

Separation and identification of photodegradation products of benzoic acid by capillary zone electrophoresis

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Abstract

A capillary zone electrophoresis (CZE) method was developed for separation and identification of photodegradation products of benzoic acid under irradiation at a wavelength of 300 nm. Parameters such as run buffer, applied voltage and injection time were optimized for the separation of benzoic acid and its photodegradation products. Linearity, limit of detection, and repeatability of migration time as well as peak area of the method were examined. Four reaction products, including salicylic acid, 3-hydroxybenzoic acid, 4-hydroxybenzoic acid and 3,4-dihydroxybenzoic acid have been separated and identified by spiking the known compounds into the irradiated samples using the CZE method developed. The confirmation of the reaction products is one of the key steps for proposing the possible reaction mechanisms involved in the photodegradation of benzoic acid.

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1. Introduction

A wide variety of crude oils and petroleum products, mainly hydrocarbons, are emitted into the atmosphere from natural and anthropogenic sources (e.g. oil spills, crude oil refining, automobile exhaust fumes) [1–3]. Once in the atmosphere, hydrocarbons are subject to various chemical and photochemical processes that determine their transformation and ultimate fate. Both field and laboratory studies have shown that petroleum hydrocarbons undergo photochemical oxidation reactions when they are irradiated with sunlight or other light sources [3]. The photodegradation products are primarily carboxylic acids including aliphatic and aromatic acids with significant levels of benzoic acid that is presumably

generated from the decomposition of alkyl-substituted benzenes [4]. A variety of aromatic acids are also emitted directly from sources such as industrial boilers burning distillate fuel oil, tar pots heating asphalt and motor exhausts [5–7]. These acids are preferentially transferred to the atmospheric liquid phase due to their polar nature. They constitute a significant fraction of organic aerosol associated with photochemical smog, and in part are responsible for reduction in the visibility of urban atmosphere. Chemical transformations of carboxylic acids are influenced largely by metal ions (e.g. iron and copper) as catalysts, particles present in atmospheric aerosols as well as solar irradiation [8–14]. Cunningham et al. [14] studied the mechanisms of aqueous photolysis of adsorbed benzoate on iron oxyhydroxide (goethite) surfaces. Salicylic acid was identified under aerated conditions as one of the reaction products [14]. In addition to salicylic acid, other

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products were also observed in the form of broad, late-eluting bands in high-performance liquid chromatography (HPLC) analyses, which were not further characterized [14]. Confirmation of the reaction products is critical for understanding and proposing the reaction mechanisms involved in the photodegradation of benzoic acid in the atmosphere.

Because of the ionic nature of aromatic acids, it is possible to separate these compounds using capillary zone electrophoresis (CZE). A number of studies have demonstrated that aromatic acids can be separated and identified in environmental water, soil, sediment and plant samples by CZE [15–17].

The purpose of this work was to develop a CZE method that can be utilized to separate and identify reaction products of photodegradation of benzoic acid catalyzed by dissolved iron(III) species with monochromatic light at a wavelength of 300 nm. The information about intermediates and final products of the reaction will be used to propose the reaction mechanisms. The results will find direct application in evaluation of iron photocatalysis for removal of aromatic acids in the atmospheric environment.

2. Experimental

2.1. Reagents

All chemicals were used without further purification. 3,4,5-Trimethoxybenzoic acid, benzoic acid, salicylic acid, 3-hydroxybenzoic acid, 4-hydroxybenzoic acid and 3,4-dihydroxybenzoic acid were obtained from Fluka (Milwaukee, WI, USA). Other chemicals were obtained from Fisher Scientific (Pittsburgh, PA, USA).

All solutions were prepared using deionized water from a Milli-Q Gradient Water Purification System (Millipore, Bedford, MA, USA). Sodium tetraborate ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$) was used to prepare a run buffer. The pH values of the buffer were adjusted using either a 1.0 M NaOH or 1.0 M HCl solution. The stock solutions of the aromatic acids (1.00×10^{-2} M for each acid) were prepared by dissolving appropriate amounts of the acids in a 1.00×10^{-2} M NaOH solution. The mixtures of the acids in a concentration range of 3.0 – 1.00×10^2 μM were obtained by dilution of the stock solutions with deionized water.

2.2. CE instrumentation

Electrophoretic analyses were carried out using a Beckman capillary electrophoresis system (P/AC System MDQ, Beckman Instruments, Fullerton, CA, USA) equipped with a diode array detector and a fused-silica capillary ($57 \text{ cm} \times 75 \mu\text{m}$ I.D.). Data acquisition was carried out using the Karat Version 4.0 Software (Beckman Instruments) on an IBM computer. Prior to their first use, all capillary columns were conditioned by rinsing in sequence with 1 M HCl for 5 min, deionized water for 2 min, 1% (w/v) NaOH for 10 min, deionized water for 2 min and finally running buffer for 5 min. The capillary column was rinsed with a running buffer by applying a pressure of 20 p.s.i. for 2 min before each injection (p.s.i. = 6894.76 Pa). The samples were introduced by pressure sample injection (0.5 p.s.i.) at the inlet end and the direct UV detection was performed at a wavelength of 214 nm. The cathode was placed at the outlet buffer and the anode at the inlet buffer.

