Separation and Determination of Monochlorophenols by Capillary Zones Electrophoresis

Zarina Yasmin Banu

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SEPARATION AND DETERMINATION OF MONOCHLOROPHENOLS BY CAPILLARY ZONE ELECTROPHORESIS

by

Zarina Yasmin Banu

A Thesis
Submitted to the
Faculty of The Graduate College
in partial fulfillment of the
requirements for the
Degree of Master of Arts
Department of Chemistry

Western Michigan University
Kalamazoo, Michigan
December 1998
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Zarina Yasmin Banu
1998
I wish to thank the Chemistry Department of Western Michigan University for providing the necessary instrumentation, chemical supplies and financial assistance to accomplish this research. I gratefully acknowledge Dr. Ralph Steinhaus, Dr. Michael E. McCarville and Dr. Mark Yancey for their assistance. I also gratefully acknowledge Dr. James A. Howell without whose precious assistance and guidance in this research could not have been completed.

Zarina Yasmin Banu
Chlorine has been widely used in industry. One of the most common uses of chlorine is in municipal water treatment plants. Chlorine reacts with a variety of organic compounds, among these are phenol and its derivatives which are commonly found in natural waters. When these compounds react with chlorine, a variety of chlorine substituted and oxidized products result depending on concentrations, pH, and temperature. In order to study these reactions it is necessary to separate and determine the various products produced.

Earlier studies have shown 3-chloro and 4-chlorophenol cannot be separated by commercial GLC columns. GC/MS (electron impact) measurements cannot resolve the problem since ionization removes the chlorine first and the resulting mass spectrum of both compounds is that of phenol. Costly GC/FTIR and gradient elution HPLC instrumentation is adequate, but more complex mixtures likely would require a greater degree of resolution.

In view of the above problems it was felt that capillary zone electrophoresis might provide a better alternative in terms of time, cost of analysis, resolution, and possible sensitivity.
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INTRODUCTION

Background

Chlorine is widely used in industry. One of the most common uses of chlorine is in municipal water treatment. Chlorine is so active that it reacts with a variety of organic compounds. According to Dolara, chlorinated pollutants have deleterious side effects on both human and animal subjects such as memory problems, cancer and stunted growth.

Phenol and its derivatives are abundant in nature. It is widely recognized that the principle natural source of these compounds are the decomposition of humic substances. Man made sources of phenol are from coal, gas, steel and petroleum industrial wastes. Zhang has demonstrated that when phenols react with chlorine, a variety of chlorine substituted and oxidized products result depending on concentrations, pH and temperature. In order to study these reactions it is necessary to determine the quantities of the various products produced. Thus the need to separate and quantitate these species is essential.
Objective

Some of the products of chlorination with humic substances tend to be mixtures of monochlorophenol isomers. Previous studies of Zhang have indicated that it is difficult to differentiate between 3-chlorophenol and 4-chlorophenol. Both have similar boiling points (3-chlorophenol, 214° C; 4-chlorophenol, 220° C). No commercial GLC columns appear to be able to separate 3- and 4-chlorophenol. The GC/MS (EI) technique also does not permit differentiation of these compounds since they produce the same fragmentation pattern as has been reported by Zhang. Two approaches have solved this problem. Baiocchi and coworkers showed that HPLC analysis with gradient elution produces two partially resolved peaks for these isomers. Another approach takes advantage of the 3- and 4-chlorophenol have different dipole moments. Thus one can differentiate with GC/FTIR. This work has been reported by Brown and Howell.

The Merck index lists pKa values of 2-, 3- and 4-Chlorophenol as 8.48, 9.08 and 9.38, respectively. Taking advantage of these differences it was felt that perhaps capillary zone electrophoresis (CZE) might provide an effective separation and analysis procedure for these compounds.
Electrophoresis has been one of the more widely used techniques for the separation and analysis of ionic substances. In this process electrically charged molecules or ions move through a conductive liquid medium under the influence of an electric field. See Figure 1.

Figure 1. Block Diagram of Capillary Electrophoresis System.

The buffer solution moves through the capillary under the influence of the electric field. Buffer cations are pulled toward the cathode, dragging solvent with them. This phenomenon is known as electroendosmotic or
electroosmotic flow (EOF). The direction of EOF is toward the negative cathode.

The migration rate or speed of the analyte towards the detector depends on electrophoretic migration of the individual analytes, the electroosmotic mobility and the electric field gradient. The EOF is a function of pH and ionic strength.

\[ V_{\text{app}} = \mu_{\text{app}} E = (\mu_{\text{ep}} + \mu_{\text{eof}}) E \]  

Where \( V_{\text{app}} \) = apparent velocity, 
\( \mu_{\text{app}} \) = apparent electrophoretic mobility, 
\( \mu_{\text{eof}} \) = electroosmotic mobility, 
\( \mu_{\text{ep}} \) = electrophoretic mobility, 
E = electric field.

