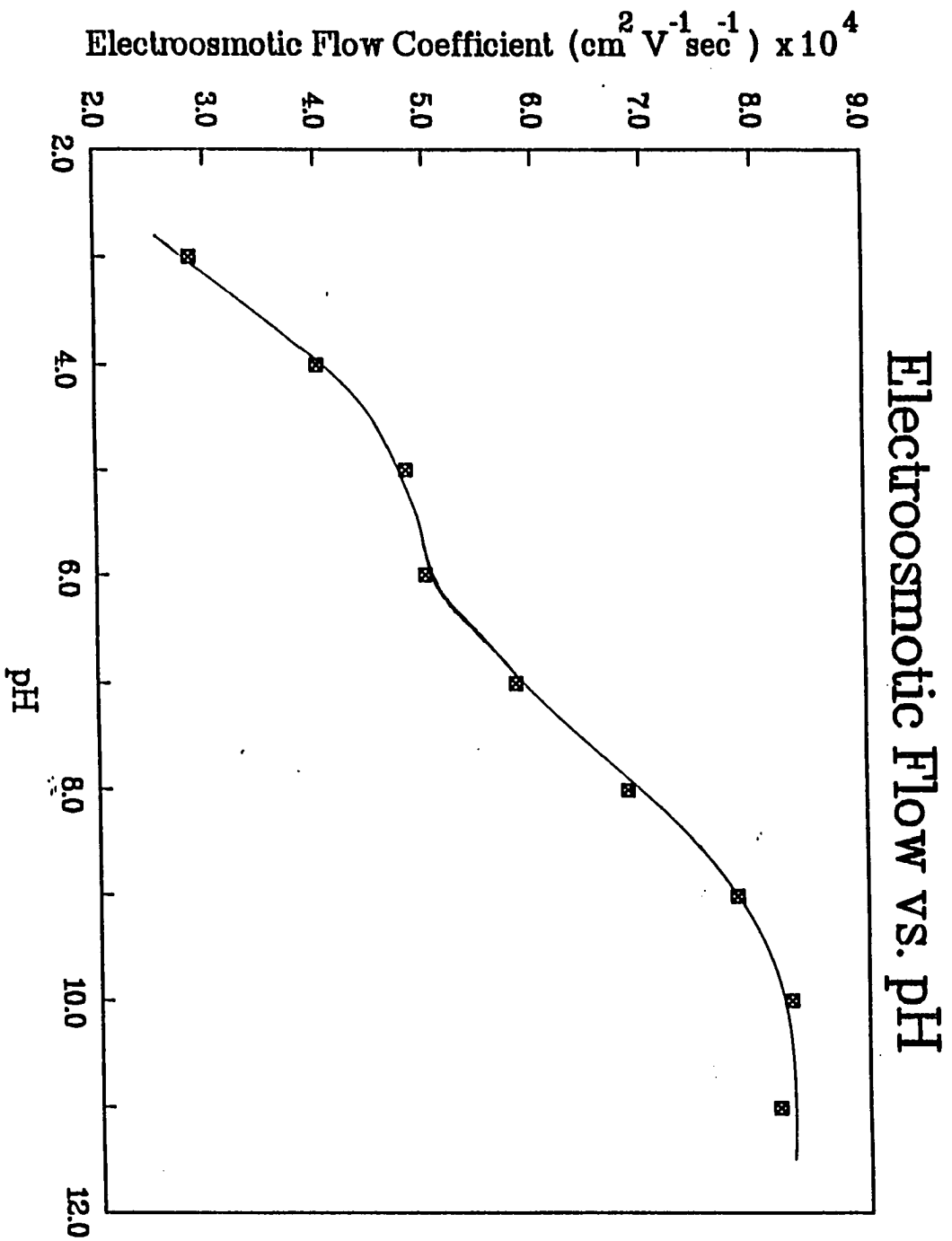
***Effect of pH on EOF:***

The effect of pH on EOF in fused-silica capillaries is well understood (10). As pH is increased, EOF increases due to the presence of more ionized silanol groups at the capillary wall and an increase in the zeta potential (Fig 1). In the range of pH 10.4-12.0 (the range used in this work) there is little change in EOF since the capillary surface is completely ionized at these pH values.

***Effect of Acetonitrile on EOF:***

The effect of acetonitrile on EOF has been studied (11-13). As the concentration of acetonitrile in the buffer solution is increased, EOF decreases as shown in Figure 2. This

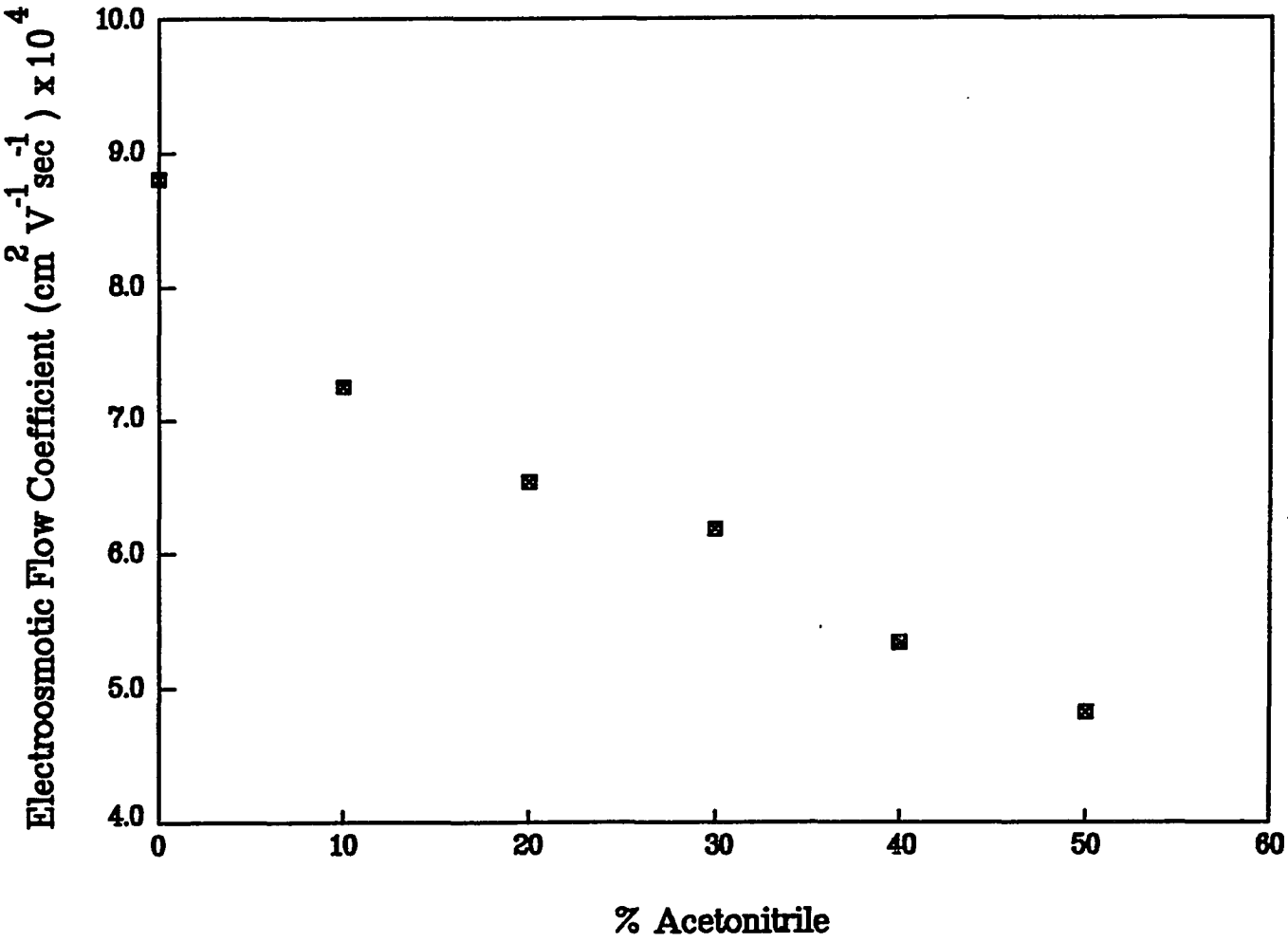
**Figure 1.** Plot of EOF vs. pH using a 1 mM borate buffer solution. Other conditions as stated previously.