2.3. Separation and identification

Separation conditions of the aromatic acids (i.e. 3,4,5-trimethoxybenzoic acid, benzoic acid, salicylic acid, 3-hydroxybenzoic acid, 4-hydroxybenzoic acid) were optimized in terms of run buffer pH, applied voltage and sample injection time using a mixture of the acids (1.00×10^2 μM for each acid, pH 3.20 ± 0.05). The dependency of applied voltage and injection time on the separation of the acids was initially conducted in the range 14–27 kV and 20–28 s, respectively. After the values for the applied voltage and the injection time were narrowed down to a certain range, separation efficiency in terms of difference in the average migration time (Δt_m) of the worst resolved compounds, repeatability of migration time (t_m) and peak area (A_p) of the compounds were further investigated. Ten replicate injections of a mixture of the acids (1.00×10^2 μM for each acid, pH 3.20 ± 0.05) were used to generate the statistical data. The best separation of the acids tested was obtained using the following conditions: a running buffer containing 13 mM sodium borate at pH 10.00 ± 0.10 , applied voltage: 15 kV, pressure sample injection time: 26 s, wavelength of UV detection:

214 nm, capillary: 57 cm×75 μm I.D. and operation temperature: 22 °C.

Identification of reaction products was carried out by spiking 50 μl of one test compound (1.00×10^2 μM, pH 3.20 ± 0.05) into an irradiated sample at a time. An increase in peak area at a given migration time for the existing compound in an electropherogram would indicate that identity of the compound spiked is the same as the existing compound in the sample.

2.4. Irradiation

A Rayonet photoreactor (Southern New England Ultraviolet Co., Branford, CT, USA) was used for irradiation of benzoic acid solutions. The reactor cavity held a 660-ml quartz reaction vessel equipped with a cold finger (Southern New England Ultraviolet Co.) where tap water was circulated through the vessel to provide cooling of a solution. The cold finger also provides a gas outlet that vents gases produced by the reaction. The surrounding lamps with a wavelength of 300 nm (Southern New England Ultraviolet Co.) provide uniform light intensity.

Two solutions were prepared for the photodegradation experiments. One solution contained benzoic acid (1.00×10^2 μM), whereas the other contained benzoic acid (1.00×10^2 μM) and 2.00×10 μM iron(III) chloride. Both solutions were adjusted to pH 3.20 ± 0.05 and were irradiated at 300 nm. The temperature of the reaction mixtures was kept at 30 ± 3 °C as tap water was circulated through the cold finger. Samples will be taken at various time intervals and analyzed by the CZE method developed in this study.

2.5. Quantitation

The stock solutions of the aromatic acids (1.00×10^{-2} M for each acid) were diluted to make mixtures of the acids at a concentration range of 3.0– 1.00×10^2 μM for each compound. Each of the solution mixtures was injected and analyzed three times using the optimized separation conditions listed in Section 2.3. The values of the peak area were averaged and the mean values were used to generate standard calibration curves. Repeatability of migration time (t_m) and peak area (A_p) was examined by 10

replicate injections of a mixture of the acids (3.0 μM for each acid). The percent relative standard deviations (RSDs) for migration time and peak area were calculated based on the results obtained from 10 replicate injections. The limits of detection for the compounds tested were estimated based on a signal-to-noise ratio of 3.

2.6. Absorption spectra

Three solutions were prepared to obtain the absorption spectra. The solutions contained 1.00×10^2 μM benzoic acid, 2.00×10 μM FeCl₃ and 1.00×10^2 μM benzoic acid with 2.00×10 μM FeCl₃, respectively. All these solutions had a pH value of 3.20 ± 0.05 . The absorption spectra of the solutions were obtained in the wavelength range 190–400 nm using a UV–visible spectrophotometer equipped with a 1-cm quartz cuvette (2401PC, Shimadzu, Columbia, MD, USA).

3. Results and discussion

3.1. Effect of pH

Five aromatic acids including benzoic acid, salicylic acid, 3-hydroxybenzoic acid, 4-hydroxybenzoic acid and 3,4,5-trimethoxybenzoic acid were tested in this study. The buffer concentration was chosen as 13 mM according to the method developed previously for the separation of seven aromatic acids in natural water samples [15]. The effect of pH on the separation of these five aromatic acids was investigated at pH 9.00, 9.29, 9.42, 9.83, 9.99 and 10.10 (Fig. 1). Among these six sets of conditions, migration of the compounds in the pH range 9.90–10.10 provided the best resolution and separation of the acids. Thus, pH 10.00 ± 0.10 was selected as the optimized buffer pH value for the separation of the acids.

