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The analysis of aqueous mixtures using liquid chromatography: electrospray mass spectrometry

Steven Kent Johnson
Iowa State University

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The analysis of aqueous mixtures using liquid chromatography - electrospray mass spectrometry

by

Steven Kent Johnson

A dissertation submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of
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Major Professor

Signature was redacted for privacy.

For the Major Program

Signature was redacted for privacy.

For the Graduate College

To my parents, for their constant support.

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ABSTRACT

The focus of this dissertation is the use of chromatographic methods coupled with electrospray mass spectrometry (ES-MS) for the determination of both organic and inorganic compounds in aqueous solutions.

The combination of liquid chromatography (LC) methods and ES-MS offers one of the foremost methods for determining compounds in complex aqueous solutions. In this work, LC-ES-MS methods are devised using ion exclusion chromatography, reversed phase chromatography, and ion exchange chromatography, as well as capillary electrophoresis (CE). For an aqueous sample, these LC-ES-MS and CE-ES-MS techniques require no sample preparation or analyte derivitization, which makes it possible to observe a wide variety of analytes as they exist in solution. The majority of this work focuses on the use of LC-ES-MS for the determination of unknown products and intermediates formed during electrochemical incineration (ECI), an experimental waste remediation process.

Ion exclusion chromatography (IEC) was used to study small carboxylic acids. The effect of pH, solvent composition, sheath gas make-up, and presence of concentrated matrix was observed. Detection limits were found to range from 40 to 200 ppb for the direct infusion of most carboxylic acids and from 2 to 8 ppm for IEC-ES-MS.

The ECI of benzoquinone is studied using IEC-ES-MS. Unknown intermediates and products are identified and observed as a function of ECI time. The ECI procedure is described in detail and electrode efficiency is evaluated. Mechanisms are also proposed for the production of maleic, succinic, malonic, and acetic acids.

The ECI of 4-chlorophenol is observed using reversed phase liquid chromatography - ES-MS to study aromatic compounds, and ion exchange chromatography - ES-MS for organic and inorganic ions. The majority of this work is accomplished with ion exchange chromatography - ES-MS, which requires a suppressor to remove sodium ions from the mobile phase. The system has proven effective in the identification of a wide range of unknown compounds in complex aqueous solutions. Detection limits range from 50 ppb to 4 ppm.

Capillary electrophoresis was also studied as a possible separation method coupled to an ES-MS. Different mobile phases are examined and tested with CE-ES-MS for the determination of carboxylic acids. Detection limits range between 1 and 10 ppm.

CHAPTER 1. GENERAL INTRODUCTION

There is tremendous potential in coupling the resolving capability of liquid chromatography (LC) with the structural information and specific detection of electrospray - mass spectrometry (ES-MS). The resulting combination, LC-ES-MS, is a powerful analytical technique capable of accurate identification of unknown compounds in mixtures. LC used with traditional absorption detection methods does not yield the selectivity of LC-ES-MS. The widely used gas chromatography - mass spectrometry (GC-MS) methods are inadequate for nonvolatile, thermally unstable, and polar compounds [1]. GC-MS often requires preparation and derivatization of the sample before it can be injected onto the column. LC-ES-MS requires no preparation or derivatization, which makes it possible to observe a wide variety of analytes as they exist in aqueous solutions.

Historical Perspective of LC-MS and Interface Developments

The combination of LC and MS offers the analytical chemist one of the most powerful techniques for the determination of unknown compounds. Attempts to couple LC and MS systems on-line by various laboratories began in the 1960's [2], but were unsuccessful due to technological problems that could not be overcome at the time. Some researchers considered off-line techniques with independent collection of liquid fractions of effluent, evaporation of solvent, and transfer of solute to MS as the only realistic method of using LC and MS together [3-5]. Successful on-line LC-MS results were not reported until the 1970's by the laboratories of E. C. Horning, F. W. McLafferty, and R. P. W. Scott [6-8]. The first successful commercially available LC-MS interface was the transport, or moving-belt, system designed by McFadden [2,9] in which the chromatography effluent is deposited on a moving stainless steel belt which transports the sample to the mass spectrometer. The effluent solvent in the transport system is removed in a vacuum before the analyte reaches the ion source and is volatilized. The moving belt system was utilized and improved for over a decade [4,5,10-13].

Direct liquid injection (DLI) is one method of on-line LC-MS [14-18]. It was first developed in the laboratory of F.W. McLafferty [14] with similar work reported later by Henion [18]. In a DLI method, the highest fraction of HPLC effluent compatible with the vacuum system of the MS is introduced into the MS ion source. The gas flow produced from the effluent of the conventional LC column was twenty times higher than typical vacuum system could withstand at the time of the early DLI experiments, so significant quantities of the effluent were split from the flow, and only a small fraction, 1-5%, went to the MS. The requirement of such a large split seriously reduced the usefulness of DLI-LC-MS. Cryogenic pumps with improved vacuum efficiency were utilized to handle higher flow rates of up to 100 μl/min which increased the use of DLI methods somewhat [16,17,19,20].

Many developments over the past twenty years have improved the performance of LC-MS. Some of the greatest improvements have resulted from advancements in micro HPLC techniques [21-26] which reduce the flow from the column to 10 - 50 μl/min and eliminate the need for splitting. Another area of significant advancements in LC-MS has been the development of effective new systems to interface LC and MS. The fundamental problem of LC-MS with conventional flow rates has been the inability of the mass spectrometer vacuum system to withstand all the solvent vapor of the evaporated effluent. Thermospray and atmospheric pressure ionization (API) sources have been developed to successfully eliminate vacuum load difficulties in LC-MS.

Thermospray

The thermospray ionization interface [27-29] significantly increased the acceptance of LC-MS, because it readily accommodates conventional reversed phase flow rates and eluent compositions, and it is a relatively simple and rugged system that does not require unusual training to operate. The technique was first developed by Vestal and co-workers [27] in 1983. In thermospray ionization an aqueous sample is passed through a heated metal capillary tube. Ions are produced by direct ion evaporation [30] of a sample ion or by a two-step process similar to conventional chemical ionization (CI), where an ion of electrolyte, typically ammonium acetate, ejected from a droplet reacts with a sample molecule in the gas phase to generate a sample ion. Thermospray uses an additional vacuum line directly at the ion source to maintain vacuum while accommodating up to 2 ml/min of effluent flow rate. The technique produces accurate molecular weight information but thermospray's limitations include difficulty in sensitive temperature control, thermal degradation, poor detection limits, and inability to handle low liquid flow.

API sources

API sources create droplets and ions from LC effluent in an atmospheric pressure region and then draw ions into a MS. The first work using API sources was done by Horning and his co-workers [31-35]. Horning used heated nitrogen gas to volatize the sample and thermal electrons of a corona discharge to ionize the sample. The development of API sources has grown rapidly over the past 15 years and three API sources will be discussed here: heated pneumatic nebulizer, liquid ion evaporation, both originally reported in 1983, and electrospray, first developed in 1984.

Heated pneumatic nebulizer

The heated pneumatic nebulizer [36-38] reported by Thomson in 1983 is an API source in which a liquid sample passes through a heated metal tube surrounded by a coaxial nebulizer gas. The combination of heat and gas flow desolvates the nebulized droplets to produce a dry vapor of solvent and analyte molecules which pass through a corona discharge created from a charged needle in the atmospheric pressure region. Heated pneumatic nebulizer ionization can operate with flow rates up to 2 ml/min and it is robust enough to tolerate volatile salts, acids, bases, and other chromatography additives. The technique is a mild ionization source, and it can not offer much structural data from fragmentation.

Liquid ion evaporation

Liquid ion evaporation, developed by Irabarne and Thomson, was reported in 1983 [30]. In this technique, the liquid sample is passed through a pneumatic nebulizer into the atmospheric pressure interface region. Charge is transferred to the droplets from a small high-voltage electrode located near the nebulizer tube. The sample is not heated and conventional flow rates are acceptable. Liquid ion evaporation is well suited for easily ionized and polar analytes, and the mild ionization at room temperature makes liquid ion evaporation very well suited for the production of doubly charged ions [39]. However, the technique can not be used for analytes that are difficult to ionize, and observed ions can experience substantial clustering due to the mild ionization conditions.

Electrospray

In electrospray ionization, the liquid sample is passed through a metal capillary tube at a high-voltage potential, where charge is transferred to the solution before droplets are formed. The technique enables spectra to be obtained from a variety of compounds and offers great flexibility in ion extraction conditions, making possible the formation of fragment, molecular, and cluster ions. The electrospray LC-MS interface will be discussed in greater detail in the next section.

Electrospray - Mass Spectrometry Historical Perspective

The first report of an electrospray-type phenomena dates back almost three hundred years ago to the experiments of Bose [40], while the first description of an actual electrospray was published by Zeleny [41] in 1917. Electrospray (ES) was not used as an ionization source until the late 1960's when Dole and co-workers [42,43] observed ion retardation and ion mobility using ES to produce gas phase macro-ions. Fenn [44] and Aleksandrov [45,46] combined electrospray ionization and mass spectrometry (ES-MS), working independently of each other in 1984. Whitehouse, Fenn, and co-workers [47] gave the first detailed description of an ES interface for LC-MS in 1985.

Although ES-MS offers a relatively simple method for ionizing LC effluent, flow-rate restrictions diminished the usefulness of LC-ES-MS. Ionspray, or pneumatically assisted ES interface, which combines ESI and pneumatic nebulization, was introduced in 1987 [48]. The ionspray interface can introduce flow-rates of 200 μl/min. Increasingly higher flow-rates up to 2ml/min can be achieved currently with minor modifications, such as liquid shield [49] and TurboIonSpray [50].

Electrospray Ionization Process

Although ES-MS is relatively new, the ESI process has been investigated extensively [51-58]. ESI requires four steps: the production of highly charged droplets, solvent evaporation, Rayleigh fission, and finally the production of gas phase ions.

Charged droplet formation

Charged droplets are generally produced by applying a high electric potential to a stainless steel capillary tube, or electrode tube. As the sample solution passes through the electrode tube, charge is transferred to the liquid. A potential difference of 3-6 kV is typically applied between the electrode tube and a counter electrode located about 1 cm away. In pure ESI, the electric stress caused by the potential difference is the sole method of droplet formation. In the more common pneumatically assisted ESI, a nebulizer gas is forced around the electrode tube to facilitate droplet formation. Liquid flow-rate and solvent composition are important factors in droplet production. Pure ESI has typical flow-rates of 1-10 μl/min, while pneumatically assisted ESI has flow rates up to 200 μl/min [48]. Organic solvents, such as methanol or propanol, are required for droplet formation and efficient evaporation, and supporting electrolyte is typically used to assure effective charge transfer to solution droplets.

Solvent evaporation and Rayleigh fission

Droplets formed at the electrode tube experience significant physical changes before reaching the mass spectrometer. In the Perkin Elmer-Sciex API/1 instrument used for this work, the charged droplets are sampled into an interface region, containing a drying, or curtain gas heated to 60°C and bordered by two charged plates as shown in figure 1. The curtain gas stimulates collision-induced dissociation of solvent clusters, and it also prevents clogging of the small, 100 μm diameter, sampling orifice opening. Organic solvents in the solution also increase the evaporation rate and reduce clogging. As the solvent of the charged droplets evaporates, the droplets shrink, and as the droplet shrinks the similar charges inside the droplet are forced closer together. Eventually, the coulombic repulsion between the similar charged ions exceeds the surface tension of the droplet. At this point, the droplet undergoes Rayleigh fission, a process in which small, highly-charged droplets are expelled from the larger initial droplets. These small expelled droplets have been measured to contain 15% of the original droplet's excess charge and only 2% of its original mass. Evaporation and Rayleigh fission can occur repeatedly, producing smaller and smaller droplets. A typical beginning droplet ($r = 1.5$ μm, charge = 8×10^{-15}C at 35°C) will go through an average of three fissions in roughly 500 μs before emitting gas phase ions [59].

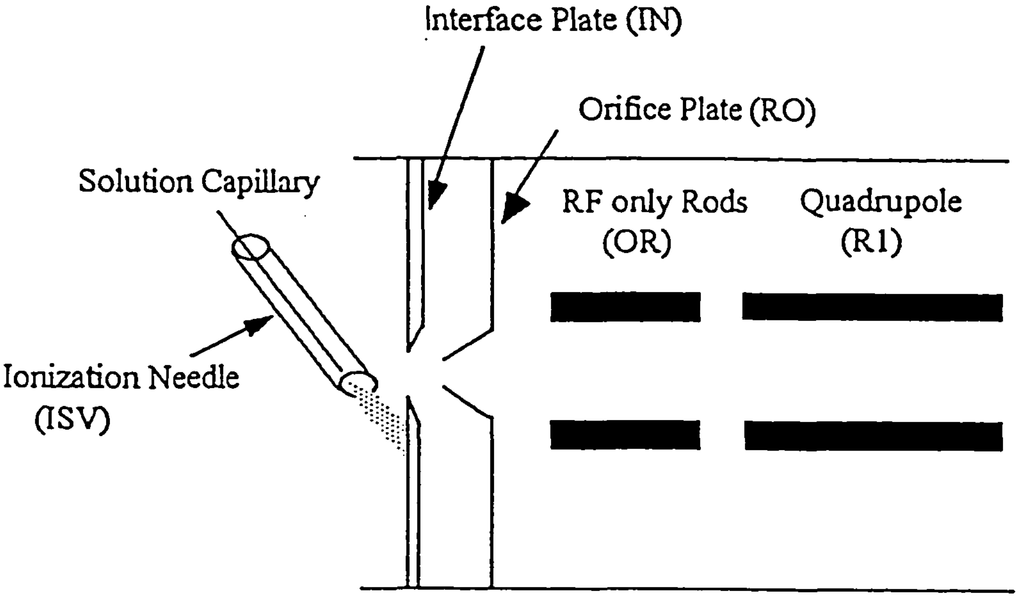

Figure 1: PE-Sciex API/1 Electrospray Mass Spectrometer

Production of gas phase ions

The mechanism that produces gas phase ions from charged droplets is uncertain [60-62]. Two theories have been suggested, ion evaporation theory (IET) [63,64] and single ion droplet theory (SIDT) [42,43,65,66]. The IET was proposed by Iribarne and Thomson in 1976 [63]. It states that a single solvated ion is emitted from the surface of the droplet when the surface charge density of the droplet is strong enough to desorb ions. The SIDT was originally proposed by Rõllgen in 1983 [65], and it states that through evaporation and Rayleigh fission the droplets will become so small that they contain only one ion. As this micro-droplet undergoes evaporation, the droplet will be converted to an ion in the gas phase. Experiments to determine the exact mechanism for gas phase ion production have been inconclusive. IET depends on significant charge density on the droplet surface, and SIDT depends on the production of extremely small droplets. Both conditions are feasible in the ESI process.

The gas phase ions produced in the interface region enter a high vacuum region containing the quadrupole mass analyzer through the 100 μm diameter sampling orifice. Most other API source designs are derived from the interface described by Whitehouse et al [47], using a two stage vacuum system, and a sample transfer capillary. The Perkin Elmer - Sciex instrument used for this work incorporates a single quadrupole mass analyzer and a channel electron multiplier for detection.

Dissertation Objectives and Organization

The emphasis of this thesis is the use of LC-ES-MS to identify intermediate and product compounds resulting from the electrochemical incineration (ECI) of organic molecules in aqueous solutions. ECI is a waste remediation process whereby oxygen atoms are transferred from water in the solvent phase to the oxidation products by direct or indirect reactions on the anode surface. ECI is a versatile, energy efficient, environmentally compatible, and low cost method for elimination of organic wastes. The LC-ES-MS techniques described for the identification of ECI unknowns require no sample preparation or analyte derivatization. Limited sample preparation reduces the possibility of altering the analytes, and makes it possible to analyze the sample as it exists in solution.

Chapters 2, 3, 4, and 5 of this thesis stand alone as scientific manuscripts and are either published, submitted for publication or ready for submission. Chapter 6 is a general conclusion with suggestions for future research.

Chapter 2 presents preliminary studies using ion exclusion chromatography (IEC) and ES-MS to analyzes mixtures of carboxylic acids. Compromise experimental parameters were evaluated to observe several organic acids. The effects of sheath gas, solvent composition, concentrated matrix ions, and pH of solution on sensitivity and linearity were examined. A basic isopropanol solvent with nitrogen sheath gas yielded the best results and detection limits from 40 to 200 ppb for direct infusion and 2-8 ppm for IEC-ES-MS. The presence of concentrated matrix ions can suppress the analyte signal significantly. These matrix effects are minimized by chromatographic separation; the use of internal standards compensates for most of the remaining matrix effects. In most cases the sample is injected directly onto the column with little or no preparation. A small additional flow of organic solvent with pH adjustment is added to the sample after separation and before reaching the ES-MS.

Chapter 3 examines the ECI products and intermediates of benzoquinone using the ion exclusion chromatography - ES-MS set-up described in chapter 2. Unknown intermediates and products were identified and studied as a function of ECI time. The ECI procedure is described and electrode efficiency is evaluated. Mechanisms are proposed for the production of maleic, succinic, malonic, and acetic acids.

Chapter 4 presents the study of intermediates and products during the ECI of chlorophenol. In this study a TurboIonSpray ionization source is used with a mass spectrometer. The majority of the unknown determination is accomplished using ion exchange chromatography coupled to the TurboIonSpray mass spectrometer. A suppressor column is also placed between the ion chromatography column and the electrospray interface to eliminate sodium cations from the eluent. Reversed phase chromatography was also used with ES-MS for the determination of unknown ECI samples. The major intermediates and products of ECI were maleic, succinic, malonic, and acetic acids, as well as chloride, chlorate, and perchlorate ions.

Chapter 5 details a make-up liquid sheath flow system for coupling capillary electrophoresis with electrospray mass spectrometry for the determination of carboxylic acids. Maleic, succinic, malonic, and glutaric acids were separated using capillary electrophoresis with an aqueous mobile phase containing 1,2,4,5-benzenetetracarboxylic acid, 2,6-naphthalenedisulfonic acid, methanol, and diethylenetriamine. Optimum mobile phase composition and separation conditions are reported and the system is evaluated using analytical figures of merit such as resolution and detection limits.

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CHAPTER 2. DETERMINATION OF SMALL CARBOXYLIC ACIDS BY ION EXCLUSION CHROMATOGRAPHY WITH ELECTROSPRAY MASS SPECTROMETRY

A paper published in Analytica Chimica Acta[1]

Steve K. Johnson, Linda L. Houk, Jianren Feng, Dennis C. Johnson, R. S. Houk[2]

Abstract

Ion exclusion chromatography (IEC) is used with electrospray mass spectrometry (ES-MS) to analyze a mixture of formic, glyoxylic, oxalic, 2-hydroxyisobutyric, and maleic acids. Compromise experimental parameters were evaluated. Ion extraction voltages can be found that produce reasonable signals from negative parent ions ($[M-H]^-$) of these compounds as well as succinic, malic, 1-hexanoic, malonic, and tricarballylic acids. The effects of sheath gas, solvent composition, concentrated matrix ions, and pH of solution on sensitivity and linearity were examined. A basic isopropanol solvent with nitrogen sheath gas yield the best results. Detection limits are 40 to 200 ppb for direct infusion of sample into the ES-MS and 2 to 8 ppm for IEC-ES-MS. The presence of concentrated matrix ions can suppress the analyte signal significantly. These matrix effects are minimized by chromatographic separation and internal standards. In most cases, the sample is injected directly onto the column with little or no preparation. A small additional flow of organic solvent with pH adjustment is added before the sample reaches the ES-MS.

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[2] Author for correspondence

Introduction

A rapid and sensitive method for the determination of carboxylic acids in environmental and biological samples is needed. Applications for such a procedure are numerous. Recent research has implicated carboxylic acids in the acidification of environmental water, such as rain and snow [1,2]. Carboxylic acids are monitored in sludge from sewage treatment plants, as these compounds destroy methane-producing bacteria [3]. Other studies of environmental interest include the decomposition of toxic aromatic hydrocarbons to CO_2 via carboxylic acid intermediates [4,5]. Characterization of carboxylic acids in biological fluids also helps identify inborn errors of metabolism [6,7,8].

Currently, the most sensitive method for the identification and quantification of carboxylic acids is gas chromatography - mass spectrometry (GC-MS), with typical detection limits around 1 ppm for low molecular weight compounds [9,10]. However, GC-MS usually requires time consuming separation and/or derivitization procedures to remove the analyte from the sample matrix before injection into the GC [6]. Electrospray mass spectrometry (ES-MS) [11,12] can potentially measure polar, labile solutes in aqueous solutions without these sample preparation steps. Several studies have demonstrated the use of LC-ES-MS for large biological molecules [13,14,15,16], but much less work has been published using LC-ES-MS for molecules of low molecular weight [17]. In the present work small carboxylic acids are determined by IEC-ES-MS with very little sample preparation. An aqueous sample is simply injected onto the IEC column. The chromatographic system is coupled directly to the mass spectrometer.

However, because of substantial differences in flow rates between the two systems, there are some challenges to be addressed.

There has been some prior work on LC-MS of carboxylic acids. Pacholec et al. joined high performance liquid chromatography (HPLC), in particular IEC, with thermospray MS to characterize mixtures of organic acids [18]. Detection limits of 10 ppm were observed for glycolic, acetic, propionic, and butyric acids. However, a hydroxyacid and three dibasic acids produced no usable spectra. Detection limits in the part per billion range were achieved by Tsai et al. using an elaborate post-column derivatization and moving belt interface with HPLC-MS [19]. Sub part per billion detection limits have been achieved using solid phase extraction previous to LC-ES-MS for a variety of compounds [20,21]. The concentrations of the solutions entering the LC-ES-MS in these cases range from 1-30 ppb after the solid phase extraction step.

Several other recent ES-MS studies are related to the present work. Mallet et al [22] reported preliminary results concerning ES-MS analysis of a mixture of hydroxy acids at 10^{-5}M in a waste stream. Stronger signals were seen for carboxylate anions $[M-H]^-$ than for positive ions. Solutions containing just individual acids provided stronger ion signals for $[M-H]^-$ than mixtures. These workers concluded that capillary electrophoresis with ES-MS should prevent this signal suppression problem and also separate components at the same nominal m/z value, although no capillary electrophoresis ES-MS results were described. Finally, Murphy and co-workers [23,24] described ES-MS of lipid hydroperoxides, keto acids, and polyhydroxy unsaturated fatty acids. These compounds readily produced carboxylate anions from methanol-water solutions at concentrations of approximately 1 ppm without derivatization. Murphy's studies dealt primarily with determination of structure by collision-induced dissociation (CID) spectra.

Because IEC is the method of choice for separating small aliphatic carboxylic acids [25], an IEC column was coupled with ES-MS in the present work. IEC offers the following desirable features: sensitivity, high capacity, and simplicity [26]. A strong anion or cation exchange resin, with a functional group attached to the surface, separates weakly ionized solutes. The charge of the functional group on the resin is the same as that of the partially ionized analyte. For carboxylic acids, the resin has sulfonate groups or other negative functional groups which repel negative ions. Anions of strong acids, such as HCl and H_2SO_4, are repelled by these functional groups and elute without retention. Neutral or partially ionized compounds can penetrate the negatively charged resin zone into the occluded liquid phase and are partitioned in this fashion [27,28]. This process is sometimes called the Donnan exclusion principle.

In IEC of anions, the eluent is a dilute solution of strong acid which protonates some of the carboxylic acid molecules. In general, acids with higher pK_a values are protonated more extensively and are retained longer on the column [27,28]. Hydrogen bonding and adsorption also affect retention time. In general, retention time increases with molecular weight [29].

The main intent of the present work is to develop rapid analytical methodology to determine small carboxylic acids that could be produced by electrochemical incineration of toxic organic wastes such as benzene and phenol [30]. Therefore, this study focusses mainly on the determination of formic acid, glyoxylic acid, oxalic acid, 2-

hydroxyisobutyric acid, and maleic acid. Some experiments were also done with succinic acid, 1-hexanoic acid, tricarballylic acid, and malonic acid. Detection limits were examined by direct infusion into an ES-MS and by joining IEC to ES-MS. This study also compares signal differences with methanol and 2-propanol as the solvent and with nitrogen and oxygen sheath gases for ES-MS. Straub and Voyksner reported variations in ion response of adenosine 5-monophosphate in different solvents with oxygen as a sheath gas [31]. Electrospray extraction conditions are examined, calibration curves, pH effects, and matrix interferences are discussed. Because chemical separation procedures for diacids are more time consuming than those for volatile monoprotic acids [6], a variety of compounds were tested. These acids are especially challenging for ES-MS because their m/z values are in the range m/z = 46 to 116 where background spectral peaks for solvent ions complicate the observed spectra.

Experimental Section

Reagents and samples

Water was distilled and then deionized (18MΩ cm^{-1} at 25°C) with a Barnstead Nanopure-II system (Newton MA). The mobile phase was 0.4 mM trifluoroacetic acid (TFA, Fisher Scientific, Fair Lawn, NJ) in water. Aqueous TFA at this concentration produced a pH of 3.5 after the LC column. Either methanol or 2-propanol (Fisher Scientific, Fair Lawn, NJ) and ammonium hydroxide (Malinckrodt, Paris, KY) were added post-column through an HPLC micropump to the sample stream to facilitate ionization of analyte, droplet formation and evaporation of eluent. The acids used were obtained from the following suppliers: a) formic and oxalic (Fisher Scientific, Fair Lawn, NJ); b) malic, malonic, tricarballylic, 2-hydroxyisobutyric, 1-hexanoic, succinic, and maleic (Aldrich, Milwaukee, WI); c) glyoxylic (Janssen Chimica, Geel, Belgium). A 10,000 ppm stock solution of each carboxylic acid was prepared. Aliquots of stock solution were frozen separately to avoid sample decomposition. An aliquot of the stock solution was thawed and refrigerated for no more than 30 days, prior to use. For separations, a sample solution containing 50 ppm of each acid was prepared by diluting aliquots of the stock solutions with 0.4 mM TFA. Solutions with each individual analyte were also made by diluting aliquots with 0.4 mM TFA. All solutions were filtered to minimize plugging of the ES-MS orifice. The TFA eluent was filtered under vacuum with a Magna-R nylon membrane (0.22 μm pore diameter, Fisher). This filtration step also degassed the eluent. Sample solutions were filtered with Costar μstar filters (0.22 μm pore size) directly before injection onto the column.

IEC conditions

The IEC system was composed of a micropump, a sample injector with a 50 μL injection loop, and an ion exclusion column. The components and conditions for the separation are summarized in Table I. The acidic eluent used in IEC must have a pK_a that is less than the pK_a of the analyte acids for good separations [27,28]. The eluent must also evaporate easily in the ES-MS. Dilute solutions of strong inorganic acids like phosphoric acid and sulfuric acid are commonly used for IEC with absorbance detection. In our experience, these eluents produce complicated background spectra and greatly suppress signals from carboxylate anions in ES-MS. The weaker acid TFA at 0.4 mM

Table I. Chromatographic Components and Operating Conditions

Pumps	Dionex model APM-2 analytical pump (Sunnyvale, CA) Beckman model 112 micropump (Fullerton, CA)
Injection Valve	Rheodyne 7010 high pressure sample injector (Cotati, CA)
Column	Dionex IonPac IEC-AS6 ion exclusion column (Sunnyvale, CA)
Stationary Phase	Cross-linked styrene/acrylate/divinylbenzene functionalized with sulfonate and carboxylate
Mobile Phase	0.4 mM trifluoroacetic acid in water
Sample Flow Rate	400 μL min^{-1}
Injection Volume	50 μL

gave useful chromatographic separations and $[M-H]^-$ sensitivity without severe problems from background ions. The column was equilibrated with the mobile phase prior to use.

ES-MS conditions

An API/1 (Perkin-Elmer SCIEX, Thornhill ON, Canada) single quadrupole mass spectrometer was used. This apparatus uses a curtain gas interface (Fig. 1 inset) and has been described previously [32,33]. The IonSpray source was operated in the negative ion mode. Table II summarizes the instrumental operating conditions of the ES-MS. These conditions were kept fairly consistent, but a few parameters (marked by an asterisk in Table II) needed optimization from day to day.

Table II. ES-MS Operating Conditions

Ionization Source	IonSpray (nebulizer assisted electrospray)
Nebulizer Gas Pressure	40 psi, zero grade (99.998%) N_2
Nebulizer Gas Flow Rate	0.6 L min^{-1}
Sample Flow Rate	15 μL min^{-1}
Curtain Gas Pressure	80 psi, carrier grade (99.999%) N_2
Curtain Gas Flow Rate	0.7 L min^{-1}
Curtain Gas Temperature	60°C
Discharge Needle Voltage	-4000V*
Interface Plate Voltage	-450V*
Orifice Plate Voltage	-120V*
RF Only Quadrupole Voltage	-100V*
Mass Analyzer Quadrupole Voltage	-95V*
CEM Detector Voltage	+2800V
Operating Pressure of Quadrupole Chamber	3.5 x 10^{-5} Torr
Dwell Time	100 ms
m/z Values Monitored	m/z=45 (formate) m/z=73 (glyoxylate) m/z=89 (oxalate) m/z=103 (2-hydroxyisobutyrate) m/z=115 (maleate)

*Typical values cited. These parameters were adjusted daily to maximize ion signal and chromatographic quality, and differed slightly from day to day.

Operating conditions were optimized to provide maximum $[M-H]^-$ signal during infusion of simple solutions. These same conditions were also used when the IEC separations were employed. There was no obvious indication of interaction or cooperativity between the various experimental parameters.

Apparatus

The experimental set-up is shown in Figure 1. Eluent flows from the first HPLC pump through the column at 400 μL min^{-1} to a splitter. The ES-MS operates best with approximately a 15 μL min^{-1} liquid flow rate, so the eluting flow from the column must be split before it enters the ES-MS. This split is accomplished using a T-connector with different lengths and diameters of capillary tubing arranged to deliver the desired 40:1 split. The dimensions of these capillaries are indicated in Fig. 1. Approximately 390 μL min^{-1} flow to a UV-visible absorbance detector (λ=214 nm, Spectroflow 757, Kratos Analytical) through a 20 cm length of 200 μm diameter PEEK tubing. A 20 cm length of 200 μm diameter PEEK tubing then leads from the absorbance detector exit and is joined to another (100 μm ID $\times$ 7 cm long) capillary, which is used as a flow constrictor. The capillary length of the flow constrictor is varied to deliver the appropriate 10 μL min^{-1} flow to a mixing union. The 10 μL min^{-1} flow is then mixed with pH-adjusted 2-propanol (see below) at 5 μL min^{-1} from a second HPLC pump. The eluent/solvent solution leaves the mixing union as a 15 μL min^{-1} stream and enters the ES-MS source through a capillary. The capillary tube passes into the stainless steel electrospray needle and stops approximately 1 mm before the needle exit. The charge on the needle is transferred to the sample, while a sheath gas sprays the solution as an aerosol out of the needle and into the vicinity of the sampling orifice of the mass spectrometer.

The IEC separation uses an aqueous eluent, whereas the electrospray process prefers some organic solvent. Ammonium hydroxide is also added post-column to convert the carboxylic acids into anions, which improves the anion signal for electrospray. Strictly speaking, pH is an ambiguous term for mixed organic-aqueous solvents. The pH values cited below are those measured during optimization experiments using infusions after addition of aqueous NH_4OH only to the aqueous IEC solvent. The 2-propanol was then added after the pH measurement steps. During actual IEC separation, both 2-propanol and aqueous NH_4OH were added post-column in a single liquid flow, hence the term "pH-adjusted propanol" used in the preceding paragraph. The 2-propanol was 33% of the total flow.

Data acquisition

For the separation of carboxylic acids, ions were measured at five m/z values by selected ion monitoring during the chromatographic separation. Compromise operating conditions were obtained using a standard solution containing all five acids and maximizing $[M-H]^-$ signal while peak hopping over all five m/z positions. Chromatograms were recorded in real time with PE Sciex Tune 2.4 software, stored on the hard disk, and later analyzed using PE Sciex Mac Spec 3.22 software. All spectral scans were collected by averaging 10 consecutive scans. Detection limits were calculated to be the concentration or mass of analyte necessary to produce a net signal equivalent to three times the standard deviation of the background.

Results and Discussion

ES-MS extraction conditions

The observed spectra are quite sensitive to ion extraction conditions [34,35,36]. The voltage difference between the orifice plate and the RF only quadrupole has the greatest effect on the observed ions. A large voltage difference here imparts a high kinetic energy to the ions, which then fragment extensively via collisions in the supersonic jet. With a small voltage difference, more clusters are observed.

These effects are discussed below for two typical compounds: 1-hexanoic acid, which has one carboxyl group , and succinic acid, which has two. Figure 2 demonstrates the effect of changing extraction conditions for succinic acid. The voltage on the orifice plate is set at three different values: (a,b)-105V, (c,d)-120V, and (e,f)-140V, while the RF only quadrupole is kept at -100V. Figure 2a shows spectra obtained under mild collision conditions. The difference between the voltages on the orifice plate and the RF only quadrupole is only 5V. Most of the peaks at low m/z values are background ions from the TFA and propanol as shown also in Figure 2b. A low collision energy yields cluster ions like TFA · $[TFA-H]^-$ (m/z=227), succinate · TFA (m/z=231), and succinate · succinic acid (m/z=235).

Figure 2c shows the succinate spectra observed under normal extraction conditions, and Figure 2d shows the accompanying background at these conditions. The voltage difference between the orifice plate and the RF only quadrupole is now increased to 20V. In the negative ion mode, under these conditions, the major ion observed is $[M-H]^-$. For succinic acid (MW=118), the $[M-H]^-$ peak is observed at m/z=117. $[TFA-H]^-$

is observed at m/z=113 from the eluent. Succinate yields a small peak at m/z=73 due to loss of CO_2, and $[TFA\text{-}H]^-$ also loses CO_2 to yield the peak at m/z=69.

Figures 2e and 2f show the spectra obtained under harsh collision conditions with a difference of 40V between the orifice plate and the RF only quadrupole. Parent ions are less abundant and fragmentation is more extensive.

Figure 3 shows the change in signal intensity for various ions from succinic acid as a function of the voltage difference discussed above. Again, this experiment is performed by varying the orifice plate voltage while holding all other conditions constant. Succinate is selected here to typify the general behavior of dicarboxylic acids. Line (a) in Fig. 3 shows the signal for the parent ion $[M\text{-}H]^-$ at m/z=117. The $[M\text{-}H]^-$ signal maximizes with a voltage difference of -5 to +25 V. Line (b) is the signal at m/z=73, the fragment ion $CH_3CH_2COO^-$ formed by the loss of CO_2, and line (c) is m/z=99, which is a fragment ion due to the loss of H_2O. As the voltage difference becomes more than 20 to 25 V, these fragments become more abundant at the expense of signal for $[M\text{-}H]^-$. The large voltage difference results in more energetic collisions during extraction, which produce more fragment ions. A voltage difference of 25 V (i.e., orifice voltage of -125 V) is sufficient to dissociate the clusters at m/z=231 and 235, as described above.