Anions have electrophoretic mobility toward the anode while cations have electrophoretic mobility toward the cathode and neutral molecules have no electrophoretic mobility and thus move only with the EOF. Because of electroosmotic flow all solutes are carried through the capillary. Thus neutral species can be separated from charged molecules. Anions and cations can be separated in a single electropherogram when injection occurs at the anode. Otherwise two separate electropherograms
must be carried out, one with polarity set to analyze cations, the other with the polarity switched for anions.

The surface silanol (Si-OH) groups of fused silica capillary are ionized to negatively charged silanoate (Si-O⁻) groups at pH above about 3.0. Baker describes how silanoate groups attract positively charged cations from the buffer, which form an inner layer of cations at the capillary wall. See Figure 2.

<table>
<thead>
<tr>
<th>electroosmotic flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>High pH</td>
</tr>
<tr>
<td>Cathode (-)</td>
</tr>
<tr>
<td>O⁻ Si⁻ O⁻ Si⁻ O⁻ Si⁻ O⁻ Si⁻ Anode (⁺)</td>
</tr>
<tr>
<td>O⁻ H⁺ O⁺ O⁻ H⁺ O⁺</td>
</tr>
<tr>
<td>+ - + + - +</td>
</tr>
</tbody>
</table>

| Low pH              |
| Cathode (-)         |
| O⁻ Si⁻ O⁻ Si⁻ O⁻ Si⁻ O⁻ Si⁻ Anode (⁺) |
| OH O⁻ OH O⁻ OH (+)  |
| + + + -             |

Figure 2. Mobility of Charged and Uncharged Molecules in an Applied Field at the Surface of a Silica Capillary.

These cations do not neutralize all of the negative charges, so a second outer layer of cations forms. The inner layer is tightly held by the Si-O⁻ groups and the outer layer of cations are held less tightly because they are
further away from the silanol groups. At an applied electric field the outer cation layer is pulled toward the negatively charged cathode. These cations being solvated, drag the bulk buffer solution with them, thus causing the electroosmotic flow. The EOF has a relative flat profile compared to laminar flow as encountered in HPLC. Thus capillary electrophoresis tends to give sharper peaks than observed with HPLC peaks.

The velocity of the EOF has been described by Baker and can be seen in Equation 2.

\[
V_{\text{EOF}} = \frac{\varepsilon \zeta E}{4 \pi \eta} \tag{2}
\]

Where \( V_{\text{EOF}} \) = velocity of electroosmotic flow,
- \( \varepsilon \) = dielectric constant of the buffer,
- \( \zeta \) = zeta potential,
- \( E \) = applied electric field in volts/cm,
- \( \eta \) = viscosity of the buffer.

At pH above three, the surface silanol groups of fused silica capillary tube are ionized to negatively charged silanoate groups. The negatively charged silanoate groups then attract positively charged cations from the buffer, which form an inner layer of cations at the capillary wall. As mentioned earlier, a more loosely bound layer of cations exist at a
somewhat greater distance from the capillary wall. There is a potential difference between the two layers of cation which is termed the zeta potential. VanOrman and workers have shown the zeta potential to be a function of pH, ionic strength and concentration of the buffer. At lower or higher pH, there is less surface ionization and a lower zeta potential. Ionization of surface silanol groups also depends on the ionic strength and thus the concentration of the buffer. At constant temperature, increasing the buffer concentration or ionic strength will reduce the electroosmotic flow due to the lower zeta potential. However if the temperature of the capillary is not controlled, an increase of the electroosmotic flow may occur due to lower viscosity. Lower buffer concentrations will give rise to shorter migration times but very low concentrations of buffer give broadened asymmetric peaks.

The flow of electric current causes Joule heating when passing through the conductive solution. Joule heating warms the solution and leads to thermal diffusion. Molecules in warmer center of the tube migrate faster than those near the cooler wall, leading to zone spreading. Capillary columns, however, are very efficient in dissipating thermal energy and thus minimize convection. Wallingford and Ewing have describe the dissipation of electric power per unit of length of the column as:
\[ \frac{P}{L} = \frac{(kCr^2V^2)}{L^2} \]  \hspace{1cm} (3)

where \( P \) = power,

\( L \) = length of the capillary,

\( k \) = molar conductance of the solution,

\( C \) = concentration of buffer,

\( r \) = internal radius of the column,

\( V \) = applied voltage.

The capillary wall thickness is also important when air cooled since the column serves as a heat sink.

In a round tube, the temperature difference between the center and the wall of the tube, \( \Delta T \), is:

\[ \Delta T = \frac{(0.239 \, Q)}{4K} \, r^2 \]  \hspace{1cm} (4)

where \( Q \) = power density watts/m\(^3\),

\( K \) = thermal conductivity,

\( r \) = radius.