3.2. Effect of applied voltage

An ideal separation is generally obtained by applying a voltage as high as possible since the number of theoretical plates in CE is proportional to the applied voltage. However, Joule heat generated

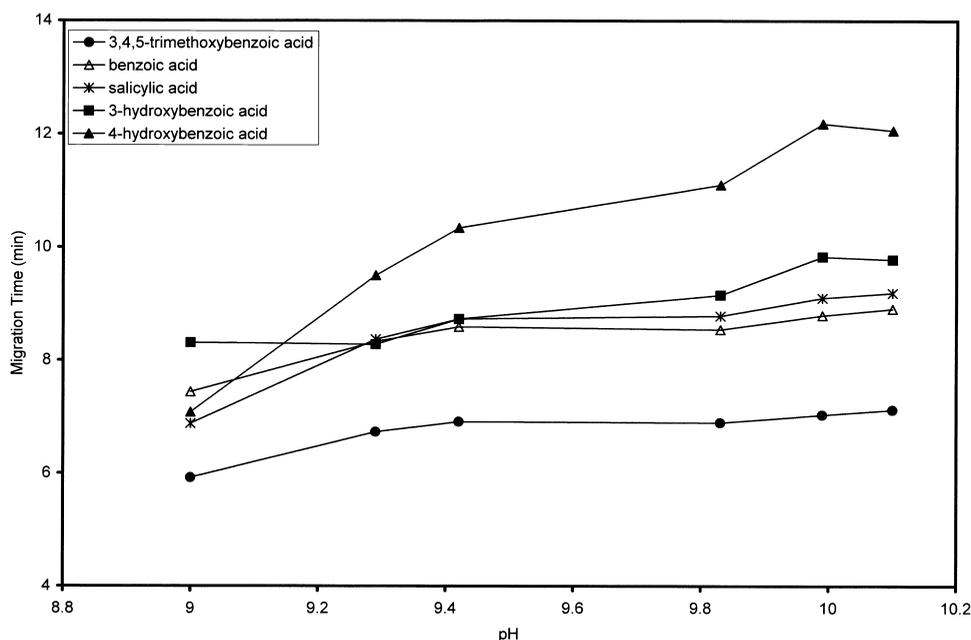


Fig. 1. pH effect on migration time of the compounds tested. Experimental conditions: concentration of sodium tetraborate (run buffer), 13 mM; concentrations of the acids, $1.00 \times 10^2 \mu\text{M}$ each; injection pressure, 0.5 p.s.i.; injection time, 25 s; applied voltage, 20 kV; wavelength of UV detection, 214 nm; capillary, 57 cm \times 75 μm I.D.; operating temperature, 22 °C. The pH of the run buffer solutions varied from 9.00 to 10.10. The optimized pH of the run buffer is pH 10.00 \pm 0.10.

within a capillary due to the voltage applied to the CE column causes the broadening of peaks, thus the separation efficiency decreases. The effect of applied voltage on migration time of the acids is shown in Fig. 2. A better separation was achieved in a voltage range of 14–16 kV. The worst resolved compounds were benzoic acid and salicylic acid. Thus, the dependency of resolution of these two compounds on the applied voltage of 14, 15 and 16 kV was further investigated. A standard mixture ($1.00 \times 10^2 \mu\text{M}$ for each acid, pH 3.20 ± 0.05) was injected 10 times and the compounds in the mixture were separated at a voltage of 14, 15 and 16 kV. Table 1 shows the difference in the average migration time (Δt_m) of benzoic acid and salicylic acid and the RSD of Δt_m as a function of the applied voltage. The value of Δt_m of benzoic acid and salicylic acid is the greatest at 15 kV, indicating the best separation of the two compounds. The RSD of Δt_m was the lowest at 15 kV, indicating the smallest deviation of Δt_m . Thus, the voltage of 15 kV was chosen as the optimized applied voltage.

3.3. Effect of sample injection time

Sample injection time has been proven to be proportional to the volume of a sample introduced into the capillary, and can be optimized in order to yield analyte peaks large enough for quantitation. The preliminary data showed that the peak area increased linearly as injection time increased. However, the peaks of benzoic acid and salicylic acid tended to be overlapped and the separation of the acids deteriorated when the injection time was greater than 26 s. The dependency of separation efficiency of these two compounds on the injection times of 24, 26 and 28 s was further investigated. Table 2 shows the difference in the average migration time (Δt_m) of benzoic acid and salicylic acid and the RSD of Δt_m . Although an increase in injection time decreases the separation efficiency with respect to the Δt_m and RSD of Δt_m as shown in Table 2, the differences in Δt_m values was relatively small. Thus, additional parameters were used to evaluate the separation efficiency of these two compounds. Table

