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## Capillary electrophoresis of triorganotin compounds with indirect fluorescence detection

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### Abstract

A capillary electrophoresis–indirect fluorescence detection method for triorganotin compounds was developed. The five triorganotin cations, viz., trimethyltin, triethyltin, tripropyltin, tributyltin and triphenyltin, can be completely separated by capillary electrophoresis with a zwitterionic buffer of 70 mM *N*-2-hydroxyethyl-piperazine-*N'*-2-ethanesulfonic acid (HEPES) at pH 4.0. The running buffer also contains 1 mM 6-aminoquinoline (6-AQ) as the background fluorophore. Indirect fluorescence detection was performed using a non-laser-based fluorometer. The concentration limits of detection for the five triorganotin compounds were in the range 8–18  $\mu\text{M}$  (as tin), which correspond to a mass detection level of 80–180 fmol tin. The relative standard deviations were between 3.5 and 8.6%.

**Keywords:** Detection, electrophoresis; Triorganic compounds; 6-Aminoquinoline; Organotin compounds

### 1. Introduction

Organotin compounds are widely used in the plastics industry as stabilizers and in agriculture as insecticides, fungicides and biocides [1]. The utility of tributyltin (TBT) and triphenyltin (TPhT) in marine anti-fouling paints is particularly significant [2,3]. As trialkyltin and triaryl tin are among the most toxic of the tin compounds, and as their annual consumption grows steadily, there is increasing concern over their presence in the environment.

Different methods have been proposed to separate and quantitate organotin compounds. The most commonly used separation technique is gas chromatography (GC) with flame photometric detection (FPD) [4,5], electron-capture detection (ECD) [6,7] or atomic emission detection (AED) [8,9]. GC methods

have high-resolution power and can be connected to very sensitive detectors. However, in order to enhance the volatility and detectability of ionic organotin species, sample derivatization is typically necessary prior to analysis. Both hydride generation with  $\text{NaBH}_4$  [10,11] and alkylation via a Grignard reaction [4,12–14] are commonly used. Alternatively, the more labile alkyltin halides can be readily chromatographed with a GC provided that hydrogen halide is added to the carrier gas stream at a significant level [5,7]. This strategy eliminates tedious prior preparations of derivatives off the column. High-performance liquid chromatography (HPLC) has also been employed for the separation of organotin compounds. Although tedious derivatization and clean-up procedures are generally not required by HPLC methods, detection of alkyltin presents a problem because its lack of chromophore precludes the direct use of UV detection. Several alternative

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methods, such as indirect UV detection [15], complex reaction detection [16,17] and element-specific atomic spectroscopic detection [18,19] have been employed to overcome this problem.

Recently, there has been an increasing interest in the separation and determination of ionic and ionizable compounds using capillary electrophoresis (CE) owing to its high efficiency and simplicity [20]. Because of the small capillary dimensions encountered and the minuscule sample zone generated in CE, on-column detection methods, such as UV absorption and fluorescence detection, are the most commonly used. However, direct detection of organotin compounds by UV absorption or fluorescence is difficult because most of the organotins possess poor chromophoric and/or fluorophoric properties. Indirect detection, which is based on the principle of displacement of a visualization buffer component by the analyte to yield a decrease in the background signal, may alternatively be employed [21]. Both direct and indirect UV detection of alkyltin compounds separated by CE [22] and micellar electrokinetic chromatography (MEKC) [23] have recently been reported. Analysis of di- and trimethyltin by CE coupled with a microwave-induced plasma atomic emission detector (MIP-AED) has also been demonstrated [24]. On the other hand, fluorescence detection of organotins has not yet been applied to CE.

In this paper, we describe an indirect fluorescence-detection method for CE of triorganotin compounds. The application of indirect fluorometry to CE has been reviewed by Yeung and Kuhr [25] and Amankwa et al. [26]. To our knowledge, this is the first report on using fluorometry for CE of triorganotins. Fluorescence detection in CE has most often been carried out with lasers as the excitation source. While lasers can produce spectacular detection performance, they also suffer from a number of disadvantages such as high cost and complex operation. Besides, the choice of available excitation wavelengths is rather limited. In this work, a commercial fluorometer built with a 200-W xenon-mercury arc lamp as the excitation source was used for fluorescence detection. The effect of buffer pH, concentration of background electrolyte (BGE) and content of organic modifier on both resolution of triorganotins and detector response are discussed. The sensitivity and detection limits are also reported.