This relationship has been discussed by Tsuda and coworkers. It is seen that the temperature difference between center and wall of the tube will be reduced if the radius of the tube is decreased. Again, at constant voltage, if the radius is decreased, resistance of the capillary tube will be increase and the current will decrease with less heat being generated.
There are several modes of capillary electrophoresis, capillary zone electrophoresis (CZE), capillary gel electrophoresis (CGE), capillary isoelectric focusing (CIEF) and capillary isotachopheresis (CITP).

Capillary zone electrophoresis is the most widely used mode of electrophoresis because of its efficiency, the ability to reduce convection and its speed of analysis. The principle of the separation in this technique is based on the charge to size ratio of the solute. In CZE, the capillary is filled with a buffer as well as the inlet and outlet vials. After a sample is injected into the capillary filled with buffer and a voltage is applied, the solutes migrate through the capillary as zones.

Capillary gel electrophoresis separates ionic solutes primarily on the basis of their size. Capillaries are filled with a polymer which causes separation of solutes. Hjerten and Zhu have discussed capillary isoelectric focusing which separates ionic solutes on the basis of their different isoelectric points. A pH gradient is established in the capillary, the solutes are focused, mobilized to pass through the detector. Capillary sotachopheresis is a moving boundary technique in which the sample is sandwiched between a leading and terminating buffer in a capillary.
Description

Power Supply Requirement

The power supply is necessary to provide a high electric field across the capillary. Constant voltage, constant current, or constant power mode may be used. The constant voltage mode is the most widely used since the velocity of an ion is directly proportional to the electric field (Equation 1). Typically CE power supplies have maximum specifications of 30 kV, 300 \(\mu\)A and 6 W.

Injector

There are several techniques used to inject a sample into the capillary tube. The most common are the hydrodynamic and electrokinetic methods. With hydrodynamic injection, the sample is introduced by immersing the capillary inlet into a vial containing sample and either pressurizing the inlet vial containing the sample or applying a vacuum to the outlet vial for a prescribed time period. Hydrodynamic injection may also be done by siphoning. Siphon injection, also called gravity injection, is done by raising the sample vial relative to the outlet vial for a prescribed time. In the electrokinetic injection process, a low voltage (1 to 10 kV) is applied to the sample vial, causing the sample components to migrate into the capillary due to electroosmotic and electrophoretic
mobility. Neutral solutes have no electrophoretic mobility and are pulled into the capillary only by the electroosmotic mobility. The electrokinetic injection process draws more positive ions than negative ions and neutral molecules into the capillary. This process can discriminate between the ions and molecules.

Detector

A variety of detectors have been used in capillary electrophoresis including UV/Vis absorbance, fluorescence, mass spectrometry, conductivity, amperometric and atomic spectroscopy.

UV/Vis absorbance detectors function by monitoring the light absorbed by the solute molecules from the incident beam. They are not appreciably flow or temperature sensitive, have a good dynamic linear range, and are somewhat selective.

When energy in the form of light is absorbed by a molecule, some of that energy may be given off as heat and some as light of a lower frequency. A small number of compounds absorb radiation of one wavelength and subsequently emit fluorescent radiation. Baker states that approximately 10% of organic compounds can be detected by using a fluorescent detector. The sensitivity of a fluorescence detector is proportional to the intensity of the incident light. Due to its high intensity light and the ability to accurately focus the light down to the capillary
inner diameter, laser fluorescence gives high sensitivities.

A mass spectrometer detector is a universal detector which detects all solutes that have a molecular weight within the instrument's mass range. It has relatively good sensitivity. In this spectrometer, a sample is broken down into charged fragments or ions which often have a charge of plus one. The mass spectrometer analyzes the fragments and produces a mass spectrum, a plot of mass-to-charge ratio versus intensity for each ion.

The basis of the conductivity detector is the monitoring of the change in conductivity as ionic species exit the column. These ions are then monitored with a d. c. conductivity cell. Conductivity is proportional to the buffer concentration. Thus higher concentrations of buffer solution give higher background noise which makes it difficult to detect trace levels of solute.

Amperometric detectors measure the current that results from oxidation or reduction of electroactive solute at a working electrode surface. Oxidation or reduction of the solutes result when a potential is applied across a supporting electrolyte between a working and a reference electrode. The current that flows through the working electrode is proportional to the number of electron transfers and thus to the concentration of the solute.

Atomic spectroscopy is based on absorption, emission or
fluorescence by atoms or elementary ions. The frequency of radiation is proportional to the energy needed for the transition of an electron from one energy level to another. In this process the solute is converted into a mist of finely divided droplets by a jet of a compressed gas. Then the vaporized molecules are dissociated into atoms. These ground state atoms may be excited by the thermal energy of a flame or plasma or by the radiation of an external elemental light source. Sensitivity is high and response is specific for a given element.

Capillary Tube

Polyimide-coated fused silica capillaries which are 30-100 cm long with inner diameters from 50 to 75 µm and outer diameter 360-375 µm are typically used. The narrow capillary diameter facilitates the dissipation of heat generated by the electrical resistance of the electrolyte inside the capillary. The polyimide coating alleviates the considerable fragility of uncoated fused silica tubing.