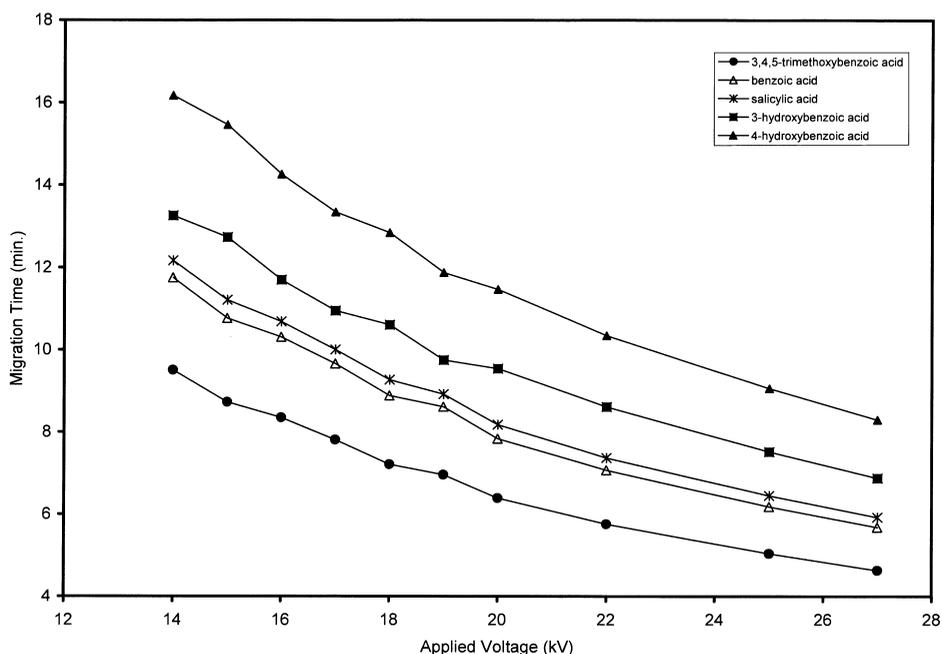


Fig. 2. Effect of applied voltage on migration time of the compounds tested in a range of 14–27 kV. Experimental conditions as in Fig. 1 using the optimized run buffer pH of 10.00 ± 0.10 . A range of voltage of 14–16 kV was chosen to be further investigated for obtaining the optimized applied voltage.

3 shows the RSDs for both migration time and peak area of benzoic acid and salicylic acid. The lowest RSD for t_m was obtained at the injection time of 26 s, whereas the lowest RSD for peak area (A_p) was obtained at 28 s. A compromise was made to choose 26 s as the optimized injection time. A representative electropherogram of the acids ($3.0 \mu\text{M}$ for each acid) under the optimized separation conditions, listed in

Section 2.3, shows that all compounds tested are well separated (Fig. 3).

3.4. Quantitation

The calibration data were obtained using the optimized CZE method. The calibration data were used to plot the concentration of the compounds

Table 1

Dependence of separation efficiency for benzoic and salicylic acid in terms of difference in the average migration time (Δt_m) and the percent relative standard deviation (RSD in Δt_m) on the applied voltage

Applied voltage (kV) ^a	Δt_m (min)	RSD in Δt_m
14	0.675	3.85
15	0.702	3.26
16	0.593	5.94

^a The statistical data were calculated based on 10 consecutive runs. Experimental conditions: 13 mM sodium borate at pH 10.00 ± 0.10 ; injection pressure, 0.5 p.s.i.; injection time, 25 s; UV detection, 214 nm; and operation temperature, 22 °C.

Table 2

Dependence of separation efficiency for benzoic and salicylic acid in terms of difference in the average migration time (Δt_m) and the percent relative standard deviation (RSD in Δt_m) on the injection time

Injection time (s) ^a	Δt_m (min)	RSD in Δt_m
24	0.472	3.43
26	0.448	4.81
28	0.391	18.4

^a The statistical data were calculated based on 10 consecutive runs. Experimental conditions: 13 mM sodium borate at pH 10.00 ± 0.10 ; applied voltage, 20 kV; injection pressure, 0.5 p.s.i.; UV detection, 214 nm; and operation temperature, 22 °C.

Table 3

The percent relative standard deviation (RSDs) in migration time (t_m) and peak area (A_p) for benzoic acid and salicylic acid with respect to the injection time

Compound	Injection time (s) ^a					
	24 s		26 s		28 s	
	RSD t_m	RSD A_p	RSD t_m	RSD A_p	RSD t_m	RSD A_p
Benzoic acid	0.869	3.85	0.547	2.96	0.744	2.08
Salicylic acid	0.795	4.06	0.641	3.08	1.14	2.06

^a The statistical data were calculated based on 10 consecutive runs. Experimental conditions: 13 mM sodium borate at pH 10.00±0.10; applied voltage, 20 kV; injection pressure, 0.5 p.s.i.; UV detection, 214 nm; and operation temperature, 22 °C.

versus peak area. A linear relationship was observed in a concentration range of 3.0–1.00×10² μM of the compounds tested. The repeatability of migration time and peak area was examined by 10 replicate injections of a solution containing five acids (3.0 μM for each acid) at pH 3.20±0.05. The RSDs for migration time (t_m) and peak area (A_p) were in the range of 1.2–1.4 and 4.9–14, respectively. The limits of detection (LODs) were determined based on a signal-to-noise ratio of 3. The data of calibration,

repeatability and limit of detection are summarized in Table 4.

3.5. Product identification and reaction mechanism

The reaction mixtures containing 1.00×10² μM benzoic acid or 1.00×10² μM benzoic acid–iron(III) chloride (2.00×10 μM) at pH 3.20±0.05 were irradiated at 300 nm. Samples were taken from the reaction systems as a function of reaction time and

