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# Capillary electrophoretic separation of organophosphonic acids using borate esterification and direct UV detection

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## Abstract

One major challenge in the analysis of small ions by capillary zone electrophoresis (CZE) is detection. The most common commercially available detector for CZE is based on UV absorbance. For many small molecules, however, little UV absorbance occurs above 210 nm, limiting the usefulness of this detection method. Carbohydrates, alcohols and amides have been separated and directly detected by complexation with sodium borate. It has been shown that these complexes have UV absorbances in the range of 220 to 280 nm, whereas the analytes alone are UV transparent at energies less than 200 nm. Separation and direct detection of organophosphonic acids using sodium borate as both a buffer and derivatization agent is demonstrated. Detection limits on the order of nanograms are reported with separations that exhibit 10 000 to 1 740 000 theoretical plates. The ultraviolet, infrared, nuclear magnetic resonance and mass spectra of various borate/phosphonic acid esters are explored.

## 1. Introduction

Organophosphorus compounds are used as pesticides, herbicides and fertilizers, and thus are released to the environment in multi-ton levels each year [1,2]. These organophosphorus compounds degrade in the environment and often produce phosphonic acids and phosphonic acid esters [1-3]. Traditionally, these compounds have been analyzed by derivatization gas chromatography (GC), ion chromatography (IC) and ion-pairing reversed-phase high-performance liquid chromatography (HPLC) [4-6]. Direct analysis of real-world samples using IC has proven to be difficult due to interference by common ions such as chloride and fluoride and by the limited resolution offered by IC. Capillary zone electrophoresis (CZE) has been shown to have higher

resolution and better detection limits for many ionic compounds as compared to IC [7,8]. CZE separations often exhibit 50 000 to over 1 000 000 theoretical plates [9,10]. IC usually produces separations with no more than 5000 theoretical plates [11]. The theoretical resolution of a separation varies as the square root of the number of theoretical plates, thus increasing the number of theoretical plates from 5000 to 500 000 would increase the resolution by a factor of ten [12]. Thus, it is possible that some of the limitations encountered in IC analyses can be overcome by using CZE [13,14].

One major challenge in the analysis of small ions by CZE is detection. The most common, commercially available detector for CZE is based on UV absorbance. In the case of some small molecules, almost all UV absorbance occurs below 210 nm making this detection method difficult at best. A number of other detectors

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based on amperometry, refractive index, mass spectrometry or fluorescence have been used to detect non-UV-absorbing compounds but most are not commercially available or common [15–17].

One method for detecting non-UV-absorbing compounds is to form complexes in solution where the resulting complex is UV absorbing. Carbohydrates, alcohols and amides have been separated and detected by forming borate–carbohydrate complexes [18–24]. It has been shown that these complexes have UV absorbances in the range of 220 to 280 nm, whereas the carbohydrates alone are UV transparent above 200 nm. In this study, the results of phosphonate–borate interactions are discussed along with their potential application for separation and detection of organophosphonic acids.

## 2. Experimental

The CZE system used in all experiments was a Beckman Model 2100. Buffers, cleaning solutions and analytes were placed in 4-ml vials and sealed with a flapped rubber cap. Boric acid was obtained from Argent Chemical Labs. (Redmond, WA, USA), sodium borate was obtained from Mallinckrodt (St. Louis, MO, USA), and sodium dodecyl sulfate (SDS) was obtained from Sigma (St. Louis, MO, USA). Methyl-, ethyl-, propyl-, butyl- and *tert.*-butylphosphonic acids (MPA, EPA, PPA, BPA and *t*-BPA, respectively) were obtained from Aldrich (Milwaukee, WI, USA). Buffers and analytes were prepared by dissolving the appropriate reagents in distilled, deionized water using standard volumetric techniques. The pH of the buffers were adjusted by addition of either HCl or NaOH stock solution. The cleaning procedure for the fused-silica capillaries was a rinse of 0.1 M NaOH followed by distilled water. Bare fused-silica 50  $\mu\text{m}$  I.D. capillaries were used in all experiments. Standards of methylphosphonic acid methyl ester (MMPA), methylphosphonic acid ethyl ester (EMPA), methylphosphonic acid isopropyl ester (IMPA) and methylphosphonic acid pinacolyl ester (PMPA) were synthesized. Concentration

levels of analytes and buffers are given later with their results.