A fused silica capillary is transparent to UV and visible light so capillary itself can be used as the detector cell when UV/Vis or fluorescence detection is used. The cell window can be made by burning or scraping off a small section of the polyimide outer coating of the capillary. Increased sensitivity may the achieved with a bubble cell or a "Z" capillary cell as depicted in Figure 3. Recently Hewlett Packard introduced a high
sensitivity cell (light path 1.2 mm and 12 nl volume) which exhibits a 10 fold increase S/N giving sensitivities comparable to those observed in HPLC.

Figure 3. Common UV/Vis Cells for CZE.
EXPERIMENT

Equipment

In this study a Bertan High Voltage power supply, Series 230-R was used. It provides constant voltage with a maximum of 30 kilovolts. The electrodes and cell assembly have been described by Sutton. This unit was operated at 19.5 kV in order to avoid excessive noise arising from leakage currents. Equation (1) indicates that the velocity of the solute is directly proportional to applied voltage. Thus if too low of a voltage were used, a lengthy separation would occur.

Capillary columns (Supelco, part no. 77501) used were 75µm internal diameter and 375 µm outer diameter. Column lengths were 65 centimeters for each electropherogram. Supelco Inc. supplied the capillary columns with the detection window 65 cm from the injector.

An Acutect Model 500 UV/Vis detector (Thermo Separation Products) was used in this study. The wavelength range was from 190 nm to 800 nm with manual drive and a mechanical wavelength indicator. The detector has the following properties: band width 6 nm, wavelength accuracy ± 1nm, and wavelength precision ± 0.1 nm. It uses a standard deuterium lamp with a concave holographic grating monochromator and
double-beam optics. One can select 12 selectivity ranges from 2.0 AUFS to 0.0005 AUFS. A 1.0 V/AU analog output, independent of range control but dependent on auto-zero function is available. Noise of $\pm 2 \times 10^{-5}$ AU/30 sec from 220-280 nm with 1.0 sec rise time is typical. An auto zero circuit capable of offsetting greater than 1.5 AU with standard flow cell is provided.

The buffer reservoir and sample reservoir both were small wide mouth glass bottles (4 dram). The bottles were made airtight with a cap which was sealed with the electrode and the capillary tube inserted through a rubber septum. This permitted purging and conditioning of the capillary pneumatically as well as pneumatic sample injection. A laboratory jack (Precision Scientific Co.) was used for gravity injection.

Reagents

Preparation

Starch Solution, 1% w/v: Dissolve 1.0 g. starch in 100 ml Milli Q water. Boil for 5 minutes and keep the clear supernatant liquid after it has cooled to room temperature.

Sodium Thiosulfate Solution: Weigh 0.3982 gram anhydrous Na$_2$S$_2$O$_3$ and about 1 gram NaOH into a 1.00 L volumetric flask. Dissolve with Milli Q water, cool and dilute to volume. Leave overnight before use.
Potassium Iodate: Dissolve 0.0461 gram KIO₃ with 10 ml concentrated HCl in a 500 ml volumetric flask and dilute to volume.

Chlorine Aqueous Stock Solution: The gas from the tank (AGA, 99.5% purity) was gently bubbled into chilled Milli Q water until saturated.

**Procedures**

Standardization of Sodium Thiosulfate Solution: Skoog, West and Holler describe the standardization process of sodium thiosulfate solution: 15.00 ml of 0.428 millimolar KIO₃ was mixed with excess KI (ca. 1 g) and then titrated with Na₂S₂O₃ until solution became light yellow. Several drops of starch solution were added and titration continued until the solution became colorless.

Standardization of Chlorine Solution: 25 ml Cl₂ stock solution was diluted with ice cold 0.1 molar sulfuric acid into 250 ml volumetric flask and diluted to volume. Excess KI (ca. 1 g) was dissolved in approximately 10 ml MilliQ water in an Erlenmeyer flask and then 2.00 ml of diluted Cl₂ solution was added. This solution was titrated with standardized sodium thiosulfate solution.
Reaction Mixture: After the concentration of the chlorine solution was determined, aliquots were mixed with a 0.047 M phenol 0°C for 24 hours.

Extraction and Sample Preparation: Aqueous reaction mixtures were mixed with methylene chloride at volume ratio 8:1 (aqueous: organic) in separatory funnels. The organic phase was transferred into a Kaderuda-Danish (K-D) apparatus. A steam bath was used to concentrate the methylene chloride extracts. Then a gentle steam of nitrogen gas was blown into the methylene chloride extracts and taken to dryness. This material was dissolved in borax buffer (0.1M, pH 9.24) and injected into capillary zone electrophoresis apparatus for identification.

All reagents used in this study are listed in Table 1.