## 2. Experimental

### 2.1. Apparatus

The CE system was assembled in-house. A high-voltage power supply (Model PS/MJ30P0400-11; Glassman High Voltage, Whitehouse Station, NJ, USA) was used to generate the potential across the capillary. Fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) were of 50  $\mu\text{m}$  I.D.  $\times$  360  $\mu\text{m}$  O.D.  $\times$  64 cm total length. Before use, the capillary was washed (pressurized flow) with 0.1 M NaOH for 15 min, followed by a 5-min rinse with water and a 2-min flush with the running buffer. The capillary was then equilibrated with the buffer under an electric field 200 V/cm for 2 h. Samples were injected by electromigration method.

Indirect fluorescence detection was performed with a commercial spectrofluorometer (Model FL-750BX; McPherson, Acton, MA, USA) equipped with a high sensitivity accessory (HSA). The HSA consists of a CE flowcell module, special collection optics, filter holder and a photomultiplier tube (PMT). A 200-W xenon-mercury arc lamp was used as the excitation source. The excitation wavelength was set at 404 nm and the fluorescence emission was monitored after passing a 515 nm cut-off filter. The PMT of the spectrofluorometer was operated at ca. 600 V. The electrophoregrams were recorded with either a strip-chart recorder (Model U-228; Pantos, Kyoto, Japan) or an integrator (Model C-R3A; Shimadzu, Kyoto, Japan).

### 2.2. Chemicals

Trimethyltin (TMT) chloride, triethyltin (TET) chloride, tripropyltin (TPT) chloride, tributyltin (TBT) chloride, triphenyltin (TPhT) chloride, 2-(*N*-morpholino)ethanesulfonic acid (MES) and *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) were purchased from Merck (Darmstadt, Germany). 6-Aminoquinoline (6-AQ), fluorescein, 3-(*N*-morpholine)-2-hydroxypropane-sulfonic acid (MOPSO) and *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES) were obtained from Aldrich (Milwaukee, WI, USA). All other chemicals were of analytical-reagent grade.

Stock solutions of triorganotin standards at a concentration 3.0 mM (as tin) were prepared in

HPLC grade methanol and were kept at 4°C in the dark. Working standard solutions of lower concentrations were freshly prepared by appropriate dilution with the running buffer. The electrophoresis BGE containing one of the zwitterionic buffers and 6-AQ was prepared with distilled–deionized water, obtained using a NANOpure II system (Barnstead, Dubuque, IA, USA). The BGE was adjusted to the desired pH with dilute (ca. 0.1 M) HCl and was filtered through a 0.45- $\mu\text{m}$  membrane filter prior to use.

### 3. Results and discussion

It is generally believed that the performance of fluorometers built with non-laser excitation sources is several orders of magnitude poorer than the laser-based systems [27]. In order to test the performance of the commercial fluorometer used in the present work, a solution of 1  $\mu\text{M}$  fluorescein was electrokinetically injected into the CE system at 24 kV for 2 s. The obtained electropherogram is shown in Fig. 1. From the peak size and the background noise level, the concentration limit of detection (CLOD) for fluorescein was estimated to be ca. 100 nM, based on an  $S/N=3$ . This value corresponds to a mass LOD of 700 amol, which is about three orders of magnitude higher than that obtained with an argon ion laser-based fluorescence detector built in our laboratory [28].

In indirect fluorometry, a fluorescing running buffer is used. A reduction in the background fluorescence intensity occurs when a sample zone elutes past the detector, because the analyte ions displace the fluorescent buffer ions. For the separation and detection of cations, such as the triorganotin species studied in this work, an electrophoretic buffer containing a cationic fluorophore should be employed. 6-Aminoquinoline (6-AQ;  $pK_a=4.9$ ) was selected as the background fluorophore because it mainly exists as a cation under acidic condition. Besides, the excitation maximum (~400 nm) of 6-AQ at pH 4.0 well matches one of the intense mercury resonance lines (404 nm) of the xenon–mercury arc source. Fig. 2 shows the excitation and emission spectra of 6-AQ at pH 4.0. While a bathochromic shift of the excitation maximum was observed with a decreasing solution pH, the emission

