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# Investigation of a pulsed-laser thermo-optical absorbance detector for the determination of food preservatives separated by capillary electrophoresis

Karen C. Waldron*, Jianjun Li

*Département de Chimie, Université de Montréal, C.P. 6128, Succursale Centre-Ville, Montréal, Québec H3C 3J7, Canada*

**Abstract**

Thermo-optical absorbance (TOA) detection using a KrF excimer waveguide laser for detection of benzoic acid, dehydroacetic acid and sorbic acid separated by capillary electrophoresis (CE) was studied. Detection limits were, on average, ten times better than those for on-column UV absorbance methods with CE, and two or more times better than those for UV absorbance with HPLC. The influence of increased laser power on TOA detection sensitivity was found to be strong for benzoic and dehydroacetic acids but quite weak for sorbic acid. It was discovered that photoisomerization of sorbic acid (2,4-hexadienoic acid) occurred readily in the detection volume at moderate laser powers ($P_{ave}$=3 mW) and increased with slow electroosmotic flows (<6 cm/min). The TOA method described here shows improved detection sensitivity for CE analyses of compounds having only weak absorptivities (<5% of maximum) at $\lambda$=248 nm, and thus demonstrates its utility for determination of a variety of analytes in a single separation.

*Keywords:* Pulsed-laser thermo-optical absorbance detection; Food preservatives; Benzoic acid; Dehydroacetic acid; Sorbic acid

## 1. Introduction

Benzoic acid, dehydroacetic acid and sorbic acid (and their salts) are antimicrobial agents added to a variety of food products, ranging from soft-drinks to cheese. These preservatives prevent biological deterioration of food and, along with antioxidants that prevent chemical deterioration, represent just a fraction of the total number of usable food additives. Although the Canadian government continues to regulate benzoic and sorbic acids, they are generally not considered a health risk at the current levels of use. Dehydroacetic acid, though, is not permitted as a food additive in Canada. Other than random spot-checks at regional laboratories to verify compliance with food and drug regulations, the Health Protection Branch (HPB) of Health Canada typically only does systematic analyses following consumer complaints. However, since these preservatives are regulated and restricted to certain foods [1,2] their determination and quantification are necessary.

Both the HPB and the Inspection Branch of Agriculture Canada use a variety of accepted AOAC (Association of Official Analytical Chemists) analytical methods for analysis of food additives, but liquid chromatography with UV absorbance detection

*Corresponding author.

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is the most common. Several LC methods for similar analyses have appeared in the recent literature [2–5], as well as GC–MS techniques for food preservative determination [6]. The increased number of articles describing capillary electrophoresis (CE) and micellar electrokinetic chromatography (MEKC) for food additive analyses [5,7–10] attests to its rapid, simple and inexpensive nature for analysing small amounts of sample. At least two groups, Massart's [5] and Trenerry's [9,10], have compared CE methods to HPLC (using UV absorbance) for determination of food additives in terms of repeatability, reproducibility, accuracy, linearity, sensitivity and separation efficiency.

Benzoic (BA), dehydroacetic (DHA) and sorbic acid (SA) preservatives are added to foods individually or as mixtures in concentrations up to 0.1% (by food weight), as permitted by legislation. Therefore, the simultaneous and sensitive determination of these chemicals is required. In both LC and CE, absorbance detection at a single wavelength is typically used. Unfortunately, the absorbance maxima and absorptivities of BA, DHA and SA are not well matched so they are analysed individually or a compromise detection wavelength near 233 nm is used [3,4,10]. For the very short pathlengths encountered in CE ($\leq$75 mm), absorbance-based measurement of analyte at a wavelength away from its maxima can be costly in terms of detection limit. A scanning UV detector or photodiode array would obviously circumvent the wavelength selection problem, but not the signal dependence on pathlength problem. Another option is thermo-optical absorbance (TOA) detection in the UV. The TOA technique is well suited to detection in capillary tubes because the signal is not dependent on the optical pathlength of the excitation beam. Although excitation is typically at a single wavelength, TOA has previously demonstrated excellent detection sensitivity for analytes that have absorbance maxima as far as 20 nm away from the excitation wavelength [11,12]. In this work the use of capillary zone electrophoresis–thermo-optical absorbance (CZE–TOA) for simultaneous separation and determination of three food preservatives is presented and the effect of the TOA detector on sorbic acid detection is investigated.