The loss of CO_2 and H_2O upon CID is expected with anions from dicarboxylic acids [23]. Succinate, for example, has one carboxyl group to contain the negative charge while the other carboxyl group splits off as CO_2. The resulting fragment ion at m/z=73 is the major ion observed with harsh extraction conditions, as shown in Figure 2e. Parent ions with only one carboxyl group do not exhibit the same fragmentation properties. 1-Hexanoic acid (MW=116) produces a parent ion $[M-H]^-$ at m/z=115 but shows no fragment ions for the loss of CO_2 or H_2O at either m/z=71 or 97. Figure 4 shows that the 1-hexanoate parent ion remains the major ion observed at the same harsh conditions as in Figure 2e. No fragment ions are observed because, if the carboxylate group is lost, no other functional group remains to contain the negative charge.

Variation of the extraction conditions can provide interesting information about the clustering and declustering properties of a compound, i.e., whether the compound loses H_2O or CO_2. However, excessive formation of fragments and clusters complicates the spectra and adversely affects detection limits. For this study, conditions similar to those in Figure 2c were chosen to give the negative parent ion $[M-H]^-$ as the major sample ion, which provides the best detection limits and reasonably simple spectra.

pH effects

The effects of pH on the generation of analyte ions were studied. The eluent must be acidic for good separation in the IEC column, but a basic solvent is desirable for production of negative ions from the weak acids. Thus, the pH of the ES-MS sample must be adjusted after the chromatographic separation.

The signals for each of five carboxylate anions were monitored during chromatographic separations with the pH adjusted to either 4 or 9 after the column and before the MS. The reader should note that these pH values were measured separately only for the aqueous component of the total flow. 2-propanol was added at 33% of after the pH adjustment to enhance the spray properties.

The results are shown in Figures 5 and 6. Of these 5 compounds, only oxalic acid ($pK_1=1.04$) and maleic acid ($pK_1=1.75$) yield useful signal at pH=4.0 (Fig. 5). They are relatively strong acids and have pK_a values well under the nominal pH of the solution. More basic conditions (pH=9.0, Fig. 6) improve the $[M-H]^-$ response for glyoxylic ($pK_a=3.30$), formic ($pK_a=3.55$), and 2-hydroxyisobutyric acid ($pK_a=3.72$), but the sensitivity for $[M-H]^-$ from maleate falls to only about 33% of that at pH=4.0. Thus, a compromise is required in the pH value used to produce anions of these compounds by electrospray.

Calibration curves were also measured for each solvent at pH=4 and pH=9. The majority of these carboxylic acids gave similar and fairly straight ($r^2\approx0.995$) calibration curves up to 10 ppm, but formic acid at pH=9 gave a better curve than at pH=4, as shown in Figure 7. Thus pH=9 was selected to provide reasonable linearity and signal response for a wider range of carboxylic acids than was the case at pH=4.0.

Solvent and sheath gas effects

The solvent and sheath gas can affect the signal intensity and stability of the electrospray current [31,39]. A proper solvent suppresses electrical discharge and evaporates easily from the analyte ions. In this study the solvent, either methanol or 2-propanol, was added to the analyte flow after elution from the column with either nitrogen or oxygen as a sheath gas.

The solvent and sheath gas effects were examined either with all other experimental conditions held constant or with specific optimization for each solvent/gas combination. In each case, a solution containing the five carboxylic acids (oxalic, maleic, glyoxylic, formic, 2-hydroxyisobutyric acids) at pH=9.0 was studied. While O_2 sheath gas yields the lowest background signal, N_2 produces $[M-H]^-$ signals that are higher by a factor of 2. At pH=9.0, 2-propanol gives higher signal than methanol regardless of sheath gas used. This latter effect is also shown in Figure 7. Thus, 2-propanol solvent and N_2 sheath gas was the best combination. The reasons why 2-propanol and N_2 work better than methanol and O_2 are not clear.

Detection limits

The detection limits observed for both direct infusion of sample into the ES-MS and for IEC-ES-MS under these conditions are shown in Table III. The detection limits for direct infusion range from 40 to 200 ppb, while IEC-ES-MS detection limits are between 2 and 8 ppm. The poorer detection limits with the IEC separation can be attributed to the large 40:1 split of the sample before the ES-MS. These detection limits were obtained by selected ion monitoring for the parent anions of each of the five acids shown in Table III. Naturally, the detection limits would be worse if more m/z values

Table III. Detection Limits for Carboxylic Acids

	Infused (no split)		IEC (40:1 split)	
Sample	ppb	pg	ppm	pg
Formate	80	20	3	800
Glyoxylate	200	50	8	2000
Oxalate	80	20	3	800
HIBA	80	20	3	800
Maleate	40	10	2	500

are included in the selected ion monitoring program or if the mass analyzer is scanned to observe unknown acids at other m/z values.

Matrix effects

In ES-MS, the presence of concentrated matrix ions in the sample can change the analyte signal significantly [40,41]. Citrate, a carboxylate compound in citrus fruits, was selected to study these possible matrix effects. Figures 8a and 8b show malonate (m/z=103) and tricarballylate (m/z=175) signals respectively, as citric acid concentration is increased. In each case, the malonic and tricarballylic acids are present at 100 ppm, in a 33% 2-propanol solution at pH=9.0. Ion signals for both malonate (Fig. 8a) and tricarballylate (Fig. 8b) decrease greatly (approximately 10 fold) as citric acid concentration is increased to 5000 ppm.

The ratio of (malonate signal)/(tricarballylate signal) is plotted as a function of citrate concentration in Fig. 9. Note that the vertical scale is much more sensitive than that in Fig. 8. This signal ratio decreases by about 25% as the citrate concentration increases. From 1000 ppm to 5000 ppm citric acid, the analyte signal ratio changes by only about 10%. Thus, the signals from these two anions, malonate and tricarballylate, are suppressed to about the same extent by the citrate matrix, so they could readily serve as internal standard pairs.

This internal standardization scheme does not necessarily work well for all pairs of carboxylate anions, however. Malate was also added to these solutions as a potential internal standard. The ratios (analyte signal)/(malate signal) varied by some 75% as citric acid concentration increased up to 5000 ppm. Thus, a potential internal standard compound must be evaluated on a case-by-case basis for the particular analytes of interest. Naturally, an isotopically-labeled analog of the analyte would be expected to be the most appropriate internal standard. Otherwise, it is apparent that an internal standard can not eliminate matrix effects, so the IEC separation is still needed. The signal from an analyte that is well separated from eluting matrix compounds should not be suppressed by the matrix. If an analyte elutes relatively close to a matrix compound, that analyte ion signal experiences suppression. An internal standard can be used in this case to minimize the matrix effect observed. For ES-MS using IEC with a make-up solvent flow, the internal standard can be added to the solvent make-up flow. The internal standard added to the solvent flow will not be subject to chromatographic separation, but will experience the same matrix effect and declustering conditions as the analyte ion in the ES-MS.

Conclusions

The analytical merits of IEC-ES-MS have been demonstrated. A difficult carboxylic acid mixture has been separated. Detection limits of 40 to 200 ppb for direct infusion and between 2 and 8 ppm for IEC-ES-MS were observed. It may be possible to improve the IEC-ES-MS detection limits by using "turbo ion spray", i.e. a heated gas flow near the electrospray needle that allows large liquid flow rates (up to 1 ml/min) to be introduced to the ES-MS. IEC-ES-MS should be useful to separate and identify weak carboxylic acids in moderately complex solutions, such as those produced by electrochemical degradation of organic wastes.

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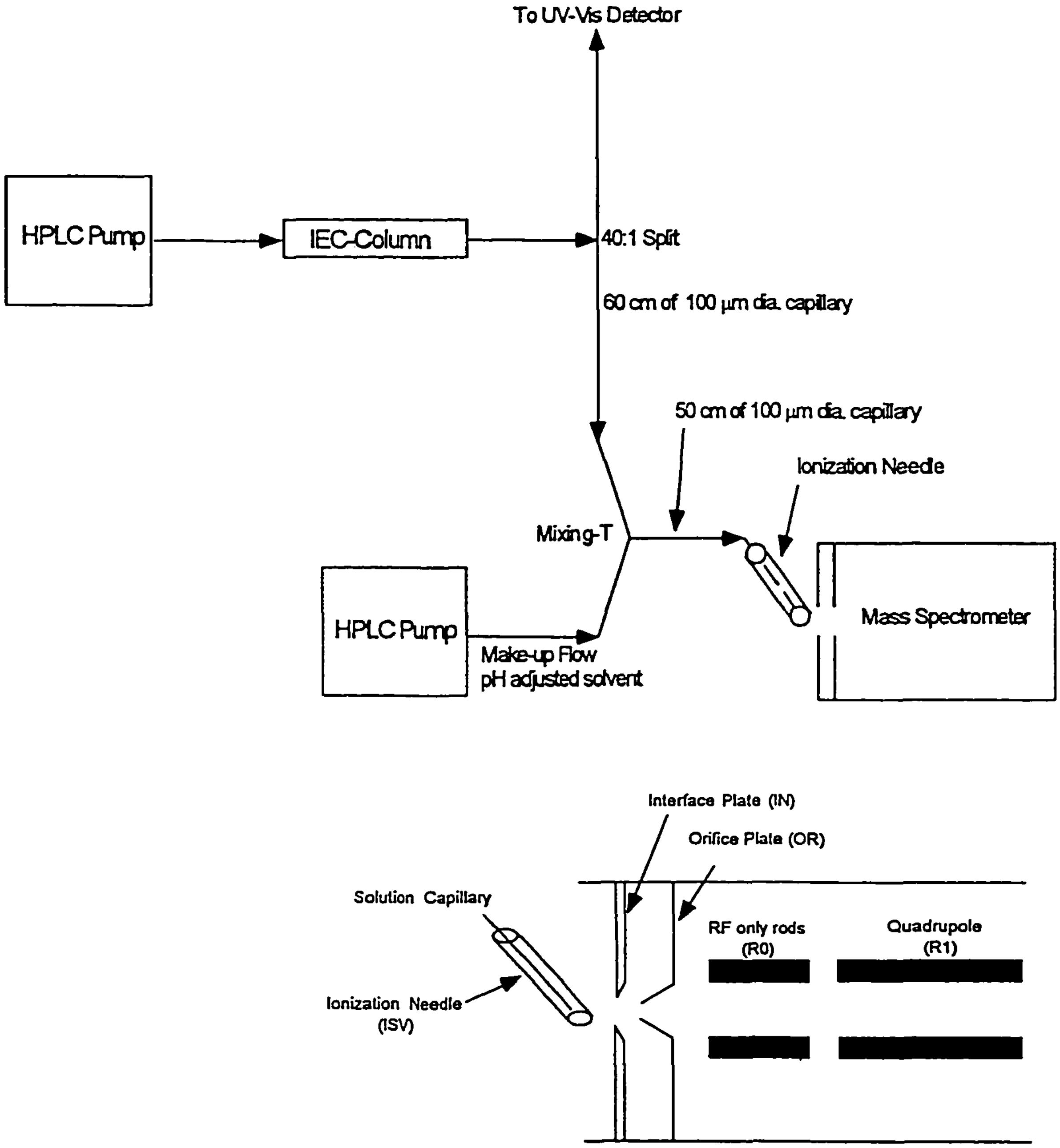


Figure 1. Experimental set-up

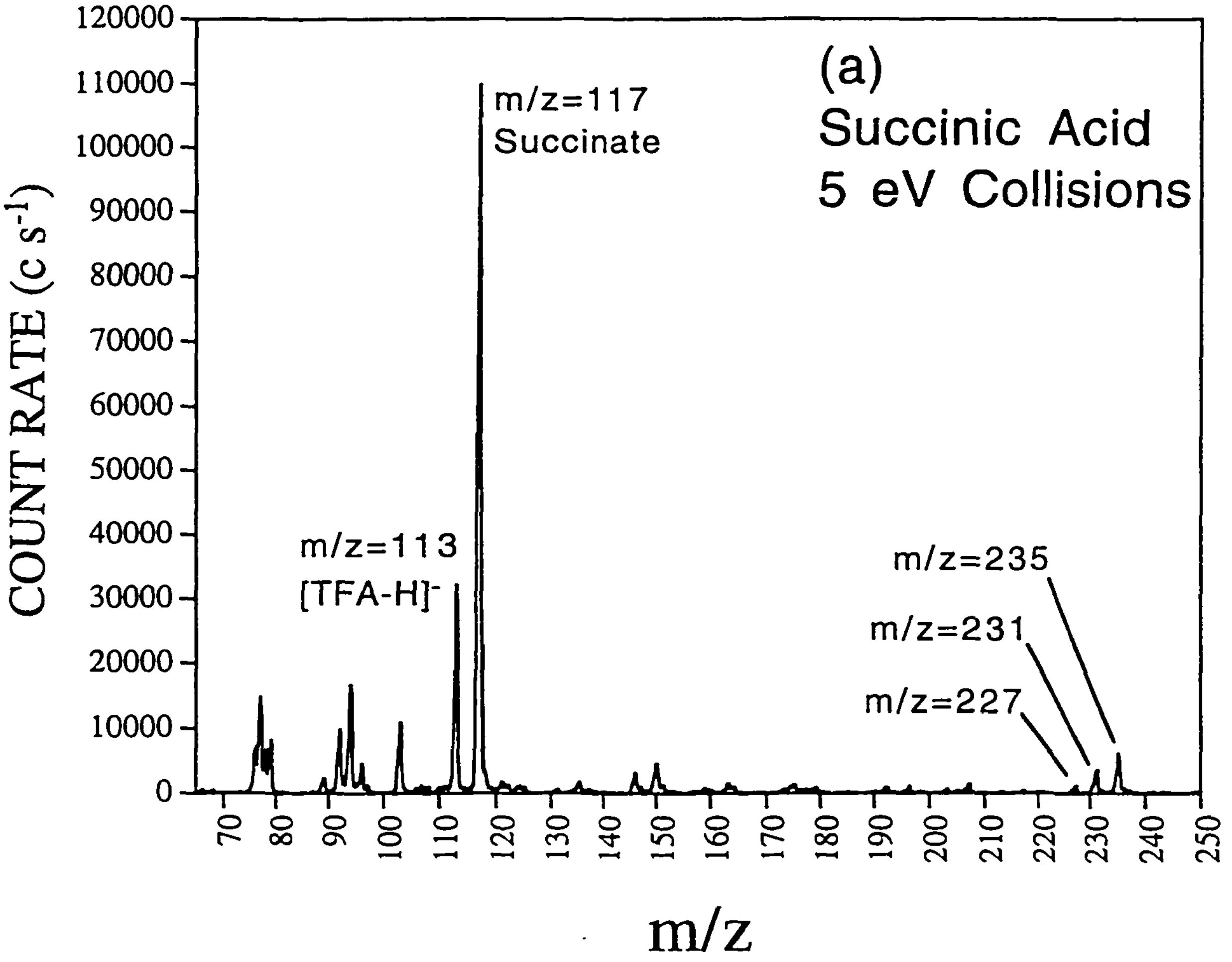


Figure 2. Effect of extraction voltages on spectra from 10 ppm succinic acid. Figures 2a and 2b low energy collisions. Figures 2c and 2d average energy collisions. Figures 2e and 2f high energy collisions. The RF only quadrupole (see Fig. 1) is at -100 volts.

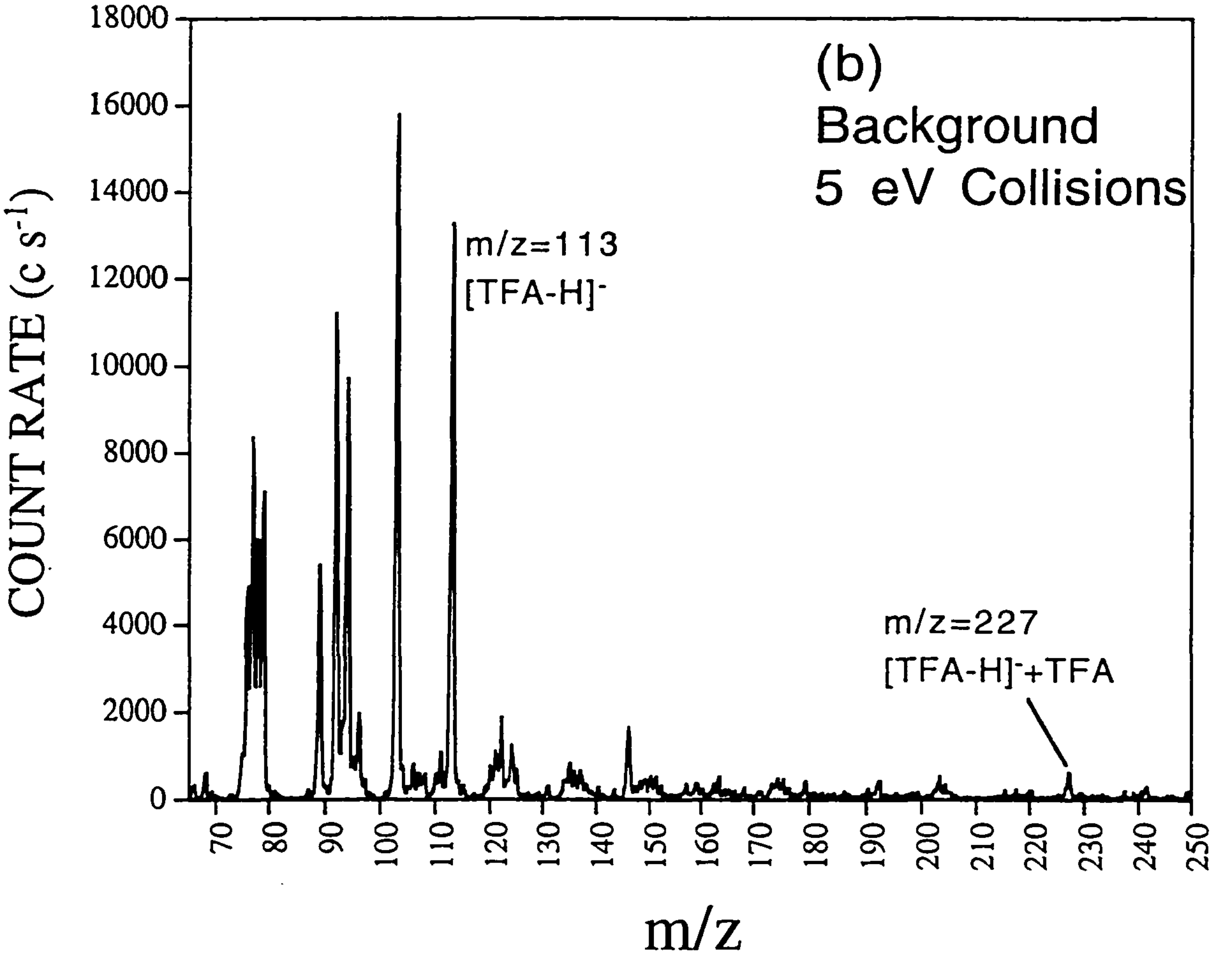


Figure 2. Continued

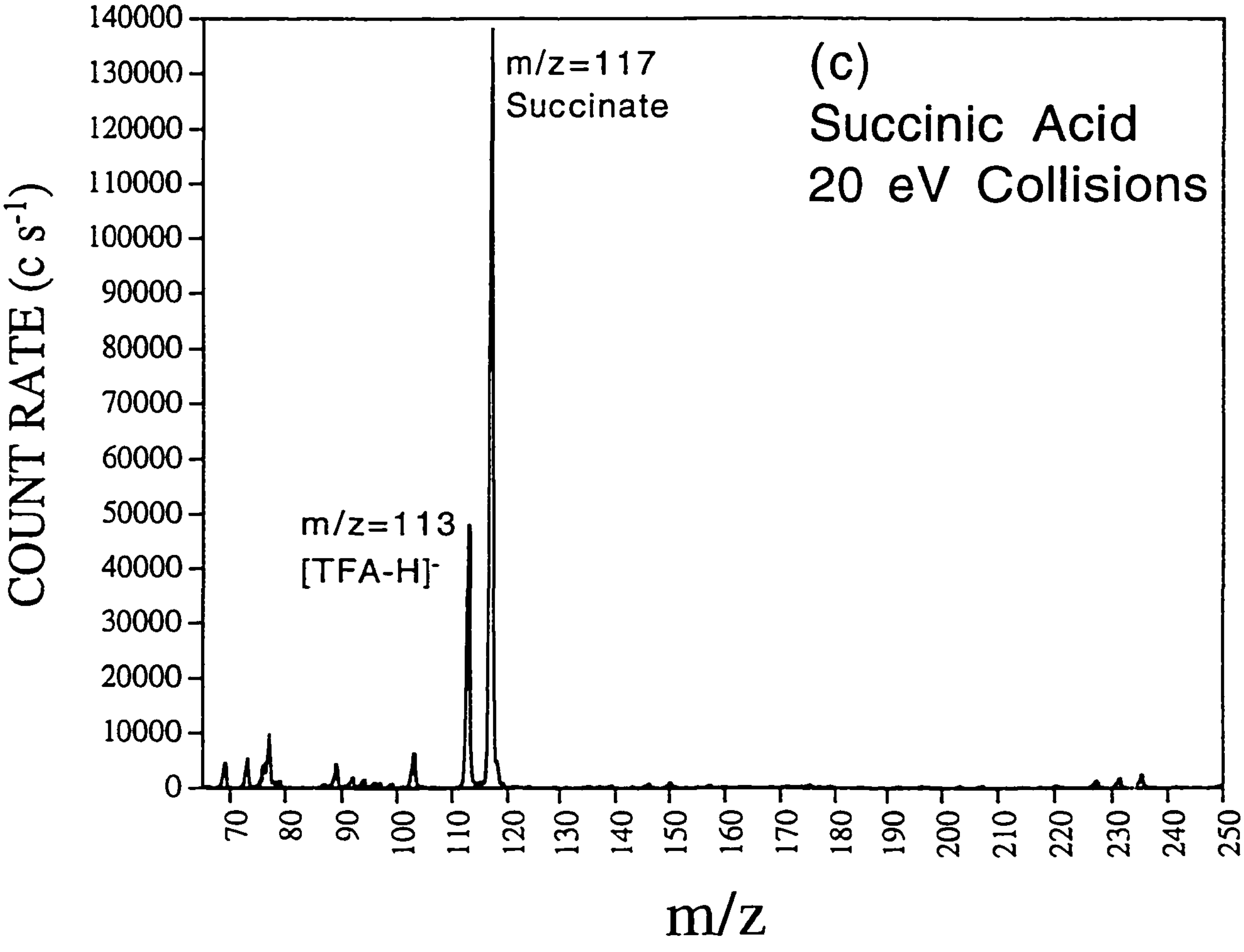
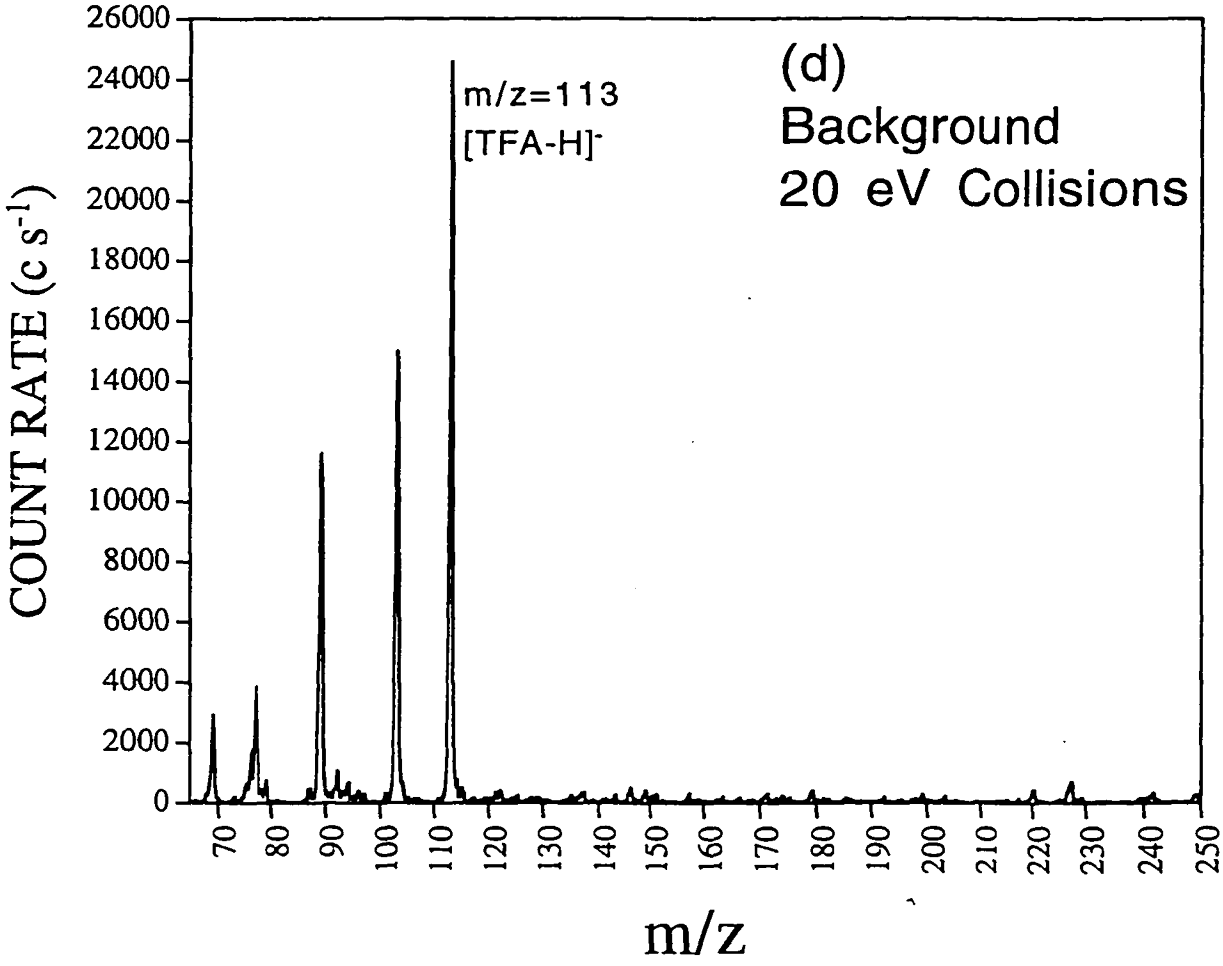


Figure 2. Continued



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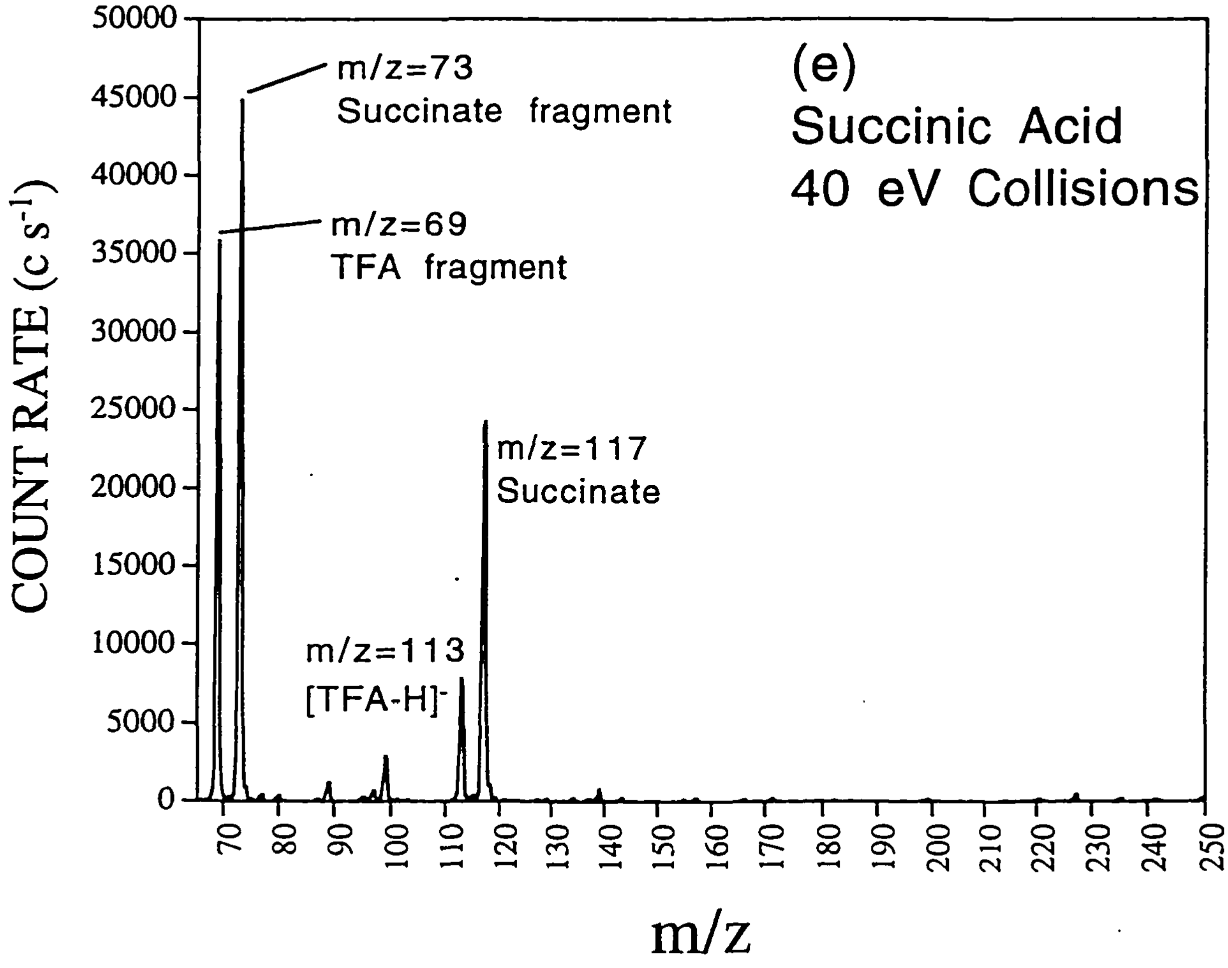

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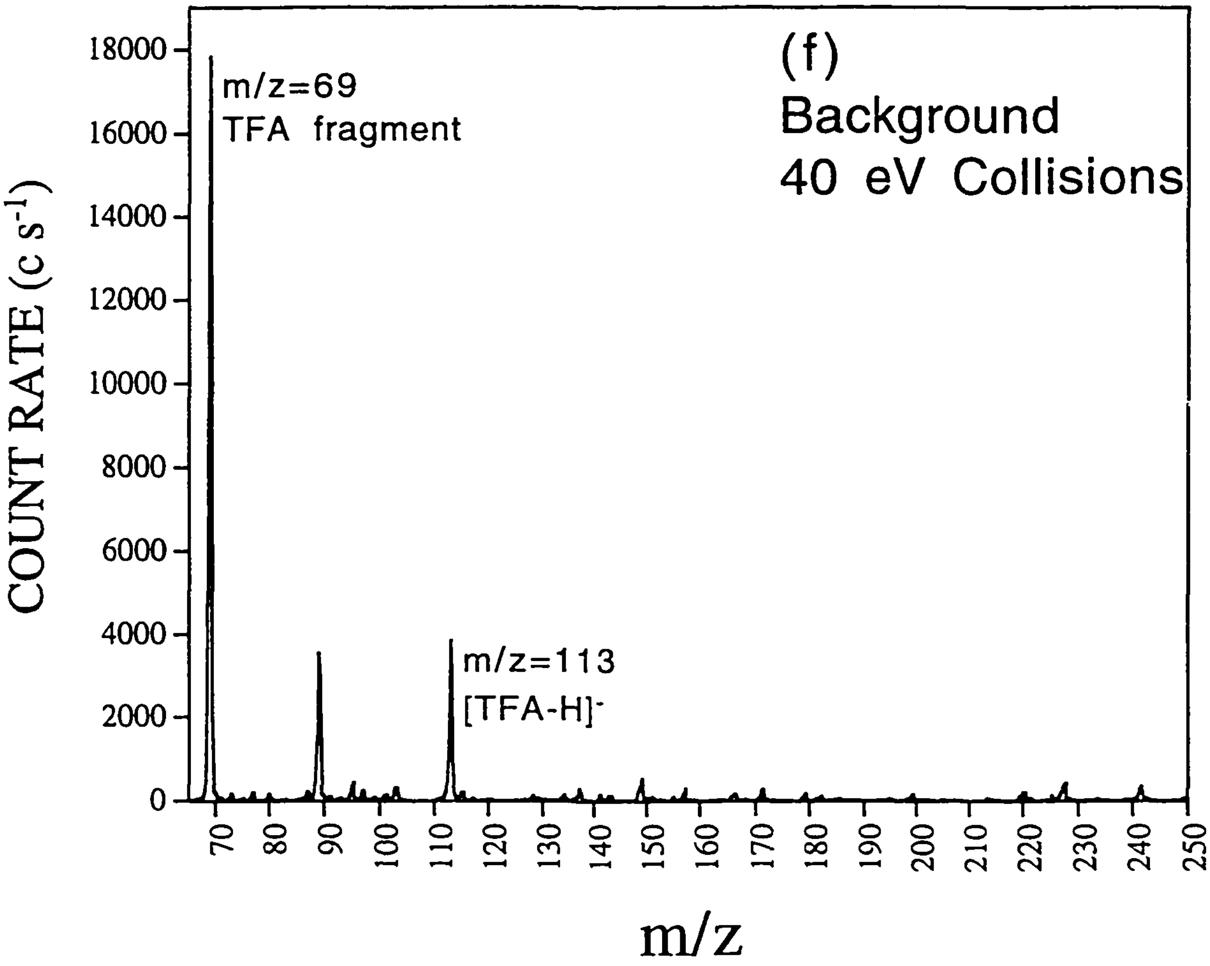

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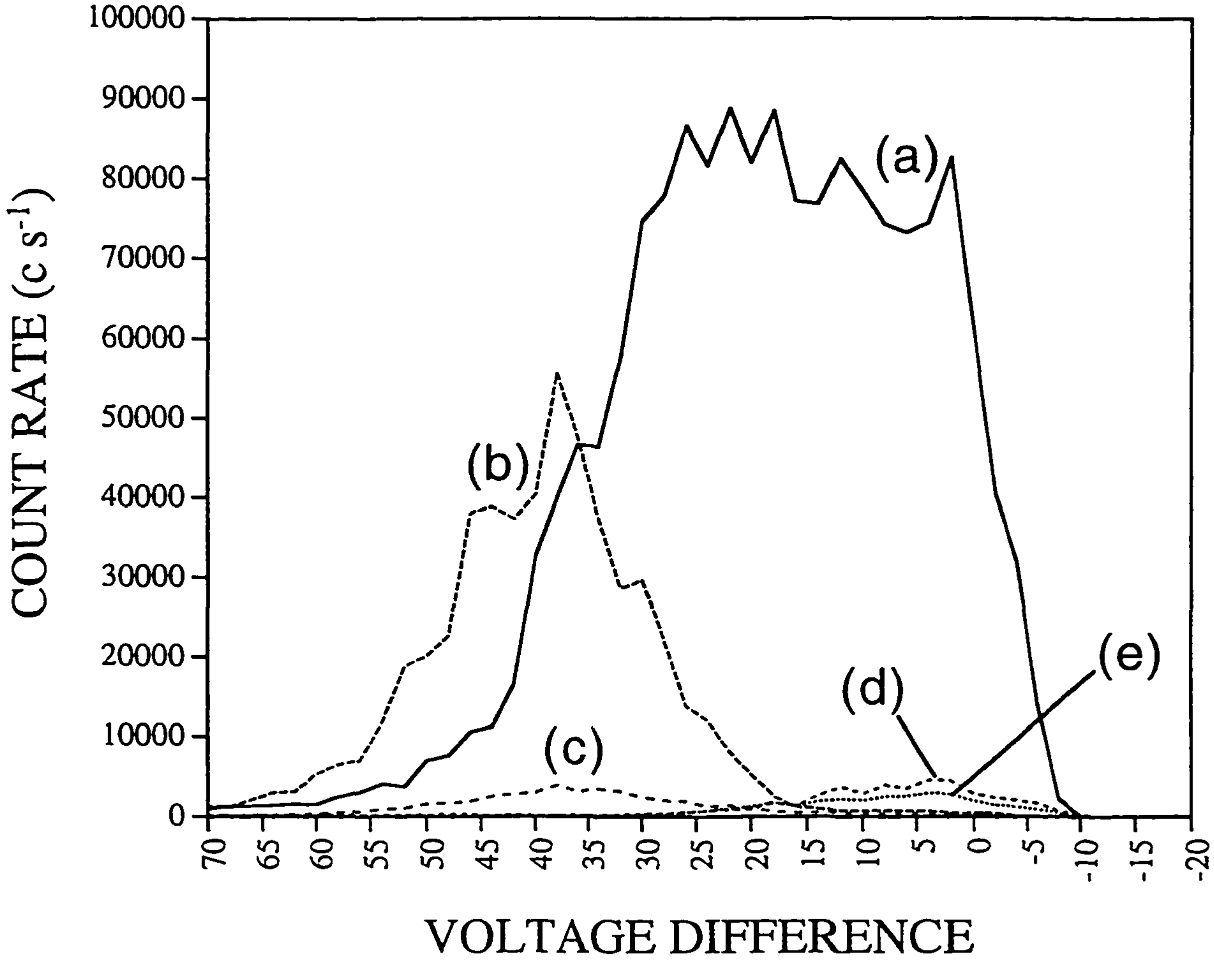


Figure 3. Effect of orifice voltage on ion signals from 10 ppm succinic acid. (a) m/z=117, (b) m/z=73, (c) m/z=99, (d) m/z=235, (e) m/z=231. The RF only quadrupole (see Fig. 1) is at -100 volts.