Phosphonate–borate compounds were identified using infrared (IR) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy, and mass spectrometry (MS). NMR spectra were recorded on a Varian (Sugar Land, TX, USA) VXR 300 at 300 MHz for  $^1\text{H}$  and 121 MHz for  $^{31}\text{P}$ . External references of tetramethylsilane and trimethylphosphine oxide were used, respectively. MS analyses were performed using an in-house modified TAGA 6000 mass spectrometer (Sciex, Thorn Hill, Canada) using direct-infusion electrospray ionization in negative ion collection mode [25]. UV spectra of the analytes were recorded on a Varian Model 200 UV–Vis spectrometer. Scanning rate was 20 nm/min, slit width was 2 nm. IR spectra were recorded on a Nicolet (Madison, WI, USA) 740 Fourier transform IR system at 4  $\text{cm}^{-1}$  resolution using a SpectraTek (Stamford, CT, USA) Circle Cell with a ZnSe rod.

## 3. Results

Some simple organophosphates only absorb UV radiation below 220 nm (Fig. 1), making detection of these compounds by UV absorbance difficult. This problem has also been encountered in the analysis of carbohydrates. The possibility of forming phosphate–borate complexes that would have lower-energy electronic states was investigated. In preliminary experiments, it was noted that higher than expected absorbivities for phosphonic acid esters were observed in borate buffers of pH 9 or greater (Fig. 2). In this example, a significant increase in UV absorbance was observed between 200 and 250 nm for EMPA in a pH 9 sodium borate solution compared to EMPA dissolved in distilled water. Similar changes in the UV spectra of the simple organophosphonic acids were observed but these shifts were not as great as those observed for the esters. The observed changes in the UV spectra of phosphonic acids and their esters led to further investigations of the possi-

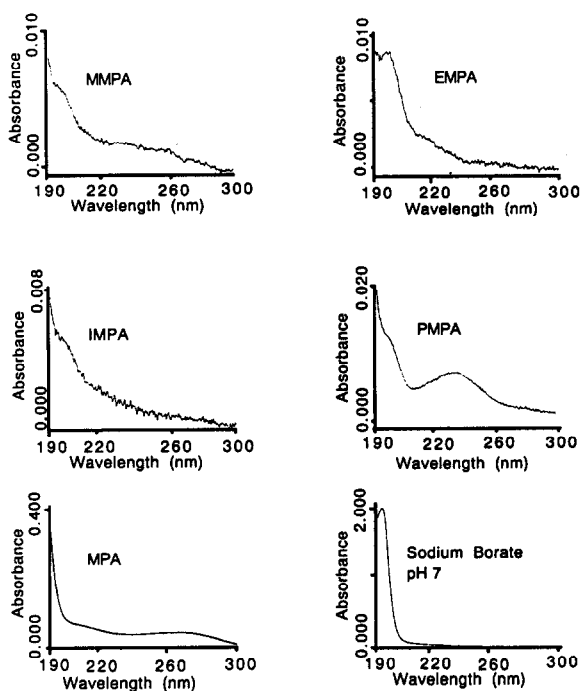


Fig. 1. Ultraviolet spectra of various organophosphonic acids dissolved in water. See Experimental section for compound identification.

bility of complex formation with the borate buffer.

Boric acid is a Lewis acid and does not

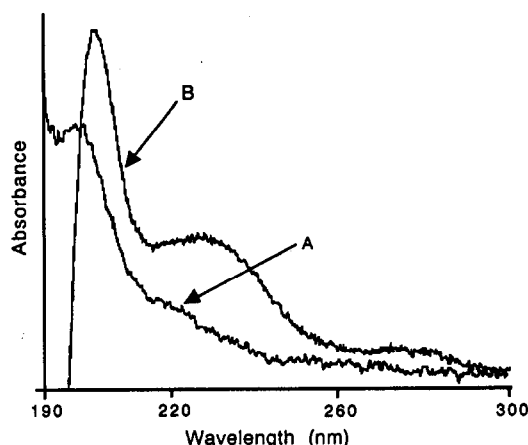
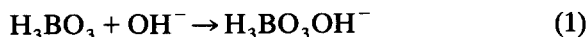