Electroosmotic Flow vs. pH

**Figure 2.** Plot of EOF vs. percentage of acetonitrile added to a 1 mM borate buffer solution at pH 11.0. Other conditions as stated previously.

# Electroosmotic Flow vs. % Acetonitrile



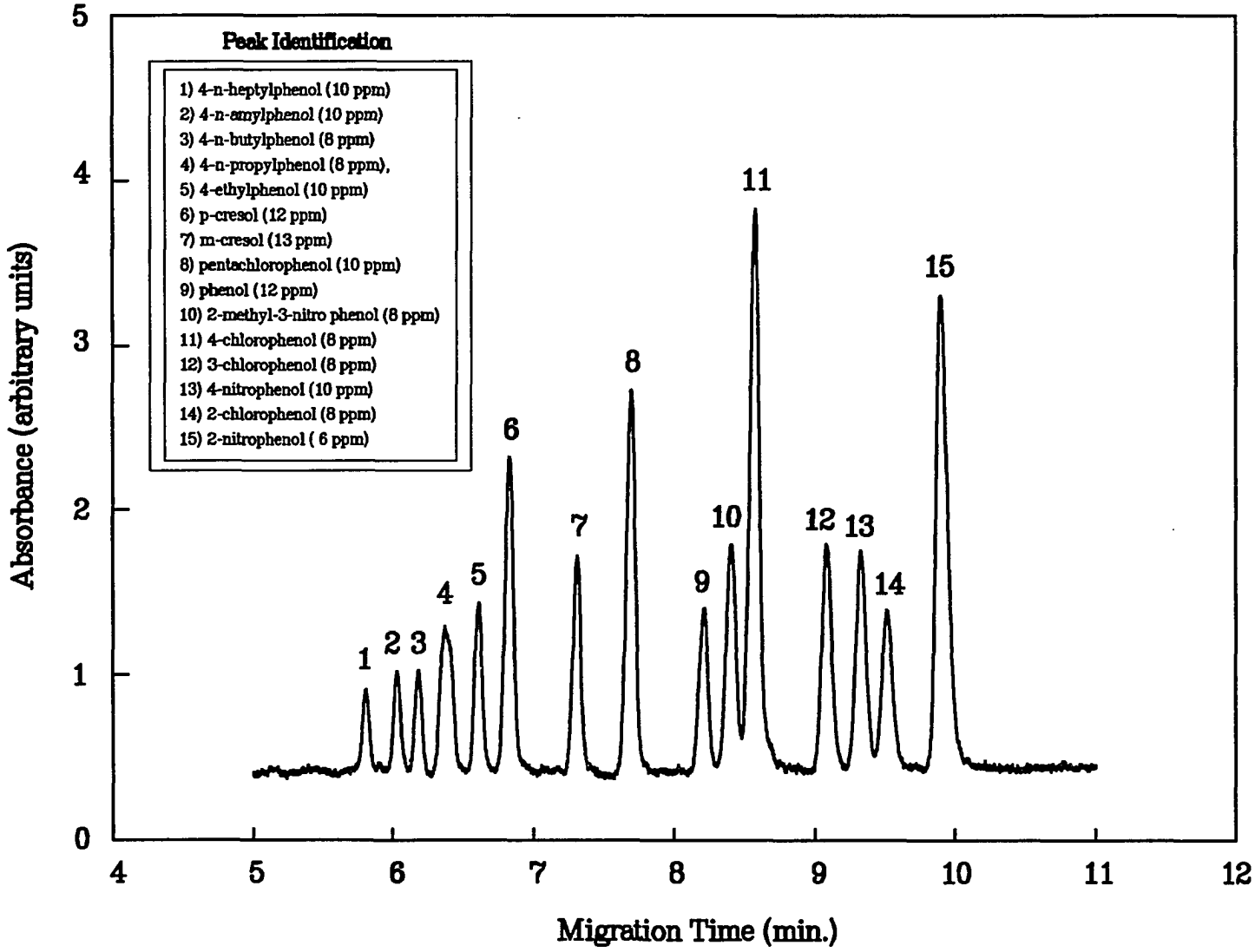
decrease in EOF is inversely proportional (eq 1) to the increase in bulk viscosity observed when acetonitrile is added to the buffer.