Procedure

One of the most important parameters in capillary zone electrophoresis separations is the selection of a suitable buffer. The efficiency, resolution and possibly sensitivity of the separation depend on pH and concentration of the buffer. This can be seen from equation 2.

As stated earlier the pKa values of 2-, 3- and 4-chlorophenol as 8.48, 9.08 and 9.39 respectively. A 0.1M solution of borax gives a pH of 9.24. At this pH the percent of the phenols dissociated can be calculated
<table>
<thead>
<tr>
<th>Chemical Name</th>
<th>Grade</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium Tetraborate decahydrate</td>
<td>A.C.S.</td>
<td>Fisher Scientific.</td>
</tr>
<tr>
<td>2-Chlorophenol</td>
<td>99 + %</td>
<td>Aldrich</td>
</tr>
<tr>
<td>3-Chlorophenol</td>
<td>98%</td>
<td>Aldrich</td>
</tr>
<tr>
<td>4-Chlorophenol</td>
<td>99 + %</td>
<td>Aldrich</td>
</tr>
<tr>
<td>Methyl Orange</td>
<td>A.C.S.</td>
<td>Allied</td>
</tr>
<tr>
<td>Methylene Chloride</td>
<td>HPLC</td>
<td>Fisher Scientific</td>
</tr>
<tr>
<td>Starch</td>
<td>A.C.S.</td>
<td>J. T. Baker</td>
</tr>
<tr>
<td>Sodium Thiosulfate, anhydrous</td>
<td>A.C.S</td>
<td>J. T. Baker</td>
</tr>
<tr>
<td>Potassium Iodate</td>
<td>A.C.S.</td>
<td>Spectrum</td>
</tr>
<tr>
<td>Potassium Iodide</td>
<td>U. S. P.</td>
<td>Mallinckrodt</td>
</tr>
<tr>
<td>2,6-Dichlorophenol</td>
<td>99%</td>
<td>Aldrich</td>
</tr>
<tr>
<td>2,4,6-Trichlorophenol</td>
<td>99%</td>
<td>Aldrich</td>
</tr>
<tr>
<td>Hydrochloric acid</td>
<td>A.C.S.</td>
<td>Fisher Scientific</td>
</tr>
<tr>
<td>Sulfuric acid</td>
<td>A.C.S.</td>
<td>Curtin Matheson</td>
</tr>
<tr>
<td>Sodium Hydroxide</td>
<td>A.C.S.</td>
<td>Fisher Scientific</td>
</tr>
<tr>
<td>Phenol</td>
<td>U.S.P.</td>
<td>Spectrum</td>
</tr>
</tbody>
</table>
as described by Skoog, West, Holler. The percent dissociation for the 2-, 3- and 4- isomers are 5.9, 1.5 and 0.79 respectively. Thus one should anticipate the order of emergence from the capillary to be 4-Cl phenol, 3-Cl phenol and 2-Cl phenol. Furthermore this buffer does not exhibit a significant absorbance in the wavelength region from 200 to 800 nm as can be seen in Figure 4. Also the buffer does not tend to dissociate at voltages less than 30 kV.

An ultraviolet-visible (UV/Vis) absorbance detector is based on Beer's law:

\[ A = abc \]  

(5)

Where

- \( A \) = absorbance,
- \( a \) = absorptivity,
- \( b \) = the path length of the light path (capillary i.d) in the sample,
- \( c \) = the solute concentration.

A UV/Vis absorbance detector was used to monitor the electrophoretic migration times in this investigation. The absorption spectra of these compounds can be seen in Figure 5, 6 and 7. While greater sensitivities might be achieved by selecting a wavelength less than 240 nm, 288 nm was chosen for better selectivity even though a
Figure 4. Absorbance Spectrum of 0.1 M Bobax Buffer Solution pH 9.24 (b = 1.00 cm).
Figure 5. Absorbance Spectrum of 0.0017 M 2-Chlorophenol Solution in Borax Buffer, pH 9.24 (b = 1.00 cm).
Figure 6. Absorbance Spectrum of 0.0017 M 3-Chlorophenol Solution in Borax Buffer, pH 9.24 (b = 1.00 cm).
Figure 7. Absorbance Spectrum of 0.0018 M 4-Chlorophenol Solution in Borax Buffer, pH 9.24 (b = 1.00 cm).
significant lose of sensitivity was to be expected.

The output of the detector was connected to a Shimadzu CR 601 Chromatopac integrator which can measure either peak area or peak height. Since absorbance detectors are concentration detectors, technically one should operate the integrator in the peak height mode. However since sample plug flow is continuous, being controlled by the EOF which remains essentially constant throughout an electropherogram, peak area measurements are equally valid. This was confirmed by the data shown in Figure 8, 9, and 10.

A typical separation of a mixture of the three isomers using 0.10M borax buffer at 19.5 kV, can be seen in Figure 11. When 2-, 3- and 4-chlorophenol solutions were analyzed in succession, the migration times changed with each electropherogram. This variation in migration times can be seen in Table 2.