Fig. 2. UV spectra of EMPA. A = 10 mM EMPA in distilled water; B = 10 mM EMPA dissolved in 11 mM sodium borate at pH 9.2.

dissociate in water. The "acid" property is due to the following reaction:



Since this reaction does not increase the total number of ions in solution, a concentrated solution of boric acid has approximately the same conductivity as distilled water. Using high (up to 1 M) boric acid concentrations facilitates the formation of borate complexes with carbohydrates [18-22]. In order to investigate the hypothesis of forming a phosphate-borate complex or compound, IR, NMR and MS were performed on samples of MPA in high-concentration borate solutions. The mass spectrum of MPA in a 1 M boric acid solution is shown in Fig. 3. The  $(\text{MPA} \cdot \text{H}_2\text{BO}_3)^-$  ion is observed along with  $(\text{MPA} - \text{H})^-$ . From these mass spectral results it is not possible to determine if the association of borate with MPA occurred in solution or was produced in the gas phase during sample injection. Thus, the result was consistent with, but not conclusive evidence for, forming a solution-phase complex or compound [26].

The IR spectrum of MPA along with its salts have been reported by Van der Veken and Herman [27,28]. When MPA is dissolved in water, the observed IR spectrum is pH dependent due to the relative concentrations of each MPA salt. The IR spectrum of borate is complicated in a similar manner with the addition of

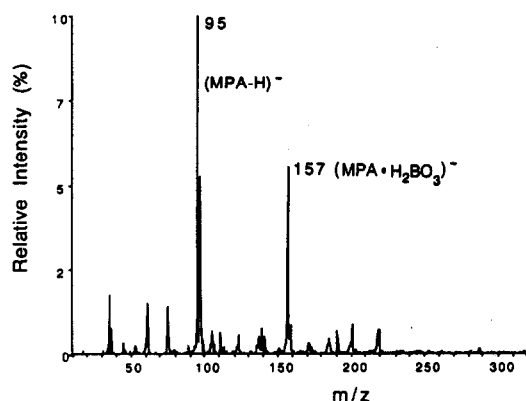


Fig. 3. Mass spectrum of MPA in 1 M boric acid. Direct infusion using electrospray sample introduction. Negative ion collection mode.

Table 1  
Infrared absorbances of MPA

IR Absorbance ( $\text{cm}^{-1}$ )		Description
Present study	Van der Veken [27]	
1318	1319	$\text{CH}_3$ rock in MPA
1313	1312	$\text{CH}_3$ rock in $\text{MPA}^-$
1150	1150	P=O stretch
1058	1060	$\text{PO}_2$ <i>sym</i> stretch in $\text{MPA}^-$
1009	1005	$\text{P}(\text{OH})_2$ <i>antisym</i> stretch in MPA
958	950	$\text{P}(\text{OH})_2$ <i>sym</i> stretch in MPA
927	925	$\text{P}(\text{OH})_2$ <i>sym</i> stretch in $\text{MPA}^-$

polymeric forms of borate present in solutions where the total concentration of borate is above 25 mM [29,30]. Table 1 shows the observed IR absorbances of MPA in distilled water along with the normal mode assignments. The observed absorbances agreed with those previously reported [27,28]. Fig. 4 shows the IR spectra of MPA with and without sodium borate: spectrum A shows the spectrum of MPA in distilled deionized water and spectra B and C show MPA in pH 7.7 and pH 9 borate, respectively. When these spectra are compared, the P=O stretch at  $1150\text{ cm}^{-1}$  is observed to decrease and eventually disappear as the pH of the borate solution is