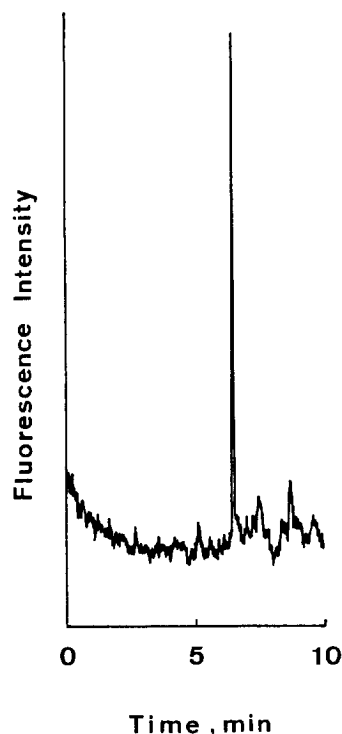


Fig. 1. Electropherogram of 1  $\mu\text{M}$  fluorescein. Column, 50  $\mu\text{m}$  I.D.  $\times$  360  $\mu\text{m}$  O.D.  $\times$  64 cm total length (55 cm to the window); 15 mM borate buffer (pH 9.0); separation voltage, 400 V/cm (7.6  $\mu\text{A}$ ); sample injection, 2 s at 24 kV;  $\lambda_{\text{ex}}=488$  nm, emission monitored with a 515 nm cut-off filter; PMT voltage, 650 V.

maximum (540 nm) was very stable. In order to block the scattered excitation light and to achieve high fluorescence transmission, a 515 nm cut-off filter was placed in front of the PMT.

In this study, aqueous triorganotin cations were separated under coelectroosmotic CE conditions, that

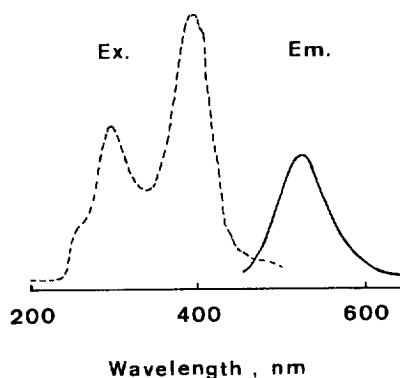


Fig. 2. Excitation and emission spectra of 6-AQ at pH 4.0.

is, the mobility vectors of cationic analytes and electroosmotic flow (EOF) have the same direction. Since the capillary wall is negatively charged, adsorption of cationic species onto the capillary surface due to columbic and hydrophobic interactions is a problem. Various salts, zwitterions, and surfactants have been used in buffers to compete with analytes for adsorption sites and to minimize analyte-wall interactions [29–31]. Alternatively, permanently coating the capillary wall with a variety of hydrophilic polymers to block the adsorption sites has also been attempted [32]. Due to its simplicity and viability, dynamic modification of the capillary wall with a zwitterionic buffer is used in the present work.

Several zwitterionic buffers were examined, which include MES, MOPSO, TES and HEPES. HEPES was found to be the optimal, judging from the separation efficiency and the background stability. With a  $pK_a$  of 7.56, HEPES will exist mainly as neutral species in an acidic aqueous media. The HEPES molecules will dynamically coat the capillary wall, and hence reduce both the surface charge and the EOF velocity. Its zwitterionic character also generates a low electrophoretic current. With an excitation wavelength 404 nm, the background fluorescence intensity of 6-AQ was found to vary with the pH of the HEPES buffer, as shown in Fig. 3. The maximum intensity appears at pH 4~5. In order to keep 6-AQ mainly as cationic species and also to achieve the maximum sensitivity, all later studies were performed with an electrophoresis buffer at pH 4.0.

The effect of the HEPES concentration on the migration time of the five triorganotin compounds, viz., TMT, TET, TPT, TBT and TPhT, is shown in Fig. 4. With increasing HEPES concentrations, the effective mobilities of triorganotins decrease and migration times increase. This is mainly due to the decreasing  $\zeta$ -potential of the capillary, caused by adsorption of the neutral HEPES zwitterions onto the capillary wall, which in turn lowers the EOF velocity. Separation also improves as HEPES concentration increases. A typical separation pattern of the five triorganotin compounds in a 70 mM HEPES buffer at pH 4.0 is shown in Fig. 5. The electrophoretic current under a field strength 300 V/cm was only 4  $\mu$ A and complete separation of five tri-