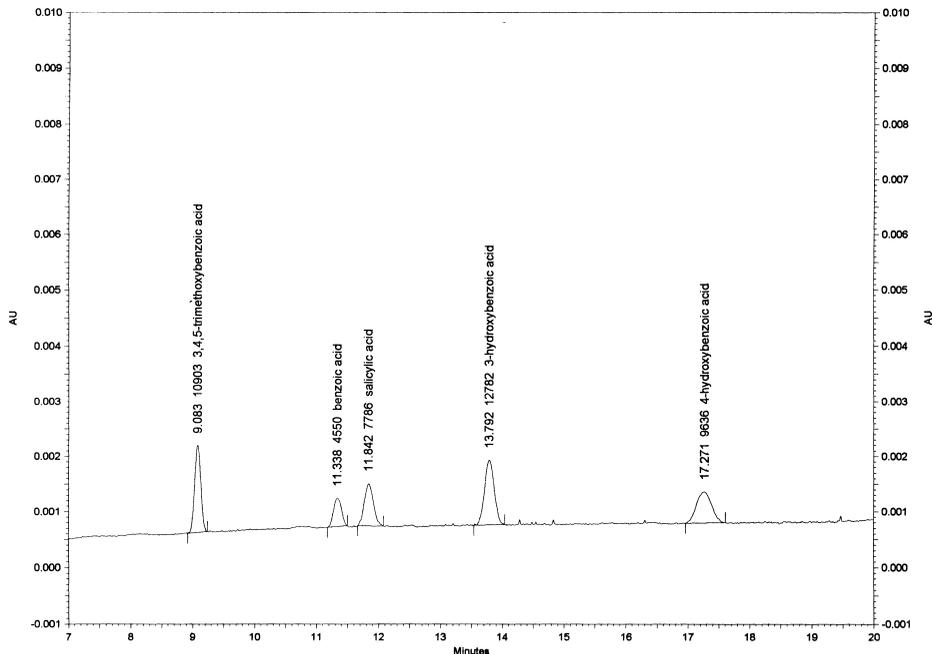


Fig. 3. Separation of five aromatic acids using the optimized CZE conditions: 13 mM sodium borate at pH 10.00±0.10; applied voltage, 15 kV; injection pressure, 0.5 p.s.i.; injection time, 26 s; UV detection, 214 nm; and operation temperature, 22 °C.

Table 4
Calibration data, repeatability and limits of detection for the compounds studied

Compound	Linear range (μM)	Slope	R^2	Repeatability ^a (RSD, $n = 10$)		LOD (μM) ^b
				t_m	A_p	
3,4,5-Trimethoxybenzoic acid	3.0–1.00 $\times 10^2$	3.4 $\times 10^3$	0.99	1.2	4.9	0.39
Benzoic acid	3.0–1.00 $\times 10^2$	1.3 $\times 10^3$	1.0	1.4	9.5	1.1
Salicylic acid	3.0–1.00 $\times 10^2$	1.9 $\times 10^3$	1.0	1.3	14	0.82
3-Hydroxybenzoic acid	3.0–1.00 $\times 10^2$	3.5 $\times 10^3$	0.99	1.2	7.2	0.52
4-Hydroxybenzoic acid	3.0–1.00 $\times 10^2$	2.6 $\times 10^3$	0.99	1.3	8.8	1.1

^a Percent relative standard deviations (RSDs) were calculated based on the migration time (t_m) and peak areas (A_p) by making 10 replicate injection of a solution containing five acids (3 μM for each compound) at pH 3.20 \pm 0.05, using the optimized CZE conditions: 13 mM sodium borate at pH 10.00 \pm 0.10; applied voltage, 15 kV; injection pressure, 0.5 p.s.i.; injection time, 26 s; UV detection, 214 nm and operation temperature, 22 °C.

^b The limits of detection (LOD) were determined based on a signal-to-noise ratio of 3.

analyzed by the CZE method developed in this study. The results showed that the simultaneous presence of benzoic acid, iron as a catalyst and light was necessary for the reaction to take place within a reaction time frame of 210 min. This observation indicates that iron plays a critical role on photodegradation of benzoic acid. The appearance of four new peaks was observed for a sample taken from the benzoic acid–iron(III) chloride system at a reaction time of 210 min (Fig. 4a). In general, the identity of a product can be established by comparing the migration time of the product with that of a known compound spiked into the sample. Enhancement of an existing peak at a given migration time in an electropherogram provides evidence that identity of the product in the sample was likely the same as that of the compound spiked. Identification of the reaction products was carried out by spiking 50 μl of one test compound (1.00 $\times 10^2$ μM , pH 3.20 \pm 0.05) into the sample taken from the benzoic acid–

iron(III) chloride system at the reaction time of 210 min. Four reaction products including salicylic acid, 3-hydroxybenzoic acid, 4-hydroxybenzoic acid and 3,4-dihydroxybenzoic acid were identified by comparing their migration times with those of the known compounds (Fig. 4a–e). Table 5 gives the concentrations of the products estimated using the calibration data listed in Table 4. Since compounds with similar charge-to-mass ratios could possibly present in the sample, they could appear at the same migration time. Thus, identity of the reaction products found in this study need to be further confirmed by other analytical methods such as HPLC.