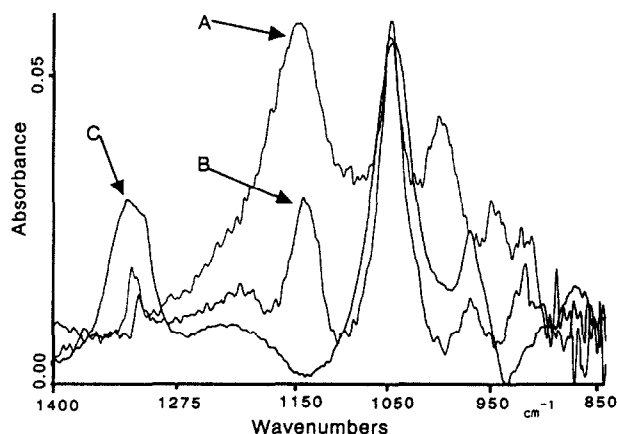


Fig. 4. IR spectra of 21.9 mM MPA. A = MPA in distilled water; B = MPA in pH 7.7, 260 mM borate; C = MPA in pH 9, 270 mM borate. Resolution is  $4\text{ cm}^{-1}$  with 200 scans co-added.

increased. The intensity of the  $\text{PO}_2$  symmetric stretch at  $1058\text{ cm}^{-1}$  remains almost constant with only slight broadening observed. The  $\text{CH}_3$  rock at  $1318\text{ cm}^{-1}$  increases in intensity, broadens and moves to higher frequency.

The above changes in IR spectra are consistent with the formation of a borate ester (a borate compound, rather than a complex) such as shown in Fig. 5. This proposed structure is based on the loss of the P=O absorbance, as evidenced from the increased intensity of the  $\text{CH}_3$  rocking absorbance and maintenance of the  $\text{PO}_2$  symmetric stretch absorbance. The IR absorbances observed for the borate ester are shown in Table 2. Boric acid is known to react with both phosphoric acid and acetic acid to form stable compounds [31]. Thus, the formation of a stable compound is consistent with previous observations.

Similar results as those obtained for MPA

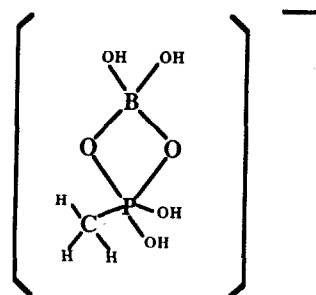


Fig. 5. Proposed MPA-borate compound.

Table 2  
Infrared absorbances of the methylphosphonic acid-borate compound

IR (cm <sup>-1</sup> )	Description
1322	CH <sub>3</sub> rock with hydrogen bonding to the borate molecule
1055	PO <sub>2</sub> sym stretch
978	PO <sub>3</sub> motion
870	CH <sub>3</sub> motion

were observed when EPA, PPA, BPA and t-BPA acids were dissolved in high-pH borate solutions. Fig. 6 shows the IR spectra of BPA acid in distilled water and pH 9 sodium borate. Again, the P=O stretch disappears and a CH<sub>2</sub> rocking motion appears.

To further investigate the structure of the borate compound, <sup>31</sup>P and <sup>1</sup>H NMR spectroscopy were performed. Fig. 7 shows the <sup>31</sup>P NMR spectra of MPA under various conditions. The signal from the phosphorus atom shifted 8.8 ppm when Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub> was added to the solution of MPA. Addition of KOH instead of Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub> produced a different shift to further down field. The proton NMR showed the same shift when either Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub> or KOH was added (data not shown). This result is interpreted to indicate that little to no hydrogen bonding is occurring in the

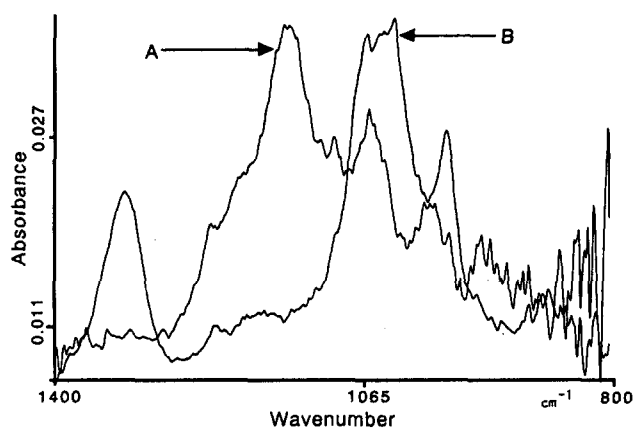


Fig. 6. IR spectra of butylphosphonic acid. A = Butylphosphonic acid in distilled water; B = butylphosphonic acid in 250 mM Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub> at pH 9.