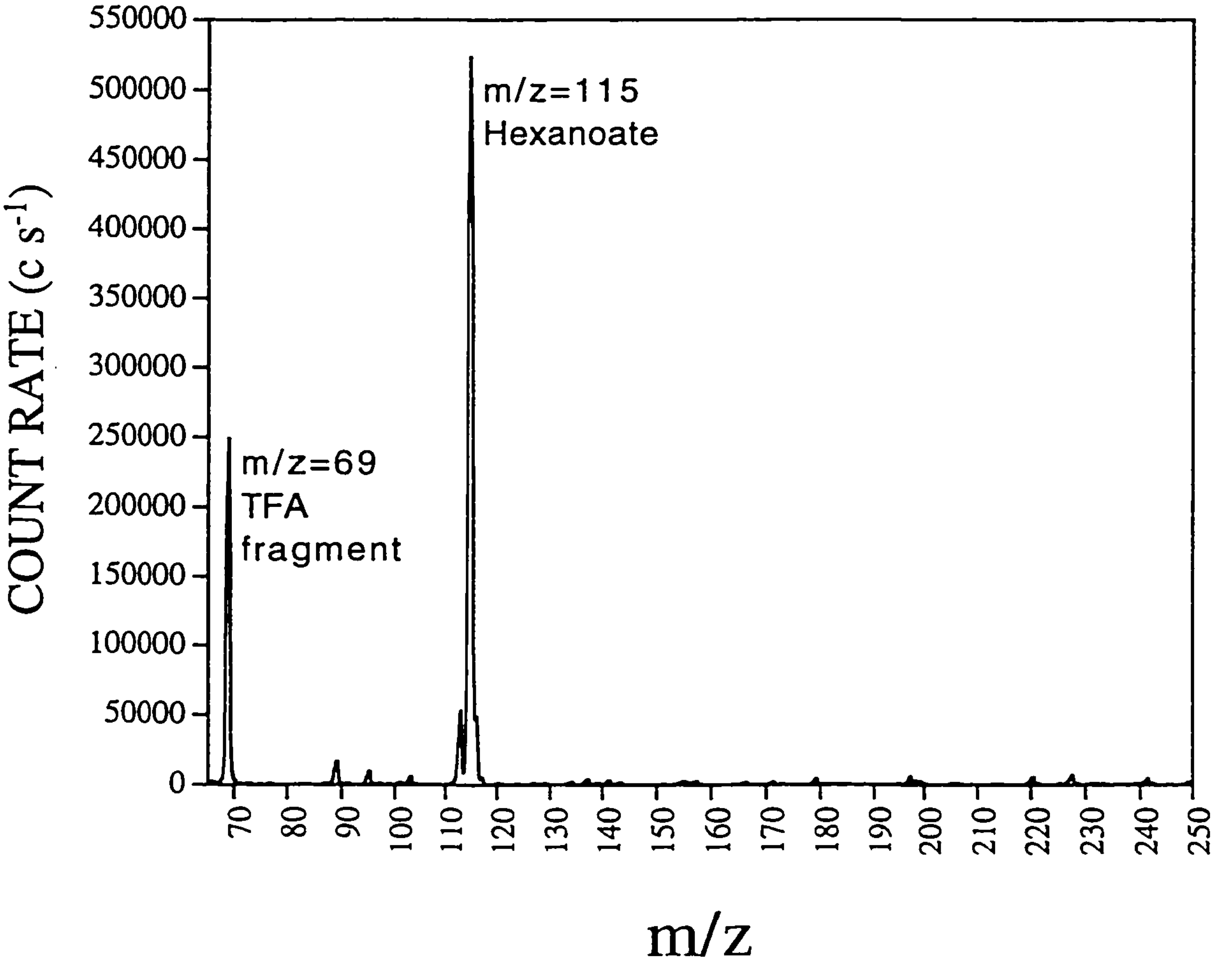


Figure 4. Spectrum from 10 ppm 1-hexanoic acid with harsh extraction conditions. the difference between the orifice voltage and RF only quadrupole voltage is 40 volts.

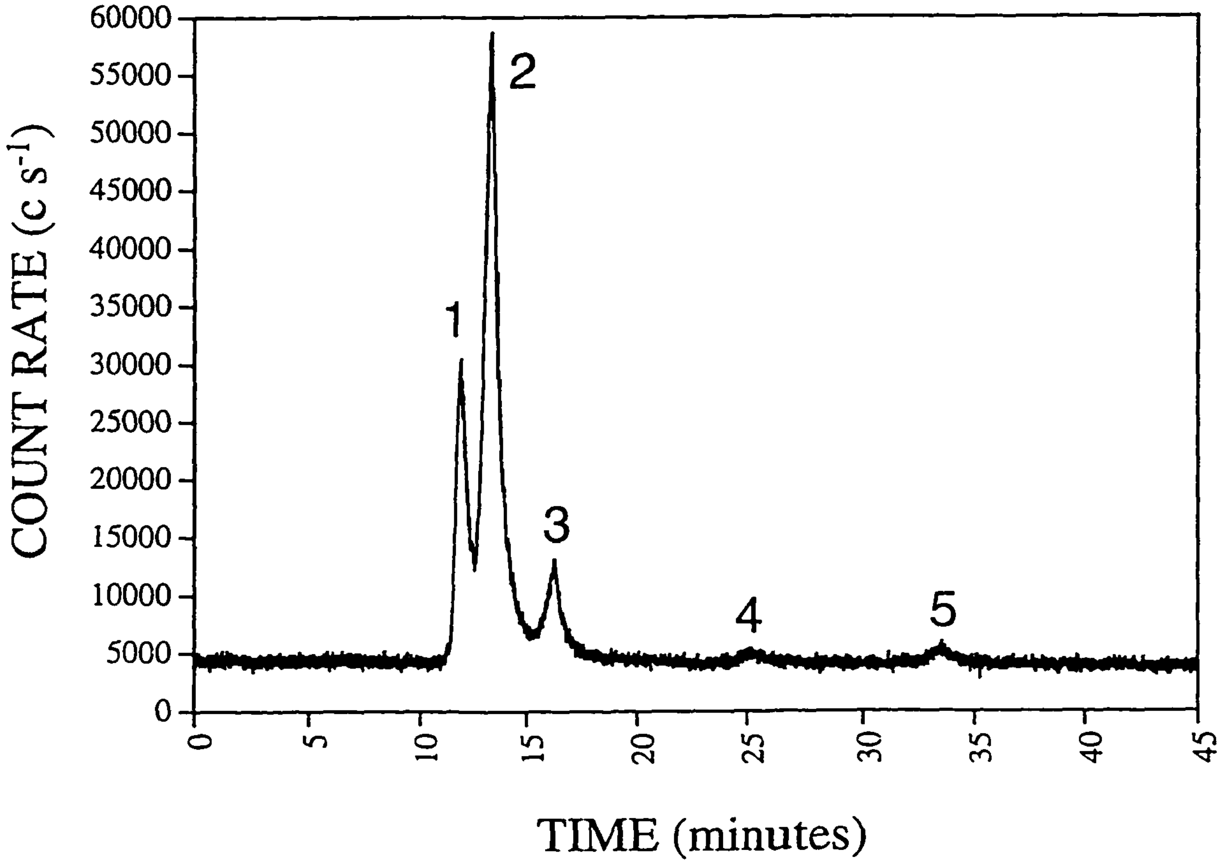


Figure 5. Total ion chromatogram for the separation of 50 ppm oxalic acid (1), maleic acid (2), glyoxylic acid (3), formic acid (4), 2-hydroxyisobutyric acid (5) at pH=4.0 with 0.4 mM TFA and 33% 2-propanol.

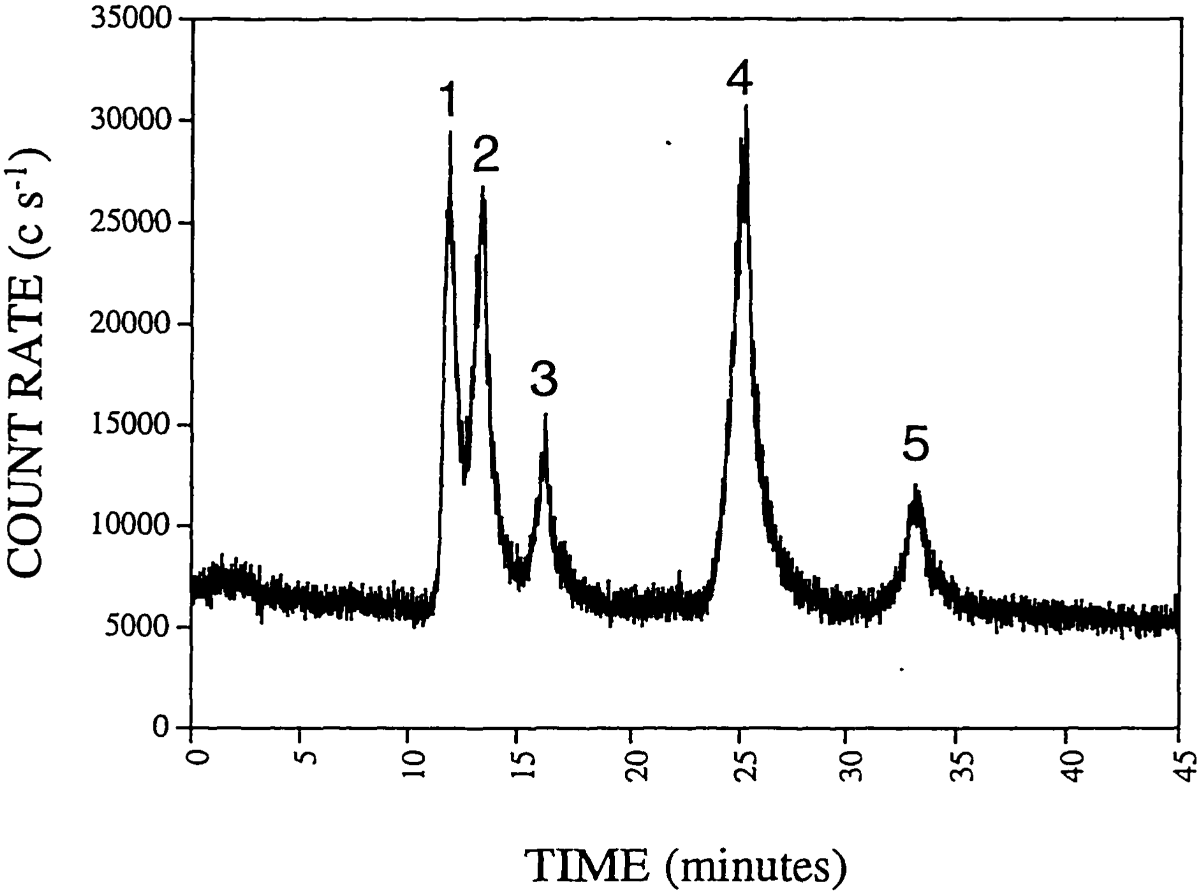


Figure 6. Total ion chromatogram for the separation of 50 ppm oxalic acid (1), maleic acid (2), glyoxylic acid (3), formic acid (4), 2-hydroxyisobutyric acid (5) at pH=9.0 with 0.4 mM TFA and 33% 2-propanol.

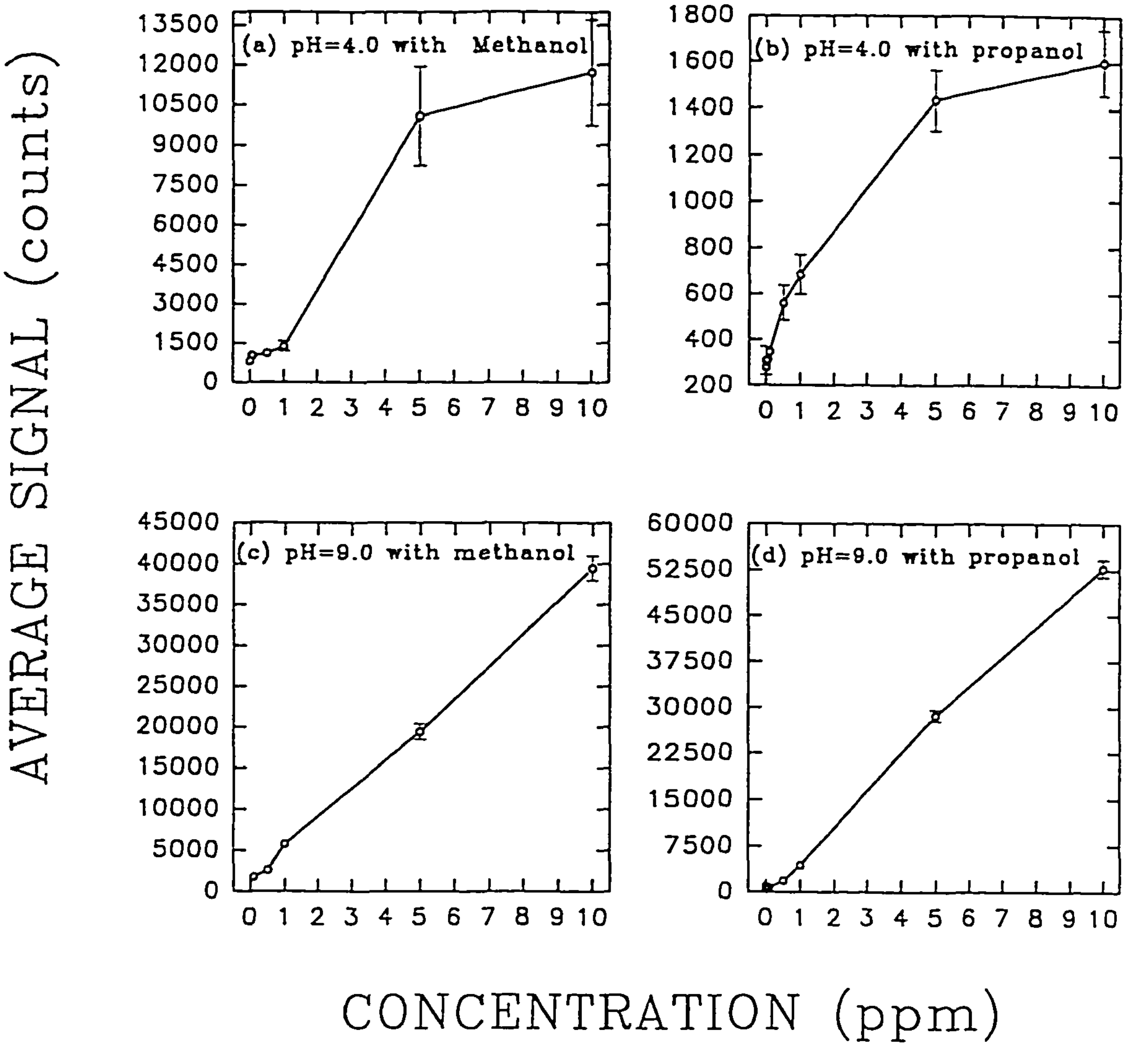


Figure 7. Calibration curves for formate. Figure 7a: pH=4.0 with methanol solvent. Figure 7b: pH=4.0 with 2-propanol solvent. Figure 7c: pH=9.0 with methanol solvent. Figure 7d: pH=9.0 with 2-propanol solvent. The error bars represent the standard deviation of 100 measurements.

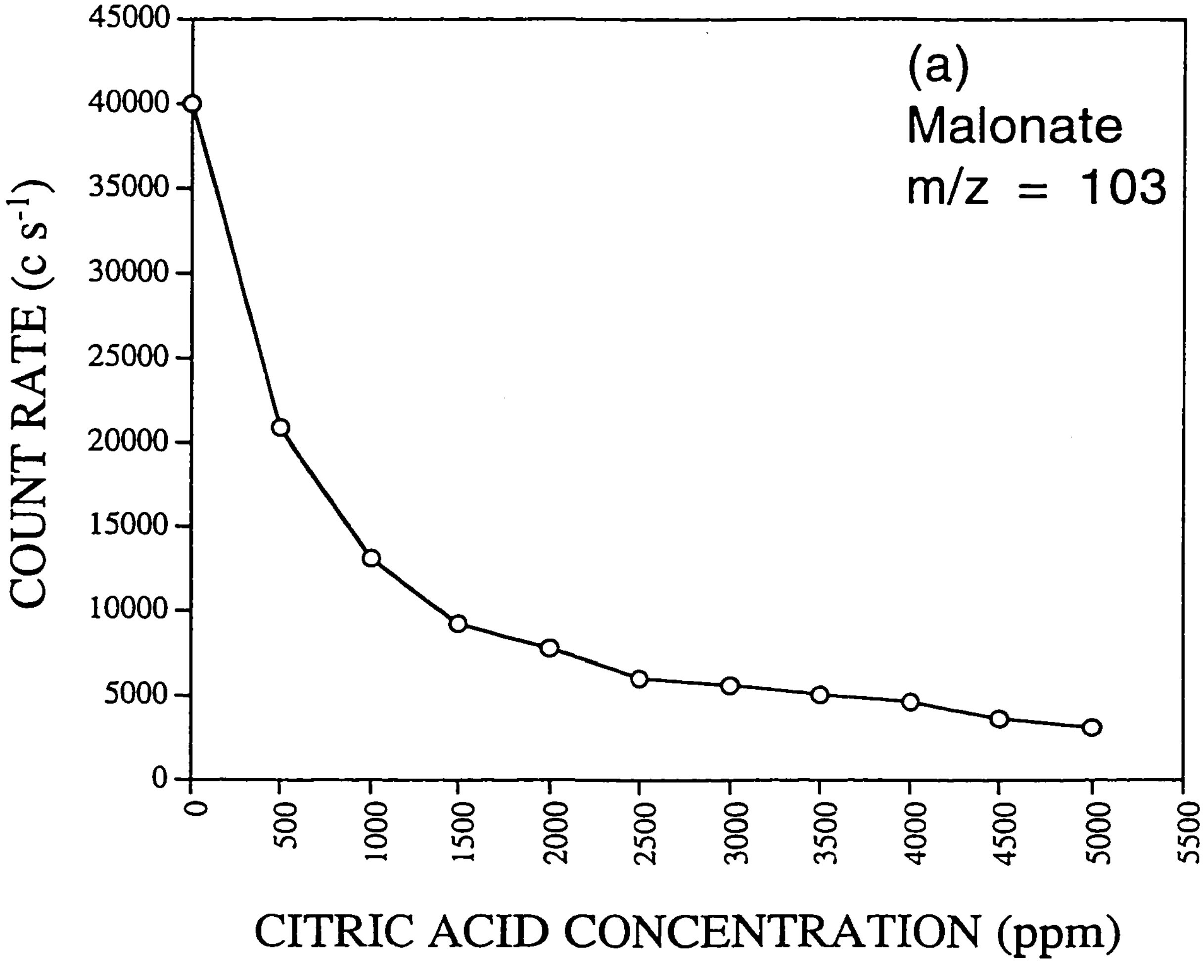
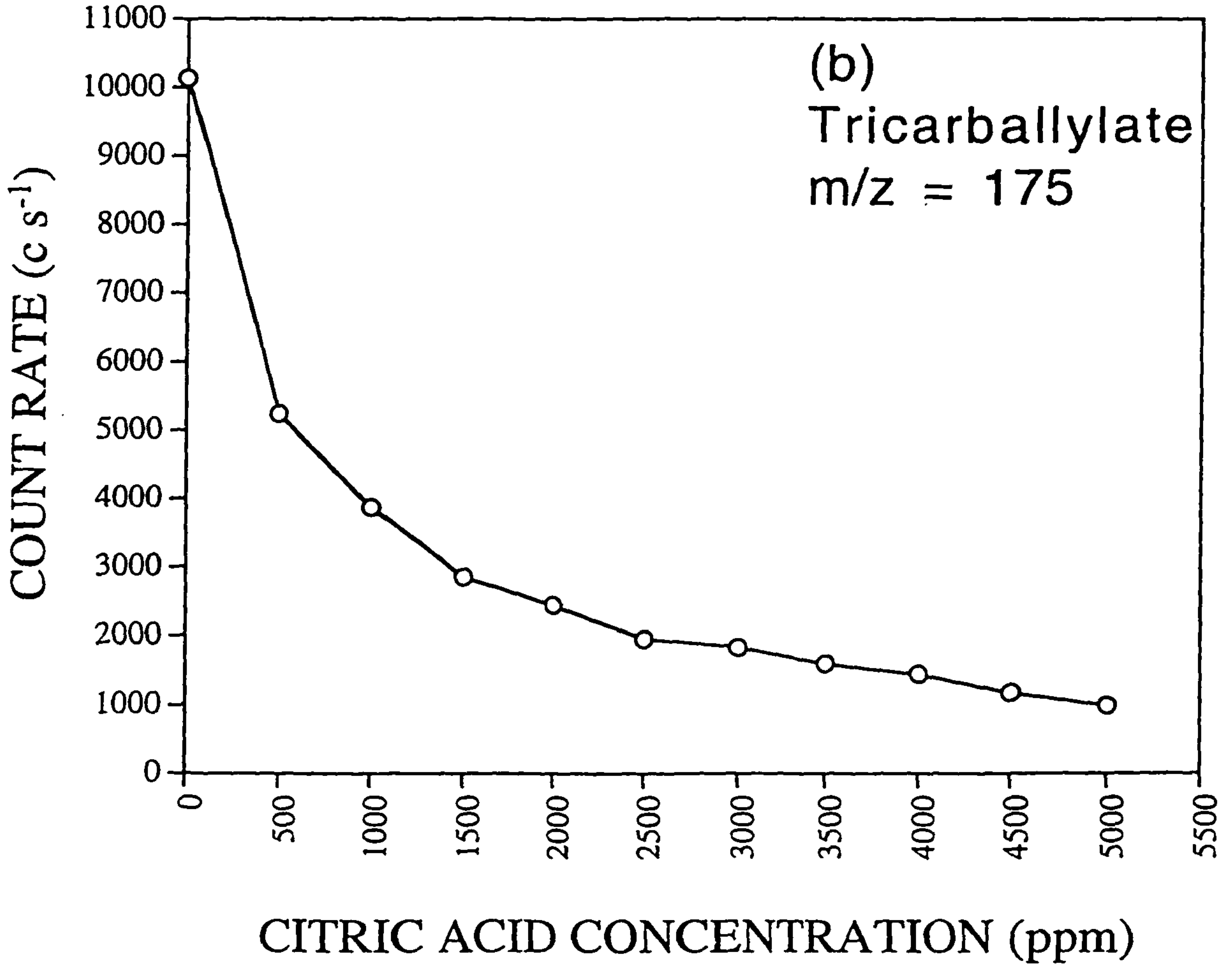


Figure 8. Effect of citric acid concentration on ion signals from (a) 100 ppm malonic acid and (b) 100 ppm tricarballylic acid.



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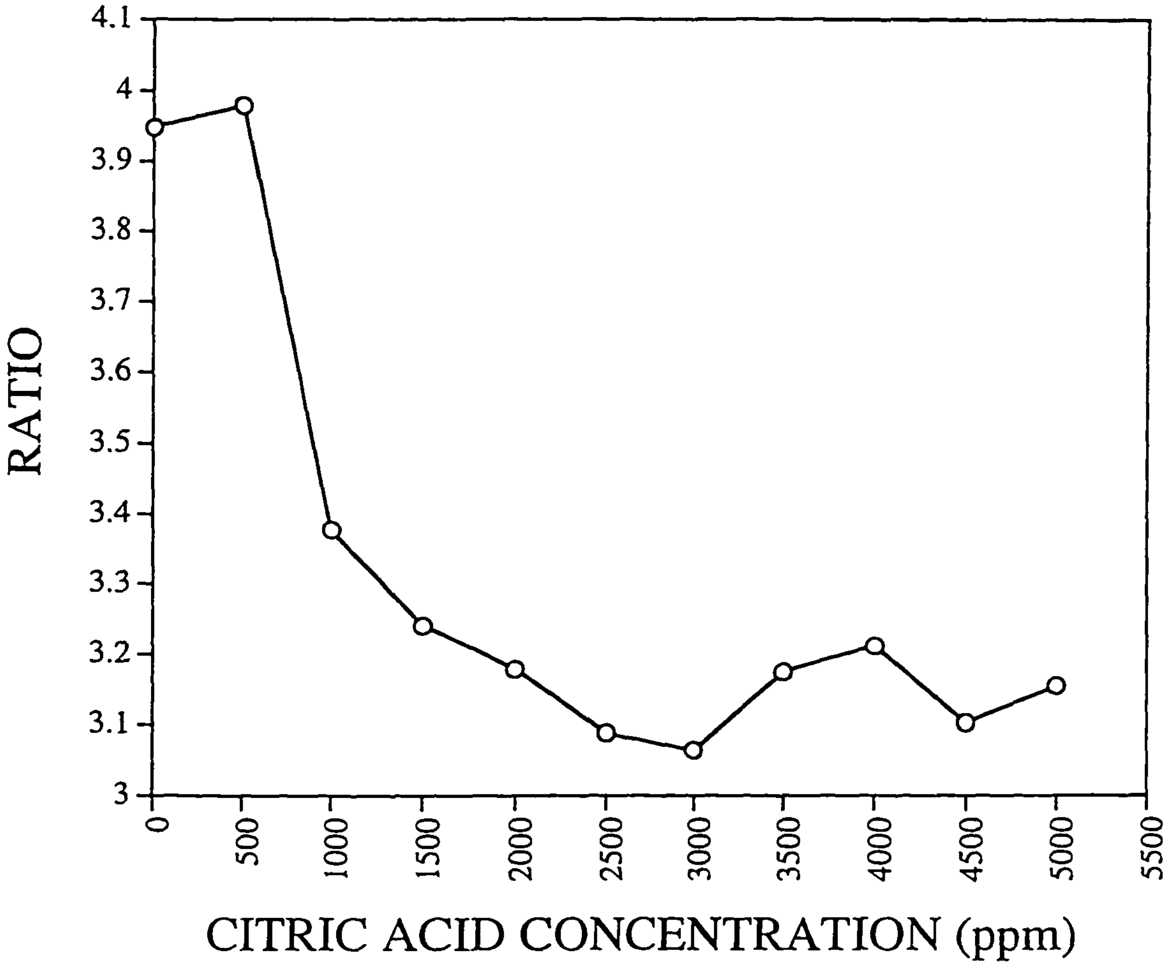

Figure 9. Ratios of malonate to tricarballylate ions as citric acid is increased.

CHAPTER 3. ELECTROCHEMICAL INCINERATION OF BENZOQUINONE IN AQUEOUS MEDIA USING A QUATERNARY METAL OXIDE ELECTRODE IN THE ABSENCE OF A SOLUBLE SUPPORTING ELECTROLYTE

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Steve K. Johnson, Linda L. Houk, Jianren Feng, R. S. Houk, Dennis C. Johnson[1]

Abstract

Electrochemical incineration of *p*-benzoquinone was evaluated as a model for the mineralization of carbon in toxic aromatic compounds. A Ti or Pt anode was coated with a film of the oxides of Ti, Ru, Sn and Sb. This quaternary metal oxide film was stable; elemental analysis of the electrolyzed solution indicated the concentration of these metal ions to be 3 μg/L or less. The anode showed good reactivity for the electrochemical incineration of benzoquinone. The use of a dissolved salt matrix as the so-called "supporting electrolyte" was eliminated in favor of a solid-state electrolyte sandwiched between the anode and cathode. This substitution permitted the electrolysis solution to be analyzed by electrospray-mass spectrometry (ES-MS); however, as a consequence, electrolysis periods were excessively long. Total organic carbon (TOC) and chemical oxygen demand (COD) decreased to 1 - 2 mg/L after 64 hours of electrolysis. The solution pH changed from 5 to 4. Phenolic and carboxylic acid intermediate products such as hydroquinone, maleic acid, fumaric acid, succinic acid, malonic acid, acetic acid and formic acids were identified and quantified using solid phase micro-extraction with gas chromatography with mass spectrometric detection (GC-MS) or liquid

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[1] Author for correspondence

chromatography (LC) with conductivity detection, absorbance detection, or electrospray mass spectrometry (ES-MS). Less than 1% of the carbon in benzoquinone was converted to acetone and acetaldehyde.

Introduction

Public awareness of the discharge of industrial wastes has resulted in governmental and private development of efficient, economical and safe procedures for the destruction of toxic organic waste. Alternatives to the traditional use of thermal incineration include supercritical water oxidation, photochemical degradation, sonochemical oxidation and electrochemical incineration.

Supercritical water oxidation is performed above the critical point of water (374 °C, 218 atm) in the presence of O_2 or H_2O_2 [1]. Organic species only slightly soluble in water are miscible with supercritical water [2]. The literature contains descriptions of reaction mechanisms, kinetics and engineering aspects of supercritical water oxidation applied to numerous organic pollutants including: phenol [3-6], 1,3-dichlorobenzene and benzene [6], pyridine [2,7], acetic acid [2,8], 1,4-dichlorobenzene [9], chlorophenols [2], pulp and paper mill sludge [1], and explosives [10]. Major reaction products are water, carbon dioxide and inorganic salts. Supercritical water oxidation is well suited for destruction of large volumes of toxic organic waste; however, for disposal of small quantities of toxic organic waste, supercritical water oxidation is not considered feasible economically. Therefore, evaluation of less costly methods is appropriate.

Recently, interest in photochemical degradation of toxic organic waste in aqueous media has expanded rapidly. The primary oxidant is the photogenerated hydroxyl radical formed on semiconductor metal oxide surfaces [11]. Typically, TiO_2 powder is the semiconductor used because it is inexpensive, insoluble under conditions used in photochemical degradation, stable and non-toxic [12]. The literature of photochemical degradation describes applications to chlorophenols, dichloroacetate and oxalate [13], 4-chlorophenol [14-17], humic acids [11], dichlorophenols [18], benzene [19], phenol [20], dimethoxybenzene [21] and toluene [22]. Applications of photochemical degradation appear most suitable for solutions having low turbidity [23].

Sonochemical oxidation has been used for degradation of phenol [23] and humic acids [24]; and of 4-chlorophenol, 3,4-dichloroaniline and 2,4,6-trinitrotoluene [25]. The primary reaction in sonochemical oxidation is the pyrolysis of solute present in bubbles generated by acoustical cavitation. Secondary reactions also occur as a result of interactions of solute with hydroxyl radicals and hydrogen atoms produced by the sonication of water [23].

Electrochemical incineration is an alternative to the degradation methods just described. This is a waste remediation process whereby oxygen atoms are transferred from H_2O in the solvent phase to the oxidation product(s) by direct or indirect reactions on the anode surface. This procedure is attractive for low-volume applications such as confined living spaces, e.g., spacecraft, and research laboratories. Kaba *et al.* described successful electrochemical incineration of waste biomass using Pt and PbO_2 electrodes [26]. They reported the major advantages of electrochemical incineration over thermal incineration include: absence of CO and NO_x generation, and low operating temperatures.

Because of the high cost of Pt and the toxicity of lead salts, Kotz *et al.* [27], and Comninellis and Pulgarin [28], compared PbO_2 and Pt electrodes to SnO_2-film electrodes doped with Sb(V) ("Sb-SnO_2"). Both Swiss groups demonstrated that phenol is removed from aqueous solution more efficiently with Sb-SnO_2 anodes than with Pt and PbO_2 anodes. Their work also indicated that for Pt anodes, oxidation stops with the formation of small carboxylic acids, e.g., maleic, fumaric and oxalic. More recently, Comninellis and Battisti [29] compared Pt, IrO_2/Ti, and Sb-SnO_2/Ti anodes and proposed a mechanism for the electrolysis of organic compounds. These and other descriptions of electrochemical incineration literature are reviewed by Rajeshwar *et al.* [30]. Advantages of electrochemical incineration discussed by Rajeshwar *et al.* include: versatility, energy efficiency, amenability to automation, environmental compatibility and low cost.