## 2. Experimental

### 2.1. Materials

Benzoic acid, dehydroacetic acid, sorbic acid and sodium tetraborate were purchased from Sigma (St. Louis, MO, USA) and used without further purification. Sodium monohydrogenphosphate was from Anachemia (Montreal, Canada). Cetyltrimethylammonium bromide (CTAB) and HPLC-grade methanol were purchased from Aldrich (Milwaukee, WI, USA). Sodium hydroxide was obtained from BDH (Toronto, Canada). In-house distilled water was passed through a multi-cartridge Millipore water filtration–deionization system before using. Nylon syringe filters, 0.2 $\mu$m pore size, were purchased from Chromatographic Specialties (Brockville, Canada). Fused-silica capillary tubing coated with polyimide was purchased from Polymicro Technologies (Phoenix, AZ, USA). Platinum wire and 600-ml microcentrifuge tubes were obtained from Fisher Scientific (Montreal, Canada).

### 2.2. Buffer, standard and sample preparation

Water, filtered through the Millipore system, was used to make all buffers and standards. A 1.2-ml volume of buffer was re-filtered through a syringe filter for use in the electrophoresis buffer reservoirs. CZE running buffer consisted of 25 m*M* sodium phosphate ranging in pH from 6 to 11. MEKC running buffer consisted of 7.5 m*M* sodium phosphate, 7.5 m*M* sodium tetraborate, 15 m*M* CTAB, pH 8. Stock solutions of 1 mg/ml BA, 1 mg/ml DHA and 1 mg/ml SA were prepared in water. Further dilutions were made in running buffer. Food samples for analysis were diluted 2–25-fold in running buffer, depending on the analyte concentration, and then passed through the syringe filter.

### 2.3. Apparatus

The CE–TOA detection system was built in-house, similar to the design described by Waldron and Dovichi [12], as shown in Fig. 1. All components were assembled on an optical breadboard. Separations were performed at room temperature in a

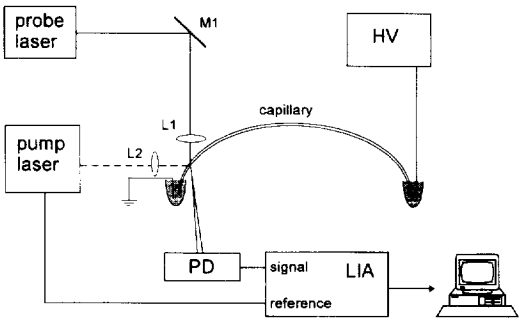



Fig. 1. Schematic of capillary electrophoresis–thermo-optical absorbance detection system (CE–TOA). M1=mirror; L1=microscope objective; L2=plano-convex lens; HV=high voltage power supply; PD=photodiode; LIA=lock-in amplifier. All other components are described in detail in the text.

45-cm-long 50-$\mu$m-I.D., 190-$\mu$m-O.D. fused-silica capillary tube with high voltage provided by a Spellman CZE1000R power supply (Plainview, NY, USA). Samples and standards were injected hydrodynamically at a height of 6 cm for 30 s. On-column detection was performed 40 cm from the high voltage end of the capillary, where a 1-cm length of polyimide coating had been burned off.