These variations probably arise due to changes in the column surface and variations in the buffer. This problem was solved by using a marker, which was added to the standard and sample solutions. A relative migration time was calculated for each of the components in the standard or sample by dividing the migration time of the solute by the migration time of the marker. The relative migration times became the parameter of
Figure 8. Linearity of Area and Height vs. Concentration of 2-Chlorophenol.

Figure 9. Linearity of Area and Height vs. Concentration of 3-Chlorophenol.
choice in interpreting the electropherograms. The criteria for selecting a marker were: 1. must absorb at 288 nm, 2. have an electrophoretic mobility similar to the analytes and 3. be soluble in the buffer. After examining numerous compounds it was found that methyl orange was ideally suited. It was water soluble. It gave one peak at 288 nm, it gave an anion at pH 9.24, and it gave a peak between 2-chlorophenol and 4-chlorophenol. Reproducibility of relative migration times using methyl orange as a marker can be seen in Table 3. A typical electropherogram of the three chlorophenol isomers with methyl orange can be seen in Figure 12.

Figure 10. Linearity of Area and Height vs. Concentration of 4-Chlorophenol.
1 = 4-Cl Phenol (0.032 mM)
2 = 3-Cl Phenol (0.11 mM)
3 = 2-Cl Phenol (2.35 mM)

0.1 M Borax Buffer, pH = 9.24
Gravity Injection: 3.0 inches
4.0 seconds
19.5 kV

UV/Vis Detector: 288nm

Figure 11. Electropherogram of 2-, 3- and 4-Chlorophenol Solution.
Table 2
Migration Time of 2-, 3- and 4-Chlorophenol

<table>
<thead>
<tr>
<th>Compound</th>
<th>Sample 1</th>
<th>Migration Time (min)</th>
<th>Sample 2</th>
<th>Sample 3</th>
<th>Sample 4</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Cl Phenol</td>
<td>11.9</td>
<td>11.8</td>
<td>11.6</td>
<td>11.8</td>
<td></td>
<td>0.12</td>
</tr>
<tr>
<td>3-Cl Phenol</td>
<td>5.98</td>
<td>5.15</td>
<td>5.47</td>
<td>6.05</td>
<td></td>
<td>0.43</td>
</tr>
<tr>
<td>4-Cl Phenol</td>
<td>4.05</td>
<td>4.62</td>
<td>4.97</td>
<td>4.35</td>
<td></td>
<td>0.40</td>
</tr>
</tbody>
</table>

Table 3
Relative Migration Times of Chlorophenols

<table>
<thead>
<tr>
<th>Compound</th>
<th>No. Detn's</th>
<th>Average (min)</th>
<th>Standard Deviation</th>
<th>Coeff. of Var. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Cl Phenol</td>
<td>10</td>
<td>1.216</td>
<td>0.0081</td>
<td>0.67</td>
</tr>
<tr>
<td>3-Cl Phenol</td>
<td>10</td>
<td>1.068</td>
<td>0.007</td>
<td>0.65</td>
</tr>
<tr>
<td>4-Cl Phenol</td>
<td>10</td>
<td>0.884</td>
<td>0.0027</td>
<td>0.30</td>
</tr>
</tbody>
</table>

Before attempting quantitative measurements it was necessary to establish a reliable injection procedure. As stated earlier the most common modes of injection for capillary zone electrophoresis are electrokinetic and hydrodynamic. Since electrokinetic injection might give
1 = 4-Cl Phenol (2.3 mM)
2 = 3-Cl Phenol (2.6 mM)
3 = 2-Cl Phenol (2.54 mM)
4 = Methyl Orange (0.05 mM)
0.1 M Borax Buffer, pH = 9.24
Gravity Injection: 3.0 inches
4.0 seconds
19.5 kV
UV/Vis Detector: 288nm

Figure 12. Electropherogram of 2-, 3- and 4- Chlorophenol Solution With Methyl Orange Marker.
rise to a sampling bias the hydrodynamic injection technique was chosen. Hydrodynamic injections were made by placing the capillary inlet in the sample vial, and pressurizing the sample vial or by elevating (siphoning) the sample vial relative to the destination vial for a fixed time interval.

The first injection process was carried out by raising the sample vial 3.0 inches for 4.0 seconds and then returning the vial to its original position. Subsequently the sample vial was replaced with a buffer vial and voltage was applied. Table 4 shows the coefficient of variation in area of three isomers.