The major challenge for future development of electrochemical incineration is the discovery of nontoxic anode materials and electrolysis conditions that can achieve conversion of toxic organic waste to innocuous products with high current efficiencies. Other desirable electrode properties include low cost, lack of toxicity, high stability and high activity. The matter of current efficiency is especially pertinent because the desired O-transfer reactions require the anodic discharge of H_2O to produce adsorbed hydroxyl radicals (OH_{ads}). However, a high surface excess of the OH_{ads} species leads to evolution of O_2, an undesired product. Previous work in this laboratory has demonstrated that electrodes comprised of Fe(III)-doped β-PbO_2 films on Ti substrates ("Fe-PbO_2/Ti") are quite stable in acetate buffered media (pH 5) and offer significantly improved catalytic activity over pure β-PbO_2 film electrodes for conversion of CN^B to CNO^B under potentiostatic conditions [31] as well as the anodic degradation of *p*-benzoquinone under galvanostatic conditions [32].

The present focus of our research related to electrochemical incineration is directed away from the use of a PbO_2 film electrode to a quaternary metal oxide consisting of a SnO_2 film doped with varying amounts of the oxides of antimony, titanium and ruthenium. The cathode is a porous stainless steel cylinder. In this study, a Nafion membrane is used as a solid-state electrolyte sandwiched between the anode and cathode. Use of Nafion, a perfluorinated membrane, precludes the need for addition of soluble inorganic salts to function as supporting electrolytes. A dramatic increase in lifetime of the anodes has been observed to result from omission of added electrolytes. Furthermore, the low ionic strength of the electrolysis solution facilitates the use of ES-MS for determination of ionic products and there is little or no electrolyte to remove from the remediated solution.

Experimental Section

Reagents and samples

All chemicals were reagent grade (Fisher Scientific) and water was purified in a Nanopure-II system (Barnstead, Newton, MA). Quaternary metal oxide films were prepared from a solution comprised of 0.4 M $SnCl_2 \bullet 2H_2O$, 0.03 M $SbCl_3$, 0.08 M $RuCl_3$ and 0.02 M $TiCl_4$ in a 1:1 mixture of 12 M HCl and *i*-propanol. This composition was chosen on the basis of patents claiming high stability for TiO_2-RuO_2-SnO_2 [33] and RuO_2-Sb_2O_3-SnO_2 [34] films on Ti substrates in saline solutions. *p*-Benzoquinone (Fisher

Scientific) was purified by sublimation and used for preparation of 100 mg/L stock solutions in water. Carboxylic acids (Aldrich) were dissolved in water to prepare 1000 mg/L standard stock solutions, which were then frozen until needed to prevent microbial degradation.

Electrolysis apparatus

Quaternary metal oxide films were prepared by a thermal procedure in which Ti or Pt substrates were alternately painted with the solution of the four metal salts followed by heating above the flame of a Bunsen burner for ca. 15 s. After ten wetting-heating cycles, the electrode was annealed in a muffle furnace for 1 h at 600°C. The resulting quaternary metal oxide films on Ti and Pt substrates are designated here as "Ru-Ti-Sb-SnO_2/Ti" and "Ru-Ti-Sb-SnO_2/Pt", respectively. Preliminary work made use of a Ti substrate (10 cm^2 working area), in the form of a rectangular plate, and a Pt cathode. Subsequently, a Pt wire (0.62-mm o.d., 24-cm length, 4.7-cm^2 working area) was used as the substrate for quaternary metal oxide films. In the latter case, a rectangular piece of Nafion 117 membrane (2 cm x 4 cm) was placed around the cathode and wrapped tightly with the quaternary metal oxide-coated wire anode, as shown in Fig. 1. The cathode was prepared from a rod of type-360 stainless steel (6.4-mm o.d. and 3.5-cm length) drilled with 20 holes (3-mm dia.) positioned normal to the axis of the rod. Other anodes include those described by Feng *et al.* [31,32].

The electrolysis cell was assembled from a 50-mL three-necked pyrex flask. Teflon stoppers were machined to fit the outer two ports of the cell. One stopper allowed entry of the electrode assembly. The other stopper allowed passage of a hypodermic needle to add water or introduce a solid phase microextraction fiber for headspace analysis. A tapwater-cooled condenser was inserted in the center neck of the cell to decrease evaporative water loss during long electrolysis periods. All glassware was washed in 1 M KOH in a 1:1 mixture of ethanol and water, then in 2 M H_2SO_4, then rinsed with water and then dried overnight at 100°C. The power supply was a potentiostat/galvanostat (model 363, EG&G Princeton Applied Research, Princeton, NJ) operated in the galvanostatic mode.

Electrolyses

Electrolyses were performed under galvanostatic control at 1.0 A (ca. 0.2 A/cm^2) on 50-mL aliquots of benzoquinone stock solution. At the conclusion of each electrolysis, deionized water was added to bring the volume in the cell back to 50 mL, i.e., the starting volume.

Surface characterization

Micrographs and elemental analyses of the electrodes were obtained using a Hitachi S-246N scanning electron microscope (SEM, Mountain View, CA) equipped with a LINK ISIS energy dispersive X-ray spectrometer (EDS, Oxford). The response for the analyte elements was calibrated by the ZAF procedure with matrix effects corrections, as provided by the manufacturer.

Chemical analyses

The elemental content of the initial benzoquinone solution and one that had been electrolyzed for 64 h was determined by inductively coupled plasma-mass spectrometry (ICP-MS). The apparatus and typical operating conditions have been described by Hu *et* *al.* [35]. A semiquantitative analysis mode was used for calibration. Spectral scans were measured in separate m/z windows typically 50 daltons wide. Analyte signals were adjusted for blank signal, isotopic abundance and approximate degree of ionization [36] and then compared to those for known concentrations of Co, La or Tl, whichever was in the particular m/z window of interest. Scans of the full m/z range showed that matrix effects were negligible.

In addition to the starting solution, samples representing eight electrolysis periods in the range 0.5 - 64 h were analyzed for TOC, COD, pH, and inorganic and organic anions. TOC was determined at the University of Iowa's Hygienic Laboratory, which is EPA approved. Samples were analyzed by a DC190 TOC Analyzer (Dohrmann, Santa Clara, CA) using a combustion infrared method. COD was determined by titration with $KMnO_4$ as described by Feng *et al.* [32] or by a Hach DR2000 analyzer (Loveland, CO).

Quinone and phenolic compounds were separated with a reverse phase Zorbax SBC18 column (25-cm length, 4.6-mm dia.) developed by Rockland Technologies (Chadds Ford, PA) and detected by absorption at 240-nm using a Kratos Analytical Spectroflow photometer (Ramsey, NJ) or with a Perkin-Elmer SCIEX API/1 ES-MS (Thornhill ON, Canada) based on a single quadrupole mass spectrometer. The ES-MS was equipped with a Perkin-Elmer SCIEX TurboIonSpray heating probe (Thornhill ON, Canada). The TurboIonSpray employs a heated gas flow near the electrospray needle which increases evaporation of solvent and allows liquid flow rates up to 1 mL/min. TurboIonSpray eliminates the need to split the eluent stream from the HPLC, eliminates some background peaks, improves detection limits where background peaks are eliminated, and allows use of low organic solvent levels. Methanol and water at a 1:1 ratio was the eluent used at a flow rate of 0.3 mL/min. In addition, inorganic and organic anions in the electrolysis solutions were monitored by direct infusion into the ES-MS. Carboxylic acids were identified using an ICE-AS6 ion-exclusion column from Dionex (Sunnyvale, CA) coupled to ES-MS as described by Johnson *et al.* [37].

The inorganic and organic anions were quantified using an AS11 anion- exchange column with an ED40 conductivity detector from Dionex. A sodium hydroxide and methanol gradient elution program as described in the literature accompanying the column provided the needed separation of the analytes of interest.

Polyacrylate and carbowax-divinylbenzene coated SPME fibers from Supelco (Bellefonte, PA) were used to extract constituents in the benzoquinone solution and in the headspace. Solid phase microextraction fibers underwent thermal desorption in a Varian 3400 gas chromatograph (Palo Alto, CA) equipped with a DB-1 or DB-5 column from J&W Scientific (Folsom, CA), and coupled to a Finnigan TSQ-700 triple quadrupole mass spectrometer (San Jose, CA).

Aldehydes and ketones were collected with Sep-Pak (DNPH-Silica) cartridges manufactured by Waters Chromatography (Marlborough, MA). The HPLC analysis of the eluent in the Sep-Pak cartridges was performed as described in the manufacturer's instructions accompanying the cartridges.

Results and Discussions

Comparison of electrode materials

Table 1 presents a comparison of the performance of seven electrode materials applied for anodic degradation of benzoquinone in acetate buffer. Included are values of COD following electrolysis for specified time periods and brief comments pertaining to the electrolysis solution or the electrode surfaces. The COD in these solutions was determined by titration with standard $KMnO_4$, a procedure that ignores contribution from the acetate/acetic acid components. The Au anode was least effective, requiring 48 h to decrease the COD to 582 mg/mL, *i.e.*, a 46% decrease from the original value. The Ru/Ti anode was slightly more effective than Au with a COD of 28 mg/L after 48 h. The PbO_2/Ti anode decreased the COD to 12 mg/L after 24 h; however, the Fe-PbO_2/Ti anode decreased the COD to 8 mg/L after only 10 h. The Ru-Ti-Sb-SnO_2/Ti anode was somewhat less efficient than the Fe-PbO_2/Ti anode, producing a COD of 6 mg/L after 24 h. The glassy carbon anode exhibited significant degradation within 10 h and corrosion of the Ti surface in the Sb-SnO_2/Ti anode was observed after only 0.5 h.

Comments are frequently offered by environmentalists that use of toxic lead-based anodes is not acceptable for electrochemical incineration applied to potable waters. Therefore, even though Ru-Ti-Sb-SnO_2/Ti anodes were slower to oxidize benzoquinone than Fe-PbO_2 anodes, the former was chosen for further evaluation in this project.

Quaternary metal oxide films corroded slowly when operated at large current densities (> 100 mA/cm^2) and ambient temperatures (25 - 35 °C). The U-tube shape of the Ru-Ti-Sb-SnO_2/Ti anode permitted circulation of thermostated water. With this

Table 1. Electrode Materials Comparison

Electrode ($10\ cm^2$)	Current (mA/cm^2)	Time (h)	COD[a] (mg/L)	Observations
None	n.a.	0	1071	Brown-black solution.
Au	10	48	582	Deep yellow solution.
Ru/Ti	10	48	28	Yellow solution.
Glassy carbon	10	10	-	Carbon particles suspended in solution.
PbO_2/Ti	10	24	12	Colorless solution.
Fe-PbO_2/Ti	10	10	8	Colorless solution.
Sb-SnO_2/Ti	10	0.5	-	Apparent corrosion of Ti substrate.
Ru-Ti-Sb-SnO_2/Ti	10	24	6	Colorless solution.

[a] COD determined by titration with $KMnO_4$.

electrode, the quaternary metal oxide films exhibited less corrosion when operated at higher temperature. Typically, corrosive losses were not visible nor detectable by gravimetry following 70-h electrolysis periods when the Ru-Ti-Sb-SnO_2/Ti tubular anode were operated at 200 mA/cm^2 and 60 °C. The observed benefit from a higher operating temperature is not understood; however, it is known that increased temperature increases the rate of water discharge and, therefore, causes the anode potential to be decreased [38].

Morphology and elemental composition of quaternary metal oxide film

A freshly prepared quaternary metal oxide film on Ti that had not been used for electrolysis was examined by scanning electron microscopy. The micrograph (not shown) indicated a moderately uniform film corresponding to an aggregation of small crystallites with individual diameters <2 μm. The results of energy dispersive spectroscopy for this surface confirmed the presence of Sb, Ru, Ti and Sn. An elemental analysis of two different regions of the electrode surface yielded the following percent compositions: Sb = 7 and 8%, Ru = 9 and 9%, Ti = 10 and 21%, and Sn = 34 and 39%. In comparison, the relative concentrations of metallic components of the solution used for thermal preparation of quaternary metal oxide films were: Sb = 6%, Ru = 14%, Ti = 3% and Sn = 77%.

Performance of the solid-state electrolyte

The Nafion membrane eliminated the need for added soluble salts to serve as supporting electrolytes, which facilitated direct analysis of product solutions using ES-MS. These analyses can only be performed on solutions of low ionic strength to prevent build-up of salt deposits that plug the orifice cone in the ES-MS. The Nafion 117 membrane also prevented film formation on the anode surfaces during electrochemical incineration of 10 mM benzoquinone solutions over periods of several weeks. Comninellis and Pulgarin reported formation of organic films on Pt electrodes applied for anodic degradation of phenol and stated that film formation was exacerbated by high pH, low current density, high temperature and high phenol concentrations [39]. Similar problems of film formation with loss of electrode activity have been encountered in this laboratory during amperometric detection of phenol at Pt electrodes [40] and electrolysis of benzoquinone at Pt (unpublished).

Construction of electrolysis cells using a solid-state electrolyte required that the membrane be sandwiched tightly between porous anode and cathode materials [41]. Undoubtedly, for applications to solutions having zero ionic strength, *i.e.*, very low conductivity, electrolysis occurred only on those small portions of the electrode surfaces that were in simultaneous contact with solution and membrane. The result was a severely attenuated working area of the electrodes with a corresponding increase in the effective current density. We observed cell voltages >10 V as compared to <5 V for the presence of acetate buffer (pH 5).

Whereas this loss of effective electrode area, with a resulting increase in cell voltage, is seen as a disadvantage of this cell design, it is probably the explanation for the absence of organic film build-up on our anode surfaces. The higher effective current density resulted in an elevated rate of H_2O discharge at the working portions of the anode with a corresponding large flux density for OH radicals that are believed to be the source of O-atoms transferred to the product(s) of the electrochemical incineration reaction(s). Therefore, the lifetime of organic radicals was greatly diminished with the beneficial decrease (or elimination) of radical polymerization to form surface films. The smell of $O_3(g)$ also was detected above the electrode assemblies constructed with the Nafion membranes. This can be expected when high current densities are applied at noble electrodes [42]. It is not known to what extent the evolution of some O_3 assists in promoting the desired electrochemical incineration.

A disadvantage of using the solid-state electrolyte in place of added soluble electrolyte was a significant increase in the electrolysis time needed to decrease COD values to specified levels. For example, addition of 0.1 M acetate buffer (pH 5) to our cell resulted in a ca. 50% decrease in time required to achieve >90% decrease in COD.

COD, TOC, and pH results

Figure 2 contains plots showing the change in COD and pH as a function of electrolysis time during the electrochemical incineration of a solution containing 100 mg/L benzoquinone. Values for COD, obtained with the Hach DR2000, steadily decreased from an initial value of 190 mg/L to 2 mg/L during a 64-h electrolysis period. Values of TOC (not shown) decreased to 1.2 mg/L during this same period. The pH of the electrolysis solution decreased sharply to a minimum of *ca.* 3 at 2 h followed by a gradual increase to a final value of *ca.* 4. Whereas the rate of CO_2 evolution is maximum immediately following the onset of electrolysis, ionization of the resulting H_2CO_3 ($pK_{a,1} = 6.3$) is not sufficiently strong to explain the sharp drop in pH. The most probable explanation is the formation of carboxylic acids by the first steps in benzoquinone degradation. Comninellis and Pulgarin reported the presence of maleic, fumaric and oxalic acids following electrolysis of phenol solutions [28,39].

Dissolved products of benzoquinone solutions

Benzoquinone is very reactive in water and undergoes condensation reactions. The identity of these condensation products is highly dependent upon starting concentration and pH [43]. Products include dibenzofuran, biphenols, a trimer of molecular weight (MW) 290, plus a higher MW polymer. Condensation occurs rapidly in alkaline media but only slowly in neutral and acidic media. Following addition of benzoquinone to pure water, the color of the solution changes from light yellow to a tea color within 72 hrs and to coffee brown within one week. Because a similar color transition is observed during the initial phase of the electrochemical incineration of benzoquinone, an attempt was made to look for the compounds named above. None of these compounds was detected using a solid phase microextraction fiber in combination with GC-MS for a 10 mM benzoquinone solution.

In the electrolysis solutions (100 mg/L benzoquinone), only hydroquinone and resorcinol were detected on the solid phase microextraction fibers even though the sensitivity for phenols was increased by derivatization with acetic anhydride. Derivatization was not performed on the 10 mM benzoquinone solution. Results obtained using the polyacrylate fiber during electrolysis were consistent with the presence of hydroquinone and resorcinol following 0.5, 1 and 2 h. At 4 h, hydroquinone was not detected and at 8 h, resorcinol was not detected.

ES-MS was also used to look for dibenzofuran, biphenols and other phenolic compounds. Although ES-MS could detect these compounds in standard solutions, dibenzofuran and biphenols were not observed before or during the electrolysis of solutions containing 100 mg/L or 10 mM benzoquinone. However, phenol was detected in a stock solution of week old 10 mM benzoquinone that had not undergone electrolysis.

ES-MS identified *p*-benzoquinone and hydroquinone in electrolysis solutions at 4 h. Chromatograms are compared in Figure 3 for a benzoquinone solution after 1 h of electrolysis (A) and a standard solution (B). In addition to the standards shown in the chromatogram, selected ion monitoring was used to look for 2-hydroxybenzoquinone. Kurien and Robins [44] reported that benzoquinone in dilute aqueous solution was converted to hydroquinone and 2-hydroxybenzoquinone *via* a benzene-1,2,4-triol intermediate product. A peak was not seen in the chromatogram for 2-hydroxybenzoquinone but a signal was obtained at $m/z = 123$ whenever benzoquinone or hydroquinone were eluted from the column. 2-Hydroxybenzoquinone was not commercially available and was too unstable to be synthesized and stored. However, 2,5-dihydroxybenzoquinone was detected by ES-MS.

Because resorcinol was detected in the electrolysis sample by the solid phase microextraction method but not when using ES-MS, resorcinol might have formed in the sample preparation step of the microextraction method. A high pH was required for the derivatization with acetic anhydride and benzoquinone is very reactive under these conditions. Another possible explanation is that the solid phase microextraction method was more sensitive to resorcinol than LC-ES-MS.

The Zorbax SBC18 HPLC column works well for the separation of phenols in conjunction with ES-MS. One method of separating phenols is ion suppression chromatography which uses a phosphate buffer (pH 4) to suppress ionization. Phosphate buffers are known to suppress the ES-MS signal, as discussed by Johnson *et al.* [37]. Methanol and water are commonly used as a solvent for ES-MS analyses; fortunately the SBC18 column retained the phenols with only this eluent. Because detection limits improve as column diameter decreases, the use of a SBC18 column (3-mm i.d.) was tested to improve the sensitivity of the analytical technique. However, resolution between hydroquinone and its isomers was lost unless trifluoroacetic acid was added to the eluent at such a high concentration that signal suppression occurred in the ES-MS. Detection limits for HPLC-ES-MS of phenols were as follows: hydroquinone and benzoquinone = 100 μg/L, phenol and 2,5-dihydroxybenzoquinone = 300 μg/L, and resorcinol and pyrocatechol = 50 μg/L. When the same chromatographic conditions were coupled to an absorbance detector, the limits of detection for all six compounds were ca. 20 μg/L.

Table 2 shows the acidic intermediate products detected during the electrochemical incineration of 100 mg/L benzoquinone. The major identified intermediate products were formic, acetic, maleic, succinic and malonic acids. Maleic acid concentrations peaked at 2 h and, by 8 h, had decreased to <500 μg/L. The presence of succinic, malonic, acetic and formic acids persisted after 32 h of electrolysis. Fumaric acid was detected in the first 4 h of electrolysis; however, concentrations were always less than 500 μg/L. The inorganic anions chloride and sulfate were present as impurities in the starting solution (1 mg/L). As a result of the anodic oxidation of chloride, chlorate was found in most electrolysis solutions at low levels (*ca.* 500 μg/L). Because the detection limit for perchlorate was ca. 5 mg/L with the anion-exchange column, it could not be detected with the conductivity detector. However, perchlorate was detectable by ES-MS in all samples after ca. 4 h at concentrations estimated to be <5 mg/L.

It was not possible to identify all of the ions detected by direct infusion of the sample into the ES-MS. Because of the numerous reactions occurring in the electrolysis solution and fragmentation and clustering occurring in the electrospray ionization process,

Table 2. Acidic Intermediates

Compound	Peak Concentration (mg/L)	Electrolysis Time (h)
p-Hydroquinone	1	1
Formic acid	5	0.5
Fumaric acid	<1	0.5
Maleic acid	9	2
Malonic acid	1	8, 16
Succinic acid	10	8
Acetic acid	8	64

the interpretation of these data was difficult and necessitated the coupling of the ES-MS with LC.

A Dionex AS11 anion-exchange column with a conductivity detector was used to quantify the anions listed in Table 2. Fifteen peaks were detected and a typical chromatogram is shown in Figure 4. Because more peaks could be detected with the anion exchange column than with the ion exclusion column using ES-MS detection, the identities of all peaks shown in Figure 4 have not been established. The anion-exchange column could not be coupled with ES-MS because the sodium hydroxide eluent was not compatible with ES-MS. Using LC-ES-MS, acetic acid, formic acid, chloride, succinic acid, malonic acid, maleic acid, fumaric acid and sulfate were identified. Fumaric acid was quantified using LC with absorbance detection.

Figure 5 compares remediation rates for four compounds generated during electrochemical incineration of benzoquinone. Whereas benzoquinone and maleic acid quickly undergo a redox reaction in the electrolysis solution, succinic and acetic acids were only slowly oxidized by electrochemical incineration. At 64 h, acetic acid was the only significant organic compound remaining in solution. Malonic acid levels were never higher than 1 mg/L and, therefore, it is apparent that malonate is oxidized more rapidly than either succinate or acetate. Formic acid levels gradually dropped throughout the course of the electrolysis from 5 mg/L in the solution electrolyzed for 0.5 h.

Gas phase products

The first attempt to analyze the gas phase above the benzoquinone solution during anodic oxidation was to measure the yield of CO_2 using Pt mesh electrodes and compare the CO_2 yields with that from the thermostatically controlled Ru-Ti-Sb-SnO_2/Ti tubular anode. The tubular electrodes were described in Feng *et al.* [32]. Concentrations of CO_2 were determined gravimetrically [45] and yields with Pt mesh electrodes were 63% without use of an antifoam and 72% with an antifoam. Carbon dioxide yields using the quaternary metal oxide tubular electrodes were 74% for electrolysis periods in the range 48 - 72 h. As stated earlier, there was evidence that some of the organic intermediate products were swept out of the solution by co-evolution of CO_2 and O_2 with the result of CO_2 yields $<100\%$.

Results from analysis of the headspace above the coiled electrode assembly using the solid phase microextraction fiber indicated the presence of acetaldehyde and benzoquinone. Therefore, Sep-Pak cartridges were used to quantify aldehydes and ketones emitted from the electrolysis solution. The Sep-Pak cartridges concentrated aldehydes and ketones from the gas stream. After a 48-h electrolysis period, <1% of the carbon in benzoquinone appeared to have been oxidized to acetaldehyde and acetone. No formaldehyde was detected in the gas stream.

Because some of the small carboxylic acids generated by electrolysis are volatile, the condenser above the electrolysis cell was rinsed to see if any acids might adhere to it. Indeed, small peaks for acetic and formic acids were obtained using absorbance detection; however, no attempt was made to quantify these acids.

Metal ions in electrolyzed solution

Conceivably, metals from the quaternary metal oxide film, the Pt substrate, or the stainless steel cathode could be dissolved into the product solution. This is an issue of concern in consideration of metal oxide films for remediating organic waste solutions. Therefore, the elemental content of a benzoquinone solution after a 64-h incineration period at a well-used electrode was determined by ICP-MS. The estimated concentrations (μg/L) are: Ti = 0.5, Cr = 0.5, Mn = 1, Ni = 3, Zn = 32, Ru = 2.4, Sn = 1, Sb = 1 and Pt = 0.6. The concentrations determined following electrolysis using a newly-prepared electrode ranged from ten to one hundred times larger than those values reported here for a well-used electrode.

Chromium, Sn and Pt are elements of major environmental concern and these were present at very low levels (0.5 - 1 μg/L). Unexpectedly, Zn was at 32 μg/L. To determine the source of Zn, the solution used for preparation of the quaternary metal oxide film was analyzed by ICP-MS. Indeed Zn was present at 260 μg/L. The source of the Zn is speculated to be the 12 M HCl used to prepared that solution (see section 2.1); however, that reagent was no longer available and confirmation of this speculation was not possible. The count rates for the Fe peaks at $m/z = 54$ and 56 were approximately the same as for the unremediated blank and, therefore, virtually no Fe dissolved from the stainless steel counter electrode during electrolysis.

Electrochemical incineration mechanism

Figure 6 presents a suggested mechanism for the oxidation of benzoquinone to maleic acid. If benzoquinone is absorbed onto the electrode surface and gives up an electron, a neighboring adsorbed OH radical then attacks the benzoquinone. If this process repeats itself at the para position, the ring could open to form maleic acid and ethene. No ethene was detected in the headspace analysis; however, Tomilov *et al.* reported that ethene is oxidized to CO_2 at Pt but oxidized to acetaldehyde, acetone and propionaldehyde on Au or Pd electrodes [46].

The mechanism in Figure 6 suggests that maleic acid is reduced to succinic acid at the cathode followed by oxidation to malonic and acetic acid at the anode. Kanakum *et al.* reported on the electroreduction of maleic and fumaric acids to succinic acid at a lead cathode [47]. In our laboratory, an electrolysis of succinic acid resulted in the appearance of malonic acid followed by acetic acid. Tomilov *et al.* reported that alcohols can be oxidized to the corresponding carboxylic acids if the reactants are not reduced at the cathode [46]. It is possible that use of divided cells to prevent access of maleic acid to the cathode might decrease the time for total electrochemical incineration.

Conclusions

Quaternary metal oxide films applied to Ti or Pt substrates exhibited high and persistent activity as anode materials for the electrochemical incineration of benzoquinone. Use of a Nafion membrane, sandwiched between the anode and cathode, eliminated the need for addition of soluble salts and, thereby, permitted product solutions to be analyzed by ES-MS. However, the low ionic strength of the solutions resulted in a substantial decrease in the working area of the electrodes with a corresponding increase in the electrolysis period needed to bring the COD effectively to a zero value.

Numerous ionic intermediate products formed during the electrochemical incineration of benzoquinone were identified and quantified. The major intermediate products identified were *p*-hydroquinone, formic acid, fumaric acid, maleic acid, malonic acid, succinic acid and acetic acid.

In future work, a cation suppressor with the anion-exchange column and ES-MS is expected to permit the confirmation of all anions in the electrolysis solution. Future work also will seek to shorten the electrolysis time by attempting to find a cathode that does not reduce maleic acid so the pathway can be avoided that results in generation of succinic and acetic acids that are degraded slowly.

A distinct advantage of using the solid-state electrolyte in large-scale applications of electrochemical incineration is the production of a final product which is essentially pure water that can be disposed into sanitary sewage systems without the need for desalting or pH adjustment.

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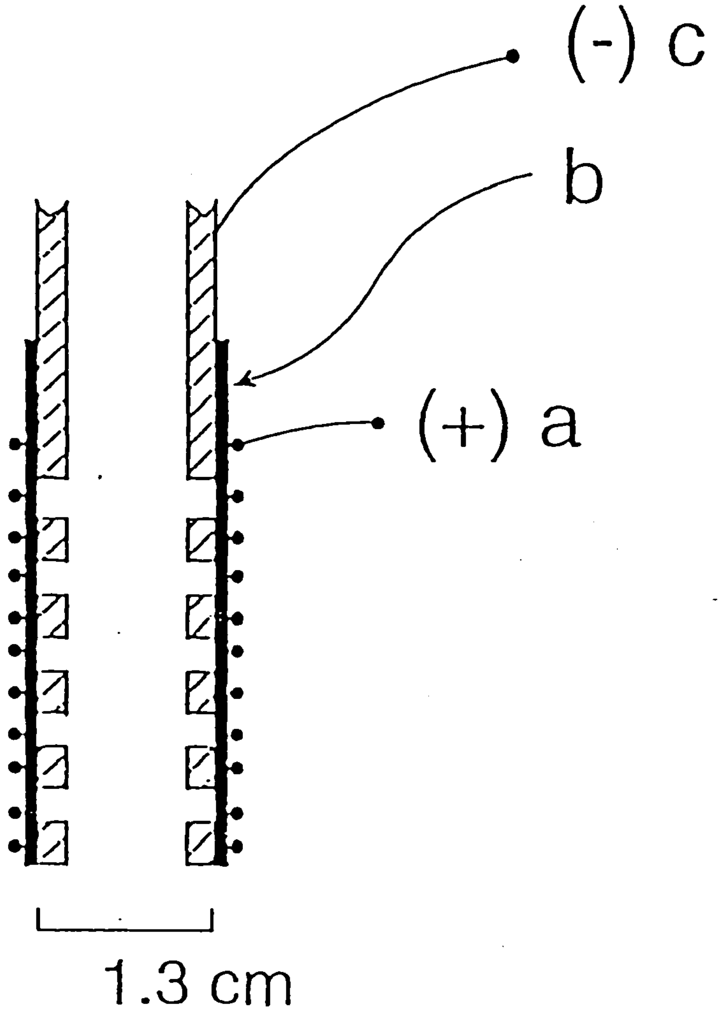


Figure 1. Two dimensional view of electrode assembly: (a) quaternary metal oxide film on coiled Pt anode (0.62 mm thick); (b) solid state electrolyte (0.2 mm thick); and (c) stainless steel cathode (1 mm wall).

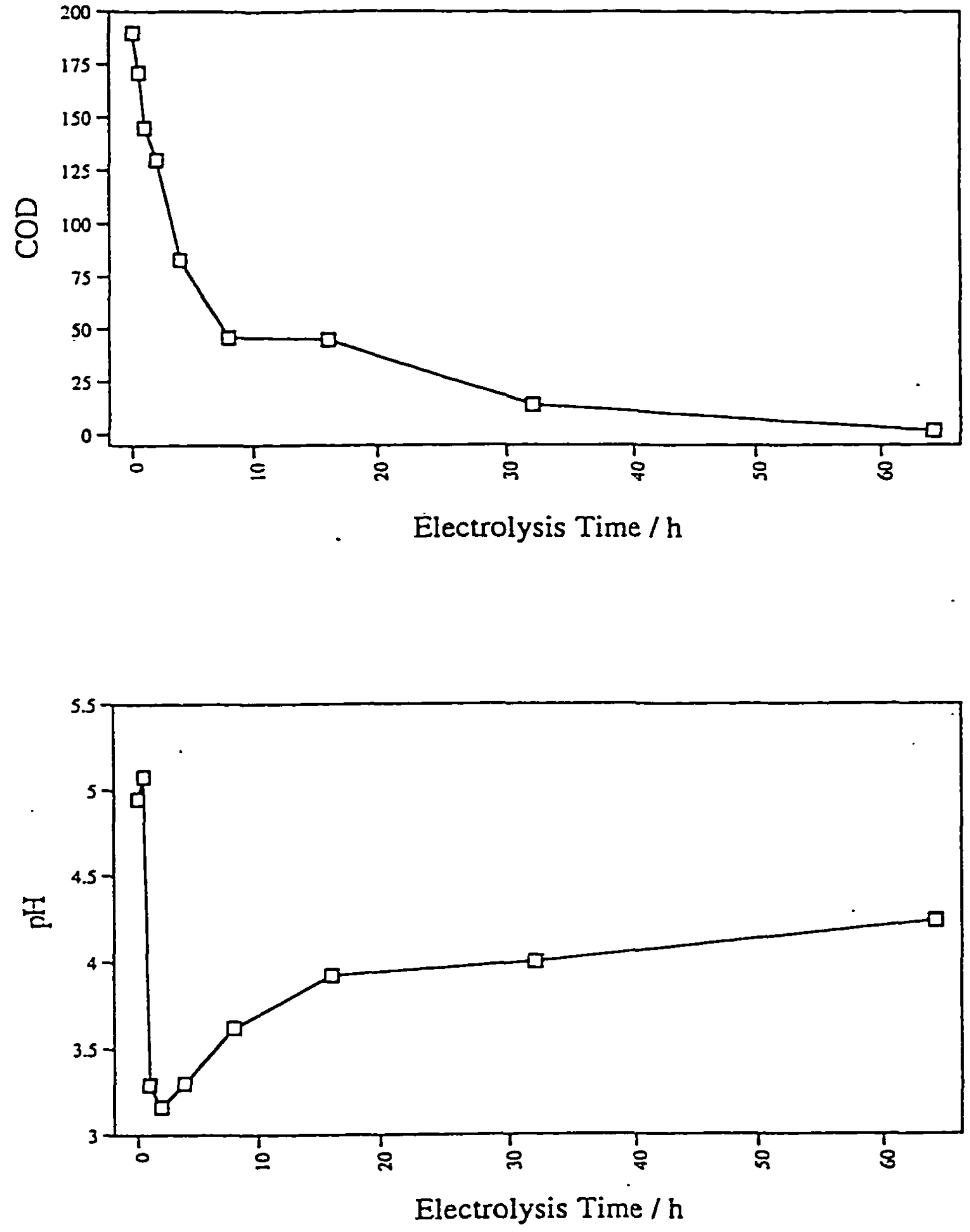


Figure 2. Plots of COD and pH *vs.* time during electrochemical incineration of 100 mg/L benzoquinone using a Pt anode covered with the quaternary metal oxide film.