The role that iron plays in the photodegradation of benzoic acid and the identification of the reaction products provide useful information on proposing reaction mechanisms. Benzoic acid has two absorption bands at wavelengths of 228 and 274 nm, respectively (Fig. 5). Therefore, direct photodegradation of benzoic acid would be difficult to proceed at 300 nm because the absorption bands of benzoic acid do not significantly overlap with the wavelength (300 nm) of the lamps. Indirect photodegradation, however, may occur as iron(III) hydroxide complexes $\text{Fe}(\text{OH})_2^+$ and $\text{Fe}(\text{OH})_4^-$ have weak absorption bands that extend into the wavelength region of 290–400 nm [9]. The iron hydroxide complexes are considered to be one of the major photochemical precursors to hydroxyl radical (OH^\cdot) formation [18]. A recent study shows that in situ photochemical generation of hydroxyl radicals in clouds and fog

Table 5
Estimation of the concentrations of major reaction products generated from the benzoic acid–iron (III) chloride system irradiated at 300 nm for 210 min, using the calibration data listed in Table 4

Compounds	Concentration (μM)
Benzoic acid	40.9
Salicylic acid	4.8
3-Hydroxybenzoic acid	3.3
4-Hydroxybenzoic acid	3.8

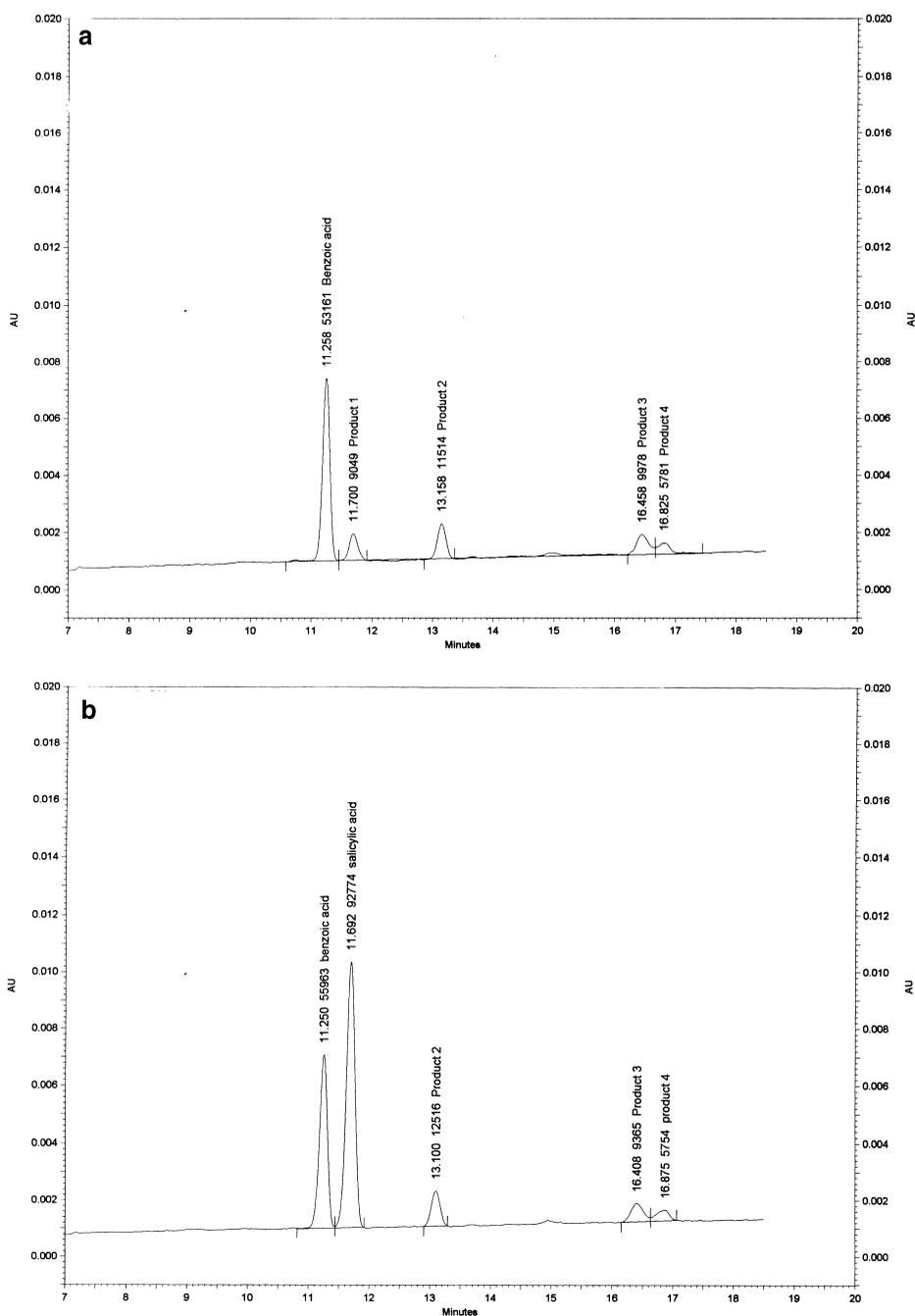


Fig. 4. Identification of the reaction products by spiking known compounds into the sample taken from the benzoic acid–iron (III) chloride system irradiated at 300 nm for 210 min. (a) Sample without spiking; (b) spiked salicylic acid; (c) spiked 3-hydroxybenzoic acid; (d) spiked 4-hydroxybenzoic acid; and (e) spiked 3,4-dihydroxybenzoic acid. The separation was carried out using the optimized CZE conditions.