Table 4

Gravity Injection

<table>
<thead>
<tr>
<th>Isomer</th>
<th>No. Detn's</th>
<th>Average Area (µV-sec.)</th>
<th>Standard Deviation</th>
<th>Coeff. of Var. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Cl Phenol</td>
<td>5</td>
<td>1779</td>
<td>299</td>
<td>16.8</td>
</tr>
<tr>
<td>3-Cl Phenol</td>
<td>5</td>
<td>1776</td>
<td>289</td>
<td>16.3</td>
</tr>
<tr>
<td>4-Cl Phenol</td>
<td>5</td>
<td>1382</td>
<td>224</td>
<td>16.2</td>
</tr>
</tbody>
</table>

The coefficient of variation of area of these three chlorophenols was about 16 %, implying poor reproducibility by the gravity injection process.
Subsequently the injection was done with pressure. Here the sample vial was pressurized by nitrogen gas in a tank. The pressure was about 1.0 psi and sampling time was 4.0 seconds. Table 5 shows the data for this injection technique.

Table 5
Pressure Injection by Nitrogen Tank

<table>
<thead>
<tr>
<th>Isomer</th>
<th>No. Detn's</th>
<th>Average Area (µV-sec.)</th>
<th>Standard Deviation</th>
<th>Coeff. of Var.(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Cl Phenol</td>
<td>6</td>
<td>15845</td>
<td>1449</td>
<td>9.1</td>
</tr>
<tr>
<td>3-Cl Phenol</td>
<td>6</td>
<td>8776</td>
<td>712</td>
<td>8.1</td>
</tr>
<tr>
<td>4-Cl Phenol</td>
<td>6</td>
<td>12928</td>
<td>1231</td>
<td>9.5</td>
</tr>
</tbody>
</table>

The coefficient of variation of about 9% was also considered to be too large. So the injection process was changed again to syringe pressure. In this case 0.5 ml of air was injected into the sample vial for 4.0 seconds. Table 6 shows the data for this injection technique. The coefficient of variation was about 2% which was considered acceptable.
Table 6

Syringe Pressure Injection

<table>
<thead>
<tr>
<th>Isomer</th>
<th>No. Detn's</th>
<th>Average Area (µV-sec.)</th>
<th>Standard Deviation</th>
<th>Coeff. of Var.(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Cl Phenol</td>
<td>6</td>
<td>13563</td>
<td>394</td>
<td>2.9</td>
</tr>
<tr>
<td>3-Cl Phenol</td>
<td>6</td>
<td>9541</td>
<td>149</td>
<td>1.5</td>
</tr>
<tr>
<td>4-Cl Phenol</td>
<td>6</td>
<td>8235</td>
<td>150</td>
<td>1.8</td>
</tr>
</tbody>
</table>
RESULTS

Quantitative analysis by capillary electrophoresis is based on the linear relation of peak heights or peak area with concentration when using a UV/Vis absorbance detector. Peak areas of the electropherogram are dependent on three factors: 1. concentration of solute, 2. injection time, 3. injection pressure. A study of these effects on peak area was undertaken by varying each independently while holding the others constant. Figure 13 shows the relationship between peak area and concentration of the solute when injection pressure and injection were constant.

Figure 13. Area vs. Concentration of Monochlorophenols.
(injection pressure = 2.1 mm Hg, injection time = 4 sec.)
When concentration and pressure were constant and injection time varied, the data shown in Figure 14 was obtained. Figure 15 was obtained when injection pressure was varied and concentration and injection time were held constant.

![Graph showing area vs. injection time with equations for 2-Cl-phenol, 3-Cl-phenol, and 4-Cl-phenol](image)

**Figure 14. Area vs. Injection Time.**
(injection pressure = 2.1 mm Hg concentration 2-Cl Phenol = 0.0017 M, 3-Cl Phenol = 0.00165 M, 4-Cl Phenol = 0.00185 M.)

From Figure 15 there appears to be an anomalous positive deviation from linearity at higher pressures. If one can operate at lower pressures, less than 6 mm Hg linear relationship between area and solute concentration, injection time and pressure can be assumed. This is consistent with theory as described by Landers. Table 7 shows typical analysis data one might anticipate using CZE to analyze monochlorophenol isomers. The limit of linear (LOL) response and the limit of quantitation (LOQ) as
Figure 15. Area vs. Injection Pressure.
(time = 4.0 sec., concentration 2-Cl phenol = 0.0017 M, 3-Cl phenol = 0.00165 M, 4-Cl phenol = 0.00185 M)

discussed by Skoog can be used to define the applicable concentration range of the method. Using this criteria, the applicable range for the CZE method to determine 2-, 3- and 4-chlorophenol is from 0.026 mM to 10 mM. The limit of detection (LOD) was arbitrarily determined to be 0.01 mM.