A KrF excimer waveguide laser (Potomac Photonics, Lanham, MD, USA), $\lambda$=248 nm, with average output power 12 mW, operated at 553 Hz was used for the pump (excitation) beam. Quartz neutral density filters (Melles Griot, Nepean, Canada) were used to attenuate the power, measured with a Scientech-372 Power Meter. The probe (detection) beam was a 0.5-mW helium–neon laser (Newport, Pierrefonds, Canada), $\lambda$=633 nm. The two lasers were arranged in a co-planar fashion, intersecting the capillary at right angles. The pump beam was focused onto the capillary lumen such that absorption of analyte induced a temperature rise in the surrounding solution, creating a refractive index gradient. The probe beam was focused about 5 mm off centre and the refracted and deflected beam was detected with a photodiode (New Focus Front-End Receiver, Sunnyvale, CA, USA) placed 17 cm from the capillary. Changes in the probe beam intensity, proportional to analyte concentration, were phase-referenced to the pump laser frequency using a Stanford Research Systems lock-in amplifier (Sunnyvale, CA, USA). Digital output from the lock-in amplifier was collected on a Pentium computer equipped with hardware (GPIB and I/O boards) and development software (LabView) from National Instruments (Austin, TX, USA). High-voltage control and data acquisition (at 4 Hz, $\tau$=1 s) were achieved using an in-house program written with LabView.

For comparison to the CZE–TOA detection system, detection limit measurements for BA, DHA and SA were made on a SpectroPHORESIS 100 capillary electrophoresis system (Thermo Separations Products, Mississauga, Canada) equipped with a variable-wavelength UV absorbance detector and using a 70-cm-long, 50-$\mu$m-I.D., 360-$\mu$m-O.D. (40 cm to detector) capillary. Injection volumes were on the order of 5 nl, injected by vacuum. UV absorption spectra of BA ($\varepsilon_{248\ nm}$=330 $M^{-1}cm^{-1}$), DHA ($\varepsilon_{248\ nm}$=4100 $M^{-1}cm^{-1}$) and SA ($\varepsilon_{248\ nm}$=21 500 $M^{-1}cm^{-1}$) were run in 1-cm cells on a Varian Instruments Cary 5E spectrophotometer.

## 3. Results and discussion

### 3.1. Effects of TOA detection on BA, DHA and SA

Thermal lens measurements in single and crossed-beam configurations for both static and flowing samples have been well documented [13]. In crossed-beam thermal lens techniques, signal is typically proportional to pump laser power, therefore, high power lasers should improve the detection of weakly absorbing species. The UV absorbance spectra for BA, DHA and SA are shown in Fig. 2. Even though benzoic and dehydroacetic acids absorb weakly at the detection wavelength ($\lambda$=248 nm) a large TOA signal was obtained. Detection limits of 0.27 $\mu$g/ml BA, 0.25 $\mu$g/ml DHA and 0.93 $\mu$g/ml SA were determined (at 3$\sigma$) for an average pump laser power of 3 mW. The SA detection limit was higher than expected based on its molar absorptivity at 248 nm, suggesting that a photoinduced reaction may be occurring during detection. To study the effect of average pump laser power on analyte TOA signal, separations were performed with detection at different powers as shown in Fig. 3. Powers higher than 3

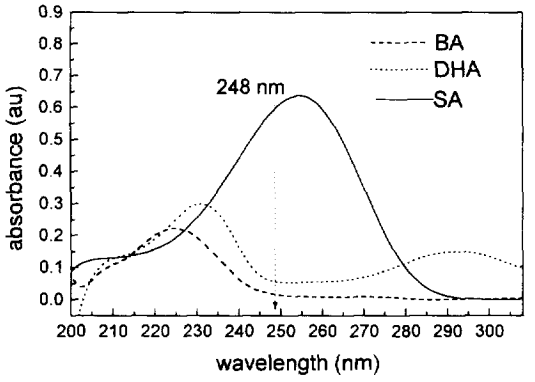



Fig. 2. Absorbance spectra of 3.3 μg/ml BA, 2.9 μg/ml DHA and 2.9 μg/ml SA in water.