***Separation of Phenolate Anions:***

As a group, chloro-, nitro-, and alkyl-substituted phenols represent a wide range of acidity. Chlorophenols themselves have a wide range of $pK_a$ values (e.g. pentachlorophenol: 4.74, 4-chlorophenol: 9.37). Nitrophenols also have a relatively wide range of $pK_a$s (e.g. 2,6-dinitrophenol: 3.71, 3-nitrophenol: 8.36). In contrast, alkyl-substituted phenols have a much narrower $pK_a$ range (e.g. *m*-cresol: 10.00, 2-*t*-butylphenol: 11.08). A mixture of chloro-, nitro-, and alkyl-substituted phenolate ions was separated as shown in Figure 3. From this separation, it is apparent that the less acidic alkyl-substituted compounds have shorter migration times than the more acidic chloro- and nitrophenols. This trend is due to the larger degree of dissociation observed for the more acidic phenols at the separation pH. A larger degree of dissociation is accompanied by a higher effective ionic charge resulting in a higher electrophoretic mobility toward the anode and longer migration times.

Since alkyl-substituted phenols have similar dissociation constants and masses, these compounds will have comparable electrophoretic mobilities at a given pH. Separations of compounds with similar mobilities are often difficult. For these reasons, the alkyl-substituted phenolate ions were chosen as test compounds for the following study of the effect of pH and acetonitrile.

**Figure 3.** Separation of a mixture of chloro-, nitro-, and alkyl-substituted phenolate ions. Buffer: 1 mM borate, 30% acetonitrile, pH 11.2; 30 kV, 30 μA, 254 nm. Other conditions as stated previously. Peaks are as identified.


Peak Identification
1) 4-n-heptylphenol (10 ppm)
2) 4-n-amylphenol (10 ppm)
3) 4-n-butylphenol (8 ppm)
4) 4-n-propylphenol (8 ppm),
5) 4-ethylphenol (10 ppm)
6) p-cresol (12 ppm)
7) m-cresol (13 ppm)
8) pentachlorophenol (10 ppm)
9) phenol (12 ppm)
10) 2-methyl-3-nitro phenol (8 ppm)
11) 4-chlorophenol (8 ppm)
12) 3-chlorophenol (8 ppm)
13) 4-nitrophenol (10 ppm)
14) 2-chlorophenol (8 ppm)
15) 2-nitrophenol ( 6 ppm)
Absorbance (arbitrary units)
Migration Time (min.)
0
1
2
3
4
5
4
5
6
7
8
9
10
11
12

***Effect of pH on Electrophoretic Mobility:***

For separations of ionizable compounds by CZE, pH is an important parameter since it determines the extent of ionization of a solute, and thus its electrophoretic mobility. The migration of a weak acid (HA) in CZE is given by (6):

$$\mu = \mu_{A^-}(K_a/[H^+])/[1 + (K_a/[H^+])] \quad (5)$$

where

$\mu$ = mobility of HA at a given pH

$\mu_{A^-}$ = mobility of the anionic form of the acid

$K_a$ = acid dissociation constant for HA

Thus, acids with similar sizes and dissociation constants (or $pK_a$s) have nearly the same mobility at a given pH. Several alkyl-substituted phenols with similar $pK_a$ values were studied. A list of these compounds and their corresponding $pK_a$s is shown in Table 1.

The phenols listed in Table 1 were divided into three groups for separation purposes: straight-chain alkyl-substituted, methyl-substituted, and propyl- and butyl-substituted phenols. Experiments on the separation of the straight-chain, alkyl-substituted phenols were used to demonstrate the effects of pH and acetonitrile on the separations.

The buffer pH was studied in the range of 10.4-12.0. At pH values lower than 10.4, little resolution was observed, due to the low degree to which most of the phenols had dissociated. Figure 4 shows the effect of varying the buffer pH on the electrophoretic mobilities of the straight-chain, alkyl-substituted phenolate ions. As expected, the electrophoretic mobilities increased with increasing pH since the phenols are more

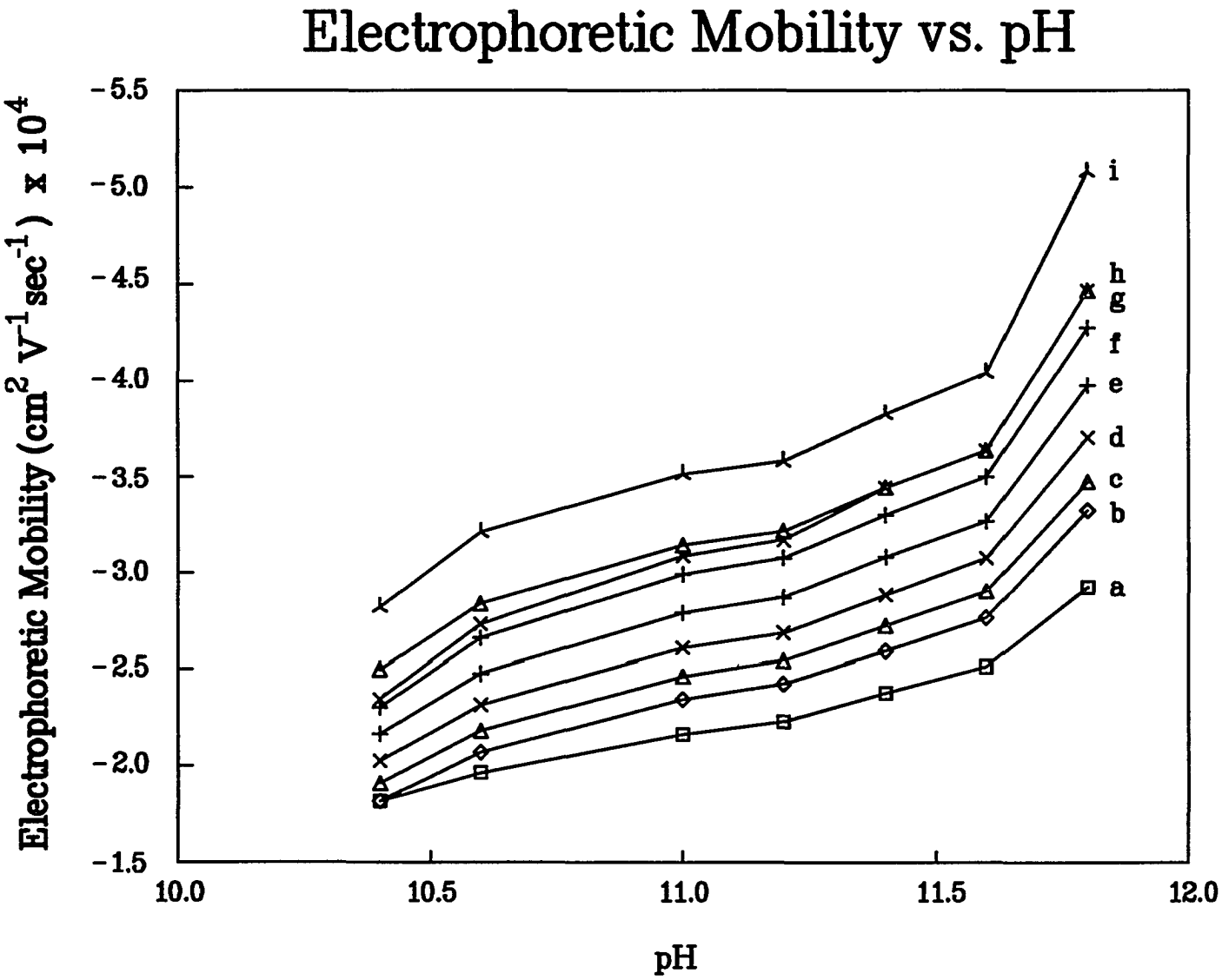
**Table 1.** $pK_a$ values for alkyl-substituted phenols