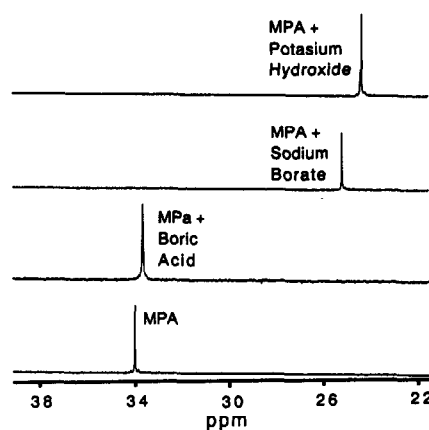


Fig. 7. <sup>31</sup>P NMR spectra of methylphosphonic acid under a variety of conditions.

borate-phosphonic acid compound [32-34]. It is concluded that the broadening and increase in intensity of the CH<sub>2</sub> wag motion observed in the infrared spectra is due to a change in the bond strength of the C-P bond resulting from the loss of the P=O bond, and not from hydrogen bonding. Further studies are required to more completely elucidate the structure of the proposed phosphate-borate ester.

These results indicate that the formation a phosphate-borate ester under the conditions used in CZE was possible. The formation of a borate ester shifts the UV absorbance to lower wavelength, facilitating detection of the phosphate. While this is in essence a derivatization technique, it can be performed in-situ and is specific to phosphates. It was suggested by the work of Hoffstetter-Kuhn et al. [35] that raising the temperature of the buffer solution may favor formation of borate complexes. Also, since most reaction rates increase with increased temperature, the effect of temperature on the formation of the borate-phosphate compound was investigated.

Fig. 8 shows electropherograms of PMPA and IMPA at various temperatures between 25 and 50°C. The spikes observed on the PMPA and IMPA peaks are reproducible and are temperature dependent. In general, narrower, more Gaussian peaks are observed at higher tempera-

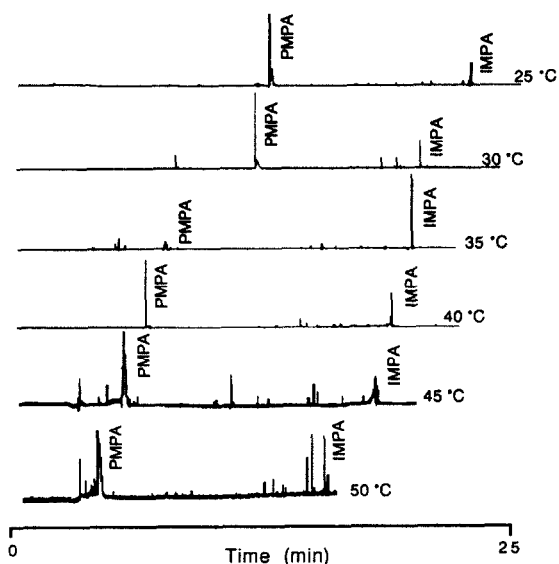


Fig. 8. CZE Separation of PMPA and IMPA in 100 mM  $H_3BO_3$ -10 mM  $Na_2B_4O_7$  at various temperatures. Column is 77 cm (67 cm to detector)  $\times$  50  $\mu$ m I.D.; 15 kV applied with 2.8  $\mu$ A, UV detection at 254 nm.

tures along with shorter elution times. Unfortunately, higher noise is also observed at higher temperatures. This is especially true at 50°C where many noise spikes are common. Note that detection is at 254 nm, which is above the absorbance region for IMPA alone.

The formation of the borate-phosphonic acid ester is especially useful in the separation and detection of MPA and other simple organophosphonic acids. Fig. 9 shows the separation of EPA from MPA. Note that detection was at 214 nm, above the normal absorbance of either MPA or EPA. This demonstrates that the UV spectra of phosphonic acids are shifted sufficiently for direct detection at 214 nm. Fig. 10 shows a separation using indirect UV detection of the same compounds. The electroosmotic flow has been reversed in the indirect UV detection method but it is still possible to compare the number of theoretical plates observed for both methods. The number of theoretical plates for EPA and MPA using both methods are shown in Table 3. Two to three orders of magnitude more theoretical plates were observed for the direct detection method than for the indirect detection method for these compounds.