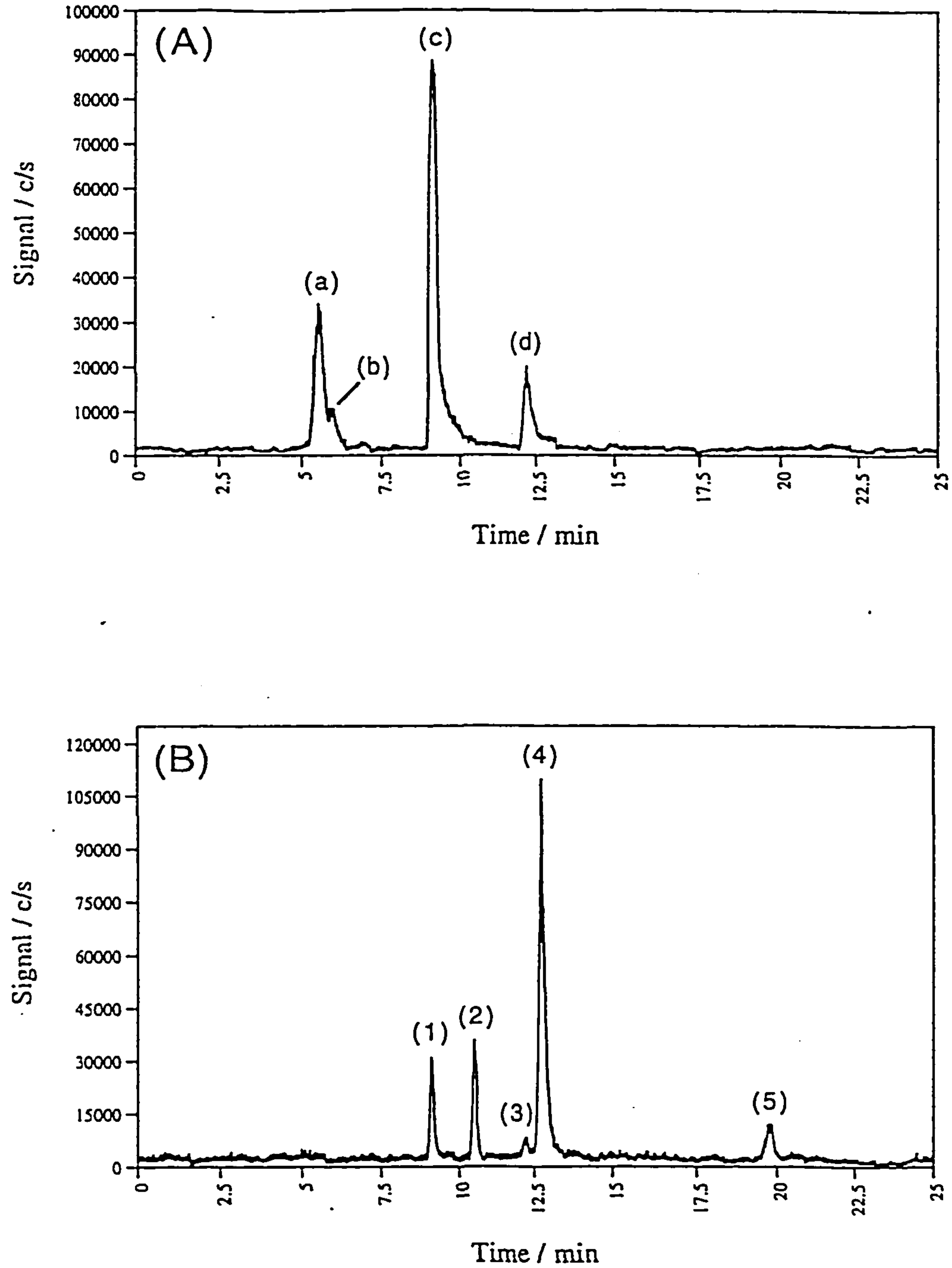


Figure 3. Total ion signal in LC-ES-MS obtained for (A) a 1-h electrolysis solution of 100 ppm benzoquinone and (B) a standard solution. Peaks in A: (a) maleic acid, (b) succinic acid, (c) hydroquinone and (d) benzoquinone. Peaks in B (1 ppm each): (1) *p*-hydroquinone, (2) resorcinol, (3) *p*-benzoquinone, (4) pyrocatechol, and (5) phenol.

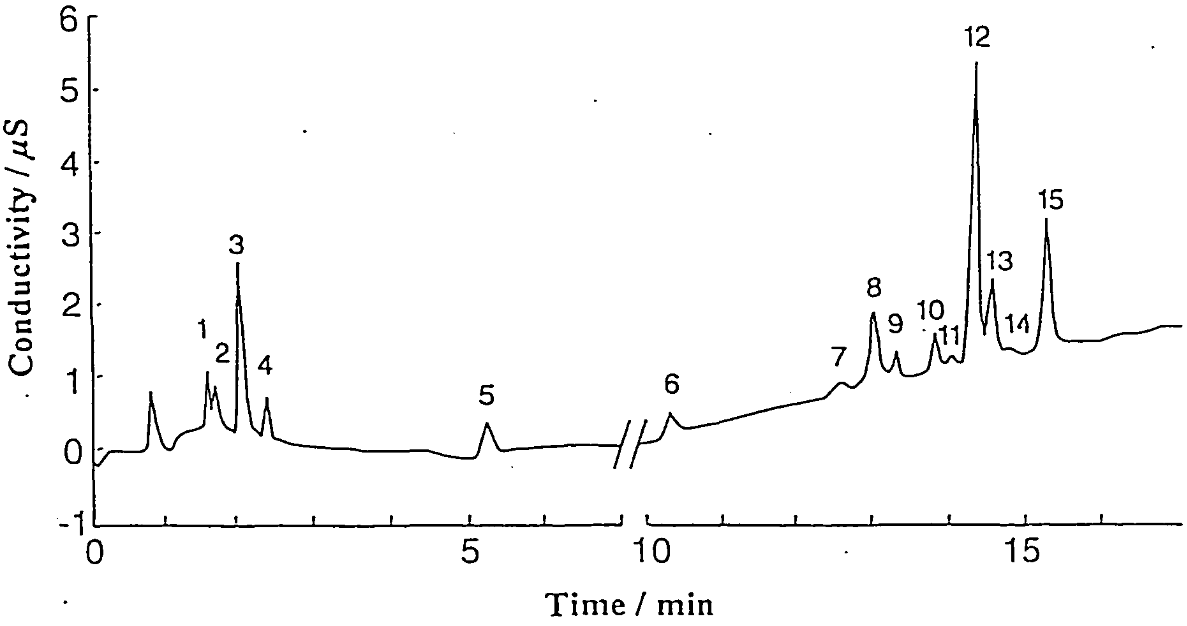


Figure 4. Chromatogram of 100 mg/L benzoquinone solution after a 2-h electrolysis period using an anion exchange column with conductivity detection. Peaks: (1) acetic acid, (2) unknown, (3) formic acid, (4) unknown, (5) chloride, (6) chlorate, (7) carbonate, (8) succinic acid, (9) unknown, (10) malonic acid, (11) unknown, (12) maleic acid, (13) unknown, (14) fumaric acid, and (15) sulfate.

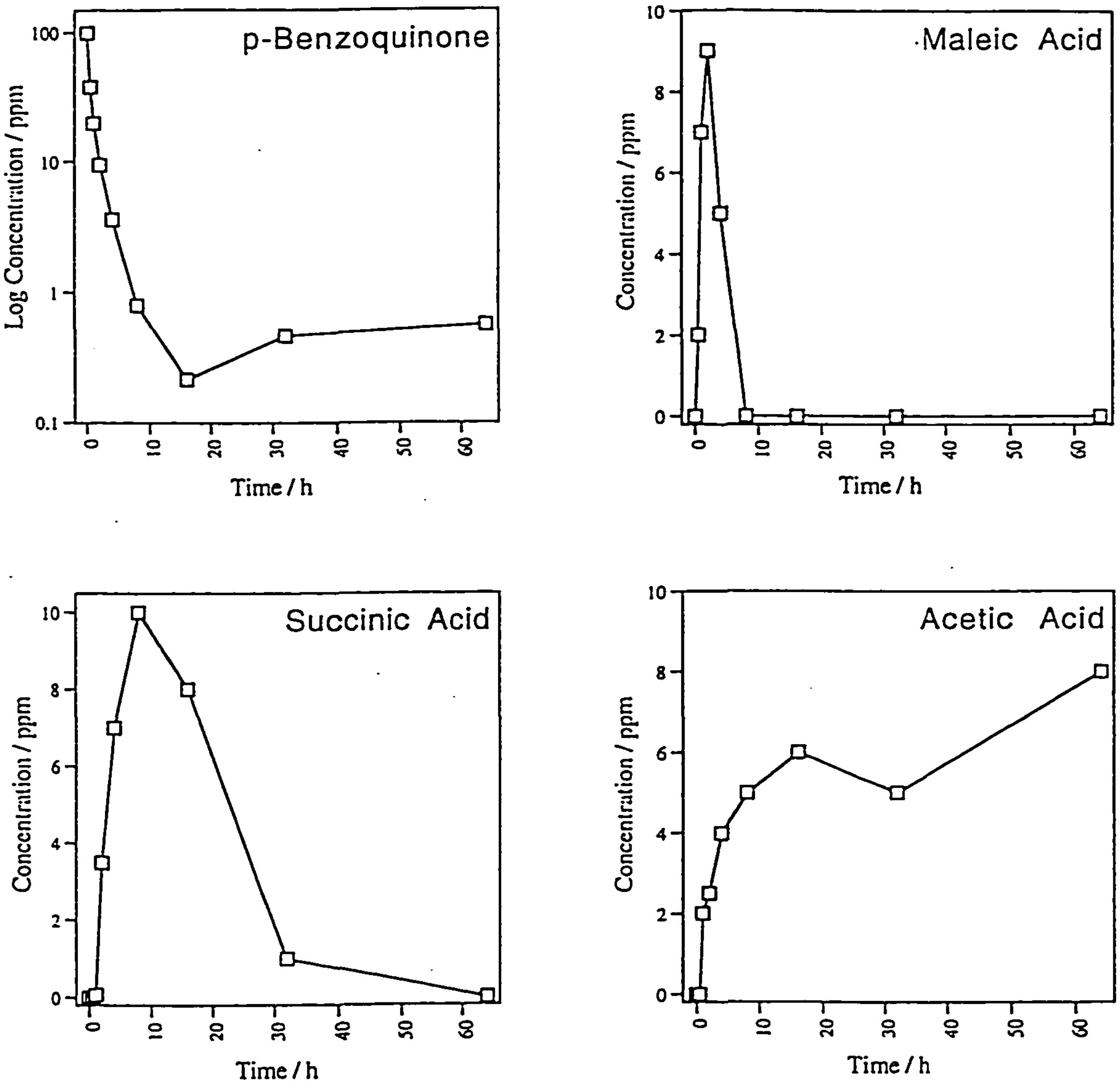


Figure 5. Change in concentration of selected reaction products *vs.* electrolysis time using a Pt anode covered with the quaternary metal oxide film, Nafion membrane, and porous stainless steel cathode.

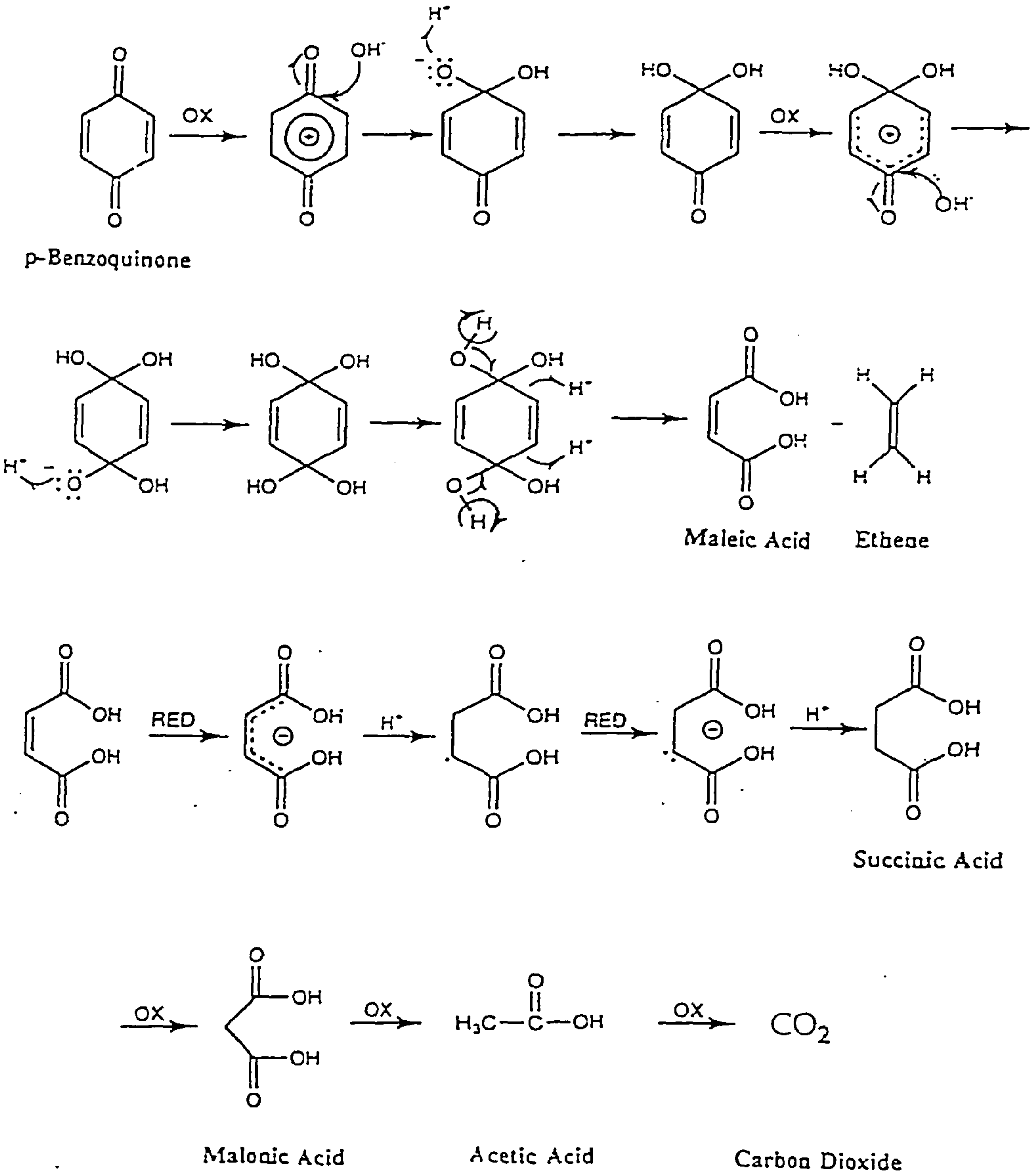


Figure 6. Suggested reaction pathway for the electrochemical incineration of benzoquinone at a Pt anode covered with the quaternary metal oxide film.

CHAPTER 4: THE DETERMINATION OF INTERMEDIATES AND PRODUCTS FORMED FROM THE ELECTROCHEMICAL INCINERATION OF 4-CHLOROPHENOL BY LIQUID CHROMATOGRAPHY - ELECTROSPRAY MASS SPECTROMETRY

A paper to be submitted to Environmental Science and Technology

Steve K. Johnson, Linda L. Houk, Jianren Feng, Dennis C. Johnson, R. S. Houk

Abstract

Electrochemical incineration (ECI) of 4-chlorophenol is achieved in an aqueous medium using a platinum electrode coated with a quaternary metal oxide film containing Ti, Ru, Sn, and Sb oxides. The electrode is stable and active when used with a solid Nafion membrane without the addition of soluble supporting electrolyte. Liquid chromatography (LC) methods, including reverse phase and ion exchange chromatography, are coupled with electrospray mass spectrometry (ES-MS) and used, along with gas chromatography-mass spectrometry (GC-MS), pH, chemical oxygen demand (COD), and total organic carbon (TOC) measurements to study the reaction and identify the unknown products and intermediates for the ECI of 4-chlorophenol. Twenty-five intermediates are identified and reported. The most abundant intermediates are benzoquinone, maleic acid, succinic acid, malonic acid, and inorganic anions such as chloride, chlorate, and perchlorate. After 24 hours of ECI a starting solution containing 100 ppm 4-chlorophenol yields only 1 ppm carbon while 98% of the original chlorine remains in solution as inorganic species. LC-ES-MS and direct infusion ES-MS detection limits are between 80 ppb and 4 ppm for the intermediates.

Introduction

Chlorophenols are present in waste-water as byproducts of the pulp and paper, dyestuff, pharmaceutical and agro-chemical industries. Phenols can readily be chlorinated during waste-water and drinking water treatment [1,2]. Because these halogenated compounds are toxic and resist biodegradation, research efforts are underway to develop techniques which mineralize the carbon and chlorine atoms in chlorophenols. 4-Chlorophenol was chosen as a model compound for the study of ECI, because it is widely present in the environment, it is a priority pollutant [3], and its degradation has been studied previously.

Recent studies published about anodic oxidation of halogenated compounds include work by Kesselman *et al.* [4] and Brillas *et al.* [5]. Kesselman reported that quantum yields for photodegradation of organics in aqueous solutions were low. Therefore, these scientists used Nb-doped TiO_2 electrodes for ECI to compare the effects of current density on degradation rates of organic substrates. Degradation rates for 4-chlorocatechol and 4-chlorophenol when determined at constant current (15 mA) were similar. However when the experiments were carried out at constant potential and additional amounts of these compounds were added to the electrolysis solutions, a larger current increase was seen with 4-chlorocatechol than with 4-chlorophenol. Because 4-chlorocatechol could adsorb more readily than 4-chlorophenol to TiO_2, the authors concluded that 15 - 65 % of the 4-chlorocatechol was degraded as a result of direct oxidation on the electrode surface (depending on the electrode). Brillas *et al.* compared the degradation of 4-chlorophenol in acidic media under anodic oxidation, electro-Fenton, photoelectro-Fenton and peroxicoagulation conditions [5]. Total organic carbon (TOC) was reduced substantially faster for the last three methods, where Fe^{2+} acted as a catalyst to produce hydroxyl radicals ($OH^{\cdot}$) from H_2O_2 generated at a cathode sparged with O_2(g). The authors stated that the degradation by $OH^{\cdot}$ was much more efficient in the medium than at the vicinity of the Pt anode. A constant current of 100 mA yielded higher current efficiencies than 300 mA. Oxalic acid was the main product. The authors speculated that Fe^{3+} complexed with this diacid and that hydroxyl radicals did not have a high capability to oxidize this complex but UV light did. 4-chlorocatechol was the initial oxidation product. Maleic and fumaric acids were detected in the product solution by ion exclusion chromatography.

Vinodgopal *et al.* described the electrochemically assisted photodegradation of 4-chlorophenol [6]. A thin film of TiO_2 was applied to an optically transparent anode. A divided cell was used to observe the effects of N_2 vs O_2 saturation of the photooxidation solutions. 4-Chlorocatechol was the major intermediate in the O_2-saturated solutions. Hydroquinone predominated in the N_2 saturated solutions but its further degradation was retarded in the absence of O_2. Three pathways by which 4-chlorophenol can be oxidized at an irradiated semiconductor particle were also presented. Haque and Rusling also studied 4-chlorophenol with photoelectrodes and reported that 4-chlorophenol was completely mineralized to CO_2 and Cl^- [7].

Stucki *et al.* reported on the performance of Sb-doped SnO_2 anodes for ECI of wastewater [8]. The oxidation of organic compounds was 5 times more efficient than observed for Pt anodes. SnO_2 produced less Cl_2 and therefore showed less potential to form hazardous halogenated hydrocarbons. The use of an undivided cell did not indicate any interference of the cathode with the oxidation and the rate of oxidation was not limited by mass transfer. The authors concluded that ECI is an interesting alternative to chemical oxidation using O_3 or H_2O_2.

Kaba *et al.* described the electrochemical incineration of human biomass which contained Cl^- with Pt and PbO_2 anodes in 12 M H_2SO_2 [9]. Cl_2 constituted less than 0.1 % of the evolved gases. The authors did not mention if they looked for halogenated products such as trihalomethanes. This group did find that the use of ultrasound and the addition of the Ce^{3+}/Ce^{4+} redox couple enhanced the rates of waste oxidation.

Comninellis and Nerini compared Ti/SnO_2 and Ti/IrO_2 anodes to electrolyze phenol in the presence of NaCl [10]. The presence of NaCl in the electrolysis solution catalyzed the anodic oxidation of phenol only at Ti/IrO_2 anodes. The catalytic effect was attributed to ClO^- near the anode and/or in the bulk solution. However the improved efficiency with the use of NaCl was offset by the formation of chloroform. Therefore, our study of the ECI of 4-chlorophenol included techniques to identify and quantify halogenated hydrocarbons produced as a result of this waste remediation process.

Research efforts in our laboratory have examined the increased catalytic activity of Fe(III)-doped PbO_2 films on titanium substrates [11,12]. More recently, work in our laboratory reported that benzoquinone could be incinerated in water with no supporting electrolyte using a quaternary metal oxide on a Pt anode and a porous stainless steel cathode [13]. Although values of TOC and chemical oxygen demand (COD) could be reduced to 1 ppm, electrolysis times were lengthy.

The coupling of ion chromatography with mass spectrometry provides a unique opportunity to identify and quantify inorganic and organic anions produced as a result of ECI. Electrospray ionization (ESI), first developed by Dole and co-workers [14,15], is an efficient method for the transfer of ions from liquid solution into the gas phase. Fenn and Yamashita demonstrated that ESI is a versatile ionization source for mass spectrometry by developing electrospray mass spectrometry (ES-MS) [16,17]. ES-MS ionization and extraction conditions can be adjusted to deliver protonated cations or deprotonated anions with a wide variety of samples and solutions to study inorganic [18,19], organic [20-22], and biological compounds [23,24].

Liquid Chromatography (LC) coupled with ES-MS offers rapid and selective methods for determining unknown compounds in complex mixtures. Applications for such a detection method are numerous, including the determination of pesticides and herbicides [25-27], dyes [28,29], environmental toxins [30-32], and monitoring waste streams [33]. Currently, GC-MS is the most common technique offering similar unknown determination ability, but the GC-MS analysis of aqueous samples requires sample extraction. The determination of carboxylic acids requires analyte derivitization before injection onto the column [34]. These sample pretreatment procedures increase the risk of sample contamination or changing the chemical composition of the analyte before analysis. The ES-MS and LC-ES-MS systems described here require no analyte derivatization or pretreatment, assuring that a representative sample is being observed.

Several different liquid chromatography systems have been coupled to ES-MS to take advantage of the different separation abilities. This study uses two LC methods to determine the components of a complex ECI mixture. Reverse phase chromatography is useful in the study of aromatic molecules that are not easily ionized, and ion exchange chromatography separates both organic and inorganic ions. Ion exchange chromatography was used extensively to observe identified compounds, because ion exchange chromatography offers good sensitivity when coupled to ES-MS and it can separate almost all of the identified intermediates.

Ion exchange chromatography has been coupled to mass spectrometers with atmospheric pressure ionization [35], thermospray ionization [36], particle beam ionization [37], and ESI [38]. Large sodium concentrations in the ES-MS can cause significant signal reduction, so a suppressor is used to continuously remove sodium ions present in the ion exchange mobile phase. In a membrane ion suppressor, sodium ions are replaced with hydrogen ions. The hydrogen ions combine with hydroxide ions also present in the mobile phase to form water, which causes no problems in the ES-MS.

Ion exclusion chromatography is another LC method used to separate carboxylic acids, such as those found in this study [39,40]. Ion exclusion chromatography has been coupled to ES-MS in this laboratory for the determination of carboxylic acids [41]. The technique offers greater separation of carboxylic acids than ion exchange chromatography, but unresolved peaks are usually not a concern with a selective detector like mass spectrometry. Ion exchange chromatography is able to separate a greater variety of analytes and offers better detection limits and resolution than ion exclusion, when used with ES-MS, because of the complex instrumental set-up for ion exclusion chromatography-ES-MS [41].

Experimental Section

Reagents and samples

All chemicals were reagent grade (Aldrich) and water was distilled and deionized (18 MΩ cm^{-1} at 25°C) with a Barnstead Nanopure-II system (Newton, MA). Regardless of the chromatography system, the mobile phases were always filtered under vacuum with a Magna-R nylon membrane (0.22 μm pore diameter, Fisher) and then degassed under vacuum. Standards were dissolved in water to form 1000 ppm standard stock solutions which were frozen until needed to prevent microbial degradation. ECI solutions were also frozen or refrigerated, for a period of less than twenty days, until needed. Sample and standard solutions were filtered with Costar syringe filters (0.22 μm pore size) directly before injection onto the chromatography column.

ECI apparatus

The ECI set-up and preparation of quaternary metal oxide films have been described [13]. In this study, the film was deposited on a 22 gauge platinum wire producing an anode with an area of 5.3 cm^2 coiled around a stainless steel counter electrode. The electrolysis was conducted with a constant current of 0.95 A, resulting in a current density of 0.18 A/cm^2. Nafion tubing with an inner diameter of 0.4 mm was obtained from Perma Pure Inc.

Electrospray mass spectrometer

An API/1 (Perkin-Elmer SCIEX, Thornhill, ON, Canada) single quadrupole mass spectrometer was used. The API/1 used a curtain gas interface and has been described previously [42,43]. The IonSpray source of the API/1 was operated with a Perkin-Elmer

SCIEX TurboIonSpray (Thornhill, ON, Canada) attachment. The TurboIonSpray forces a 5 L min^{-1} flow of nitrogen gas at 500°C across the aerosol stream exiting the IonSpray tube, increasing collisions and evaporation rates involved in the ionization process and allowing the use of larger flow rates (1-2 mL min^{-1}) into the ES-MS. The detection limits and signal to noise ratio are improved with TurboIonSpray for many compounds when compared to similar studies in this laboratory before the addition of the TurboIonSpray [41]. The attachment also provides much more flexibility in chromatographic flow rates and eluent compositions. The ES-MS conditions were optimized to provide $[M-H]^-$ every day, but remained fairly consistent to the instrumental operating conditions listed in table 1. Nearly the same ES-MS conditions were used for each chromatography technique and direct infusion. These parameters were adjusted daily to maximize ion signal and chromatographic quality and differed slightly from day to day.

As reported by Horlick, it was necessary to compensate for the variation of electrospray signal with the total ionic composition of the sample by using an internal standard ion [44]. In this study, chloroacrylate and propionate were used as internal standards to employ a ratio for quantitative measurements. Fairly linear calibration curves, with correlation coefficients of 0.980 to 0.999 were compiled for analyte concentrations in the range of 0.1 ppm to 50 ppm. Detection limits were determined as the concentration of analyte required to provide a net signal equivalent to 3 times the standard deviation of the blank.

Table I ES-MS Operating Conditions

Ionization Source	TurboIonSpray
Nebulizer Gas Pressure	40 psi, zero grade (99.998%) N_2
Nebulizer Gas Flow Rate	0.6 L min^{-1}
Sample Flow Rate	1 mL min^{-1}
Curtain Gas Pressure	80 psi, carrier grade (99.999%) N_2
Curtain Gas Flow Rate	0.7 L min^{-1}
Curtain Gas Temperature	60°C
Turbo Gas Flow Rate	5 L min^{-1}
Turbo Gas Temperature	500°C
Discharge Needle Voltage	-4500V[*]
Interface Plate Voltage	-300V[*]
Orifice Plate Voltage	-118V[*]
RF Only Quadrupole Voltage	-100V[*]
Mass Analyzer Quadrupole Voltage	-95V[*]
CEM Detector Voltage	+3800V
Operating Pressure of Quadrupole Chamber	3.5 x 10^{-5} Torr
Dwell Time	200 ms

[*] Typical values cited.

Reverse phase liquid chromatography

The experimental set-up for LC-ES-MS systems is shown in figure 1. Electrospray ionization requires the sample liquid to contain organic solvent such as methanol or propanol to facilitate droplet formation and solvent evaporation. A Zorbax (Rockland Technologies, Chadds Ford, PA) SBC18 column (25 cm length, 3.0 cm dia.) was used for reverse phase chromatography. The Zorbax column is operated with a 1:1 methanol/water mobile phase at a flow rate of 0.5 ml/min. These conditions match with the appropriate ES-MS conditions, using TurboIonSpray, so the effluent of the reverse phase column can be fed directly into the ES-MS as represented in figure 1a. The simplicity of the reverse phase chromatography - ES-MS set-up and the excellent match of mobile phase composition and flow rates gave reverse phase - ES-MS good detection limits and separation quality compared to other LC-ES-MS systems.

Ion exchange liquid chromatography

A Dionex IonPac AS11 anion exchange column (Sunnyvale, CA) was used with a water/methanol solvent. This separation also required a strong base such as 27 mM sodium hydroxide in the mobile phase. A self-regenerating (4mm dia.) suppressor (Dionex) was used as shown in figure 1b to remove sodium cations and replace them with hydrogen ions before entering the ES-MS. The ion exchange system also included an IonPac ATC-1 anion trap (Dionex) and an IonPac AG11 guard column (Dionex). LC-ES-MS used with ion exchange chromatography can accommodate an eluent gradient and the following program was used: Mobile phase A contained 100% water, mobile phase B was made up of 1 mM sodium hydroxide, mobile phase C was 100 mM sodium hydroxide, and mobile phase D contained 100% methanol. The gradient was held at 25% A, 25% B, and 50% D from t=0 to 2 min, progressed linearly to 10% A, 40% B, and 50% D at t=5, progressed linearly to 10% A, 15% B, 25% C, and 50% D at 15 min, and was held at that composition until the end of the separation.

Experimental conditions for the liquid chromatography methods are shown in table 2. The GPM-2 analytical pump was used for the mobile phase of each separation, and a Dionex model AMP-1 analytical pump was used to regenerate the suppressor when using ion exchange chromatography. A Cole-Parmer syringe pump (Nile, IL) was used for direct infusion analysis.

Table II Chromatographic Components and Operating Conditions

Chromatography Components	
Pump	Dionex model GMP-2 analytical pump
Injection Valve	Rheodyne 7010 high pressure injector (Cotati, CA)
Injection Volume	50 μL
Reverse Phase Chromatography	
Column	Zorbax SBC18 (25 cm length, 3.0 mm dia.)
Mobile Phase	50% Water, 50% Methanol
Liquid Flow Rate	1 mL min^{-1}
Ion Exchange Chromatography	
Column	Dionex IonPac AS11 (25 cm length, 4 mm dia.)
Mobile Phase	2.5 to 26.5 mM NaOH in 50% methanol
Liquid Flow Rate	1mL min^{-1}
Suppressor Column	Dionex ASRS-11 (4mm dia)
Regenerent	25 mM sulfuric acid
Regenerent Flow Rate	5 mL min^{-1}

Inductively coupled - mass spectrometry

Inductively coupled plasma-mass spectrometry (ICP-MS) measurements were taken using the apparatus and operating conditions described by Hu *et al.* [45]. A semiquantitative analysis was used for calibration. Spectral scans were measured in separate m/z windows typically 50 daltons wide. Analyte signals were adjusted for blank signal, isotopic abundance and approximate degree of ionization [46] and then compared to those for known concentrations of Co, La, Tl, whichever was in the particular m/z window of interest. Scans of the full m/z range showed no matrix ions, so matrix effects were negligible.

GC-MS techniques

Gas escaping from the ECI solution during electrolysis was collected by a Tekmar purge trap K (Cincinnati, OH). These sorbent column traps were then analyzed by the EPA approved University of Iowa hygienic laboratory using a semiquantitative method based on EPA method 8260. In this analysis, the sorbent column trap was heated and back-flushed with an inert gas to desorb purgeables onto a GC column. The GC was temperature programmed to separate the purgeables which were then detected with a Hewlett Packard model 5970B quadrupole mass spectrometer.

A liquid sample was also analyzed using EPA method 8260 at the University of Iowa hygienic laboratory. For water analysis, an inert gas was bubbled through the sample to transfer purgeables to the vapor phase. This vapor was swept through a sorbent column where the purgeables were trapped. The trapped purgeables were then transferred to a GC column as described above and detected with a Hewlett-Packard model 5972A quadrupole mass spectrometer.

Other techniques

Chemical oxygen demand (COD) was determined by a Hach DR200 analyzer (Loveland, CO), and total oxygen concentrations (TOC) were determined using a DC190 TOC analyzer (Dohrmann, Santa Clara, CA) at the University of Iowa's Hygienic Laboratory. pH measurements were made with an Orion Research Inc. model 601A pH meter (Cambridge, MA).

Results and Discussion

Electrode performance

Figure 2 shows values of COD for an ECI solution as a function of electrolysis time, starting with a 100 ppm solution of 4-chlorophenol. The COD concentration drops from 140 ppm to 10 ppm in the first 12 hours of electrolysis. After 24 hours of electrolysis, only 1 ppm COD remains. These data show that the ECI process readily destroys organic compounds in the solution. TOC data provide similar results as the carbon concentration drops from 59 ppm to 1.1 ppm carbon in 24 hours. The liquid product of 24 hour ECI passed EPA standards after being analyzed using method 8260 for waste water. This product contained no aromatic or halogenated compounds above the detection levels of method 8260.

Solution analysis

The pH of the electrolysis solution was monitored during ECI, and the results are shown in figure 3. The pH drops from 6 to 2 after 4 hours of electrolysis and then at 8 hours of electrolysis the pH rises to approximately 3 where it stays for the remainder of the incineration process. The drop in pH is believed to be caused by the production of carboxylic acid intermediates in the first 4 hours of electrolysis. Previous studies have also demonstrated the production of carboxylic acids during the electrolysis of benzoquinone and phenol solutions [13,47-49]. Carboxylic acids were also produced and found to reach peak concentration between 2-4 hours for ECI of 4-chlorophenol in this study.

LC-ES-MS was used to determine the unknown compounds remaining in the ECI solution. A sample chromatogram using ion exchange ES-MS for separating the ECI solution collected after 2 hours of electrolysis is shown in figure 4. The peaks reported correspond to the following compounds: (a) formate, (b) chloride, (c) chlorate, (d) 2-ketoglutarate, (e) succinate, (f) malonate, (g) fumarate, (h) maleate, (i) oxalate, (j) perchlorate. It should be noted that peaks d and e were not resolved to provided shorter analysis times. Fortunately, the selective detection ability of the mass spectrometer allows for determination of the coeluting components. The unknown components of the ECI solutions were determined using chromatograms like figure 4 and scans from direct infusion. Retention time, molecular weight, and some fragmentation data of unknown compounds were compared to those of known standards.

Intermediate concentrations as a function of ECI time

Once peaks are identified, they are observed to determine the concentration of intermediates as a function of ECI time. Figure 5 shows the concentration of 6 different compounds at different ECI times. The concentration of 4-chlorophenol is shown in figure 5a. The 4-chlorophenol concentration experiences a steep drop from 108 ppm to 1 ppm after 4 hours of ECI. Figure 5b shows the chloride ion concentration increases to a peak of 13 ppm at 2 hours of ECI. The chloride ion concentration then drops gradually to 3 ppm after 24 hours of ECI. The 4-chlorophenol and chloride graphs suggest that the chlorine atom is cleaved from 4-chlorophenol early in the ECI process, and chloride ions continue to be oxidized forming chlorate and perchlorate ions.

Benzoquinone, maleic acid, malonic acid, and succinic acid concentrations are also shown in Fig. 5. The benzoquinone concentration rises to 20 ppm after 2 hours of incineration and then drops to less than 1 ppm after 4 hours of ECI. Maleic acid peaks at a concentration of 9 ppm after 2 hours of ECI, malonic acid peaks at a concentration of 13 ppm after 4 hours, and succinic acid peaks at a concentration of 10 ppm after 8 hours of ECI.