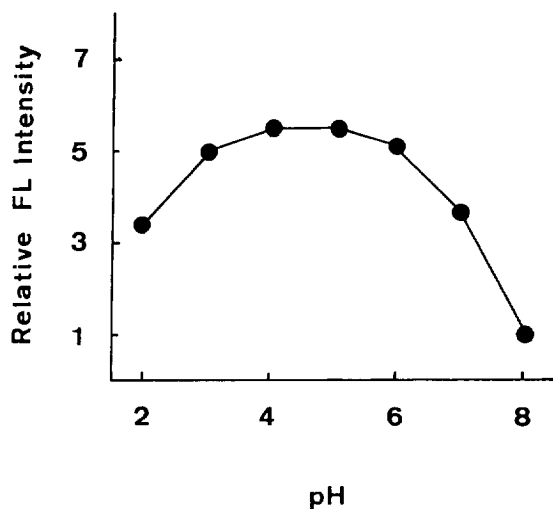


Fig. 3. Variation of the background fluorescence intensity with the buffer pH. BGE solution, 50 mM HEPES containing 1 mM 6-AQ;  $\lambda_{ex}$  = 404 nm, emission monitored with a 515 nm cut-off filter.

organotins was finished in 15 min. In Fig. 5, the system peak at ca. 24 min corresponds to the migration of EOF, which is always the last peak to elute and thus signifies the end of the electropherogram. This peak was attributed to the slight mismatch between the sample solution and the electrophoresis BGE because the stock solutions of analytes were originally prepared with methanol while the running buffer contains no methanol. The origin of the negative system peak at ca. 10 min is not clear. Similar observation of an extra system peak in CE-indirect UV detection of amino acids with a zwitter-

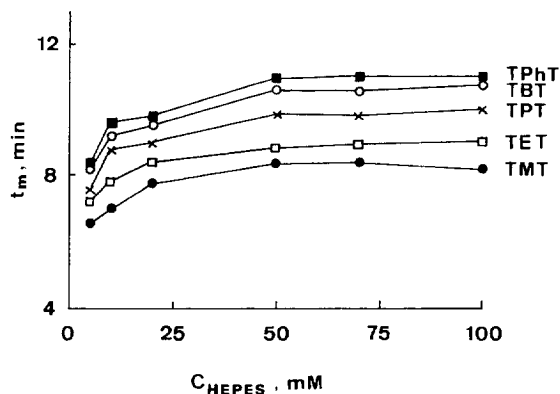


Fig. 4. Dependence of the migration times of triorganotin compounds on the HEPES concentration in BGE solution.

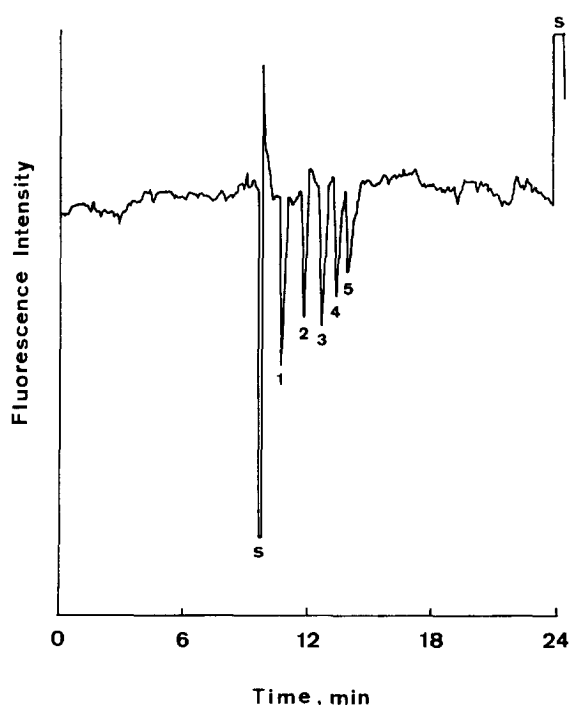


Fig. 5. Electropherogram of five triorganotin compounds with indirect fluorescence detection. BGE solution, 70 mM HEPES (pH 4.0) containing 1 mM 6-AQ; separation voltage, 300 V/cm (4  $\mu$ A); sample injection, 3 s at 18 kV;  $\lambda_{ex}$  = 404 nm, emission with 515 nm cut-off filter, PMT voltage, 610 V. Peak identities (concentration as tin): (1) TMT (150  $\mu$ M); (2) TET (100  $\mu$ M); (3) TPT (74  $\mu$ M); (4) TBT (55  $\mu$ M); (5) TPhT (40  $\mu$ M).

terionic buffer, 3-(cyclohexylamino)-propanesulfonic acid (CAPS), has also been reported [33].