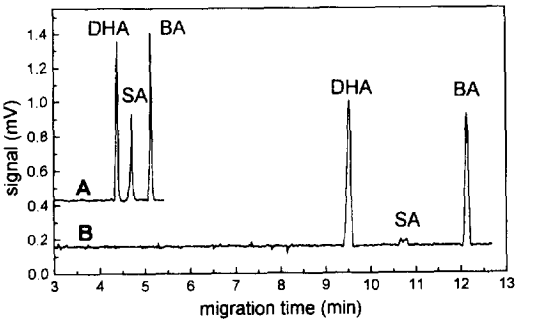



Fig. 4. Electropherograms of food preservative mixture comparing fast and slow electroosmotic flow-rates. (A) Separation at +15 kV, 43 μA, in 25 m*M* sodium phosphate, pH 11.0 buffer; (B) separation at +10 kV, 20 μA, in 25 m*M* sodium phosphate, pH 6.0 buffer.

mW were not used as they tended to damage the capillary; the use of thicker-walled capillaries are anticipated to overcome this problem. The expected increase in TOA signal with increasing laser power was observed for BA and DHA (Fig. 3) but not for SA.

Suspicion that SA was decomposing or reacting within the detection volume of the capillary was supported by the observation that the SA peak height decreased when analyte flow decreased. The electropherograms in Fig. 4 show that when the effective velocity of SA was reduced from 8.5 cm/min (Fig. 4A) to 3.7 cm/min (Fig. 4B), the SA peak was observed to decrease 92%. This reduction was consistent with SA decomposing in the detection volume when it was exposed to a longer period of illumination at the lower flow-rate. The appearance

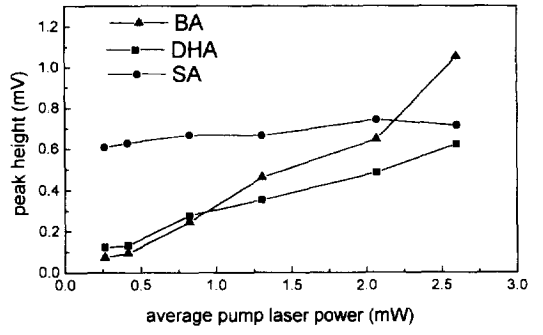



Fig. 3. Effect of laser power on peak height for BA, DHA and SA. The pump beam was attenuated with optical filters and separations run at +10 kV, 21 μA, in 25 m*M* sodium phosphate buffer, pH 11. All other conditions were as described in the text.

of two small peaks for SA in Fig. 4B suggested that two decomposition products may have been formed. All subsequent separations with SA were performed with an attenuated pump laser beam ($P_{ave}$=1 mW) which adequately reduced decomposition. The detection limit for SA was improved by a factor of two (0.47 μg/ml) at the lower laser power. Because a significant amount of heat could potentially be generated in the TOA detection volume, the nature of SA decomposition was investigated by both heating and irradiating solutions of SA.

Solutions of 50 μg/ml SA in water and in buffer (pH 10 and 2.5) were heated at 100°C for 5, 15 and 30 min. Injection of the solutions into the CZE–TOA system showed neither change in the SA peak nor evidence of decomposition. However, direct irradiation ($\lambda$=248 nm, $P_{ave}$=12 mW) of SA solutions for 5, 15 and 30 min showed increasing formation of two products, as indicated in Fig. 5B, C and D respectively. Similar results were obtained by irradiating (>1 h) a SA solution with a hand-held UV lamp operating at 254 nm. Attempts to identify the photoproducts in the mixture by UV–Vis absorbance, electron impact MS and GC–MS suggested the presence of SA isomers. To determine if more than two photoproducts existed, the irradiated mixture in water was analysed by MEKC–TOA in a CTAB buffer. The cationic surfactant CTAB was chosen for MEKC separation since sorbic acid, and presumably its photoproducts, are anionic above pH 5. The electropherogram in Fig. 6 shows the MEKC sepa-