Zhang found that chlorine reacts to produce a variety of products which have been listed in Table 8. However a problem encountered by Zhang was the inability to differentiate between 3- and 4-chlorophenol. When analysis of a similar reaction mixture reported by Zhang was carried out using the CZE procedure, five peaks were observed. Qualitative identification of compounds in a sample can be done several ways. One way is by determining migration time. In this method the
migration time of the compound of interest must be determined first using a standard. Then by injecting the sample mixture and determining if a peak appears which coincides with the relative migration time of the standard. The migration times of the chlorine-phenol reaction mixture exhibited relative migration times corresponding 2-, 3-, 4-chlorophenol, 2,6-dichlorophenol and 2,4,6-trichlorophenol. Another way to identify a compound in a sample is to inject the sample mixture and obtain an

### Table 7

**Typical Results**

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Isomer</th>
<th>Millimoles Phenol Added</th>
<th>Ave. Millimoles Phenol Found</th>
<th>No. Detn's</th>
<th>Coeff. of Var.(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2-Cl</td>
<td>0.1723</td>
<td>0.172</td>
<td>3</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td>3-Cl</td>
<td>0.2970</td>
<td>0.298</td>
<td>3</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>4-Cl</td>
<td>0.1054</td>
<td>0.107</td>
<td>3</td>
<td>2.1</td>
</tr>
<tr>
<td>2</td>
<td>2-Cl</td>
<td>0.1542</td>
<td>0.154</td>
<td>3</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>3-Cl</td>
<td>0.1799</td>
<td>0.179</td>
<td>3</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td>4-Cl</td>
<td>0.0643</td>
<td>0.065</td>
<td>3</td>
<td>2.6</td>
</tr>
<tr>
<td>3</td>
<td>2-Cl</td>
<td>0.0643</td>
<td>0.067</td>
<td>3</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>3-Cl</td>
<td>0.4085</td>
<td>0.407</td>
<td>3</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>4-Cl</td>
<td>0.1170</td>
<td>0.116</td>
<td>3</td>
<td>2.8</td>
</tr>
</tbody>
</table>
Table 8

Reaction Products of Chlorine With Phenol at Phenol to Chlorine Molar Ratio of 1:1 (0.05 M Phosphate Buffer, pH = 1.55 at 0°C) Found By Zhang

<table>
<thead>
<tr>
<th>Products</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenol, 2,4,6-trichloro (++)</td>
<td>2-Chlorophenol (+)</td>
</tr>
<tr>
<td>Phenol, 2,6-dichloro- (+)</td>
<td>3-Chlorophenol (+)</td>
</tr>
<tr>
<td>2,5-Cyclohexadiene-1,4-dione, 2-chloro- (+)</td>
<td>2,5-Cyclohexadiene-1,4-dione, 2, 6-dichloro- (+++)</td>
</tr>
</tbody>
</table>

+++: major product, > 15% of total peak area (solvent peak included)  
++: minor product, 5-15% of total peak area (solvent peak included)  
+: trace, < 5% of total peak area (solvent peak included)

electropherogram. Next the mixture is “spiked” with a standard of the suspected compound and its electropherogram is determined. Positive identification is made when one of sample peaks increases in area. When this technique was applied to the chlorine-phenol reaction mixture it was found to have the same composition as previously determined by comparing migration times. This composition seen in Figure 16.
1 = 4-Cl Phenol (0.56 mM)
2 = 3-Cl Phenol (0.46 mM)
3 = 2-Cl Phenol (0.26 mM)
4 = Methyl Orange (0.25 mM)
5 = 2,6-Dichlorophenol(*)
6 = 2,4,6-Trichlorophenol (*)

0.1 M Borax Buffer, pH = 9.24
Syringe Pressure Injection: 0.5 ml Air
4.0 seconds
19.5 kV

UV/Vis Detector: 288nm
* Standard Curve Not Available

Figure 16. Electropherogram of 2-, 3-, 4-Chlorophenol, 2,6-
Dichlorophenol, 2,4,6-Trichlorophenol
Solution With Methyl Orange Marker.
CONCLUSION

The main objectives of a capillary electrophoresis analysis are to separate and classify the sample solutes of interest which are present in a mixture and/or determine the concentration of the component in the mixture. Thus capillary electrophoresis can be used both for quantitative and qualitative analysis. Quantitative analysis can be done by comparison of peak areas or heights obtained from unknown sample with standard solutions. Qualitative analysis can be done by comparing migration times of unknown components with those of known standards.

This investigation has demonstrated that CZE can successfully separate and quantitate 3- and 4-chlorophenol over a concentration range of $1 \times 10^{-5}$ M to 0.01 M with an average coefficient of variation of 2%. Thus the CZE technique appears to be superior to both GLC and GLC/MS in the analysis of 3- and 4-chlorophenol. It also provides a much better degree of resolution than has been reported by gradient HPLC when applied to this analysis. Another important advantage of CZE is that only a few nanoliters of sample is required. A disadvantage of the CZE system used in this investigation however, results from the use of a UV/Vis detector. While the sensitivity of these detectors is adequate for many applications, trace level analysis may be problematic. However, a laser fluorescence
detector, while costly, might provide sensitivities sufficient for trace level analysis of same analytes.

The injection procedure employed in this study could be significantly improved by redesign of the sample vial and its mounting. If done properly only a few microletters of sample should be sufficient for the analysis. Pressure injection appears to be superior to siphoning and if automated should exhibit improved precision in quantitation.
BIBLIOGRAPHY