| Compound | $pK_a$ | Compound | $pK_a$ |
|---|---|---|---|
| 4-heptylphenol | NA | 2,3,6-trimethylphenol | 10.63[a] |
| 4-amylphenol | 10.25[a] | 2,4,6-trimethylphenol | 10.88[b] |
| 4-n-butylphenol | 10.28[a] | 3,4,5-trimethylphenol | 10.25[b] |
| 4-*t*-butylphenol | 10.25[a] | 2,3-dimethylphenol | 10.50[b] |
| 2-*t*-butylphenol | 11.08[a] | 2,5-dimethylphenol | 10.22[b] |
| 4-propylphenol | 10.25[a] | 2,6-dimethylphenol | 10.59[b] |
| 4-isopropylphenol | 10.25[a] | 3,4-dimethylphenol | 10.32[b] |
| 3-isopropylphenol | 10.08[a] | 3,5-dimethylphenol | 10.15[b] |
| 2-isopropylphenol | 10.43[a] | *o*-cresol | 10.26[b] |
| 4-ethylphenol | 10.00[b] | *p*-cresol | 10.26[b] |
| 2,3,5,6-tetramethylphenol | 10.77[a] | *m*-cresol | 10.00[b] |
| 2,3,5-trimethylphenol | 10.48[a] | phenol | 9.99[b] |

[a] Reference 14

[b] Reference 15

**Figure 4.** Electrophoretic mobilities of straight-chain, alkyl-substituted phenolate ions as a function of pH in a 1 mM borate buffer solution. Negative coefficients indicate mobilities toward the anode. Identification: a, 4-n-heptylphenol; b, 4-n-amylphenol; c, 4-n-butylphenol; d, 4-n-propylphenol; e, 4-ethylphenol; f, *o*-cresol; g, *p*-cresol; h, *m*-cresol; i, phenol.


Electrophoretic Mobility vs. pH
Electrophoretic Mobility (cm$^2$ V$^{-1}$sec$^{-1}$) x 10$^4$
-5.5
-5.0
-4.5
-4.0
-3.5
-3.0
-2.5
-2.0
-1.5
10.0
10.5
11.0
11.5
12.0
pH
i
h
g
f
e
d
c
b
a

dissociated at higher pH. For the mixture of phenols represented in Figure 4, the optimum pH appears to be in the range 10.6-11.4.

***Effect of Acetonitrile on Electrophoretic Mobility:***

Addition of acetonitrile to the buffer solution not only increases the solubility of the organic solutes but also affects their electrophoretic mobilities. After an approximate pH was determined for optimum resolution of a group of anions, the effect of adding acetonitrile was determined. As shown in Figure 5, the electrophoretic mobilities of the straight-chain, alkyl substituted phenolate anions decreased with increased acetonitrile concentration. This decrease is attributed to changes in the parameters in Eq. 3. Solution viscosity is increased with the addition of acetonitrile. The effective ionic charge is decreased due to a lower degree of dissociation of the phenol in the presence of acetonitrile.

Separation resolution improved with increased acetonitrile concentration. In particular, the resolution of *o*- and *p*-cresol was significantly better. A concentration of 40% acetonitrile was chosen as optimum. Only slightly better resolution was observed at higher acetonitrile concentrations and the migration times were longer. Figure 6 shows the electrophoretic mobilities as a function of pH of the same group of compounds using a buffer solution containing 40% acetonitrile. A pH of 11.2 gave the best separation as shown in Figure 7.

It should be noted that for buffer solutions containing acetonitrile, the pH values provided are "apparent" pH values. The pH values observed for solutions of 1 mM borate buffer containing acetonitrile are higher than that observed without acetonitrile. A 1 mM

**Figure 5.** Electrophoretic mobilities of straight-chain, alkyl-substituted phenolate ions as a function of concentration of acetonitrile in a 1 mM borate buffer solution at pH 11.2. Negative coefficients indicate mobilities toward the anode. Identification: a, 4-n-heptylphenol; b, 4-n-amylphenol; c, 4-n-butylphenol; d, 4-n-propylphenol; e, 4-ethylphenol; f, *o*-cresol; g, *p*-cresol; h, *m*-cresol; i, phenol.