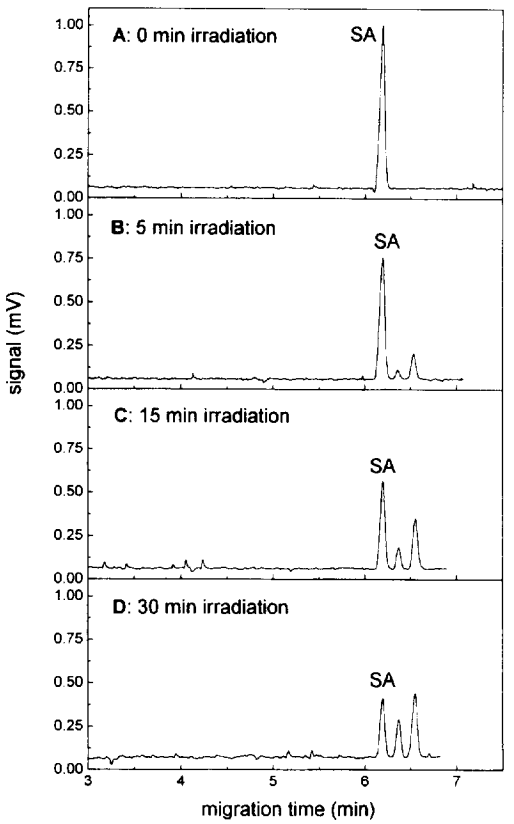



Fig. 5. Electropherograms showing the separation of SA and products formed by direct irradiation ($\lambda$=248 nm) of 500 $\mu$l of 50 $\mu$g/ml SA in water. (A) No irradiation; (B) 5 min irradiation; (C) 15 min irradiation; (D) 30 min irradiation. All separations were run at +15 kV, 43 $\mu$A, in 25 m*M* sodium phosphate buffer, pH 9.0.

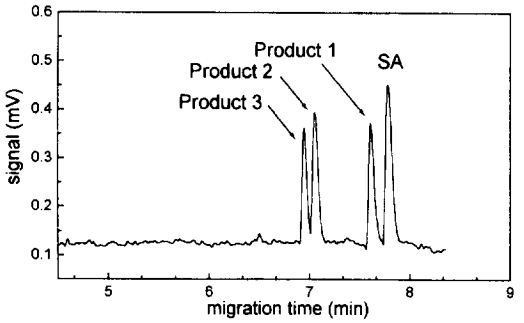
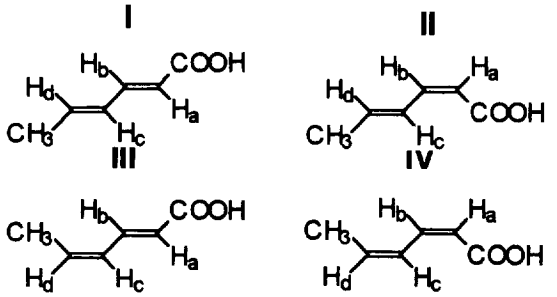



Fig. 6. MEKC electropherogram showing the separation of SA and products formed by direct irradiation ($\lambda$=248 nm) for 120 min of 500 $\mu$l of 14 $\mu$g/ml SA in methanol. Separation was run at −10 kV, in 15 m*M* CTAB, 7.5 m*M* phosphate, 7.5 m*M* borate, pH 8.0 buffer.

ration of irradiated SA solution, indicating that at least three photo-induced products were formed during irradiation. Based on the $^1$H NMR spectra of SA after irradiation, the proton $H_a$ (structure **I**) was seen to be present in two *cis* forms and two *trans* forms. It was, therefore, presumed that the *trans–trans* form of SA (starting compound) was undergoing photoisomerization to form products **II–IV** shown below. The following peak assignments for Fig. 6 were made, based on the NMR data: product 1=**III**; product 2=**II**; product 3=**IV** [14].




Armed with the evidence that photoisomerization occurs with irradiation at 248 nm, the appearance of the two small peaks for SA (Fig. 4B) detected at 3 mW laser power suggests that isomerization occurred within the detector volume and separation of the isomers had begun. The size of these two small peaks implies minimal analyte absorbance at $\lambda$=248 nm and therefore isomerization may proceed via a cyclic transition state that has no appreciable absorbance at this wavelength. The solvent and concentration dependence on the rate of SA photoisomerization, and the mechanism of formation of the photoisomers is still under investigation.