These data suggest that benzoquinone is produced soon after the chlorine is removed from 4-chlorophenol. Then benzoquinone is oxidized rapidly to form maleic acid, and maleic acid is oxidized to form malonic acid or reduced to form succinic acid. It appears that the oxidation and reduction of these carboxylic acids is not as fast as the 4-chlorophenol and benzoquinone reactions. Peak concentrations and peak ECI times, along with retention times, detection limits and the percent of total carbon and chlorine present in each intermediate at its peak concentration are shown in table 3. These findings will be discussed more thoroughly in conjunction with the proposed mechanisms in a later section.

GC-MS results

GC-MS were used to analyze gas that escapes from the reaction vessel. Sorbent column traps were used to collect gaseous products for 8 hour intervals. A GC-MS chromatogram for the gas trapped during the first 8 hours of electrolysis is shown in figure 6. Labeled compounds, bromochloromethane, 1,2-dichlorethane, and toluene-8d are internal standards added to the sample for analysis. Compounds present from the ECI sample are labeled as follows: (a) cyclopentene, (b) hexane, (c) chloroform, (d)

Table III Intermediates In 4-chlorophenol ECI Solution

Compound	m/z	t_r (min)	Peak Conc. (ppm)	Peak Time (hr)	Detection Limit (ppm)	% Total C (Cl) at Peak
4-chlorophenol ClC_6H_4OH	127	25	108	0	0.1	100
chloride Cl^-	35	19	13	2	0.05	(34.4)
chlorate ClO_3^-	83	22	30	16	0.1	(33.5)
perchlorate ClO_4^-	99	37	28	24	0.4	(26.2)
phenol C_6H_5OH	93	17	1	2	0.1	1.0
hydroquinone $C_6H_4(OH)_2$	109	2*	3	2	0.04*	2.5
benzoquinone $C_6H_4(=O)_2$	108	3*	20	2	0.05*	17.1
2-ketoglutaric acid $HO_2CCH_2CH_2COCO_2H$	145	26	4	2	0.6	2.1
malic acid $HO_2CCH_2CH(OH)CO_2H$	133	25	8	2	0.2	3.7
maleic acid $HO_2CCH=CHCO_2H$	115	27	9	2	0.08	4.8
fumaric acid $HO_2CCH=CHCO_2H$	115	26	4	2	0.1	2.1
malonic acid $HO_2CCH_2CO_2H$	103	26	13	4	0.3	5.8
oxalic acid HO_2CCO_2H	89	29	6	4	1	2.1
formic acid HCO_2H	45	11	5	4	2	1.7
succinic acid $HO_2CH_2CH_2CO_2H$	117	24	10	8	0.8	5.2
acetic acid CH_3CO_2H	59	10	2	24	0.4	3.4
4-chlorocatechol $C_6H_3(OH)_2Cl$	143	7*	?	2	0.05*	?
chloromaleic acid** $HO_2CC(Cl)=CHCO_2H$	149	32	?	4	?	?
chloroacetic acid $ClCH_2CO_2H$	93	19	1	2	2	0.3 (1.0)
dichloroacetic acid $Cl_2CH_2CO_2H$	127	23	2	4	4	0.4 (2.9)

* Separated by reverse phase chromatography-ES-MS

** Suspected intermediate

benzene, (e) cyclohexane, (f) 3-chloro-1,3-pentadiene, (g) toluene, (h) tetrachloroethane. In the first 8 hours of ECI, GC-MS reveals the formation of cyclohexane (3700 ng), chloroform (140 ng), benzene (280 ng), tetrachloroethene (12 ng), and toluene (18 ng). An unidentified peak at m/z=102 appears in the first 8 hours of ECI, and fragmentation data suggest that this peak is due to 3-chloro-1,3-pentadiene. The second 8 hours of ECI produce chloroform (45 ng), tetrachloroethene (6 ng), toluene (8 ng), and trace amounts of cyclohexane, benzene and the 3-chloro-1,3-pentadiene. The ECI process continues to produce trace amounts of these 6 compounds after 16 hours of electrolysis. The concentration of these compounds is shown in table 4.

Table IV GC-MS Data (ng/sample)

Analyte	0-8 hours	8-16 hours	16-24 hours
cyclohexane	3700	<50	<50
benzene	280	<50	<50
chloroform	140	<50	<50
toluene	18	8	3
tetrachloroethene	12	6	5
3-chloro-1,3-pentadiene[*]	?	?	?

[*] Suspected product

The production of benzene and chlorine-containing organic molecules is potentially hazardous, and the chance of these products avoiding further oxidation by escaping the incineration solution through the gas phase is cause for concern. It should be noted that volatile compounds were deliberately allowed to escape for identification purposes, and no attempt was made to keep volatile compounds in the ECI solution. It is believed that a simple water-cooled condenser would return volatile compounds to the ECI solution where they would undergo continued oxidation and be converted to less harmful products. An activated charcoal trap could also be used to prevent volatile compounds from escaping to the atmosphere. Production of large amounts of chloroform has been a concern common in similar 4-chlorophenol remediation studies. The ECI process used in this study produces only low amounts of chloroform, and little chloroform (< 1 ppm) remains in the 24 hour ECI solution.

In studies in this laboratory, using an electrode of similar construction with an area of 3.6 cm^2 and operating with a current density of 0.26 amp/cm^2 and a condenser, 98% of the original chloride remains in the liquid solution, as determined by ion exclusion chromatography with a Dionex ED40 electrochemical detector equipped with a conductivity cell. In another similar study with the Iowa State University Instrument Services, ozone was observed using capillary tubing to transfer vapor produced during ECI directly to a mass spectrometer.

Total carbon and chlorine concentrations

The carbon and chlorine concentration of the solution can be calculated using the data collected through LC-ES-MS by calculating the percent of carbon and chlorine in each of the compounds detected. Figure 7 shows the total carbon and chlorine concentrations remaining in solution vs time, as calculated from the LC-ES-MS data. The total carbon concentration drops from 65 ppm to less than 20 ppm in 4 hours. Thereafter, the total carbon concentration drops more slowly from to 10 ppm in 24 hours. These data roughly agree with the TOC results where the total carbon concentration drops from 59 to 1 ppm.

Meanwhile, the total chlorine concentration drops from 33 ppm to 23 ppm after 2 hours of ECI. After 2 hours of electrolysis, the chlorine concentration in solution stabilizes near 23 ppm even though most of the chorine is being oxidized from chloride ion to chlorate and perchlorate ions. In figure 7 it appears that there is a sudden drop in carbon and chlorine concentration after 30 minutes of ECI. There is no similar depression of concentration in the COD data. This suggests that there is an early intermediate formed before one hour of ECI that was not quantified by LC-ES-MS in this study and, therefore left out of the calculations for figure 7. Using LC-ES-MS data, 75% of the original chlorine remains in solution after 24 hours of ECI. Chlorine loss can be attributed to the escape of gaseous compounds, such as 3-chloro-1,3-pentadiene, unquantified intermediates, such as chloromaleic acid, or unidentified intermediates.

Electrochemical incineration mechanism

Figure 8 presents possible pathways for the ECI of 4-chlorophenol to form CO_2. It is shown in figure 5 that the chlorine atom is removed from 4-chlorophenol early in the incineration process and yields hydroquinone and benzoquinone upon further oxidation. The results presented in table 3 and figure 5 agree with this pathway. Benzoquinone is oxidized to produce maleic or fumaric acids, which can be oxidized to produce malonic acid, followed by acetic acid, and finally carbon dioxide. This proposed pathway agrees with similar pathways reported by Kuo and Huang using ozonation techniques [1]. Maleic acid can also be reduced at the cathode to form succinic acid, as reported by Kanakum *et al.* [50]. It has been shown in our laboratory that electrolysis of succinic acid results in the production of malonic and acetic acids, which again leads to CO_2 [13].

Another possible pathway presented in figure 8 involves the oxidation of 4-chlorophenol prior to the removal of the chlorine atom, resulting in the formation of 4-chlorocatechol, which yields oxalic acid or chloromaleic acid. A similar mechanism for the oxidation of 4-chlorocatechol to form chloromaleic acid was suggested previously [1]. Chloromaleic acid can be oxidized to produce chloroacetic acid which can react with another chlorine atom to form dichloroacetic acid. Both of these products will oxidize further to eventually produce acetic acid and carbon dioxide.

Studies in this laboratory have suggested that 1,2-benzoquinone is produced very quickly during the ECI of 4-chlorocatechol. 1,2-Benzoquinone is unstable and no standards were available for intensive studies involving its behavior during ECI. Observations of the ECI of 4-chlorocatechol suggest that 1,2-benzoquinone leads to the same carboxylic acid intermediates as 1,4-benzoquinone, but the mechanism of such a reaction is uncertain, as signified by the dashed lines and question mark in figure 8.

According to data in table 5, phenol is also produced within the first 2 hours of ECI. The pathway for phenol production is uncertain, however, reactions at the cathode are suspected to result in phenol. It has been shown previously that phenol is oxidized to form hydroquinone and benzoquinone [51], which would suggest that phenol intermediates would follow the benzoquinone pathway upon further oxidation.

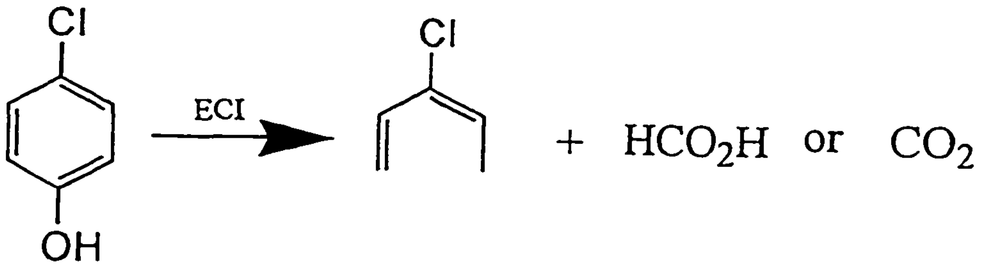
From the concentration of intermediates in table 3, it is apparent that both the benzoquinone and 4-chlorocatechol pathways exist, but the pathway through benzoquinone and maleic acid seems to be the most likely, as evident from the high peak concentration of benzoquinone. Figure 8 also suggests that the chloride ion is oxidized to produce chlorate and perchlorate ions.

Identification of intermediates by mass spectrometry

Most compounds were identified by comparing their mass spectra and retention times with those for pure standards. Two such compounds were identified despite the lack of such standards. Figure 9 shows LC-ES-MS selected ion chromatograms that indicate the presence of an anion at m/z = 149 and 151 that has the 3:1 signal ratio characteristic of one Cl atom. This ion fragments under energetic extraction conditions (voltage difference between orifice plate and RF-only quadupoles = 30 volts, compared to the normal 18 volts) to lose CO_2 and still retain the Cl atom, which indicates that the parent ion contains two carboxylate groups [41]. It is therefore assigned to chloromaleic acid ($HO_2CCH=CClCO_2H$), which is a suspected intermediate. The use of ES-MS to identify the chloromaleate anion illustrates the value of this technique for charged organic ions in solution.

The GC-MS measurement also indicated the presence of a compound at $t_R = 10.3$ min whose electron impact mass spectrum is shown in Figure 10. The peaks at m/z = 102 and 104 in a 3:1 signal ratio again indicate that the parent ion contains one Cl atom. The fragments at m/z = 74 and 76 indicate loss of C_2H_4 due to the ethene group ($-CH=CH_2$) in the parent molecule. Likewise, the peaks at m/z = 73 and 75 represent loss of C_2H_5 from the $=CHCH_3$ group. The Cl atom is retained on each of these pairs of ions. Peaks from CCl^+ also appear at m/z = 47 and 49.

Hence, this molecule is 3-chloro-1,3-pentadiene, $CH_3CH=CClCH=CH_2$. Surprisingly, this mass spectrum does not appear in either the NIST or Wiley compilations. This compound likely forms when the when the aromatic ring of 4-chlorophenol is broken at the phenolic carbon during ECI:



Both the chloromaleic acid and 3-chloro-1,3-pentadiene were found only during the first 8 hours of the electrolysis. Because there were no standards, the compounds could not be quantified, but they likely represent much of the "missing" carbon and chloride represented by the dips in total C level at 0.5 and 1 hour in Figure 7.

Elemental analysis for electrode materials

When metal oxide films are considered for the remediation of organic waste, the possibility of metals dissolving into the product solution from the film, the platinum substrate, or the stainless steel cathode, becomes a concern. Therefore, the elemental content of the ECI solution was determined by ICP-MS after incineration. The estimated concentrations (ppb) of the 8 elements observed are: Ti=5.3, Zn=5.4, Ru=22, Sn=10, Sb=25, Ba=0.2, Pt=8.0, Pb=0.6. The concentrations determined after electrolysis using a newly-prepared electrode ranged from ten to one hundred times larger than those reported here for a well-used electrode, so there is a need to "break in" an electrode before operation, to avoid much metal dissolution.

Conclusions

The ECI process described exhibited the ability to oxidatively degrade 4-chlorophenol. The majority of intermediates produced during the electrolysis were identified and studied using a variety of techniques. LC-ES-MS, particularly using ion exchange chromatography and a suppressor, was extremely important for the determination of unknowns in the solution due to its separating ability for a wide range of analytes. Pathways were proposed involving the intermediates determined.

The ECI process quickly removed chlorine from the 4-chlorophenol compound and formed carboxylic acids from benzoquinone, but the process of oxidizing the various carboxylic acid intermediates required longer ECI times. Future electrodes should be designed to consider rapid oxidation of both the starting material, 4-chlorophenol, and the intermediate carboxylic acids. Simple apparatus improvements, such as a condenser or a charcoal trap, can be used in future studies to reduce the amount of vapors escaping the ECI vessel. The reduction of escaping vapor should eliminate the production of chlorocarbons.

The ES-MS apparatus used for this work has a ESI tube of fixed size, and this tube requires the use of a narrow diameter (100 μm i.d., 170 μm o.d.) capillary for solution transport. The narrow capillary results in high back-pressure, and this high back pressure can damage the suppressor. The use of a wider ESI tube would allow for larger diameter capillary and provide lower back-pressure. Lower back-pressure will likely increase the lifetime and efficiency of the suppressor, create lower noise, and improve detection limits.

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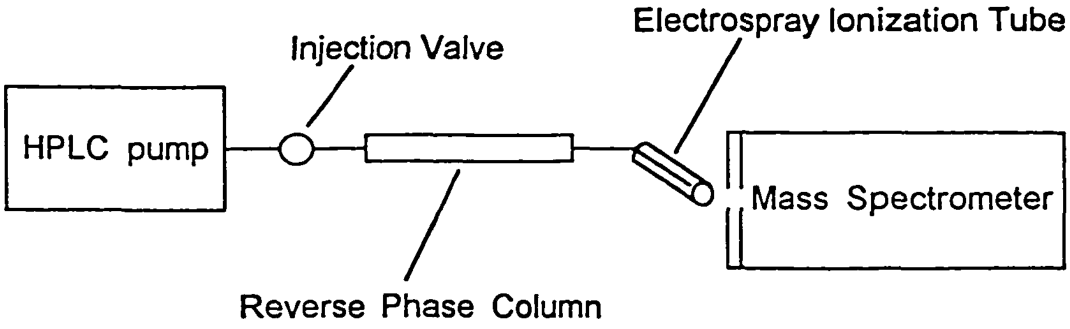
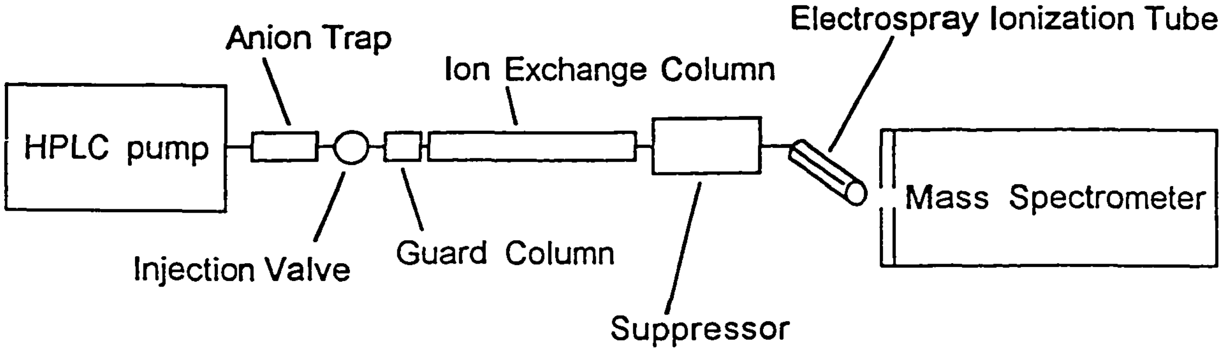


(a) Reverse phase chromatography with ES-MS



(b) Ion exchange chromatography with ES-MS

Figure 1. Experimental set-up for LC-ES-MS systems, using reverse phase (a) and ion exchange (b) chromatography.

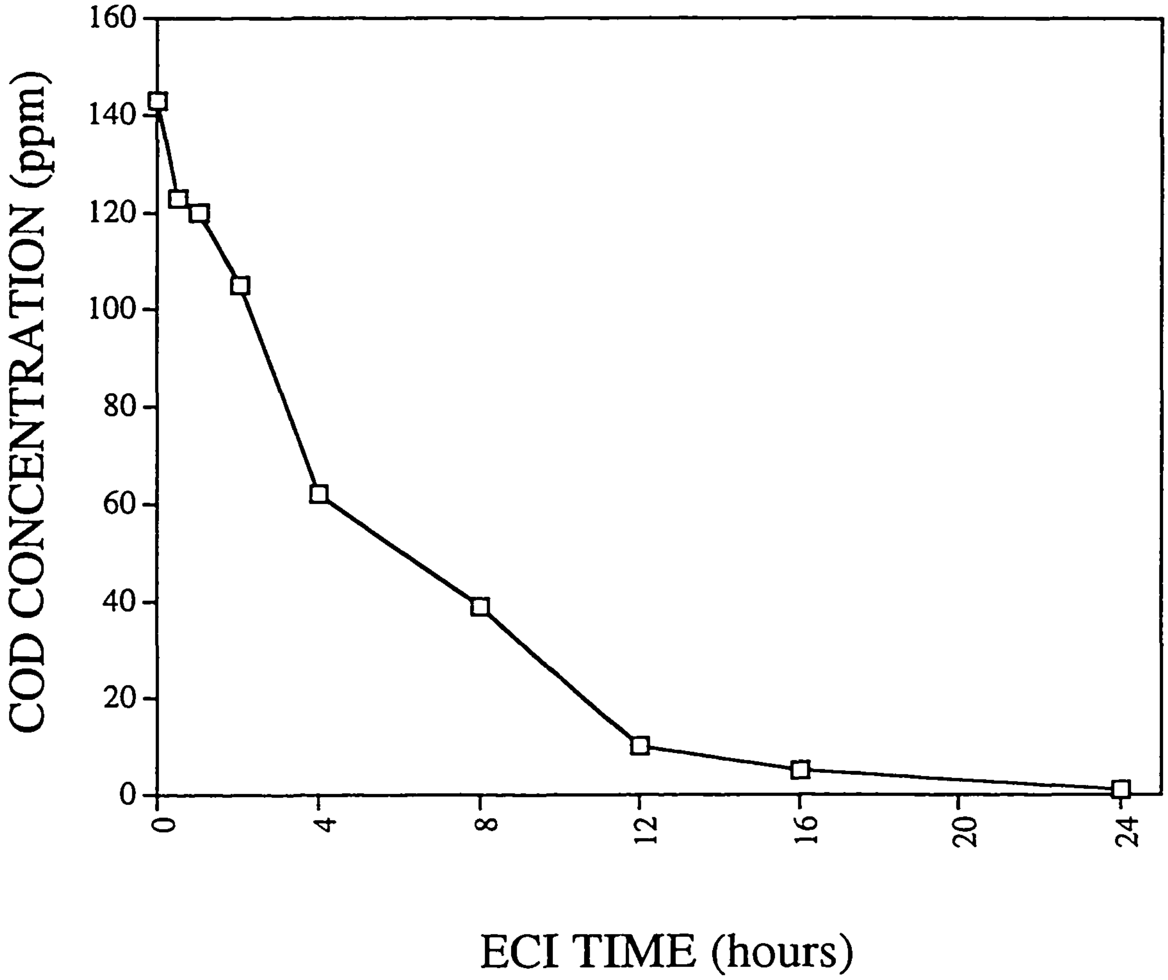


Figure 2. COD of 100 ppm 4-chlorophenol solution vs ECI time. The final COD level at 24 hours is 1 ppm.

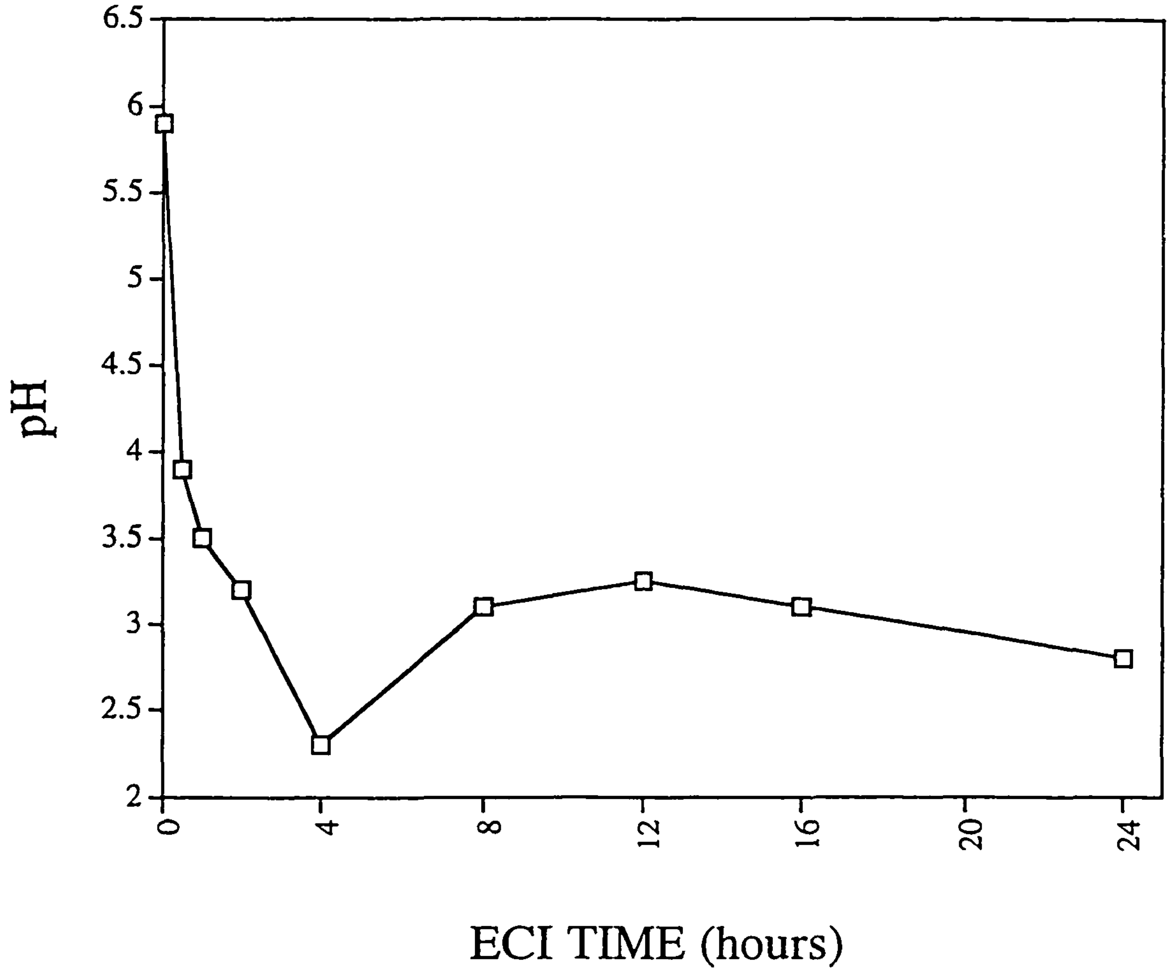


Figure 3. pH of 100 ppm 4-chlorophenol solution vs ECI time.

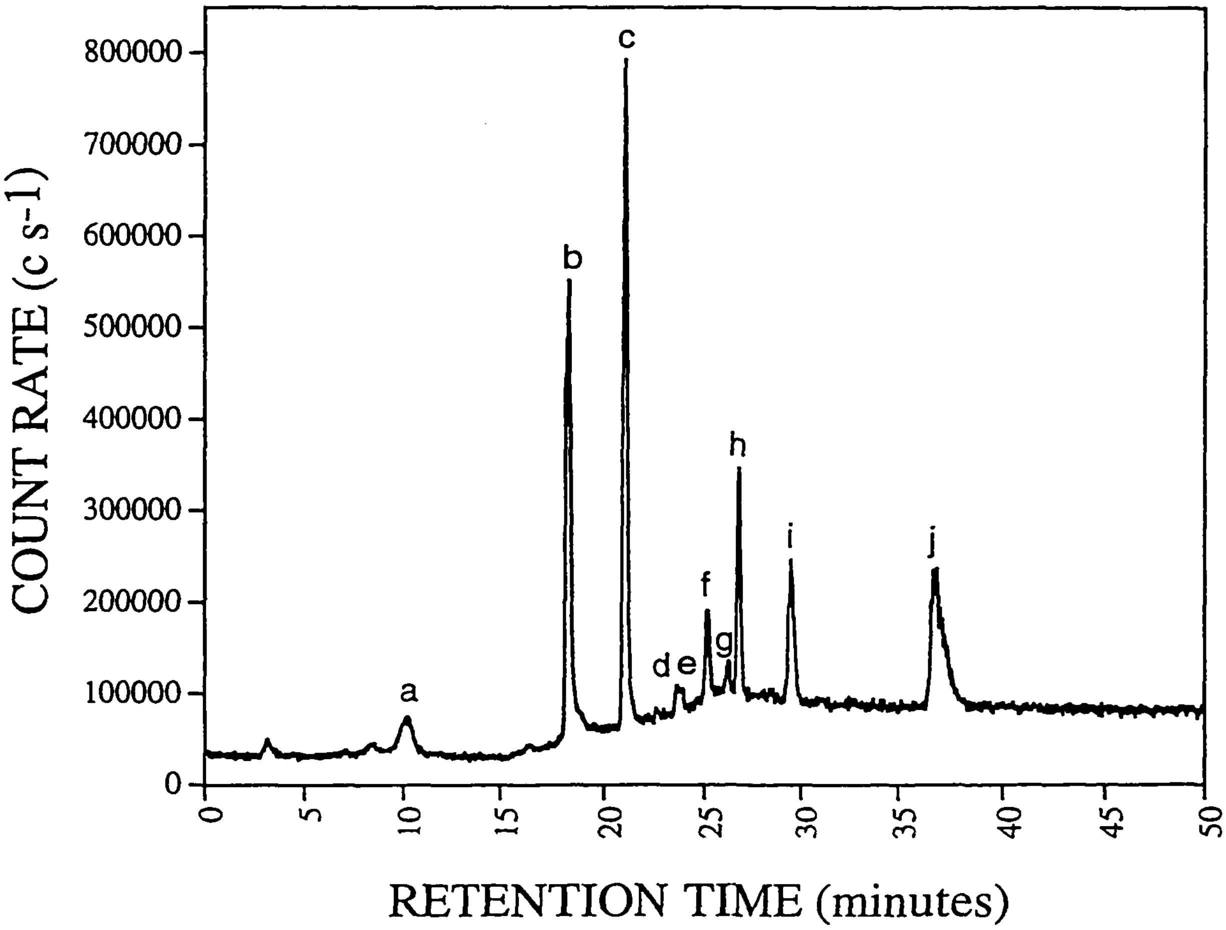


Figure 4. LC-ES-MS chromatogram for a 100 ppm 4-chlorophenol solution after 2 hours of ECI. Peak assignments are as follows: (a) formate, (b) chloride, (c) chlorate, (d) 2-ketoglutarate, (e) succinate, (f) malonate, (g) fumarate, (h) maleate, (i) oxalate, (j) perchlorate.

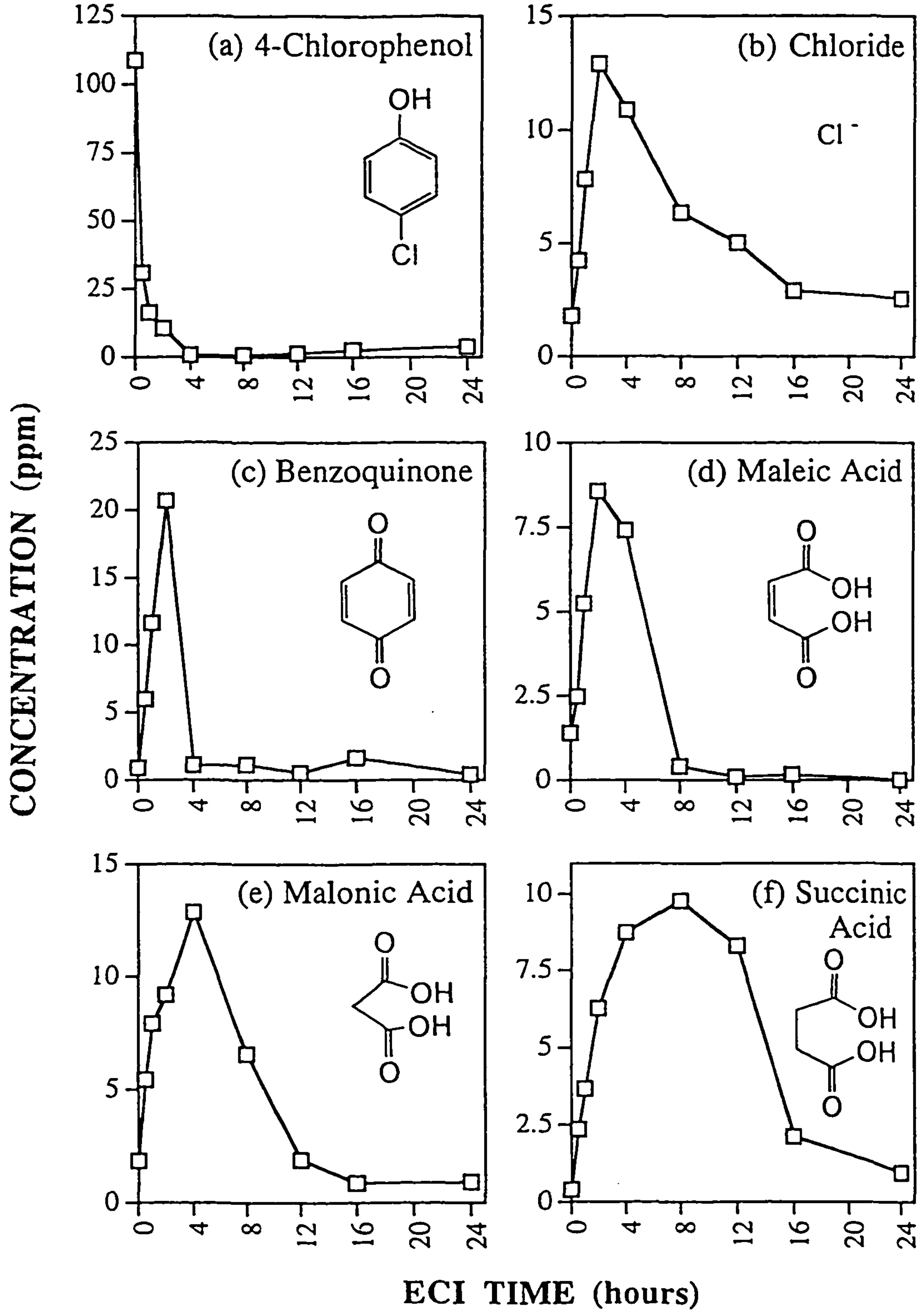


Figure 5. Concentration of selected compounds vs ECI time. (a) 4-chlorophenol, (b) chloride, (c) benzoquinone, (d) maleic acid, (e) malonic acid, (f) succinic acid.