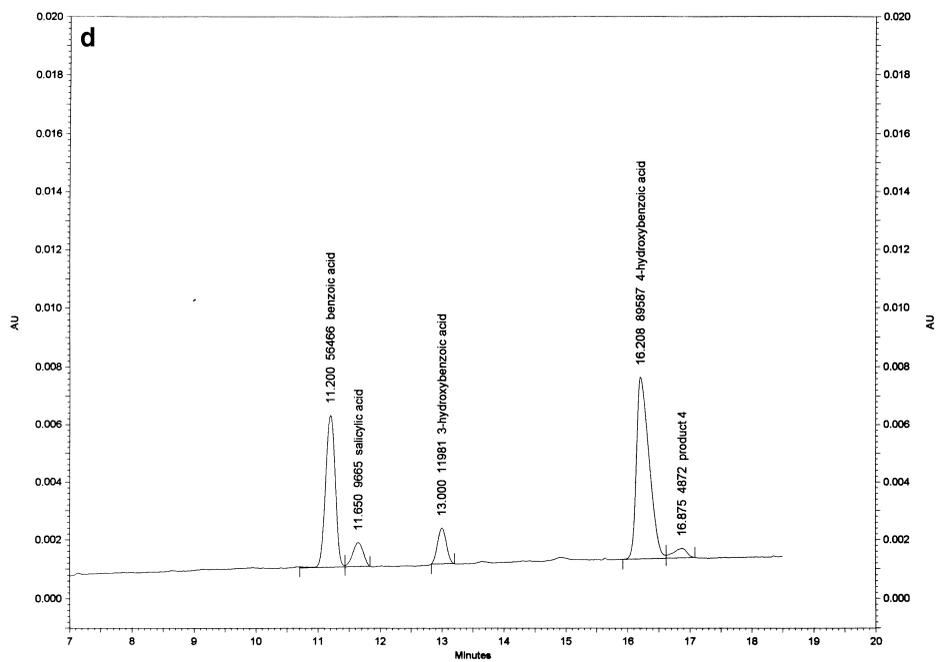
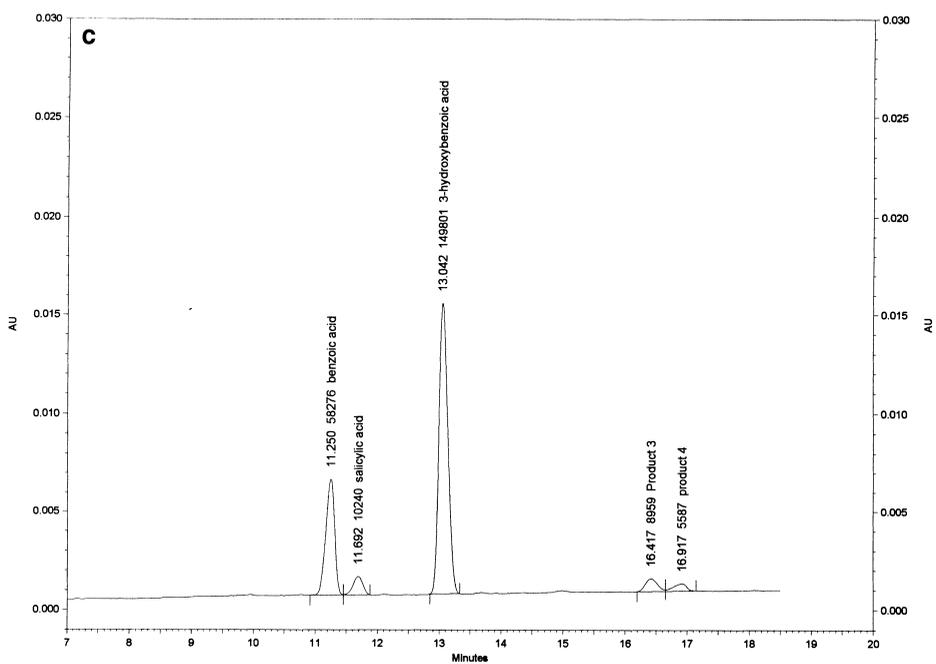


Fig. 4. (continued)

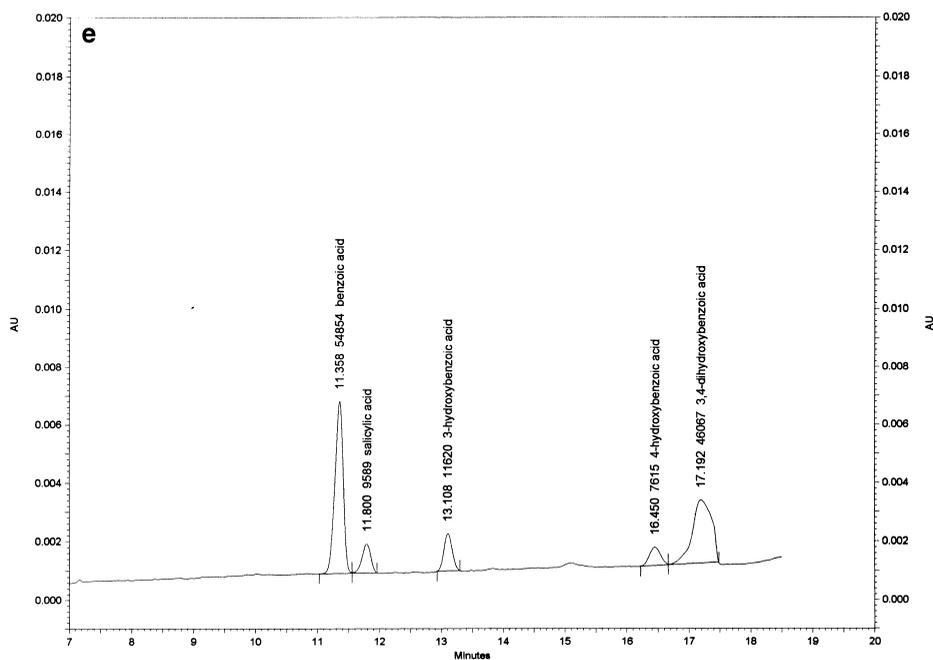


Fig. 4. (continued)