### *3.2. Determination of preservatives in food*

Antimicrobial activity is due to the undissociated forms of BA, DHA and SA [1], all of which have p$K_a$ values below 5.30. Therefore, these compounds are added to foods that have pH>5.3 in fairly high quantities (1%, w/w) in order to have enough in the undissociated form for preservation. This high level of preservatives in food would suggest that sensitive detection is unnecessary. However, analyses are greatly simplified when food samples can be diluted

generously in solvent, which requires high sensitivity detection methods. The versatility of CE makes it amenable to injecting food samples diluted in any solvent with little prior cleanup. In addition, a detection method like TOA that permits concurrent determination of several food additives can expedite analyses.

Since most foods have pH>5, CZE separation of only the dissociated forms of BA, DHA and SA were investigated. For pH values ranging from 6 to 11 in 25 m*M* phosphate buffer, the effect of pH on migration time was determined and results are presented in Fig. 7. The rates of change in migration time with buffer pH became equivalent above pH 8 for BA, DHA and SA because of the dominating effect of electroosmotic flow at high pH values. In the relationship $t_m \propto 1/(\mu_{eo}+\mu_{ep})$, electroosmotic mobility ($\mu_{eo}$) and electrophoretic mobility ($\mu_{ep}$) were in opposite directions for BA, DHA and SA. Since all three food preservatives were well separated over the entire pH range investigated, pH 10 was considered optimal to maintain speed of analysis, separation efficiency and SA sensitivity. Migration time reproducibility was within 2% R.S.D. and maximum peak efficiencies of 140 000 theoretical plates were observed.

CZE–TOA identification of BA, DHA and SA was compared to recent reports for food additive analyses. The TOA detection limits, summarized in Table 1, were typically 2–4-fold better than for LC-based methods: 0.5 $\mu$g/ml BA by HPLC [3,5]; 1 $\mu$g/ml BA and 0.5 $\mu$g/ml DHA-Na by paired-ion

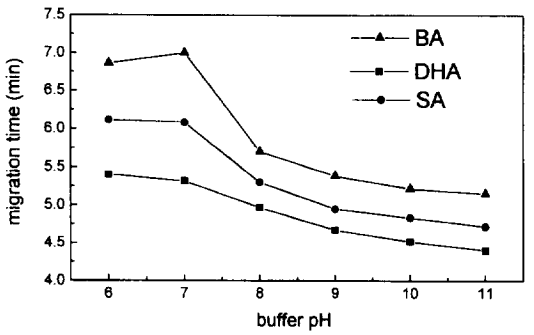



Fig. 7. Effect of buffer pH on migration time for BA, DHA, SA. Separations were run at +15 kV, in 25 m*M* sodium phosphate buffers ranging from pH 6.0 to 11.0. All other conditions were as described in the text.

Table 1
Comparison of food preservative detection limits between the commercial CE and the CZE–TOA systems

| $\lambda$ (nm) | Detection limit[a] ($\mu$g/ml) | | | | | |
|---|---|---|---|---|---|---|
| | Commercial CE[b] | | | CZE–TOA | | |
| | BA | DHA | SA | BA | DHA | SA |
| 235 | 5.4 | 1.5 | 0.64 | | | |
| 248 | 21 | 4.8 | 0.40 | 0.27 | 0.25 | 0.47 |
| 254 | 27 | 4.6 | 0.34 | | | |

[a]Signal-to-noise ratio of 3.
[b]SpectroPHORESIS 100 capillary electrophoresis system.