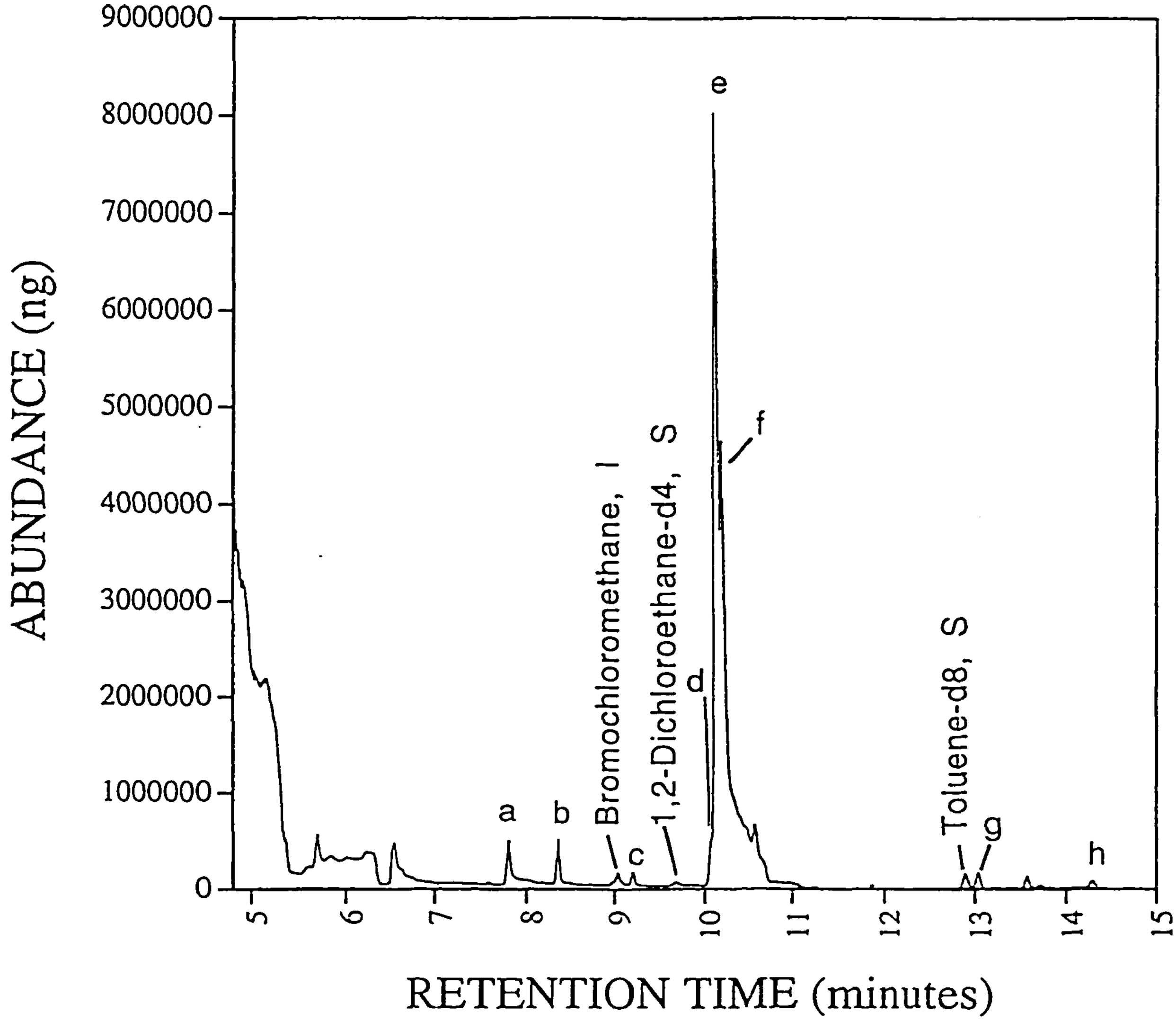


Figure 6. GC-MS chromatogram from a purge trap of gaseous products collected in the first 8 hours during the ECI of a 100 ppm 4-chlorophenol solution. (a) cyclopentene, (b) hexane, (c) chloroform, (d) benzene, (e) cyclohexane, (f) 3-chloro-1,3-pentadiene*, (g) toluene, (h) tetrachloroethene.
*Suspected intermediate

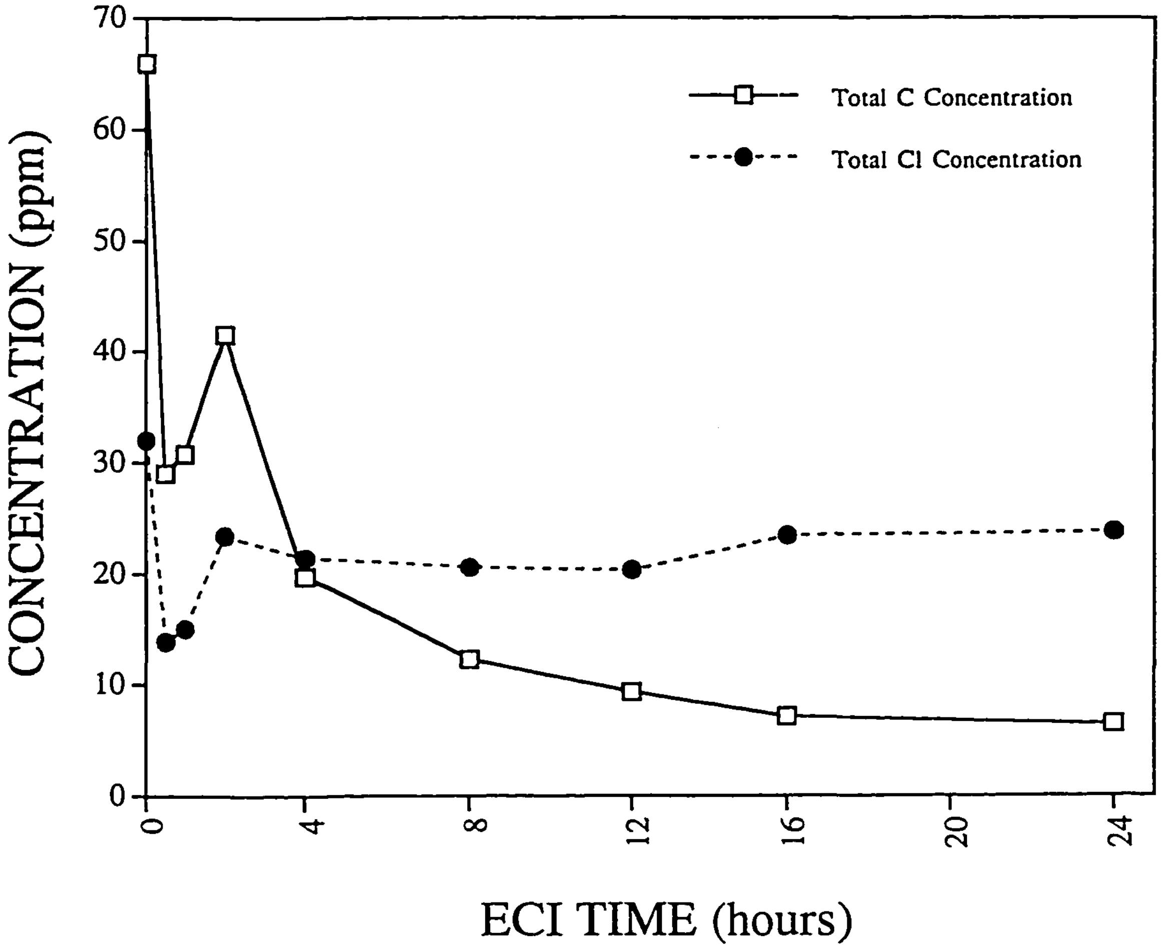


Figure 7. Total carbon and total chlorine concentrations of 100 ppm 4-chlorophenol solution vs ECI time.

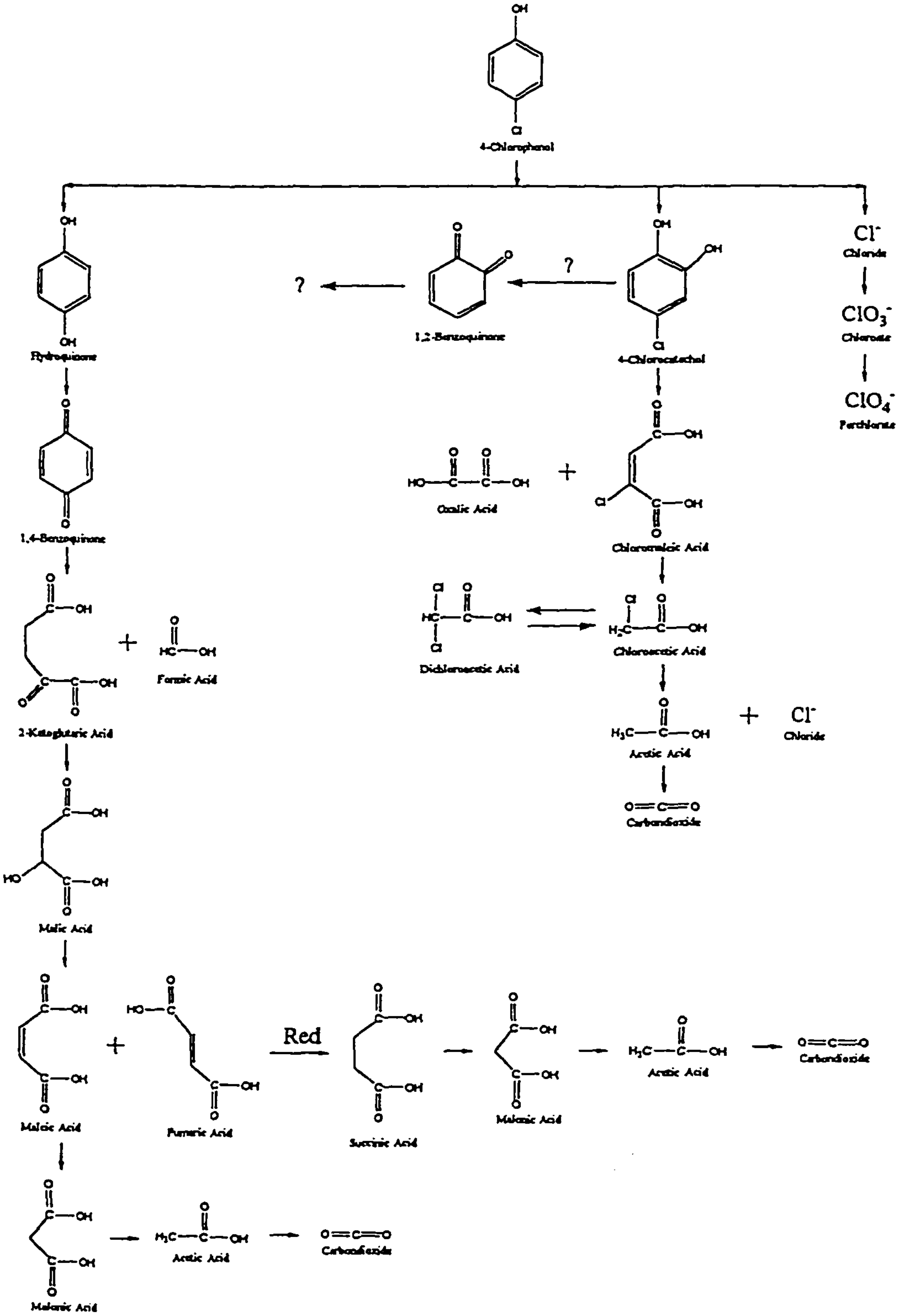


Figure 8. Proposed pathways for the ECI of 4-chlorophenol. Dashed lines indicate higher uncertainty of reactions and products

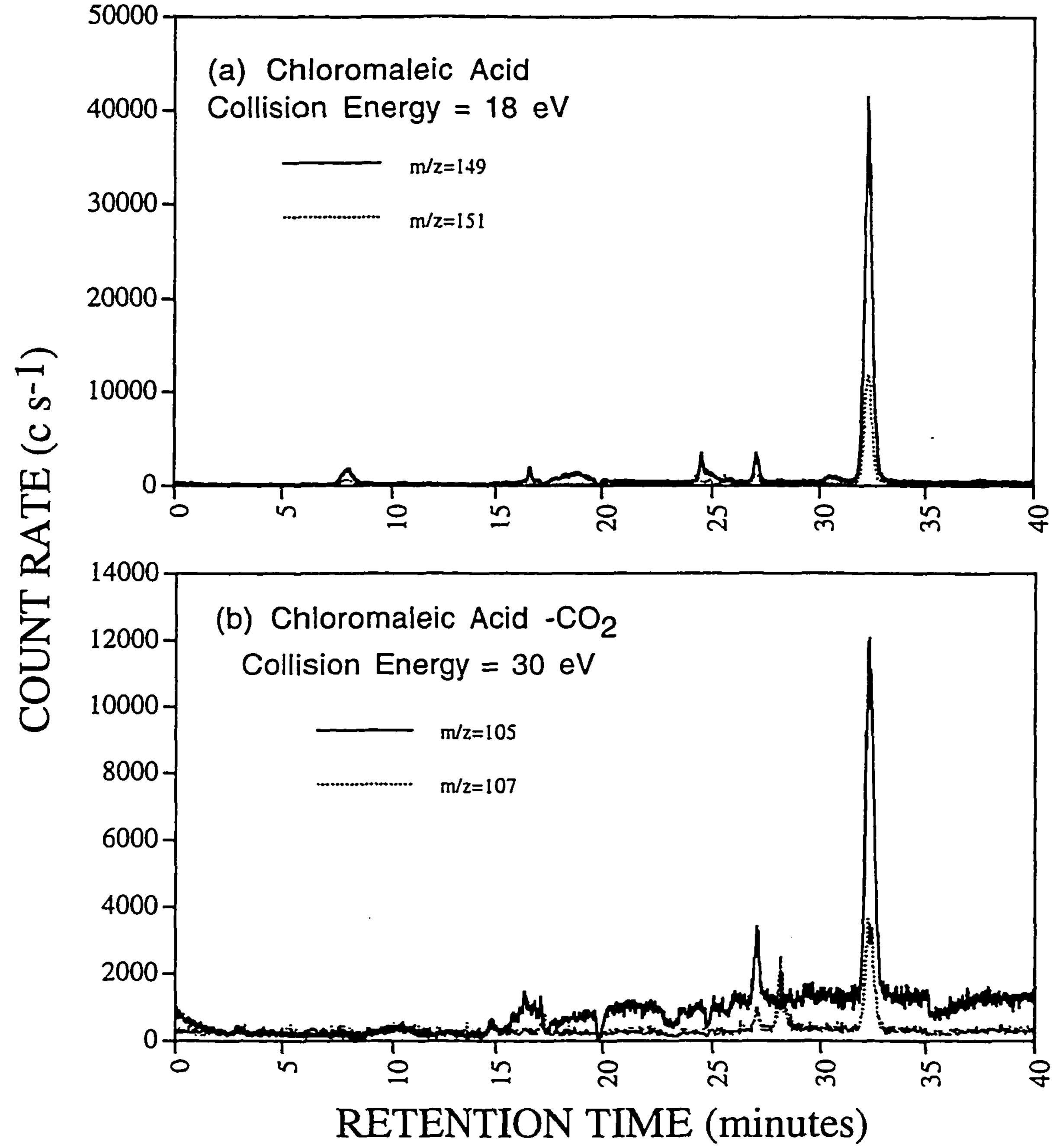


Figure 9. Chromatograms for suspected chloromaleic acid (a) and a fragment due to the loss of CO_2 (b). The sample was a 4-chlorophenol solution after 4 hours of ECI.

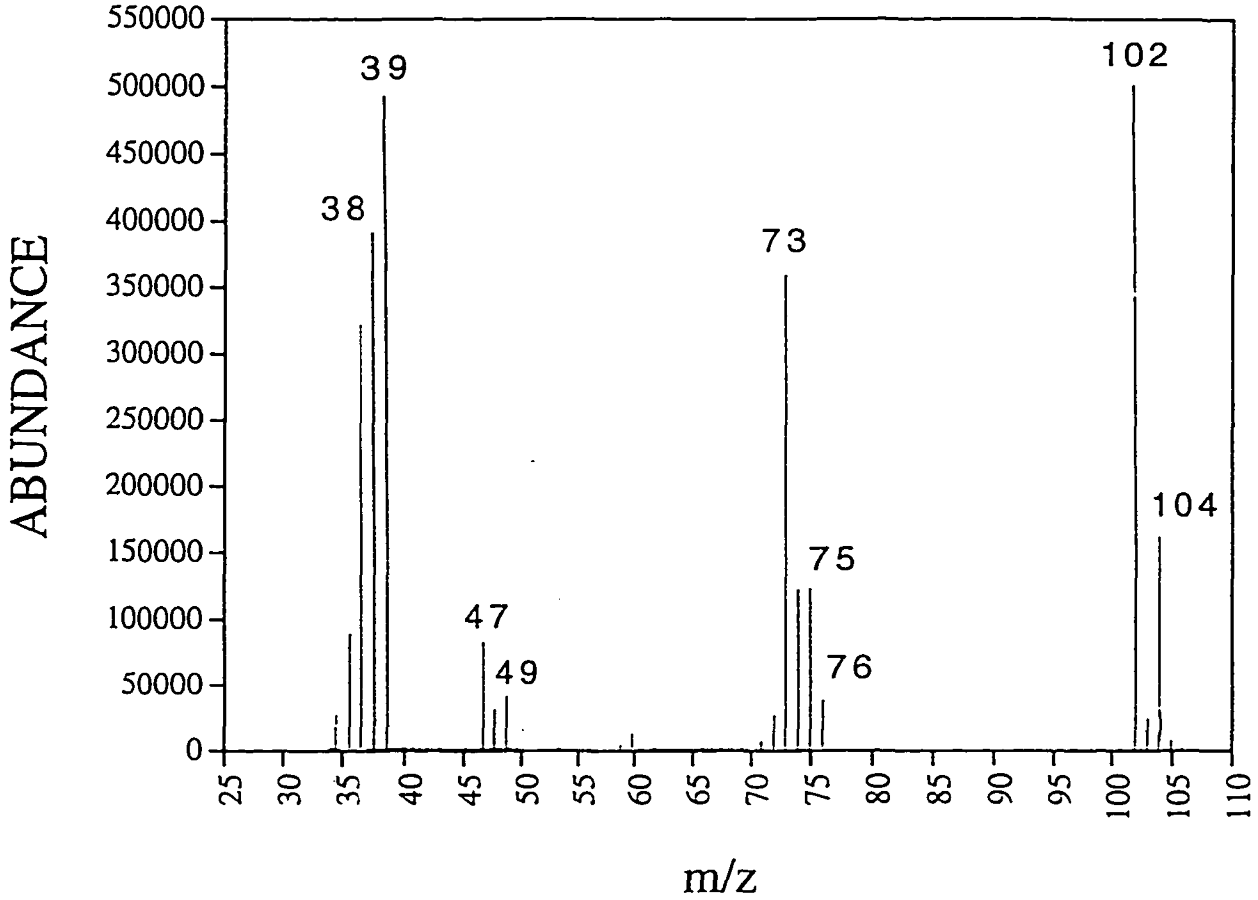


Figure 10. Electron impact mass spectrum from an unidentified GC-MS peak ($t_R = 10$ minutes). The sample is produced in the first 8 hours of ECI starting with a 100 ppm 4-chlorophenol solution.

CHAPTER 5. DETERMINATION OF SMALL CARBOXYLIC ACIDS BY CAPILLARY ELECTROPHORESIS WITH ELECTROSPRAY MASS SPECTROMETRY

A paper to be submitted to Analytica Chimica Acta

Steve K. Johnson, Linda L. Houk, Dennis C. Johnson, R. S. Houk

Abstract

Capillary electrophoresis (CE) is used with electrospray mass spectrometry (ES-MS) to analyze a mixture of succinic, maleic, formic, and glutaric acids. A make-up sheath flow is used to couple CE with ES-MS. Ion extraction voltages can be found that produce reasonable signals from the negative parent ions ($[M-H]^-$) of these compounds as well as many other carboxylic acids. The CE mobile phase consists of an aqueous solution of napthalenedisulfonate (NDS), pyromellitic acid, and methanol, with diethylenetriamine (DETA) as an electroosmotic flow modifier. Compromise experimental parameters for the CE separation and ES-MS detection are evaluated. Detection limits for CE-ES-MS determination of the sample were 1-10 ppm or 120-1200 pg. The carboxylic acid sample is injected directly into the CE column with little or no preparation.

Introduction

Low molecular weight carboxylic acids in aqueous solutions are important in environmental samples such as environmental waters and waste treatment sites, fermented juices, and biological samples [1-3]. Currently, gas chromatography - mass spectrometry

(GC-MS) is the most common method for the determination of carboxylic acids [4,5]. For GC-MS analysis, these analytes are often derivatized to increase their volatility before GC separation takes place, and preparation of the sample can be difficult and time-consuming [5-7]. With any extensive sample pretreatment, there is a risk of contaminating the sample or changing its characteristics. Liquid chromatography (LC), particularly ion exclusion chromatography, typically does not require extensive sample preparation and is an effective method for the separation of carboxylic acids [8-12]. With LC, it is possible to study aqueous carboxylic acid samples with little risk of altering the characteristics of the sample. Isotacophoresis and capillary electrophoresis have also been used to analyze aqueous carboxylic acid samples [13-18].

CE methods also do not require extensive sample pre-treatment and are attractive because they require less sample and eluent volumes. CE also has high separation efficiency and tremendous potential as a separation technique for organic and inorganic samples [19,20]. UV and fluorescence detectors are commonly used on column with CE [21-23]. Other CE detectors include laser induced fluorescence, amperometry, and conductivity [24-31]. CE can also be combined with a mass spectrometer such as electrospray - mass spectrometry (ES-MS) to form CE-ES-MS, a sensitive and selective analytical technique [27,32]. The major obstacles encountered when interfacing CE and ES-MS are flow rate differences and the incompatibility of the separation buffer with the ionization process.

In conventional capillary zone electrophoresis, using a fused silica capillary, the electroosmotic flow (EOF) is toward the negative electrode at most pH values [15].

Therefore, detection is usually carried out at the most negative electrode. Negatively charged anions, however, migrate toward the positive electrode and are typically not detected due to excessive migration times. In cases where anion migration is greater than EOF, the anions do not elute at all. Cationic surfactant is often added to the mobile phase to reverse the EOF when analyzing anions [13,32,33].

Some work has shown the effectiveness of CE separations with osmotic flow modifiers for the detection of carboxylic acids. Zare et al. separated low mass carboxylic acids from wine samples with 2-morpholinoethanesulfonic acid and tris(hydroxymethyl)aminomethane as a flow modifier, using conductivity detection [13]. Kenney used a phthalic acid and a commercially available osmotic flow modifier to separate organic acids, such as citric acid, malic acid, acetic acid, and others in food samples [16]. Shamsi and Danielson separated anions and organic acids with a mobile phase containing boric acid, diethylenetriamine (DETA) as a flow modifier, and napthalenedisulfonate as a UV-absorbing electrolyte using CE with indirect absorbance detection [18].

CE-ES-MS has been proven an effective method for the determination of organic and inorganic molecules. Olivares *et. al.* first combined CE with ES-MS for the detection of quaternary ammonium salts using a make-up liquid sheath flow [27,34]. Huggins and Henion used CE-ES-MS to separate metal ions at 30 pg levels, but they could only detect three anions at 300 pg levels because of complications from the CE additives that are needed for the CE separation [35]. Corr and Anacleto separated inorganic anions with CE-ES-MS using a pyromellitic acid buffer, which was found to be less obstructive of ES-MS detection [36]. To our knowledge no studies have shown the use of CE-ES-MS for the determination of small carboxylic acids.

The intent of this work is to develop a rapid technique for the determination of small carboxylic acids using CE-ES-MS. Of particular interest in this study are carboxylic acids found in the electrochemical incineration (ECI) of 4-chlorophenol [37]. Therefore studies focused on the separation of four carboxylic acids believed to be present in the ECI solution, succinic acid ($HO_2CCH_2CH_2CO_2H$), maleic acid ($HO_2CCH=CHCO_2H$), malonic acid ($HO_2CCH_2CO_2H$), and glutaric acid ($HO_2C(CH_2)_3CO_2H$). It should be noted that glutaric acid is no longer considered to be a product of the ECI of 4-chlorophenol. Several different separation schemes were examined, and the best results were found with a mixture of pyromellitic acid, NDS, DETA, and methanol. Optimum conditions were determined and detection limits were examined.

Experimental Section

Reagents and samples

All water was distilled and then deionized ($18 M\Omega\ cm^{-1}$ at 25°C) with a Branstead Nanopure-II system (Newton, MA). The mobile phase consisted of water, methanol (Fisher Scientific, Fair Lawn, NJ), pyromellitic acid, NDS-disodium salt, DETA (Aldrich Chemical Company, Milwaukee, WI), and was filtered with a Magna-R nylon membrane (0.22 μm pore diameter, Fisher) and degassed under vacuum. A 1000 ppm stock solution of each carboxylic acid (Aldrich) was prepared. Aliquots of stock solution were frozen separately to prevent microbial degradation. Samples were thawed and refrigerated for no more than 10 days prior to use. Aliquots of each acid stock solution were mixed and diluted with water to the desired concentration prior to use.

Capillary electrophoresis conditions

A Beckman P/ACE 2210 (Beckman Instruments, Palo Alto, CA) was equipped with an external dual polarity power supply (Beckman) for use with a mass spectrometer. Fused-silica (100-cm length × 100-μm i.d. 170-μm o.d.) was acquired from Polymicro Technologies Incorporated (Phoenix, AZ) and used as the separation capillary. Separations were carried out with -28.5 kV at the sample inlet vial and -3.5 kV at the capillary exit, resulting in a separation voltage difference of 25 kV. Separation voltage differences greater than 25 kV caused arcing in the CE. The sample was injected pneumatically using nitrogen gas (Air Products) at the inlet vial end of the capillary. After sample injection, the inlet end of the separation capillary was inserted in a vial containing buffer solution and the inlet electrode. The separation capillary passed through the Beckman cooling cartridge, exited the CE, and entered the CE-ES-MS interface with no break in the capillary.

The mobile phase contained 2mM DETA as an EOF modifier. This reagent causes the EOF and anions to move in the same direction, towards the more positive end of the separation capillary, where the ES-MS detector is located. It is important that the electrolyte has mobility similar to that of the analytes and interferes as little as possible with ES-MS detection. Many separation schemes were tested in this work including 2-morpholinoethane sulfonic acid (MES), trishyroxymethylaminomethane (TRIS), benzoate, pyromellitic acid, napthalenedisulfonate (NDS), napthalenemonosulfonate (NMS), and napthalenetrisulfonate (NTS). It was determined that the best separation and ES-MS detection was achieved with a mixture of pyromellitic acid and NDS. Corr and Anacleto demonstrated that pyromellitic acid is less detrimental to ES-MS detection than most electrolytes used to separate anions by CE-ES-MS [36]. Shamsi and Danielson showed that naphthalenesulfonates act as electrolytes to improve peak shape [18]. NDS has an intermediate mobility which makes it suitable for samples containing a wide range of analyte types.

ES-MS conditions

An API/1 (Perkin-Elmer SCIEX, Thornhill ON, Canada) single quadrupole mass spectrometer was used. This apparatus uses a curtain gas interface and has been described previously [38,39]. The IonSpray source was operated in the negative ion mode to observe anions. Table I summarizes the instrumental operating conditions of the ES-MS. These conditions were kept fairly consistent, but a few parameters (marked by an asterisk in table I) need optimization every day. Compromise operating conditions were determined to provide maximum $[M-H]^-$ signals during infusion of simple solutions. These same conditions were also used when CE separations were employed.

CE-ES-MS interface

The electroosmotic flow generated in the CE capillary is less than 1 μL/min, while the typical flow rate for the ES-MS is approximately 10 μL/min. The CE-ES-MS interface used in this study utilizes a coaxial make-up flow to provide enough liquid flow to maintain a stable spray and continuous electrical connection between the sample liquid

Table I ES-MS Operating Conditions

Ionization Source	IonSpray (nebulizer assisted electrospray)
Nebulizer Gas Pressure	40 psi, zero grade (99.998%) N_2
Nebulizer Gas Flow Rate	0.6 L min^{-1}
Sample Flow Rate	10 μL min^{-1}
Curtain Gas Pressure	80 psi, carrier grade (99.999%) N_2
Curtain Gas Flow Rate	0.7 L min^{-1}
Curtain Gas Temperature	60°C
Discharge Needle Voltage	-3500V*
Interface Plate Voltage	-400V*
Orifice Plate Voltage	-116V*
RF Only Quadrupole Voltage	-100V*
Mass Analyzer Quadrupole Voltage	-95V*
CEM Detector Voltage	+3000V
Operating Pressure of Quadrupole Chamber	3.5 x 10^{-5} Torr
Dwell Time	200 ms
m/z Values Monitored	m/z=103 (malonate) m/z=115 (maleate) m/z=117 (succinate) m/z=131 (glutarate)

*Typical values cited. These parameters were adjusted daily to maximize ion signal and chromatographic quality, and differed slightly from day to day.

and the ES ionization tube. The stable electrical connection is required for both CE separation and ES ionization. The make-up liquid also makes it possible to run and optimize the ES-MS without flow from the CE.

The CE-ES-MS interface is shown in figure 1. The inlet end of the separation capillary begins in a vial of buffer solution and the capillary proceeds through the coolant cartridge of the CE. The capillary exits the cartridge, passes through two T-unions, and runs to the ES-MS interface. At the entrance of the first T-union, a 2 cm length of PEEK tubing is glued to the outside of the capillary, so that a PEEK fitting can be used to hold the capillary in place and seal off the entrance of the T. Within the first T-union, a make-up flow of buffer solution surrounds the separation capillary in a coaxial flow of 10 μL/min, pumped from a Cole-Parmer syringe pump (Niles, IL) and a Hamilton syringe (Reno, NV). A variety of make-up flow solutions were tested, using the same components as the separation buffer at different concentrations. Solutions with higher methanol content or lower electrolyte concentrations than the separation buffer were expected to give better signal in the ES-MS. It was determined, however, that the best separations and reproducibility are obtained when the separation buffer and make-up flow solution are identical. The capillary and make-up buffer exit the first T-union enclosed within a 4-inch-long stainless steel ES ionization tube (Hamilton). The ionization tube enclosing both the separation capillary and make-up buffer continues through the second T-union, where a nitrogen nebulizer gas flow surrounds the tube. Finally, the ionization tube exits the second T-union through the ES nebulizer tube. The capillary ends inside the ionization tube approximately one mm from the tube tip where the sample is mixed with the make-up buffer. The liquid contact with the ES ionization tube provides electrical contact.

Results and Discussion

Evaluation of mobile phase

The mobile phase for the CE-ES-MS determination of carboxylic acids was evaluated by observing signal intensity and separation quality while changing the concentration of the mobile phase components. Figures 2 and 3 show the effect of pyromellitic acid concentration on maleate (Fig. 2) and glutarate (Fig. 3) ion signals. All other experimental conditions were held constant. Maleate ion signal intensity improved by nearly a factor of 10 when the pyromellitic acid concentration was increased from 2mM to 6mM (Fig. 2). Separation time also decreased as the concentration of pyromellitic acid increased. Conversely, glutarate ion signal and separation quality decreased with increased pyromellitic acid (Fig. 3). With 6mM pyromellitic acid, the glutarate signal is almost completely lost. A compromise pyromellitic acid concentration of 4mM was chosen to give reasonable results for both maleate and glutarate ions.

Figure 4 and 5 show the effects of increasing NDS concentration. NDS is typically used in CE separations as a UV absorber for indirect detection, however it has been shown that including NDS in electrolyte solutions can improve peak shape [18]. The maleate ion peak shape improves with increasing NDS concentration (Fig. 4) while malonate signal improves slightly with decreasing levels of NDS (Fig. 5). Once again, a compromise concentration, 4mM NDS, was used to acquire good spectra from both maleic and malonic acid.

The effects of methanol concentration are presented in Figure 6. Increased methanol concentration, at least up to 50% methanol, generally increases ion signal in ES-MS. However, methanol has adverse effects on the quality of this CE separation. Increased methanol concentration results in longer retention times and poor resolution in the electropherograms. A concentration of 20% methanol was determined to give the best ion signal in the ES-MS without reducing the quality of the separation.

Figure 7 shows single-ion electropherograms obtained using the compromise mobile phase conditions for the separation of the four carboxylic acid standards. Sample concentrations were 0.1 mM, and the mobile phase consisted of 4mM pyromellitic acid, 4mM NDS, 2mM DETA, and 20% methanol. Detection limits were determined to range between 1 and 10 ppm (120-1200 pg) and are shown in table 2.

Table II. Detection Limits

Analyte	Concentration	Absolute
Malonic Acid	1 ppm	100 pg
Succinic Acid	2 ppm	200 pg
Maleic Acid	10 ppm	1200 pg
Glutaric Acid	4 ppm	500 pg

Conclusions

The CE-ES-MS system developed in this study successfully determined carboxylic acids at the 1-10 ppm level. Detection limits are poor due to the small actual mass of analyte that enters the ES-MS, the substantial dilution of the analyte caused by the make-up flow, and a very high and noisy background. The system is also inconsistent and without improvements it would be difficult to use this system to identify the components of complex unknown solutions. Improvements in the system may be possible with a micro ES ionization source (MESI) [40,41]. MESI requires less liquid flow in the ionization process, which allows for the use of a lower make-up flow rate and less dilution of the sample.

Substantial background noise could be reduced by using a triple quadrupole mass spectrometer (MS-MS). All four of the acids studied are dicarboxylic acids, meaning they will readily lose CO_2 during collisions. A detection scheme searching for the loss of CO_2 from the parent ions could be employed, using MS-MS. This scheme would practically eliminate signal from all ions other than the analytes of interest.

Acknowledgements

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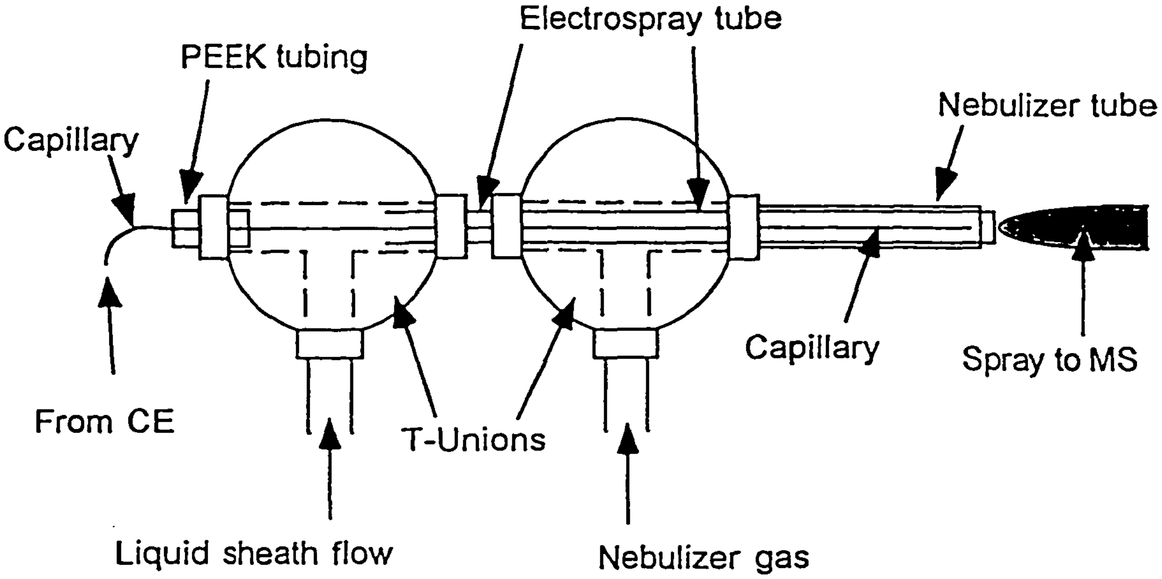

Figure 1. Schematic diagram for CE-ES-MS interface.