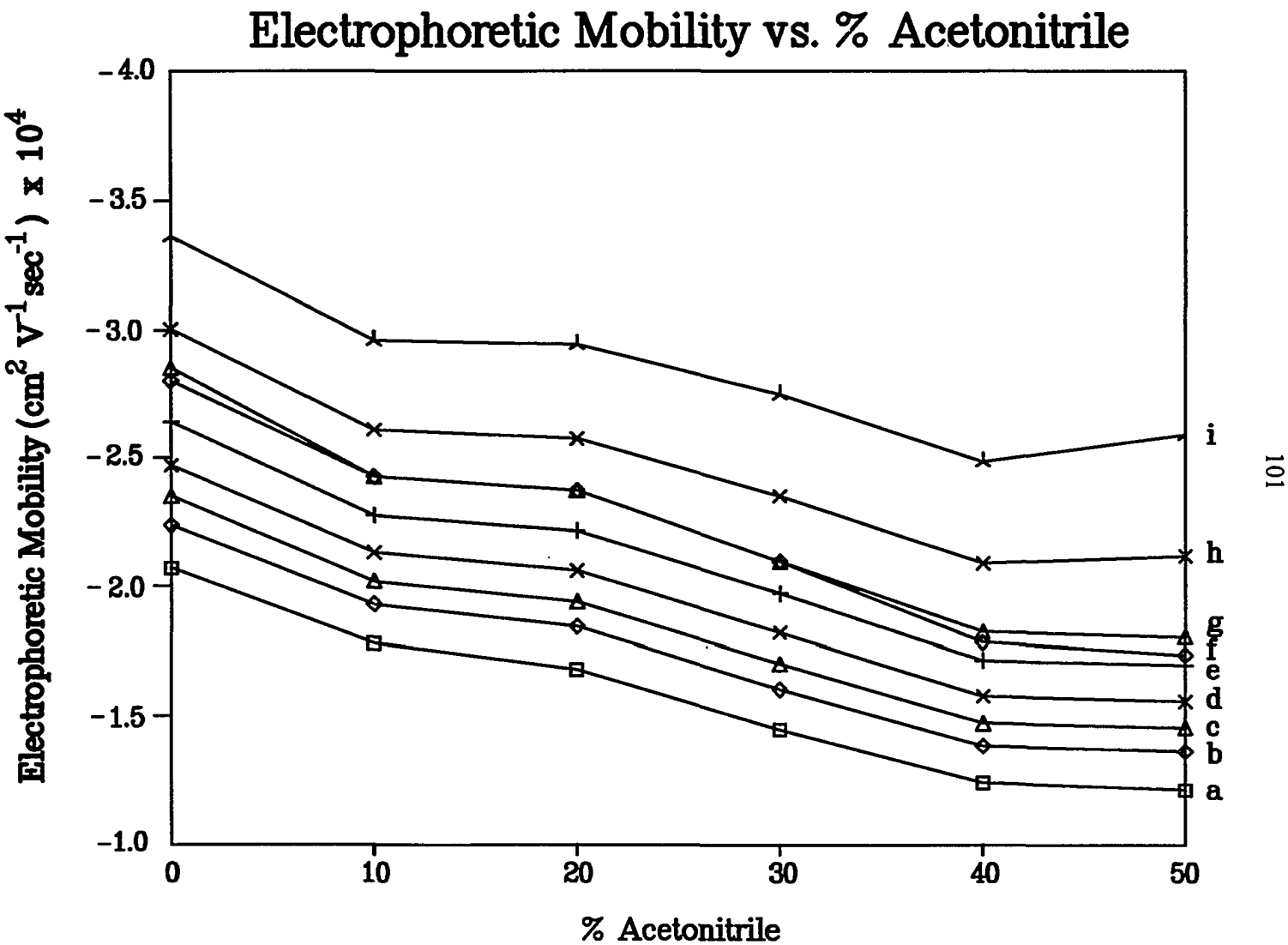

Electrophoretic Mobility vs. % Acetonitrile
Electrophoretic Mobility (cm$^2$ V$^{-1}$sec$^{-1}$) x 10$^4$
-4.0
-3.5
-3.0
-2.5
-2.0
-1.5
-1.0
0
10
20
30
40
50
% Acetonitrile
i
h
g
f
e
d
c
b
a

**Figure 6.** Electrophoretic mobilities of straight-chain, alkyl-substituted phenolate ions as a function of pH in a 1 mM borate buffer solution containing 40% acetonitrile. Negative coefficients indicate mobilities toward the anode. Identification: a, 4-n-heptylphenol; b, 4-n-amylphenol; c, 4-n-butylphenol; d, 4-n-propylphenol; e, 4-ethylphenol; f, *o*-cresol; g, *p*-cresol; h, *m*-cresol; i, phenol.