LC [4]. In addition, the CZE separations were about 2-times faster than LC separations. Compared to reported detection limits for CZE and MEKC methods, the TOA values were improved by an order of magnitude or more [5,9]. The exception was a report by Kaniansky et al. [7] for SA detected at $\lambda$=254 nm in a 300-$\mu$m-I.D. capillary with 90-nl injection where they achieved a detection limit of 0.06 $\mu$g/ml. With respect to mass detection limits, CZE–TOA results (1.3 pg BA, 1.2 pg DHA, 2.2 pg SA) were typically three orders of magnitude better than reported literature values for LC, TLC and GC–MS methods [3–6,15].

A direct comparison of TOA detection (for BA, DHA and SA) with on-column UV absorbance detection for CZE was made using the commercial system which was equipped with a ball lens to focus light through the capillary lumen [16]. Detection limits for BA, DHA and SA determined for this commercial system are presented in Table 1 alongside the CZE–TOA detection limits for comparison. It is interesting to note that the detection limits calculated for the commercial CE instrument were the same order of magnitude as those reported recently for LC determinations [3,4].

To verify the CZE–TOA technique for determination of preservatives in food samples, two diet soft-drinks (cola and lemon-lime), Smart Drink (energy soft-drink) and soy sauce were analysed. Samples were prepared as described in the experimental section. Soft-drink samples were injected onto the CZE–TOA system and the analyte peak height converted to concentration using analyte calibration curves. The soy sauce sample was analysed by standard additions because the sample

matrix caused a noisy background. The average amounts of preservative from replicate analyses were found to be: 30 μg/ml BA in the cola; 195 μg/ml SA in the lemon-lime drink; 85 μg/ml BA and 81 μg/ml SA in the energy drink; 230 μg/ml BA in the soy sauce. Manufacturers are not required by law to list the quantities of each food additive; however, the BA and SA values were in agreement with levels reported for similar products as determined by LC or CE methods. The energy drink contained several other organic acids though these did not interfere with our determination of BA and SA.

Sample matrix effects in CE separations are often a concern because they may affect the analyte signal or cause fouling of the capillary walls. The standard additions method can be used to overcome such matrix effects on the analyte signal. In addition, the luxury of simply rinsing a fused-silica capillary with a few microlitres of NaOH and HCl to regenerate the capillary surface makes it easy and cost effective for testing any type of liquid sample without prior rigorous analyte extraction. This flexibility makes CE a more attractive method for determination of food additives than LC. For solid food samples, any well documented extraction method (methanol, solid-phase, etc.) could be used taking care to minimize elution and wash volumes whenever possible.

## 4. Conclusions

Capillary electrophoresis has been slow in gaining acceptance as a routine method of analysis because of the limitations of on-column absorbance detection compared to HPLC. This work has demonstrated that TOA (thermo-optical absorbance) detection at $\lambda=$ 248 nm gives better limits of detection for CZE separation of benzoic and dehydroacetic acid, compared to HPLC with UV absorbance. When combined with previously reported detection limits of $8 \cdot 10^{-7}$ *M* phenylthiohydantoin valine ($\lambda_{max}=267$ nm) measured on a similar instrument [12], we find that TOA provides at least an order of magnitude better detection than classical CZE and MEKC absorbance methods for compounds having broad absorbance spectra with maxima between 225 nm and 270 nm. For example, at $\lambda=248$ nm, the absorptivity of BA was less than 5% of its maximum yet a detection limit of 0.27 μg/ml BA was achieved. While it is not universal, the utility of the TOA detector means that sensitive detection of many organic compounds separated simultaneously by CE is possible without requiring a multi-wavelength detection method. Absolute detection limits with the CZE–TOA technique were three orders of magnitude better than HPLC with UV absorbance detection.

Unexpectedly, sorbic acid was found to readily undergo photoisomerization when irradiated at $\lambda=$ 248 nm, causing poor detection sensitivity in CZE–TOA analysis. Further investigations involving MEKC–TOA of the SA isomers are underway to study the photoisomerization process. Because sorbic acid and its esters appear in pharmaceuticals, cosmetics and food packaging, reaction with UV light may be an important factor towards its toxicity and microbial activity.

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