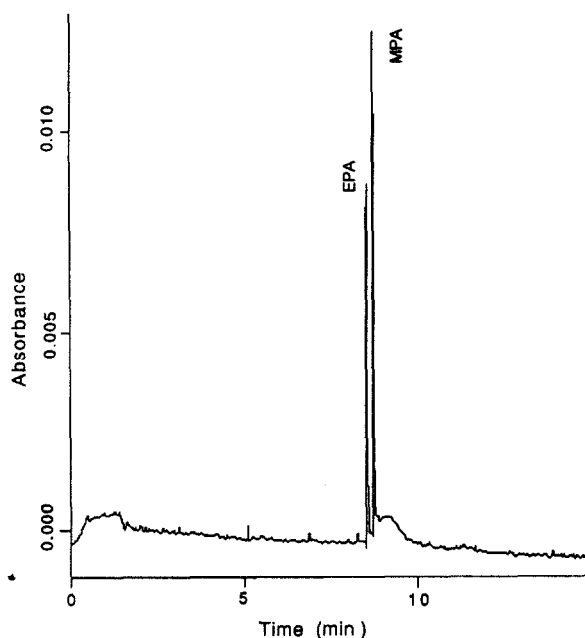


Fig. 9. Electropherogram of MPA and EPA. Buffer is 10.5 mM  $Na_2B_4O_7$  at pH 7.2. Column is 67 cm (57 cm to detector)  $\times$  50  $\mu$ m at 50°C; UV detection at 214 nm; 30 kV applied with detector at cathode.

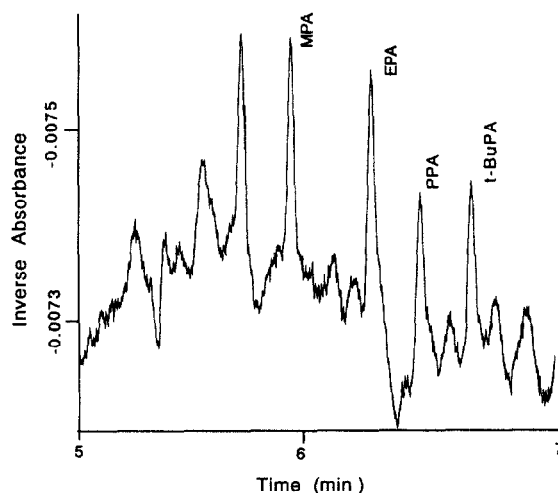


Fig. 10. Electropherogram of organophosphonic acids. Analysis performed on a Waters Quanta 4000. Column 60 cm (50 cm to detector)  $\times$  75  $\mu$ m I.D. fused-silica at 15 kV. Buffer is 5 mM sorbate, 0.5 mM OFM-BT (Waters Chromatography NICE-Pak<sup>TM</sup>, a proprietary solution), pH 6.8. Detection at 254 nm. Concentrations: MPA = 300 ppb (w/w); EPA = 300 ppb (w/w); PPA = 300 ppb (w/w); t-BuPA = 300 ppb (w/w). Electropherogram courtesy of Waters Chromatography (Marlborough, MA, USA).

Table 3  
Comparison of number of theoretical plates using direct and indirect UV detection

Compound	Detection method	No. of theoretical plates
MPA	Indirect	40 000
MPA	Direct	229 000
EPA	Indirect	55 000
EPA	Direct	1 740 000

#### 4. Conclusions

Combining the information obtained from the various spectroscopic investigations, a buffer system for direct UV detection of various organophosphonic acids was developed. Formation of borate-phosphate esters occurs at pH 7 or greater and the esters have significant UV absorbance at wavelengths greater than 200 nm. This method allows the direct separation and detection of organophosphonic acids using commercially available equipment. The observed number of theoretical plates for this technique was significantly greater than for a separation involving indirect detection.

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