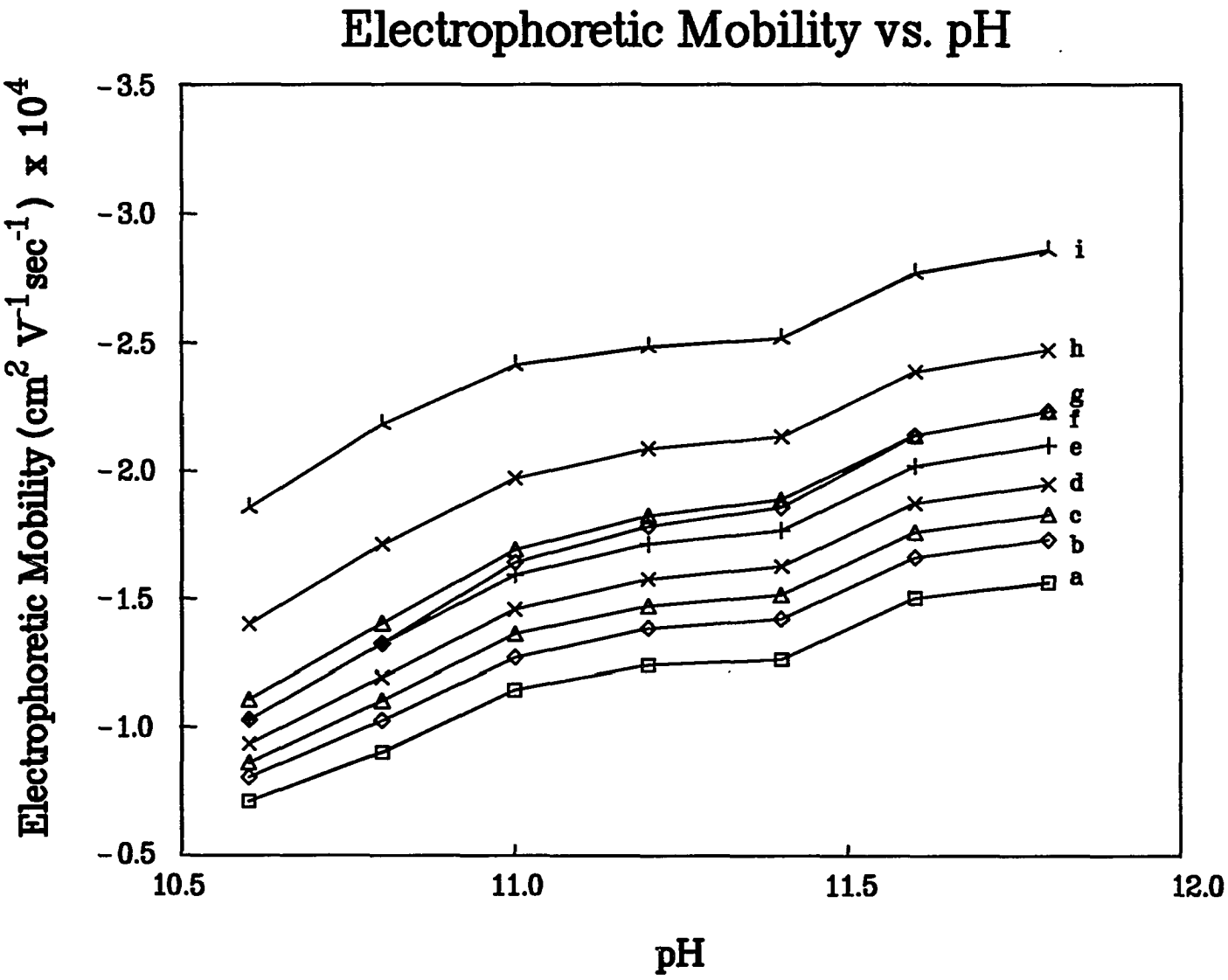
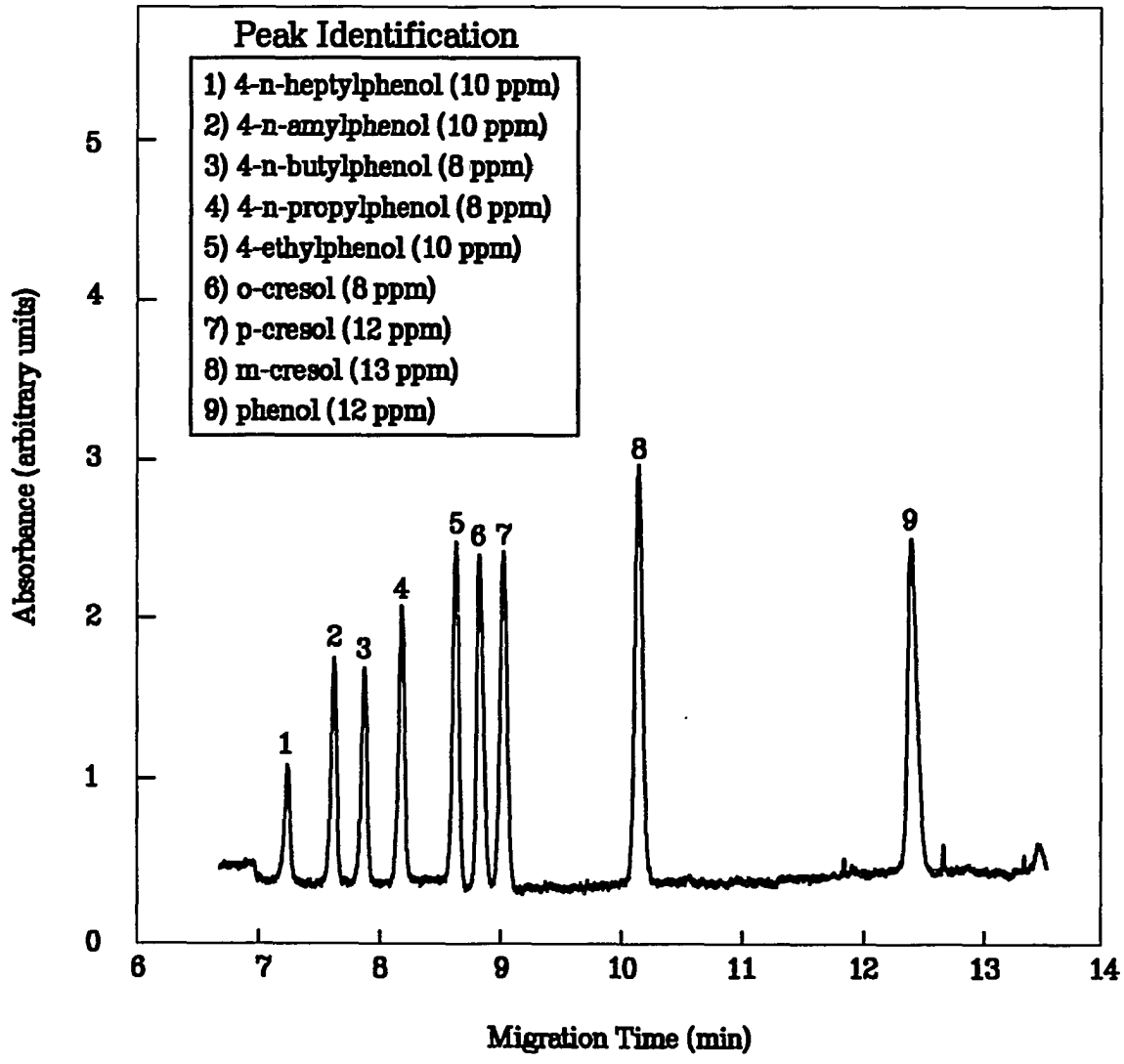

Electrophoretic Mobility vs. pH
Electrophoretic Mobility (cm$^2$ V$^{-1}$sec$^{-1}$) x 10$^4$
-3.5
-3.0
-2.5
-2.0
-1.5
-1.0
-0.5
10.5
11.0
11.5
12.0
pH
i
h
g
f
e
d
c
b
a

**Figure 7.** Separation of straight-chain, alkyl-substituted phenolate ions. Buffer solution: 1 mM borate, 40% acetonitrile, pH 11.2; 30 kV, 26 μA, 254 nm. Other conditions as stated previously. Peaks are as identified.


Peak Identification
1) 4-n-heptylphenol (10 ppm)
2) 4-n-amylphenol (10 ppm)
3) 4-n-butylphenol (8 ppm)
4) 4-n-propylphenol (8 ppm)
5) 4-ethylphenol (10 ppm)
6) o-cresol (8 ppm)
7) p-cresol (12 ppm)
8) m-cresol (13 ppm)
9) phenol (12 ppm)
Absorbance (arbitrary units)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Migration Time (min)

borate buffer solution had a pH of 8.64, whereas a 1 mM borate solution containing 40% acetonitrile had a pH of 9.96. The measured or "apparent" pH increased linearly by approximately 0.3 pH units per 10% addition of acetonitrile over the range of 0-50% acetonitrile.