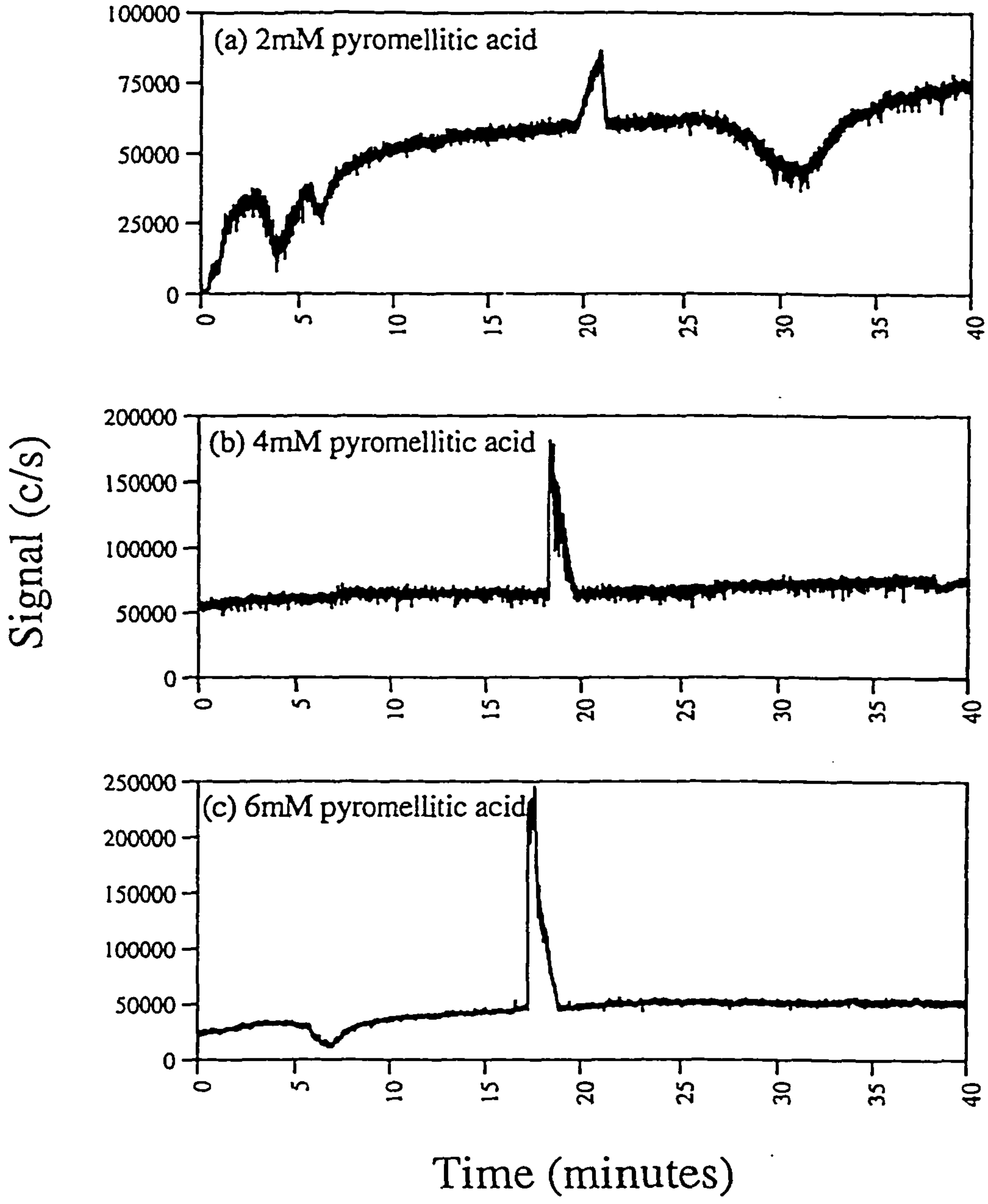

Figure 2. CE-ES-MS single-ion electropherograms for 1mM Maleate ion using a buffer containing (a) 2mM, (b) 4mM, (c) 6mM pyromellitic acid, 4mM NDS, 2mM DETA, and 20% methanol. The separation voltage difference was 25kV.

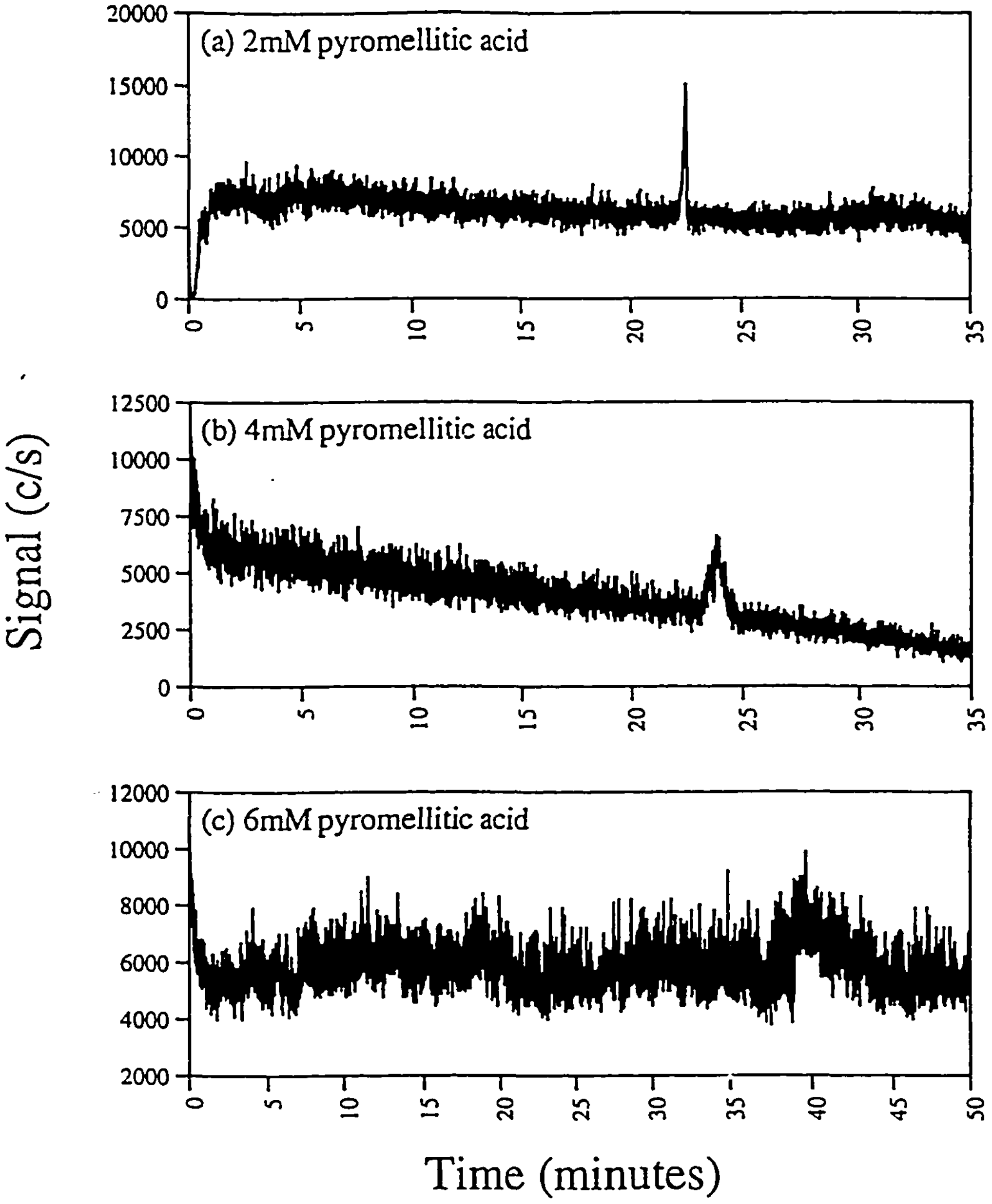

Figure 3. CE-ES-MS single-ion electropherograms for 1 mM glutarate ion using a buffer containing (a) 2mM, (b) 4mM, (c) 6mM pyromellitic acid, 4mM NDS, 2mM DETA, and 20% methanol. The separation voltage difference was 25kV.

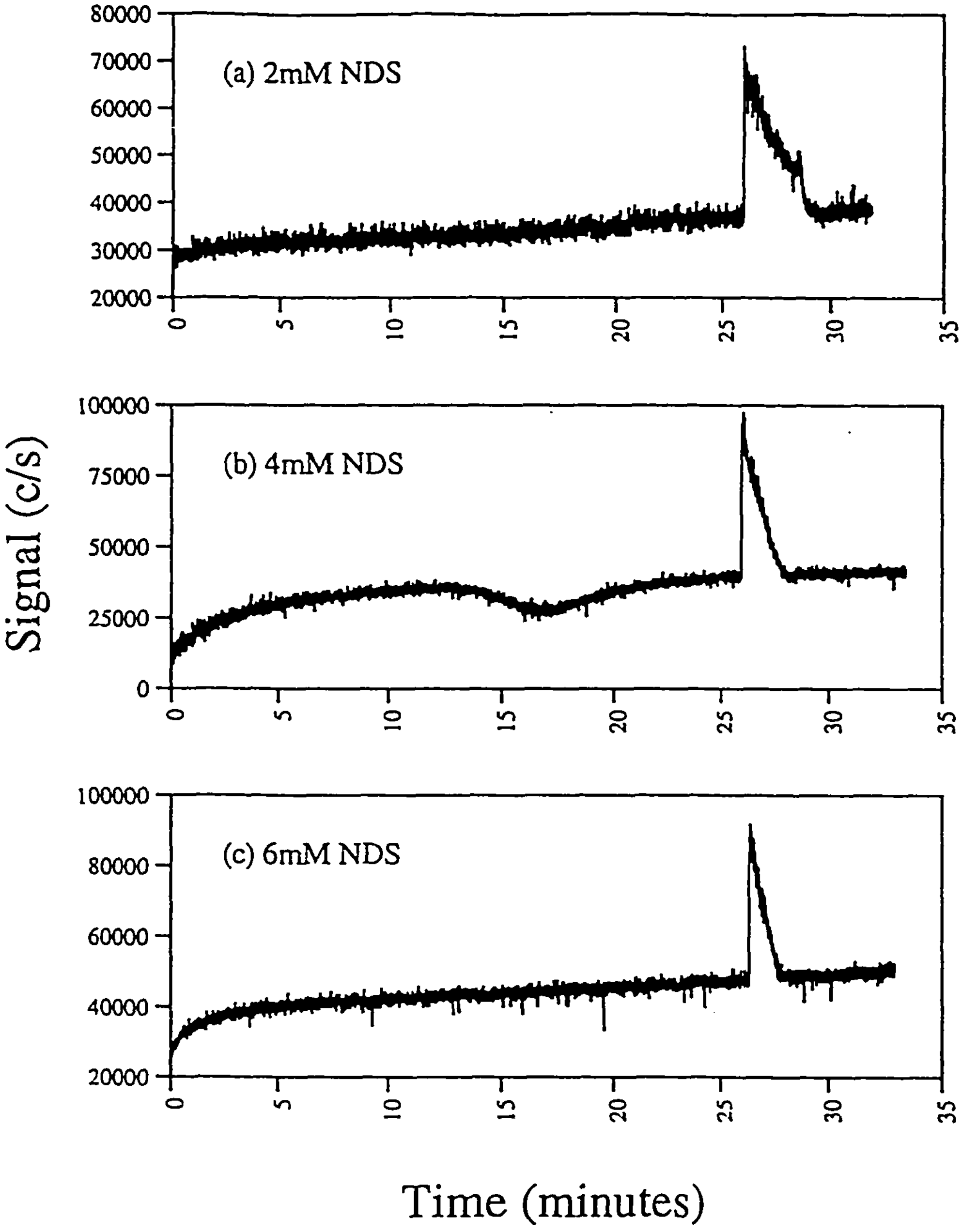

Figure 4. CE-ES-MS single-ion electropherograms for 1mM maleate ion using a buffer containing (a) 2mM, (b) 4mM, (c) 6mM NDS, 4mM pyromellitic acid, 2mM DETA, and 20% methanol. The separation voltage difference was 25kV.

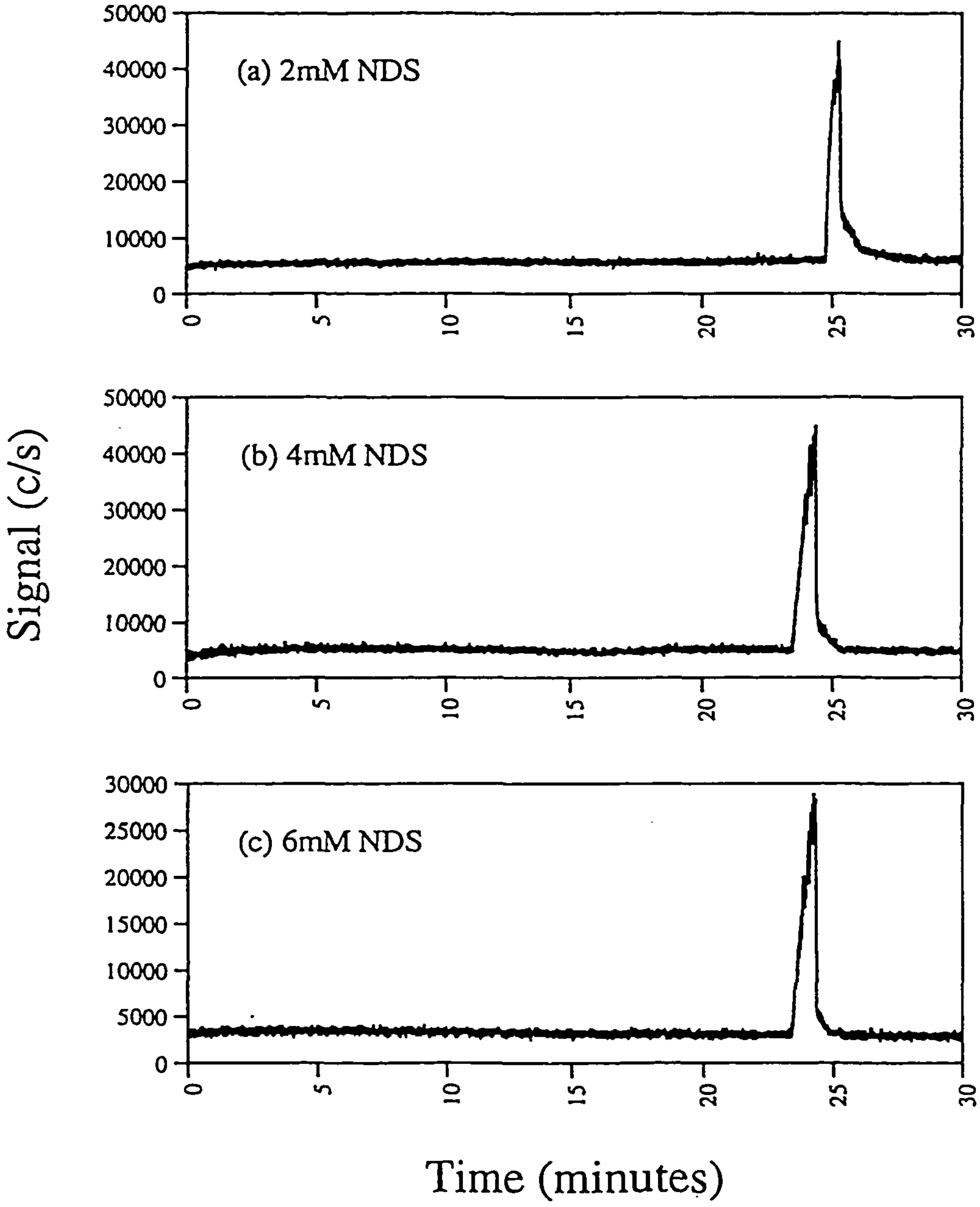

Figure 5. CE-ES-MS single-ion electropherograms for 1mM malonate ion using a buffer containing (a) 2mM, (b) 4mM, (c) 6mM, NDS, 4mM pyromellitic acid, 2mM DETA, and 20% methanol. The separation voltage difference was 25 kV.

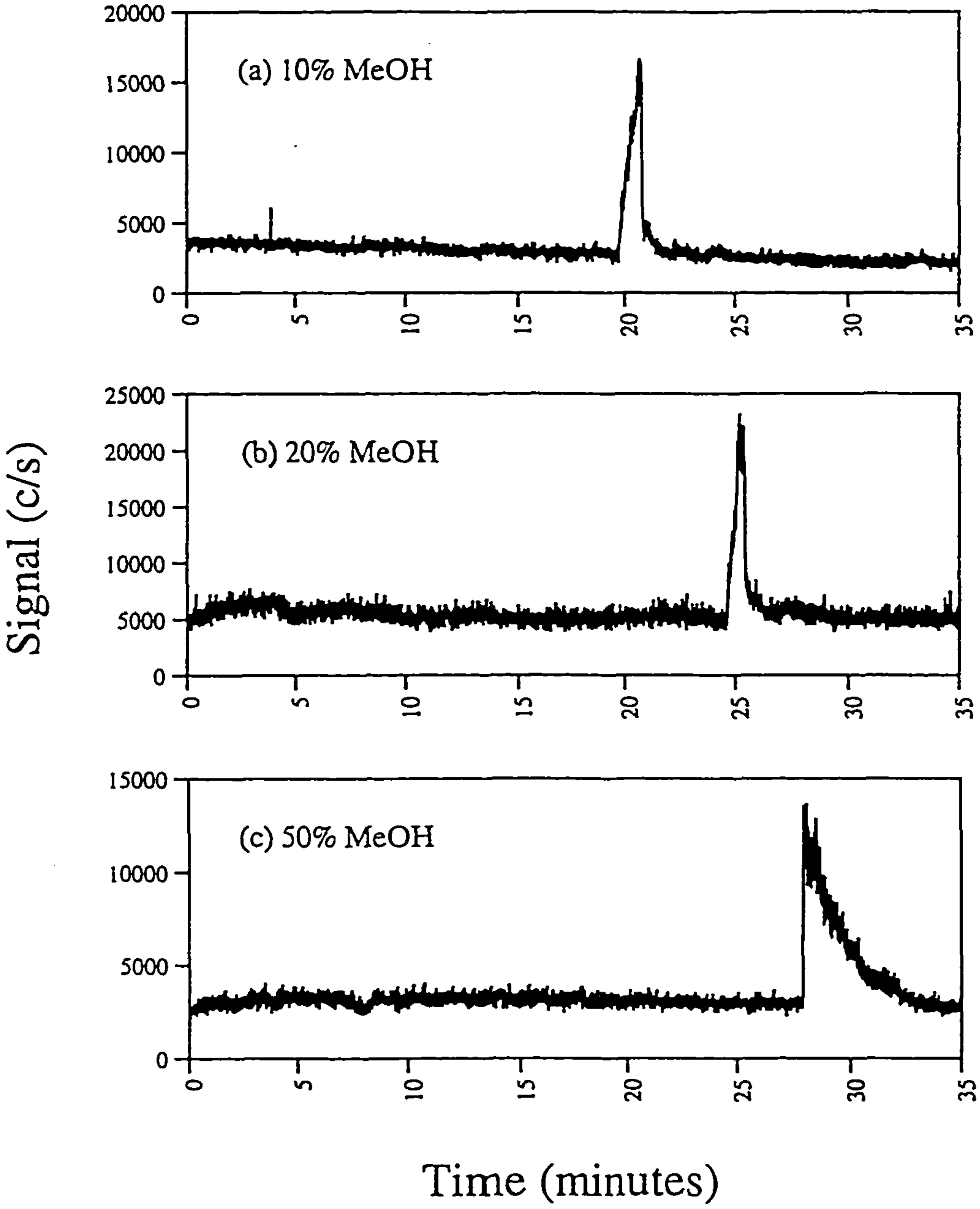

Figure 6. CE-ES-MS single-ion electropherograms for 1mM malonate ion using a buffer containing (a) 10%, (b) 20%, (c) 50% methanol, 4mM pyromellitic acid, 4mM NDS, and 2mM DETA. The separation voltage difference was 25 kV.

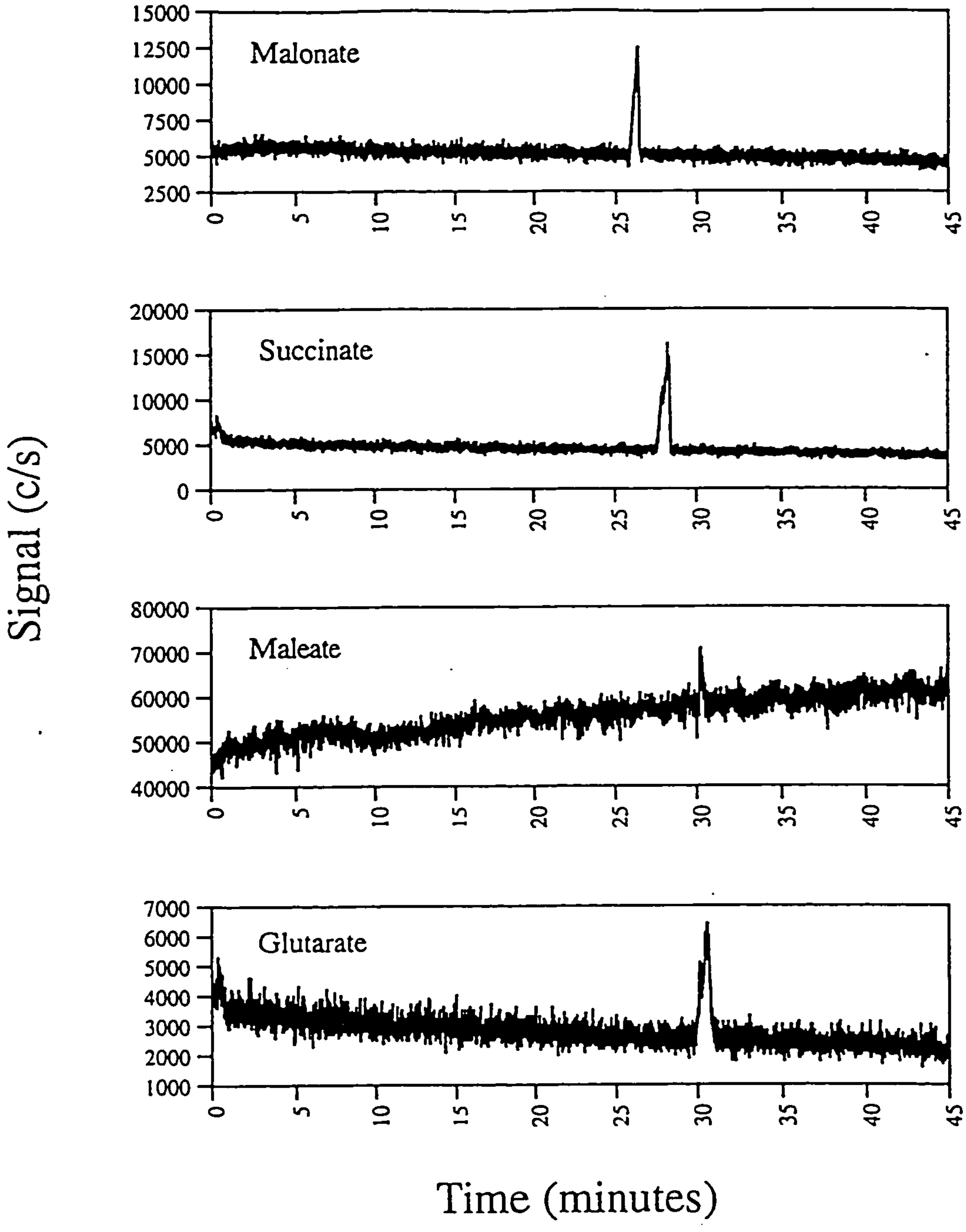

Figure 7. Single ion electropherograms for 0.1 mM malonate, succinate, maleate, and glutarate ions. The buffer contained 4mM pyromellitic acid, 4mM NDS, 2 mM DETA, and 20% methanol. The separation voltage difference was 25 kV.

CHAPTER 6. GENERAL CONCLUSION

The main focus of this dissertation has been the use of liquid chromatography (LC) coupled with electrospray mass spectrometry (ES-MS) for the determination of both inorganic and organic compounds in aqueous solution. LC-ES-MS combines retention and structural data to provide a powerful technique for the identification of unknown compounds in complex solutions. Primary focus has been given to electrochemical incineration (ECI) and the determination of ECI products and intermediates.

Chapter 2 presented the study of small carboxylic acids using ion exclusion chromatography (IEC) and ES-MS. Experimental conditions were examined and compromise conditions were determined, while considering pH, sheath gas, mobile phase composition, and matrix effect.

Chapter 3 implemented the IEC-ES-MS method to examine the complex unknown solution produced during ECI of benzoquinone. The ECI procedure and electrode were explained and evaluated. Intermediates and products were determined and examined with respect to incineration time, and mechanisms were proposed for the production of maleic, succinic, malonic, and acetic acids by the ECI of benzoquinone.

The ECI of 4-chlorophenol was studied in chapter 4. Reverse phase chromatography-ES-MS was used for the determination of aromatic compounds, while ion exclusion chromatography-ES-MS was used to study both organic and inorganic ions. The ion exchange chromatography-ES-MS system utilizes a suppressor between the separation column and the ES-MS to remove sodium ions from the mobile phase. Both

LC-ES-MS techniques offer superior separation quality and detection limits compared to IEC-ES-MS. Other techniques including, chemical oxygen demand, total organic carbon, pH and gas chromatography-mass spectrometry, were also used to study the ECI product of 4-chlorophenol. The major intermediates were determined to be benzoquinone, malonic, succinic, and maleic acids, as well as inorganic chlorine-containing anions, such as chloride, chlorate, and perchlorate. Pathways were also suggested for the ECI reaction.

Chapter 5 reported preliminary studies from coupling capillary electrophoresis (CE) with ES-MS. A sheath flow of supporting electrolyte was required for sufficient electrical connection and liquid volume. The mobile phase composition was studied to provide the best compromise results for the observation of four carboxylic acids. Although CE-ES-MS has great potential, with low sample volume and efficient separation, detection limits and reproducibility were poor.

The LC-ES-MS methods used in this work have proven effective for the identification of unknown compounds in complex aqueous solutions. Future work to improve LC-ES-MS may focus on reducing the high background noise present in the ES-MS signal. The addition of TurboIonSpray reduces some noise, but more improvement is needed. One possible area of improvement is the ionization tube and the quality of the spray. The production of small droplets in a well-focused spray can lead to improved ionization efficiency and better sensitivity. Recent work involving nanoelectrospray ionization [1] and ultrasonic nebulization ES-MS [2] seem to be achieving some signal improvements.

The ECI apparatus and procedure successfully oxidizes various organic compounds without producing environmentally hazardous intermediates and products. Most pathways for the formation of CO_2 from larger molecules involve carboxylic acid intermediates. Faster and more efficient ECI may be achieved by focusing on the oxidation of these carboxylic acids. Perhaps the addition of new metal oxides to the existing quaternary metal oxide film will result in faster waste remediation by ECI.

Advancements can also be made with novel sampling methods for ECI studies. One such sampling technique involves pulling ECI products from the electrode surface during ECI to observe the intermediates of the reaction without sampling the bulk of the solution. Sampling at the electrode surface was attempted in this laboratory with mixed results. The rise and fall of intermediate concentrations was observed, as seen for formic acid during the ECI of benzoquinone in figure 1, but the solution sampled represented mostly bulk solution, and provided no specific information about the reactions at the electrode surface. Perhaps a lower liquid flow and more precise and stable inlet placement would reduce the amount of bulk solution sampled and provide more information about the reactions at the electrode surface.

References

1 Wilm, M., Mann, M., *Anal. Chem.*, 1996, **68**, 1.

2 Straub, R., Linder, M., Voyksner, R.D., *Anal. Chem.*, 1994, **66**, 3651.

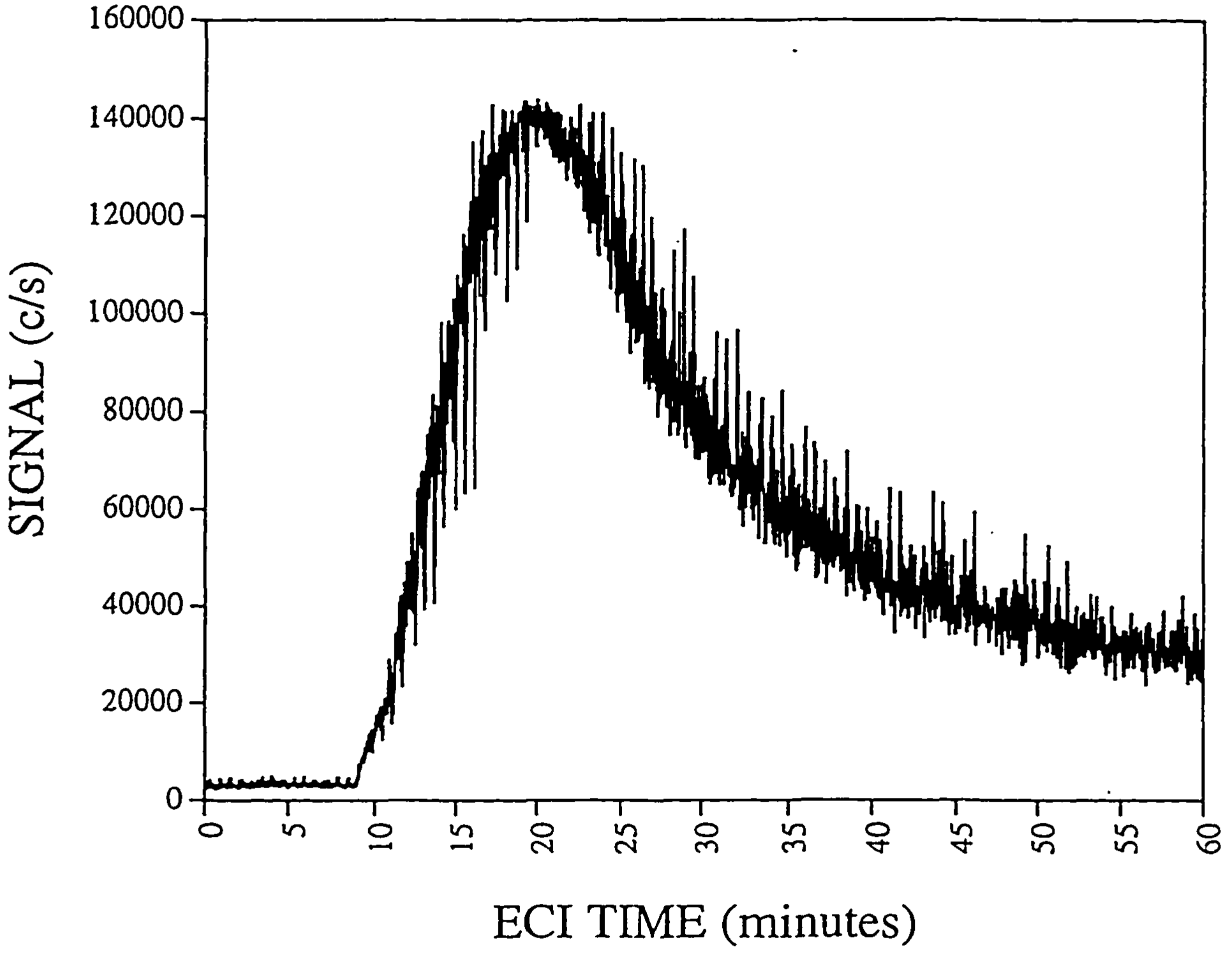

Figure 1. Real-time monitoring of formate ion (m/z=45) near the electrode surface during the ECI of 100 ppm benzoquinone.

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Finally, I would like to thank God for bringing me here, surrounding me with incredible people, giving me tremendous opportunities, and making it all possible. 1Cor 2:5 "That your faith may not rest in the wisdom of men, but in the power of God."

IMAGE EVALUATION
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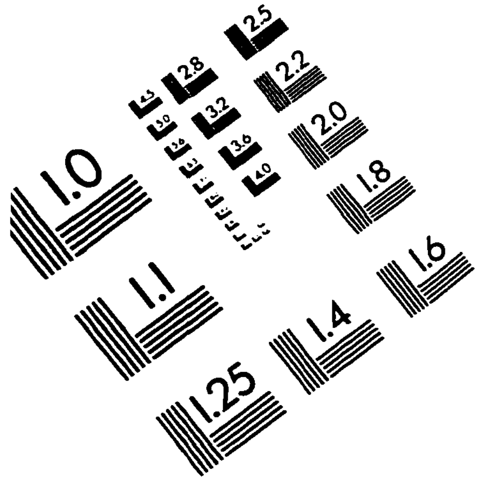

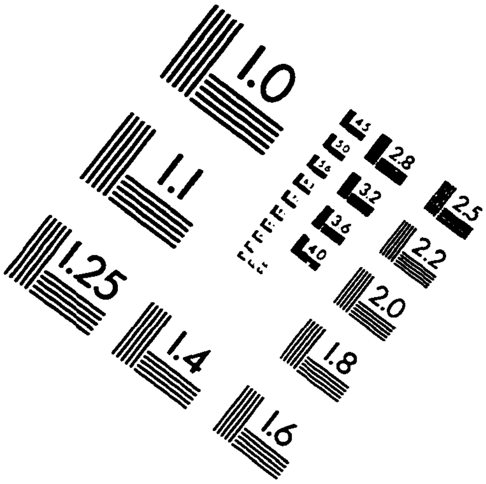

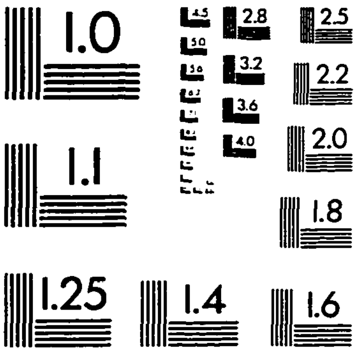

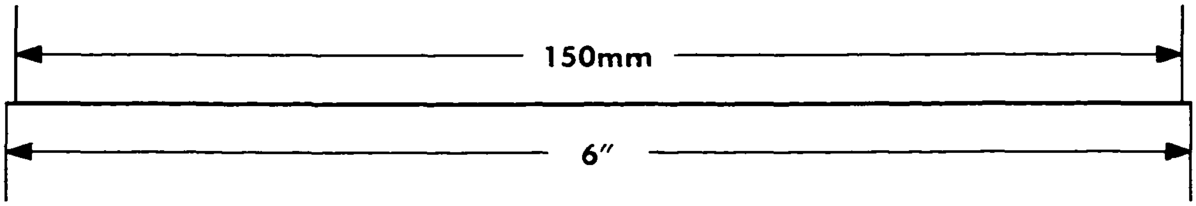

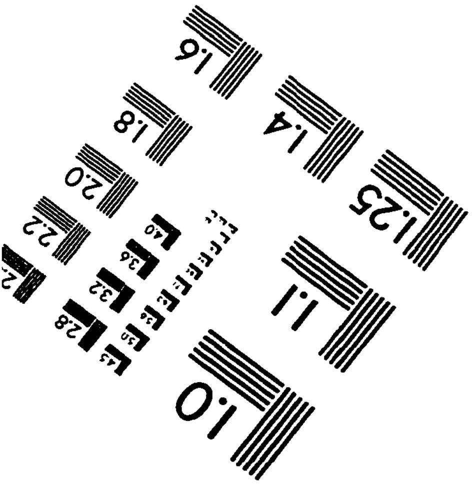

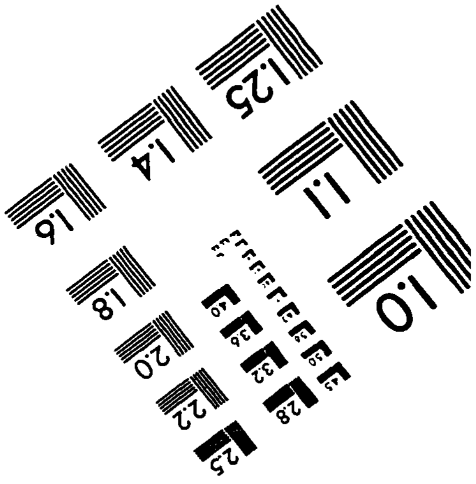

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