It has been reported that the use of mixed organic/

water running buffers with high percentages of organic modifier can significantly improve the sensitivity for some fluorescent dyes in CE, due to enhanced photophysical properties [34]. This phenomenon was not observed in the present study. Neither separation efficiency nor detection sensitivity improves as the methanol content in HEPES buffers varies in the range 0–60%. The only effect observed was a steady increase of the migration times of all analytes with an increasing methanol content. This can be explained by the reduction of EOF with an increasing fraction of organic solvent in the BGE [35].

A series of solution mixtures containing a known amount of the five triorganotin compounds was prepared, using the electrophoresis buffer as the solvent. These standard solutions were used for the construction of calibration graphs. The results are summarized in Table 1. Within the concentration range studied, a good linear correlation ( $r \geq 0.995$ ) between peak height and concentration was obtained for each analyte. The CLODs listed in Table 1 were calculated based on an  $S/N=3$ , which range from 8  $\mu$ M (as tin) for TPhT to 18  $\mu$ M (as tin) for TMT. These values correspond to a mass detection range 80–180 fmol (or 10–22 pg) tin and are more than one order of magnitude lower than those obtained with UV absorption detection [22,23]. These CLOD were obtained using an electrophoresis buffer containing 1 mM 6-AQ. In principle, the CLOD of a given indirect detection scheme can be related to  $C_M$  (the concentration of the BGE additive that is being monitored), TR (transfer ratio, defined as the number

Table 1  
Calibration data for the five triorganotin compounds

Compound	Linear range ( $\mu$ M as tin)	Correlation coefficient	CLOD <sup>b</sup> ( $\mu$ M as tin)	RSD (%) <sup>c</sup>		
				Height	Area <sup>d</sup>	Time
Trimethyltin	18–600	0.999	18	8.6	8.8	1.5
Triethyltin	17–410	0.995	17	4.7	5.2	1.3
Tripropyltin	15–300	0.996	15	3.5	4.1	2.0
Tributyltin	10–220	0.995	10	4.8	4.5	2.1
Triphenyltin	8–160	0.997	8	5.9	6.3	1.8

<sup>a</sup> Correlation coefficient.

<sup>b</sup> Concentration limit of detection;  $S/N=3$ .

<sup>c</sup> Relative standard deviation, based on seven measurements with replicate injections of each species at the concentration corresponding to five times the detection limit.

<sup>d</sup> Corrected area (area/migration time).

of molecules of the BGE additive displaced by each analyte molecule) and DR (dynamic reserve, which is equal to the signal-to-noise ratio of the background signal) by [21]

$$\text{CLOD} = C_M / (\text{TR} \times \text{DR})$$

Therefore, the CLOD can be lowered by decreasing  $C_M$ . However, the three parameters are not necessarily independent [25]. Under some circumstances as one reduces  $C_M$ , TR and/or DR can also decrease, causing no improvement in detection sensitivity. In the present study, the CLOD of triorganotins were found to be about three times higher than those listed in Table 1 if the concentration of 6-AQ in the electrophoresis buffer decreased from 1 mM to 0.1 mM.

The repeatability of the peak size and the migration time were examined by seven replicate injections of each analyte at a concentration level equal to five times the respective detection limit. The relative standard deviations (R.S.D. values) on peak height range from 3.5% for TPT to 8.6% for TMT (see Table 1). Slightly poorer precisions were obtained with peak area measurement, even with the corrected area (area/migration time). The R.S.D. values on migration time are in the range 1.3–2.1%.

#### 4. Conclusion

An indirect fluorescence detection method for CE of triorganotin compounds has been developed. The five triorganotins, viz., TMT, TET, TPT, TBT and TPhT, can be completely separated with a buffer consisting of 70 mM HEPES and 1 mM 6-AQ at pH 4.0. Indirect fluorescence detection was performed using a commercial fluorometer built with a 200-W xenon–mercury arc lamp as the excitation source. In addition to its simplicity in instrumentation, the major advantage of this indirect fluorescence-detection method is that the tedious pre- or postcolumn derivatization of analytes is not required. The CLOD of the five triorganotins studied were between 8 and 18  $\mu\text{M}$  (as tin), which correspond to a mass detection range 80–180 fmol tin. On the other hand, despite the fact that this detection scheme is over ten times more sensitive than UV absorption detection, its

concentration sensitivity is still not adequate for routine environmental analyses in which a sub-nM detection level is generally required. Besides, due to the non-selective characteristic of indirect detection mode and the complex matrices of most environmental samples, possible interferences on CE–indirect fluorometry of triorganotins also need to be further investigated. Improvement of the technique by employing off-line and on-line sample preconcentration methods is under study in our laboratory.

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