Similar experiments to those completed for the straight-chain, alkyl-substituted phenols were performed to determine optimum separation conditions for mixtures of methyl-substituted and propyl- and butyl-substituted phenolate ions. A mixture of methyl-substituted phenolate ions was separated as shown in Figure 8. A separation of a mixture of cresols, di-, tri-, and tetramethylphenols was achieved. A buffer containing 40% acetonitrile at pH 11.7 was found to give the best separation of the mixture. The observed migration order is as expected. In general, within a group (e.g. tri- or dimethyl) compounds with higher $pK_a$s have lower migration times.

The separation of several structural isomers of propyl- and butyl-substituted phenolate ions at pH 12.0 is shown in Figure 9. Again 40% acetonitrile was added to the buffer solution for the best separation resolution. The separation of 2-, 3-, and 4-isopropylphenol was observed with these conditions. The partial separation of 4-propylphenol and 4-isopropylphenol achieved at pH 12.0 is especially important since the two compounds have the same mass and $pK_a$ value.

**Figure 8.** Separation of methyl-substituted phenolate ions. Buffer solution: 1 mM borate, 40% acetonitrile, pH 11.7; 30 kV, 25 μA, 254 nm. Other conditions as stated previously. Peaks are as identified.

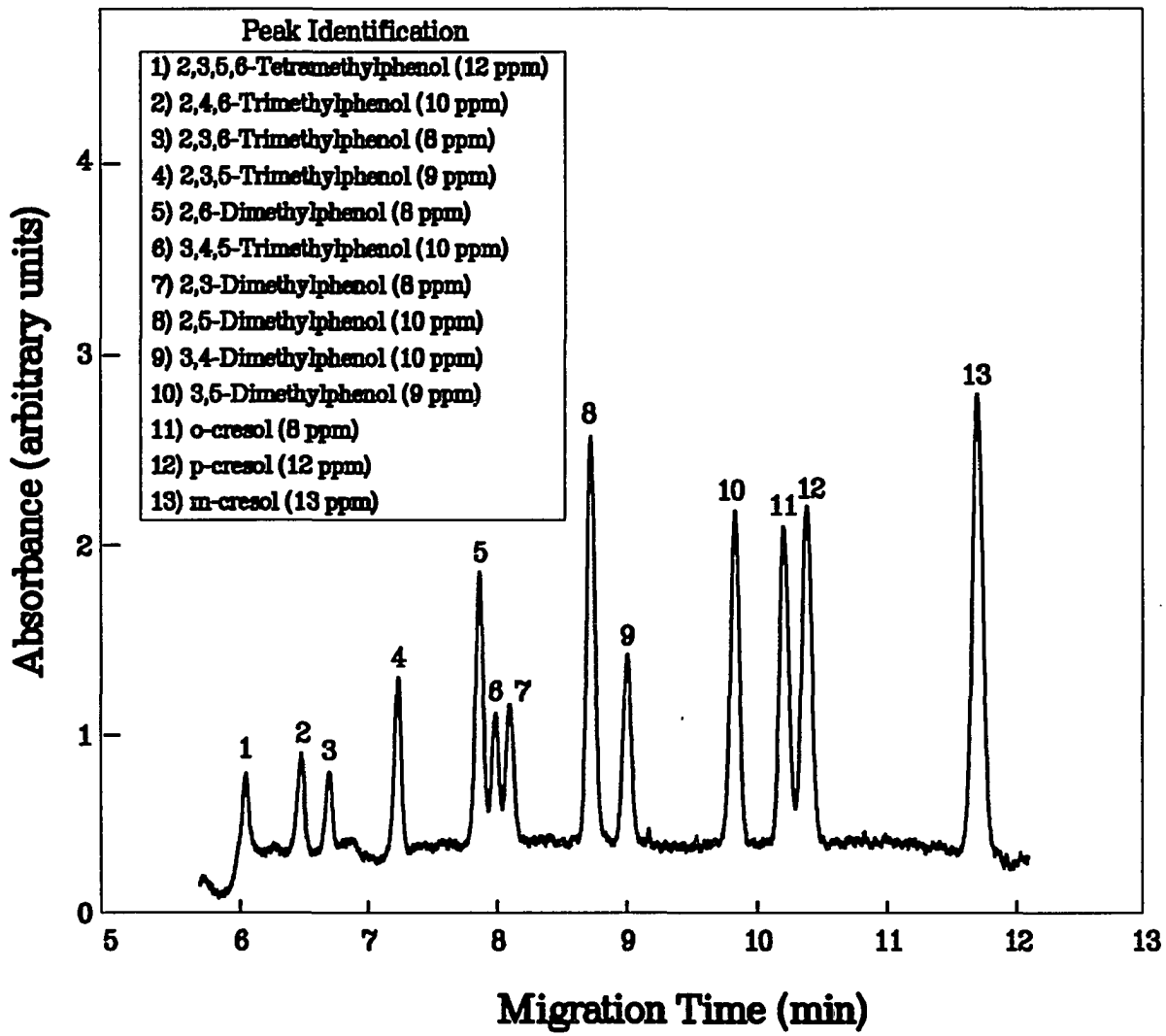
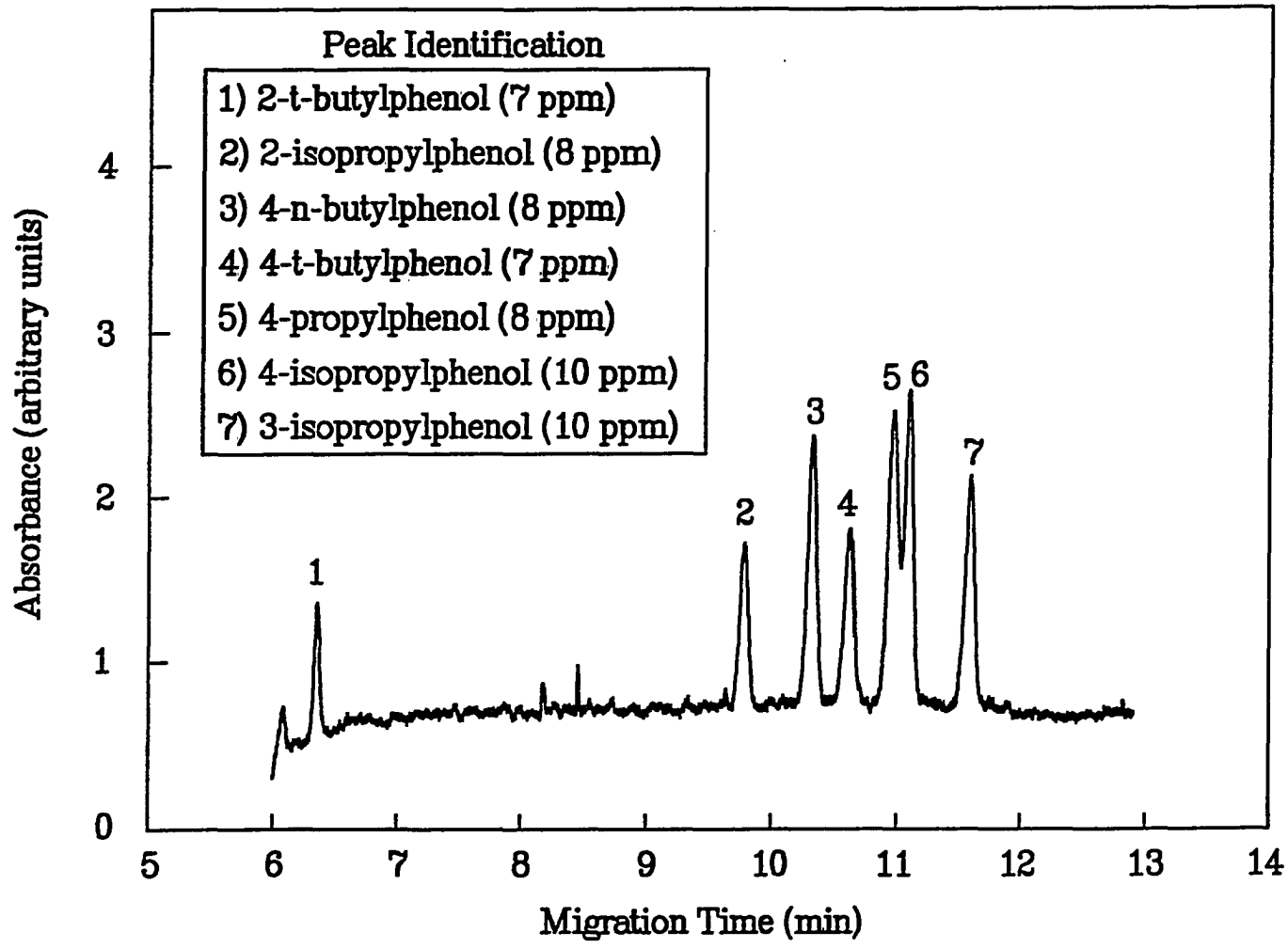