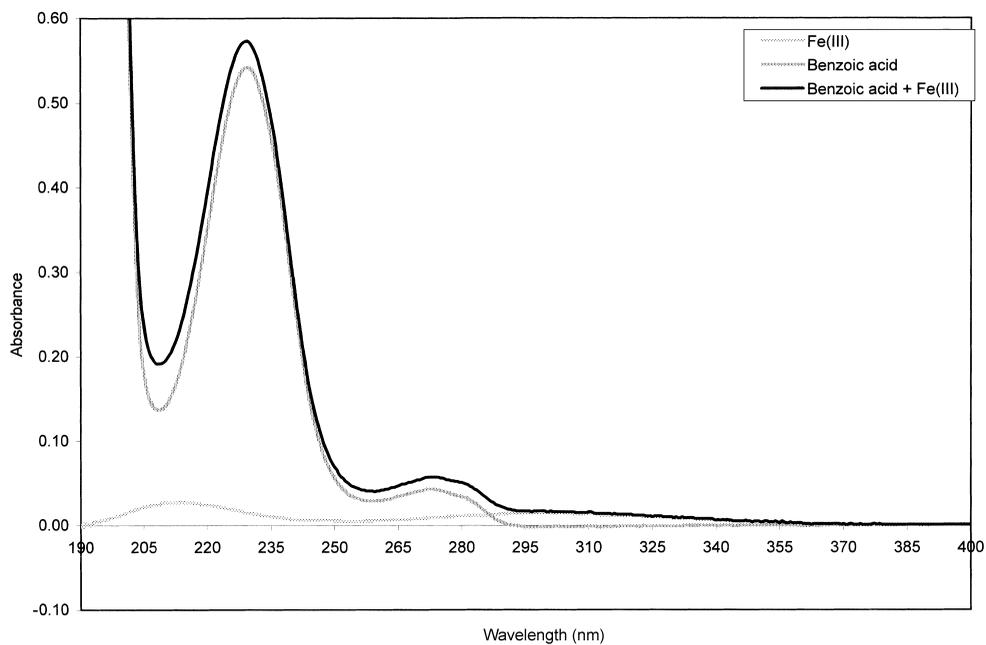
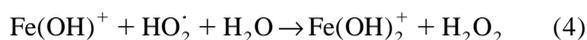
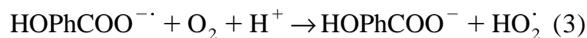
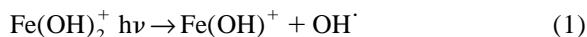


Fig. 5. Absorption spectra of benzoic acid, iron (III) chloride and a mixture of iron(III) and benzoic acid. The solutions contained $1.00 \times 10^{-2} \mu\text{M}$ benzoic acid, $2.00 \times 10^{-2} \mu\text{M}$ FeCl_3 and $1.00 \times 10^{-2} \mu\text{M}$ benzoic acid with $2.00 \times 10^{-2} \mu\text{M}$ FeCl_3 , respectively. All these solutions had a pH value of 3.20. The absorption spectra of the solutions were obtained at a wavelength range of 190–400 nm.

water may present a significant source of hydroxyl radicals in the atmosphere [19]. The hydroxyl radicals react with benzoate ions (PhCOO^-), forming hydroxycyclohexadienyl radical as an intermediate, and subsequently yielding monohydroxybenzoates (i.e. salicylic acid, 3-hydroxybenzoic acid and 4-hydroxybenzoic acid) [14]. The possible reaction mechanism is proposed as follows:



Once the monohydroxybenzoates were produced, they might be further attacked by hydroxyl radicals and/or hydroperoxyl radicals (HO_2^\cdot), generating dihydroxybenzoic acids such as 3,4-dihydroxybenzoic acid. More information is still needed to postulate a complete reaction mechanism for all products identified.

4. Conclusions

A CZE method was developed and used for separation and identification of reaction products of photodegradation of benzoic acid. Parameters such as run buffer pH, applied voltage and sample injection time were optimized so that benzoic acid and its reaction products were well separated within 18 min. A good linearity was observed in the concentration range of $3.0\text{--}1.00 \times 10^2 \mu\text{M}$ with detection limits of $0.39\text{--}1.1 \mu\text{M}$. The repeatability of migration time and peak area in terms of their percent relative standard deviations was in the range 1.2–1.4 and 4.9–14, respectively. Four reaction

products, mainly monohydroxybenzoates, were identified. The reaction mechanism under the conditions studied involves the generation of hydroxyl radical followed by its reaction with benzoic acid.

Acknowledgements

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