Peak Identification
1) 2,3,5,6-Tetramethylphenol (12 ppm)
2) 2,4,6-Trimethylphenol (10 ppm)
3) 2,3,6-Trimethylphenol (8 ppm)
4) 2,3,5-Trimethylphenol (9 ppm)
5) 2,6-Dimethylphenol (8 ppm)
6) 3,4,5-Trimethylphenol (10 ppm)
7) 2,3-Dimethylphenol (8 ppm)
8) 2,5-Dimethylphenol (10 ppm)
9) 3,4-Dimethylphenol (10 ppm)
10) 3,5-Dimethylphenol (9 ppm)
11) o-cresol (8 ppm)
12) p-cresol (12 ppm)
13) m-cresol (13 ppm)
Absorbance (arbitrary units)
Migration Time (min)
0
1
2
3
4
5
6
7
8
9
10
11
12
13

**Figure 9.** Separation of propyl- and butyl-substituted phenolate ions. Buffer solution: 1 mM borate, 40% acetonitrile, pH 12.0; 30 kV, 60 μA, 254 nm. Other conditions as stated previously. Peaks are as identified.


Peak Identification
1) 2-t-butylphenol (7 ppm)
2) 2-isopropylphenol (8 ppm)
3) 4-n-butylphenol (8 ppm)
4) 4-t-butylphenol (7 ppm)
5) 4-propylphenol (8 ppm)
6) 4-isopropylphenol (10 ppm)
7) 3-isopropylphenol (10 ppm)
Absorbance (arbitrary units)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Migration Time (min)

# CONCLUSIONS

Conditions for the separation of compounds with nearly identical electrophoretic mobilities can be determined easily when systematic studies of experimental parameters are completed. Buffer pH is the most important factor in the separation of organic acids due to the variation in the degree of dissociation as a function of pH. Adding an organic solvent, such as acetonitrile, to the buffer solution decreases electroosmotic and electrophoretic mobilities and increases resolution of separations of alkyl-substituted phenolate ions.

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# GENERAL CONCLUSIONS

A procedure was developed to determine water in soil and clay samples using liquid chromatography. Water was extracted from the sample using methanol. The water was then separated from matrix components on a cation-exchange column in the $H^+$ form via an ion-exclusion mechanism. The eluent contained a low concentration of *trans*-cinnamaldehyde and methanol. An equilibrium was established between the cinnamaldehyde and its corresponding acetal and water. When a sample containing water was injected into the system, a shift in equilibrium towards the cinnamaldehyde and an increase in absorbance proportional to the concentration of water in the sample was observed. The method was applied to a group of samples with a wide range of water concentrations. The LC results compared quite well with those obtained by a gravimetric method. The procedure was found to be a precise, specific, fast, and sensitive method for determining water in soil and clay samples.

Several experimental parameters were varied for the optimization of a variety of anion separations by CZE. A quaternary ammonium salt ($Q^+$) and several alcohols were studied to reverse EOF. CZE separations of several inorganic and short-chain organic acid anions were obtained. Low concentrations of a quaternary ammonium salt and 1-butanol were added to a chromate electrolyte solution to reverse EOF. Separations with good resolution and reproducibility were obtained using this method.

Several alkyl-substituted phenolate anions were separated by CZE. These separations were optimized by varying the pH and concentration of acetonitrile in the borate buffer

solution. The effects of pH and acetonitrile on EOF and electrophoretic mobility were investigated. Increased pH resulted in increased electrophoretic mobilities for the phenols. EOF decreased as the concentration of acetonitrile in the buffer solution was increased. The addition of 40% acetonitrile to the buffer solution increased migration times but improved resolution. Excellent separations of several alkyl-substituted phenolate anions, including structural isomers, with similar electrophoretic mobilities were achieved.

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